CSM Canadian Society of Microbiologists
SCM Société canadienne des microbiologistes

69TH ANNUAL CONFERENCE OF THE CANADIAN SOCIETY OF MICROBIOLOGISTS

Sherbrooke, Quebec
June 10-13, 2019

69e CONGRÈS ANNUEL DE LA SOCIÉTÉ CANADIENNE DES MICROBIOLOGISTES

Table of Content

Mot de Bienvenue des Membres du Comité Local d'Organisation

Nous sommes très heureux de vous accueillir à Sherbrooke pour la 69e Conférence annuelle de la Société canadienne des microbiologistes (CSM 2019). Avec plus de 500 inscriptions, CSM 2019 s'annonce déjà comme un succès.

Cette année, nous sommes honorés d'accueillir le Pr. Rodolphe Barrangou pour notre conférence d'ouverture. Pr. Barrangou, de la NC State University, est un spécialiste de classe mondiale de la biologie et de la génétique des systèmes immunitaires bactériens CRISPR-Cas. Pr. Barrangou traitera des impacts de ce champ transformateur sur la science et la société. Son intervention sera ouverte à l'ensemble de la communauté scientifique et au public, en plus de tous les participants à la conférence CSM 2019.

Notre programme scientifique couvre les trois sections de la Société canadienne des microbiologistes: Microbiologie Appliquée et Environnementale (AEM), Infection et Immunité (I&I), ainsi que Microbiologie Cellulaire et Génétique Moléculaire (MGCM). Cette année, nous avons choisi de mettre en évidence les multiples facettes de la relation entre les microorganismes, l'environnement et la santé animale et humaine à travers le concept de Santé Globale, qui sera articulé autour de cinq symposiums dédiés. Les développements récents en Microbiologie Appliquée et Environnementale sont abordés dans des sessions sur les microbes industriels et la biotechnologie, les plantes et les microbes, les microbiomes environnementaux, la chimosphère microbienne et la microbiologie alimentaire. Enfin, deux sessions sont consacrées à la biologie des systèmes et à la biologie synthétique, sujets d'actualité qui ont pris une ampleur considérable dans la microbiologie moderne ces dernières années. Nous sommes ravis d'accueillir 12 conférenciers distingués pour présenter ces symposiums.

Nous sommes très reconnaissants aux présidents de symposiums pour leurs efforts dans la sélection de conférences qui représentent la diversité de jeunes chercheurs talentueux et l'ampleur de la recherche en microbiologie au Canada. Nous tenons également à remercier les présidents de section I&I, AEM et MGCM, ainsi que les membres du comité Formation et carrières de la SCM, pour leur sélection de résumés exceptionnels pour les symposiums de section, les concours d'étudiant(e)s et les compétitions d'étudiant(e)s postdoctoraux.

Nous espérons sincèrement que votre expérience à Sherbrooke et dans les Cantons-de-l'Est vaudront le détour! Sherbrooke est réputée pour la beauté de son centre-ville, avec ses peintures murales, le lac des Nations, ses nombreux parcs et pistes cyclables, ainsi que pour la qualité de ses restaurants locaux et de ses microbrasseries. Grâce à Destination Sherbrooke, nous sommes également heureux d'offrir à tous les participants à CSM 2019 les transports gratuits en bus pendant toute la durée de la conférence. Il vous suffit de présenter le laissez-passer de la STS disponible à votre hôtel ou au kiosque d'inscription.

Nous vous souhaitons une excellente réunion scientifique, productive et engageante, ainsi qu'une agréable visite de la ville de Sherbrooke et des Cantons-de-l'Est.



Sincèrement,

Pascale Beauregard
Louis-Charles Fortier
Vincent Burrus
Coorganisateurs de la conference SCM 2019

Welcome from the Local Organizing Committee Chairs

We are very pleased to welcome you in Sherbrooke for the 69th Annual Conference of the Canadian Society of Microbiologists (CSM 2019). With more than 500 registrations, CSM 2019 is already promising to be a success.

This year, we are honored to welcome Pr. Rodolphe Barrangou as our opening keynote speaker. Pr. Barrangou, from NC State University, is a world-class specialist on the biology and genetics of CRISPR-Cas immune systems in bacteria. Pr. Barrangou will address the impacts this transformative field has had on science and society. His talk will be open to the broad scientific community and the public, in addition to all CSM 2019 attendees.

Our scientific program covers all three sections of the Canadian Society of Microbiologists: Applied & Environmental Microbiology, Infection and Immunity as well as Molecular Genetics & Cellular Microbiology. This year, we chose to highlight multiple aspects of the relationship between microorganisms, the environment and animal and human health through the One Health concept, which will be articulated in five One Health-related symposia. Recent developments in Applied and Environmental Microbiology are covered in sessions about industrial microbes and biotechnology, plants and microbes, environmental microbiomes, microbial chemosphere and food microbiology. Finally, two sessions are dedicated to systems and synthetic biology, hot topics that have gained considerable momentum in modern microbiology in the past few years. We are delighted to welcome 12 distinguished keynote speakers to introduce these sessions.

We are very grateful to our session chairs for their efforts in selecting talks that represent the diversity of talented young investigators and the breadth of microbiology research in Canada. We also want to acknowledge the efforts of the I&I, AEM and MGCM section chairs as well as the members of the CSM Education and Careers committee for their selection of outstanding abstracts for the section symposia, student competitions and postdoctoral competition symposium.

We sincerely hope that your experience in Sherbrooke and the Eastern Townships will be worth the trip! Sherbrooke is renowned for the beauty of its downtown area, with its city murals, the Lac des Nations, numerous parks and bike tracks, and for the quality of its local restaurants and microbreweries. Thanks to Destination Sherbrooke, we are also happy to offer to all CSM 2019 attendees complimentary city bus transportation for the whole duration of the conference. You only need to present the STS bus pass that will be available at your hotel or during registration.

We wish you a great, productive and engaging scientific meeting, and a delightful visit of the city of Sherbrooke and the Eastern Township area.

Sincerely,

Pascale Beauregard
Louis-Charles Fortier
Vincent Burrus
CSM 2019 conference co-chairs



Program at a Glance

Coffee Breaks and Lunch: Main lobby - Centre Culturel - B3 building (**ML-B3**)
Poster Sessions: Parterres des Foyers Mont-Bellevue (**PMB**) et Mont-Orford (**PMO**)
Exhibitors: Balcons des Foyers Mont-Bellevue (**Bellevue**) and Mont-Orford (**Orford**)
Plenary and Award Lectures: Maurice O'Bready room (**O'Bready**) – Centre Culturel - B3 building
NSERC, FOME & Professional Development Workshops: Faculté des sciences – D7 building, room 3021 (**D7-3021**)

Day 1	Monday, June 10, 2019	Location	Session
8:00 AM	Registration	ML-B3	
8:30 AM	FOME Workshop	D7-3021	Chairs: T. Noel & J. Libertucci
9:00 AM	CSM Executive Meeting	Bellevue	
11:00 AM	CSM Council Meeting	Bellevue	
1:30 PM	Professional Development Workshop	D7-3021	Chair: T. de Kievit
4:30 PM	NSERC Grant Information Session	D7-3021	Presenters: M.-C. Jobin & E. Megyeri-Lawless
6:00 PM	Welcome from CSM 2019 Co-chairs	O'Bready	P. Beauregard, L.-C. Fortier & V. Burrus
6:15 PM	Plenary Lecture	O'Bready	**Rodolphe Barrangou**
7:15 PM	Welcome Reception	ML-B3	
Day 2	**Tuesday, June 11, 2019**	**Location**	**Session**
8:00 AM	Registration	ML-B3	
8:30 AM	**S1**: One Health – Antibiotics and resistance, from animal production to public health I	Orford	Keynote: M.R. Mulvey |Chair: F. Daigle
	S2: Industrial microbes and biotechnology	Bellevue	Keynote: E. Edwards | Chair: C. Beaulieu
10:00 AM	Coffee Break	ML-B3	*Refreshments will be served*
10:45 AM	**S3**: One Health – Antibiotics and resistance, from animal production to public health II	Orford	Keynote: H. Barkema | Chair: M. Jacques *Sponsored by: Op+lait*
	S4: Systems and synthetic biology I	Bellevue	Keynote: S. Rodrigue | Chair: J.-P. Côté
12:15 PM	Lunch and Exhibitors	ML-B3	
12:45 PM	DNA Genotek workshop *A higher standard for microbiome studies: Optimizing your study design*	Bellevue	Presenter: H. Breton
1:30 PM	Student Symposium I	O'Bready	Chairs: D. Bay & J. Neufeld
3:00 PM	Poster Session I and Exhibitors	PMB PMO	*Coffee Break and Cash Bar Refreshments will be served*
3:15 PM	Op+lait workshop	Bellevue	Presenters: C.F. Prada, F. Malouin, J. Ronholm
5:00 PM	Thermo Fisher Award Lecture	O'Bready	**Joe Harrison** *Sponsored by: Thermo Fisher Scientific*
6:00 PM	Student Mixer & ASM Networking	Sciences Atrium	Atrium Sciences de la Vie D7-D8 building, 1st Floor

Day 3	Wednesday, June 12, 2019	Location	Session
8:00 AM	Registration	ML-B3	
8:30 AM	**S5**: One Health – Climate change, vector-borne diseases and emerging pathogens	Orford	Keynote: N.H. Ogden |Chair: A. Carignan
	S6: Environmental microbiomes	Bellevue	Keynote: A. Culley | Chair: A. Sellam
10:00 AM	Coffee Break	ML-B3	*Refreshments will be served*
10:45 AM	**S7**: One Health – Microbiome, microbial interactions and molecular pathogenesis	Orford	Keynote: C.F. Maurice | Chair: A. Menendez *Sponsored by: CIHR III*
	S8: Food microbiology	Bellevue	Keynote: J. Goulet | Chair: S. Labrie
12:15 PM	Lunch and Exhibitors	ML-B3	
12:45 PM	Illumina workshop *Recent advances in microbial genome sequencing and microbiome profiling using Illumina technology*	Bellevue	Presenter: M. Keddache
1:30 PM	Student Symposium II	O'Bready	Chairs: D. Bay & J. Neufeld
3:00 PM	Poster Session II and Exhibitors	PMB PMO	*Coffee Break and Cash Bar Refreshments will be served*
4:30 PM	Exhibitors Door Prize Draw	O'Bready	
5:00 PM	Armand Frappier Outstanding Student Award Lecture	O'Bready	**Yogesh Hooda** *Sponsored by: Canadian Society of Microbiologists*
6:00 PM	CSM Annual General Meeting	O'Bready	*All CSM Members welcome*
8:00 PM	Excursion to Foresta Lumina	Coaticook	*Transportation will be provided (front of B1-B2)*
Day 4	**Thursday, June 13, 2019**	**Location**	**Session**
8:00 AM	Registration	ML-B3	
8:30 AM	**S9**: One Health – Alternatives to antibiotics	Orford	Keynote: T. Louie |Chair: F. Malouin
	S10: Plants and microbes	Bellevue	Keynote: A.M. MacLean | Chair: P. Beauregard
10:00 AM	Coffee Break	ML-B3	*Refreshments will be served*
10:45 AM	**S11**: Microbial chemosphere	Orford	Keynote: C.S. Sit | Chair: J.-P. Bellenger
	S12: Systems and synthetic biology II	Bellevue	Keynote: C.A. Gross | Chair: S. Rodrigue
12:15 PM	Lunch	ML-B3	
1:30 PM	I&I Section Symposium	Orford	Chair: S. Faucher | Vice-Chair: G. Cox
	AEM Section Symposium	O'Bready	Chair: S. Short | Vice-Chair: L. Hug
	MGCM Section Symposium	Bellevue	Chair: T. Raivio | Vice-Chair: A. Hynes
3:00 PM	Coffee Break	ML-B3	*Refreshments will be served*
3:30 PM	Postdoctoral Research Symposium	O'Bready	Chair: L. Nelson
5:00 PM	CSM Murray Award for Career Achievement	O'Bready	**Yves Brun** *Sponsored by: Canadian Science Publishing*
6:00 PM	Invitation for CSM 2020 Conference	O'Bready	K. Dunfield & C.M. Khursigara
7:00 PM	CSM President's Banquet	Granada	53 Wellington Nord, Sherbrooke, QC, J1H 5A9 *Transportation will be provided (STS Bus Station)*

Conference Exhibitors

The CSM Executive and the 2019 Sherbrooke Local Organizing Committee would like to thank the following organizations for exhibiting and supporting the Canadian Society of Microbiologists and its annual conference. We encourage all conference attendees to visit the exhibitors.

DNA Genotek Inc. and CoreBiome are subsidiaries of OraSure Technologies, Inc. (NASDAQ: OSUR), and provide high-quality biological sample collection products and comprehensive services for microbiome sample processing, sequencing, and bioinformatic analysis. The company's OMNIgene product line immediately stabilizes microbiota samples, ensuring an unbiased 'snapshot' of the microbial community. Self-collection kits for stool, oral, vaginal and animal sampling are available. We also offer CoreBiome microbiome services, a complete suite of offerings including sample processing, wet lab and bioinformatics analysis. For more information about DNA Genotek and CoreBiome, visit www.dnagenotek.com.

Thermo Fisher Scientific is the world leader in serving science. Our mission is to enable our customers to make the world healthier, cleaner and safer. We help our customers accelerate life sciences research, solve complex analytical challenges, improve patient diagnostics, deliver medicines to market and increase laboratory productivity. Through our premier brands – Thermo Scientific, Applied Biosystems, Invitrogen, Fisher Scientific and Unity Lab Services – we offer an unmatched combination of innovative technologies, purchasing convenience and comprehensive services.

Beckman Coulter Life Sciences develops, manufactures and services products that simplify, automate and innovate complex biomedical testing and industrial manufacturing. More than 275,000 Beckman Coulter systems are currently used in diagnostic and research laboratories and manufacturing facilities around the world. Please visit https://www.beckman.com/ to learn more.

For over four decades, **New England Biolabs** has been shaping the landscape of bioscience research by discovering, developing and supporting superior research reagents. From our founding principles – placing the advancement of science and the stewardship of the environment as our highest priorities – to our unique corporate culture, NEB's philosophy can be distilled down to three core values: passion, humility and being genuine. A supplier-of-choice for scientists across the globe, NEB offers the largest selection of recombinant and native enzymes for genomic research. While restriction enzymes remain part of our core product portfolio, our ever-expanding catalog also includes products related to PCR, gene expression, sample preparation for next generation sequencing, synthetic biology, glycobiology, epigenetics and RNA analysis. Additionally, NEB is focused on strengthening alliances that enable new technologies to reach key market sectors, including molecular diagnostics development.

Published since 1954, the **Canadian Journal of Microbiology** is a monthly journal that contains new research in the field of microbiology, including applied microbiology and biotechnology; microbial structure and function; fungi and other eukaryotic protists; infection and immunity; microbial ecology; physiology, metabolism, and enzymology; and virology, genetics, and molecular biology. It also publishes review articles and notes, on an occasional basis, contributed by recognized scientists worldwide. The Canadian Journal of Microbiology is published by Canadian Science Publishing.

CEDARLANE® (www.cedarlanelabs.com) specializes in providing high quality reagents & consumables for your lab. We offer one of the most extensive product listings in Canada, allowing our customers the opportunity to "Consolidate & Save". In addition to the ATCC collection of bacteria, viruses, fungi and protozoa, we also offer Cell Culture Media, DNA/RNA extraction and purification kits from various samples including Plant, Soil and Bacteria/Virus. Visit us for a wide range of products including antibodies, toxins, ELISA and PCR kits for various research areas including Microbiomics, Foreign and Emerging Diseases, and Synthetic Biology.

For more than 20 years, **Illumina** has aspired to improve human health by unlocking the power of the genome. Our initial products enabled researchers to explore DNA in an entirely new way. Now, through collaborative innovation with scientists and lab directors, we are leading the way one genomic breakthrough at a time using whole-genome and whole-exome sequencing. While the rate of progress continues to accelerate exponentially as we move forward towards precision medicine, we have only just begun to discover the true impact of genomics. This realization, and the discoveries we know lie ahead in microbiology, oncology, agrigenomics, complex diseases, and beyond, are what inspire us to push the boundaries of our imagination and drive innovation.

Olympus is a global technology leader, crafting innovative optical and digital solutions in medical technologies; life sciences; industrial solutions; and cameras and audio products. Throughout our nearly 100-year history, Olympus has focused on being true to society and making people's lives healthier, safer and more fulfilling. Our Life Sciences Business is dedicated to meeting and exceeding the evolving needs and expectations of life science professionals through a comprehensive range of clinical, educational and research microscopes and microscope systems. For more information, visit https://www.olympus-lifescience.com.

Milk is an established staple in our diet, as well as a vital economic driver in our society. The dairy industry shapes our rural landscape and is constantly looking for ways to exceed quality standards and customers' expectations, through research and innovation. **Op+lait**, a strategic cluster funded by FRQNT, brings together researchers from several universities and colleges, whose mission is to enhance milk quality, from farm to factory, through state-of-the-art training of highly qualified personnel, and technology and knowledge transfer to industry stakeholders. Op+lait, for a research-driven, sustainable quality milk production!

INFORS HT is your specialist for bioreactors, shaker incubators and bioprocess software. You benefit from sophisticated systems that contribute to your success by maximizing the productivity of your cell lines or microorganisms without sacrificing reproducibility.

We offer the right solutions for your applications:

- Fermentation of microorganisms (bacteria, fungi and yeasts)
- Cell culture (mammalian cells, insect cells, plant cells and algae)
- Biofuels (biodiesel and bioethanol)
- Bioprocess integration in one software platform
- Customer-specific bioreactors and incubation shakers
- Qualification of bioreactors and incubation shakers

An eye toward solutions and personal, sustainable customer relationships are our greatest strengths.

Destination Sherbrooke is the para-municipal organisation that contributes to the promotion of Sherbrooke through sustainably developing tourist attractions. A key leader in tourism development, manager of tourist sites and promotion agent for the city in different markets, the organisation stands out for its unique mission of bringing together attractions, lodging establishments and restaurant services. Its clients are regional, national and international. In 2017, tourism generated more than 5,200 jobs and $260 million is economic spin-offs.

The **Centre for the Analysis of Genome Evolution and Function (CAGEF)** is an extra-departmental unit at the University of Toronto.

CAGEF's mission is to promote interdisciplinary research in basic and applied genome biology. To do this, CAGEF provides access to state-of-the-art genomic, proteomic, and bioinformatic research platforms.

CAGEF also promotes the training and education of students and postdocs through the development and support of innovative teaching initiatives, courses, workshops and research awards.

To Explore and Exploit Microbes. The **Waterloo Centre for Microbial Research (WCMR)** is home to all microbial related research at the University of Waterloo. As such, the WCMR focuses on microbial communities and their undiscovered scientific potential. More importantly, the WCMR supports coordinated interdisciplinary research spanning the full microbial spectrum- from basic discovery research to the application of microorganisms to solve problems of economic, health, industrial and environmental impact. The WCMR is the Canadian representative on the EU Horizon 2020 MicrobiomeSupport project.

Conference Award Supporters and Sponsors

The CSM Executive and the 2019 Sherbrooke Local Organizing Committee would like to thank the following organizations for supporting the Canadian Society of Microbiologists and its annual conference.

Diamond ($10,000+)

CIHR IRSC
Canadian Institutes of Health Research
Instituts de recherche en santé du Canada

destination SHERBROOKE.com

Platinum ($5,000)

Op+lait
Regroupement pour un lait de qualité optimale

Centre SÈVE
RECHERCHE EN SCIENCES DU VÉGÉTAL

Gold ($2,500+)

DNA genotek

illumina®

ThermoFisher SCIENTIFIC

CENTRE DE RECHERCHE | CHUS

SPERO THERAPEUTICS

UNIVERSITÉ DE SHERBROOKE

Silver ($1,500)

Bronze ($750+)

Officers of the Canadian Society of Microbiologists 2018-2019

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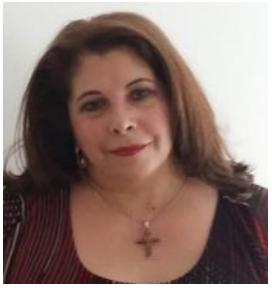

Secretariat
Mrs. Wafaa Antonious
CSM Conference Organizer
Rofail Conference and Management Services
Email: info@csm-scm.org

CSM 2019 Conference Local Organizing Committee

With special thanks to the members of our Local Organizing Committee

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Social Events, Wireless, Parking and Transportation Information

Opening Reception

Date: Monday, June 10th, 2019
Time: 7:15 PM
Location: Hall d'entrée (Main lobby) du Centre Culturel (B3)

Student Mixer and ASM/CSM Networking Event

Date: Tuesday, June 11th, 2019
Time: 6:00 PM
Location: Atrium Sciences de la Vie (1st Floor D7-D8)

Excursion Foresta Lumina

Date: Wednesday, June 12th, 2019
Time: 9:00 PM
Location: Parc de la Gorge de Coaticook
Transportation will be provided to and from the park
Bus pick-up: 8:00 – 8:15 PM, in front of the George-Cabana building (B1-B2)
Drop-off: 11:00 – 11:30 PM, Hotels and residences
Please plan to bring rain gear. Open-toed shoes are not recommended.

CSM President's Banquet & Awards Presentations

Date: Thursday, June 13th, 2019
Time: 7:00 PM
Location: Théâtre Granada
53 Wellington Nord, Sherbrooke, QC, J1H 5A9
Transportation will be provided to and from the banquet
Bus pick-up: 6:30 – 6:45 PM, STS bus stop behind the Multifunctional building (B5)
Drop-off: 10:30 PM – 12:00 AM, Hotels and residences

Poster Session I and II

Date: Tuesday and Wednesday, June 11th and 12th, 2019
Time: 3:00 – 5 :00 PM
Location: Parterres du Foyer du Mont-Bellevue et du Mont-Orford, Salon Rouge (B3 building)
Posters have to be up before 12:00 PM and taken down after 6:00 PM same day.

Poster Competition

Date: Wednesday, June 12th, 2019
Time: 3:00 – 5:00 PM
Location: Parterres du Foyer du Mont-Bellevue et du Mont-Orford, Salon Rouge (B3 building)
Presenters must to be by their poster to answer questions.

Vendor shows

Date: Tuesday and Wednesday, June 11th and 12th, 2019
Time: 12:15 – 5:00 PM
Location: Balcon du Foyer du Mont-Bellevue et du Mont-Orford (B3)

Wireless Internet Access

Centre Culturel (B3) - **Network**: ZAP
All around campus - **Network**: eduroam
For the ZAP network, please select and connect to the ZAP wireless network on your laptop or mobile device. Open your favorite browser to any page, follow the instructions and accept the conditions to gain access.

Rest/feeding room

Ladies restrooms in the conference building (B3) are equipped with changing tables. The VIP room in the same building is reserved as a rest/feeding room. Please ask one of our volunteers for access.

Parking

Please refer to the main campus map for information about Direction and Parking.
Please refer to the Université de Sherbrooke Parking Services for parking rates around campus.
Please refer to the campus map for the closest parking lot.

STS public transportation

Free public transportation is offered by Destination Sherbrooke upon presentation of the CSM2019-STS pass that will be provided to you at the CSM 2019 registration desk or at your hotel.

Workshops

DNA Genotek workshop

Tuesday June 11 - 12:45 PM - 1:15 PM
Location: Balcon du Foyer du Mont-Bellevue (B3)
Presenter: Heloise Breton

A higher standard for microbiome studies: Optimizing your study design

Empowering microbiome research by focusing on developing innovative technology has always been at the core of DNA Genotek's mission. Recently DNA Genotek and the microbiome analysis company CoreBiome joined forces to provide the research community with cutting edge yet affordable end-to-end microbiome solutions. In this workshop, we will highlight how biases can be introduced at each stage of a microbiome study (1 - donor engagement, 2 – sample processing, 3 – sequencing, 4 - data interpretation) and how optimizing workflows with the integration of DNA Genotek and/or CoreBiome products and services can help overcome these challenges.

Op+lait workshop

Tuesday June 11 - 3:15 PM - 4:15 PM
Location: Balcon du Foyer du Mont-Bellevue (B3)
Presenters: Christopher Fernandez Prada, François Malouin, Jennifer Ronholm

Research for optimal milk quality, from farm to transformation facility

Op+lait is a strategic research network dedicated to collaborative research in milk quality from farm to milk processing facility. Our scientific program is divided into three main themes: (1) On-farm interventions that affect milk quality; (2) Pathogen identification and control; (3) Milk composition and microbiota. This workshop will focus on the microbiological aspects of the various stages of milk production, such as the farm environment (bedding microbiology, fermented silage microbiology), mammary gland infection (mastitis prevention), fluctuation of the bacterial population of milk following various on-farm practices, and virulence factors of pathogens causing intramammary infections.

Illumina workshop

Wednesday June 12 - 12:45 PM - 1:15 PM
Location: Balcon du Foyer du Mont-Bellevue (B3)
Presenter: Mehdi Keddache

Recent advances in microbial genome sequencing and microbiome profiling using Illumina technology

During this workshop we will present the latest instruments and methods for bacterial whole genome sequencing and microbiome studies including high performance library preparation techniques developed at Illumina. We will discuss the difference between 16S/ITS for bacterial/ fungal sequencing and multi-kingdom shotgun sequencing for complete metagenomic profiling of bacteria, archaea, fungi, viruses, and protists. We will also introduce library enrichment techniques for targeted sequencing of microorganisms, viruses, and the detection of antimicrobial resistance genes in a variety of sample types.

A Message from the CSM FOME Committee

Welcome to CSM Forum On Microbiology Education (FOME) 2019!

It is with great pleasure that we welcome you to CSM FOME 2019, our fifth workshop on improving undergraduate microbiology education! We are excited to bring together a diverse group of microbiologists to discuss and explore innovative and effective evidence-based practices in microbiology undergraduate education.

This year, CSM FOME is excited to have the wonderful Mark O. Martin as our keynote facilitator! Mark O. Martin is currently Associate Professor of Biology at the University of Puget Sound in Tacoma, Washington. Martin is well known for incorporating social media and creative approaches in his classroom, mixing art and science in ways that students find memorable. Following the keynote workshop session, this year we are having two concurrent sessions; one will focus on assessment and the other on being an effective TA. Then, we will wrap up our CSM FOME workshop with our very popular MicroFOME sessions. These short sessions will share strategies for engaging students in authentic research, using the arts to teach students about microbiology history, and how song can be incorporated into the classroom!

The CSM FOME Committee would like to thank Vincent Burrus for all of his help and hard work. This year, organizing CSM FOME would not have been possible without him. The FOME committee would also like to thank Chris Yost, Meetings Secretary, for all of his invaluable help and continued support!

We hope you enjoy the workshop, and that when you return to your home institution, you will take with you new ideas, perspectives, and methods to enhance undergraduate microbiology education!

Sincerely,

The CSM FOME Committee

Josie Libertucci, PhD
Tanya Noel, PhD
Joe Rubin, DVM, PhD
Maria Davis, PhD
Heather Dietz, MSc

CSM FOME 2019 Workshop Schedule

8:00 – 8:30am	Refreshments and set up
8:30 – 8:45am	Welcome to FOME 2019 and Introductions
8:45 – 10:15am	Keynote by Mark O. Martin: Teaching Students to see through microbial eyes
10:15 – 10:30am	Coffee/Tea Break: Choose your session (Assessment versus TA workshop)
10:30 – 11:30am	Concurrent sessions: Effective Assessment Strategies OR Effective TA Strategies
11:30 – 11:35am	Break: Come back together
11:35 – 12:15pm	MicroFOME Sessions
12:15 – 12:30pm	Wrap up and evaluations

Keynote workshop: Teaching Students to See Through Microbial Eyes

Mark O. Martin, PhD. Associate Professor of Biology, University of Puget Sound, Tacoma, Washington, USA

Members of the microbial world—often called “germs” by the public—receive relentlessly negative publicity in the media, and are associated with disease and corruption in the minds of many nonmicrobiologists. Mark O. Martin, Associate Professor of Biology at the University of Puget Sound, is a self admitted “microbial whisperer” and advocate for "microbial centrism" outside and inside of the classroom. In his talk, Dr. Martin will demonstrate how he illustrates the essential role that bacteria and archaea play in the world around us (and within us) to his students in lecture and laboratory. This concept of microbial centrism will be shown to be true not simply in our biological relationships with what Dr. Martin calls “The Small Masters,” but to be a ubiquitous and powerful paradigm throughout the biosphere. To borrow from Pliny the Elder, “Nature is to be found in her entirety nowhere more than in Her smallest creatures.” Dr. Martin hopes to share his enthusiasm with the audience as he presents evidence that ours is a truly Microbial Planet, from deep in Earth’s crust, to high in the stratosphere, from the surface of our skin, to deep within our gut. #MicrobialCentrism is for everyone, from the beginning student to the emeritus professor!

Mark O. Martin is currently Associate Professor of Biology at the University of Puget Sound in Tacoma, Washington. Awed by bioluminescent bacteria while in junior high school, he went on to earn a B.A. in Biology at UCLA and a PhD in Biological Sciences from Stanford University. After postdoctoral work in San Diego, he went on to work in the biotechnology industry for seven years. Following that, Martin returned to academia, teaching for 8 years at Occidental College in Los Angeles, before joining the University of Puget Sound faculty in 2005. At Puget Sound, Martin teaches introductory cell and molecular biology, symbiotic associations, and his beloved microbiology.

Martin is well known for incorporating social media and creative approaches in his classroom, mixing art and science in ways that students find memorable. He has found that creative approaches in the classroom and laboratory translate into effective learning outcomes. Martin’s undergraduate research group explores the genetics of predatory and other “undomesticated” microbes, as well as novel symbioses between bacteria and terrestrial and marine animals. Over the years, Martin has sent 24 of his undergraduate research students to PhD programs across the Unites States, six of whom are currently in faculty positions.

An avowed “microbial centrist” and “microbial whisperer,” he works tirelessly in and out of the classroom to promote what he calls “#MattersMicrobial” to all who will listen. This includes not only Martin’s students, but his long-suffering wife Dr. Jennifer Quinn and tolerant sons Anson and Zachary.

Concurrent Session: Effective Assessment Strategies

Tanya Noel, PhD[1] and Joe Rubin, DVM PhD[2]

[1]*Department of Biological Sciences, University of Windsor, Windsor, Ontario, Canada*
[2]*Department of Veterinary Microbiology, University of Saskatchewan, Saskatoon, Saskatchewan, Canada*

Participants will engage in an interactive guided discussion on effective assessment strategies for large/mid-size introductory microbiology and biology classes, with a focus on best practices for creating and using multiple choice questions. Participants will compare and contrast a variety of test questions, consider item analysis results and create questions that test higher levels of student thinking (for example: application, analysis, and evaluation).

Concurrent Session: Effective TA Strategies

Maria Davis, PhD[1] and Heather Dietz[1], MSc

[1]*Department of Biology, University of Regina, Regina, Saskatchewan, Canada*

Participants will engage with a panel in a brief Q & A on strategies to be an effective TA followed by a brainstorming session in groups discussing the aspects of effective mentoring of undergraduate students in teaching labs. The session will wrap up with a short discussion on strategies for grading and creating rubrics.

MicroFOME Sessions

Bring Louis Pasteur back to life: the power of storytelling and performance art in a microbiology classroom

Josh D. Neufeld, PhD

Department of Biology, University of Waterloo, Waterloo, Ontario, Canada

The seminal contributions of pioneering microbiologists are covered within the first few lectures of most introductory microbiology classes. Although these early discoveries are profoundly meaningful, especially for the instructor, there is potential for the information to be received with disinterest by students who lack suitable contextual perspectives. This presentation will demonstrate, by example, how historical information can be animated through storytelling and complemented by performance art in order to bring microbiology pioneers "back to life" in the classroom. In addition to demonstrating the concept of teaching through story within the context of Louis Pasteur, this presentation and an associated discussion will highlight additional examples of how storytelling and in class re-enactments can animate other concepts covered by most introductory microbiology courses. In addition, we will explore the benefits and risks of using storytelling as a method for enhancing student engagement and content delivery.

Discovering arctic bacteriophages to involve undergraduate students in authentic research

Dion, M.[1,2], Trudel, L.[1], Paquet, M.-E.[1,3] and Culley, A.[1,4]

[1]*Département de biochimie, de microbiologie et de bio-informatique, Faculté des sciences et de génie, Université Laval, Québec, Québec, Canada*
[2]*Groupe de recherche en écologie buccale, Faculté de médecine dentaire, Université Laval, Québec, Québec, Canada*
[3]*Centre de recherche CERVO, Université Laval, Québec, Québec, Canada*
[4]*Institut de biologie intégrative et des systèmes, Université Laval, Québec, Québec, Canada*

Replacing traditional laboratory courses with discovery-based research is an excellent way to excite undergraduate students about science. Developing a laboratory course that fosters a sense of discovery and is also at an appropriate level for undergraduates is essential for a successful program. Bacteriophage isolation is an attractive research subject for a laboratory course because it requires little background knowledge and can be completed in a few weeks. I will present the humble beginnings of the bacteriophage discovery laboratory that was launched in 2018 at Université Laval in collaboration with Sentinel North. The goal of this laboratory course is to isolate, characterize and sequence arctic bacteriophages from Northern Canada. Second-year undergraduates isolated 73 bacterial species from environmental samples, such as microbial mats from Ward Hunt Island and freshwater from Kuujjuarapik. Then, third-year students used these species as hosts to propagate bacteriophages putatively present in the same samples. After two rounds of

propagation, they screened for the presence of bacteriophages on solid media. A total of 8 bacteriophages infecting 5 species were isolated by the 35 students in this cohort. Wild Arctic viruses remain largely uncharacterized and thus these results are very exciting, both educationally and scientifically. In the final step, bioinformatics students will analyze the sequences from these bacteriophages. With each iteration of this laboratory series, the growing collection of bacteriophages will contribute to the evolution of the curriculum. We believe that this collaborative project introduces fundamental skills in microbiology, while exposing students to the thrill of discovering microbes from uncharted biomes.

Bonus marks for a song?

Joseph Rubin, DVM, PhD

Department of Veterinary Microbiology, University of Saskatchewan, Saskatoon, Saskatchewan, Canada

Engaging students in required introductory courses can be difficult. Teaching microbiology to veterinary students at the University of Saskatchewan is both a privilege and a challenge; while our students are motivated and academically talented, they largely view non-clinical coursework as a requirement that must be met, rather than a window into an exciting field! It's a big task to both spark students' interest and keep their attention while presenting a lot of new material (dozens of bacterial species and the associated diseases, diagnostic tests and antimicrobial treatment strategies). Fortunately, bonus marks are always motivating! Each year students are given the challenge to re-imagine the lyrics of a popular song with a bacteriology theme for 2% added onto their final grade. The only constraints are that the song must accurately convey materials related to class, and that it either be performed live or posted on YouTube. On the last day of class, we have a "premier" event where videos are screened and songs are sung. I've found that over the last three years this assignment has beneficial for helping me to establish a rapport with the students and keep the class exciting. Adding a creative layer to the study of microbiology has facilitated learner engagement with the material; often times students integrate several subjects/core concepts into their songs demonstrating how much they've learned. Most rewarding for me as an educator is to see that the catchiest lyrics are remembered at final exam time! A summary of this experience will be presented.

CSM Annual Conference Scientific Program

Monday June 10, 2019		
8:00 AM	**Registration** **Location**: Hall d'entrée (Main lobby), Centre Culturel (B3)	
8:00 AM – 12:30 PM	**Forum for Microbiology Education (FOME) Workshop** *Chairs*: **Tanya Noel**, University of Windsor, Windsor, ON **Josie Libertucci**, University of Michigan, MI, USA **Location**: Marie Victorin, Faculté des sciences, room D7-3021 **8:00 AM** - **Coffee and light refreshments** **8:30 AM** - Introduction and Welcome to FOME 2019 **8:45 AM** - **Mark O. Martin**, University of Puget Sound, Tacoma, WA, USA **Teaching Students to See Through Microbial Eyes** **10:15 AM** - **Coffee Break** **10:45 AM** - **Concurrent sessions 1 and 2** Strategies for being an effective teaching assistant (room D7-2021) Assessment, the ABCs of MCQs (multiple-choice questions) (room D7-2023) **11:30 AM** - **Coffee Break** **11:35 AM** - MicroFOME sessions (room D7-3021) **12:15 PM** - Wrap up and evaluations	**8:45 AM** **Breakfast** **Location**: Balcon du Foyer du Mont-Bellevue **9:00 AM** **CSM Executive Meeting** **Location**: Balcon du Foyer du Mont-Bellevue **10:30 AM** **Coffee break** **Location**: Balcon du Foyer du Mont-Bellevue **11:00 AM** **CSM Council Meeting** **Location**: Balcon du Foyer du Mont-Bellevue **12:30 PM** **CSM Lunch** **Location**: Balcon du Foyer du Mont-Bellevue
1:30 PM – 4:20 PM	**Professional Development Workshop** *Chair*: **Teresa de Kievit**, University of Manitoba, Winnipeg, MB **Location**: Marie Victorin, Faculté des sciences, room D7-3021 **1:30 PM** - **Haley Sapers**, Caltech, Pasadena, California **2:20 PM** - **Josh Neufeld**, University of Waterloo, Waterloo, ON **3:10 PM** - **Coffee Break** **3:30 – 4:20 PM** - **Denice Bay**, University of Manitoba, Winnipeg, MB	

4:30 PM – 5:45 PM	**NSERC Grant Information Session** This presentation includes the review process of the Discovery Grants Program, the 2019 Discovery Grants (DG) and Research tools & Instruments (RTI) Competition Outcomes, NSERC program updates, and important information for the 2020 DG and RTI Competitions. *Presenters*: **Marie-Claude Jobin** (Program Officer) & **Enikö Megyeri-Lawless** (Director, Research Grants, Engineering and Life Sciences). **Location**: Marie Victorin, Faculté des sciences, room D7-3021
6:00 PM – 6:15 PM	**Welcome from CSM 2019 Co-Chairs** **Pascale Beauregard, Louis-Charles Fortier, and Vincent Burrus** Université de Sherbrooke, Sherbrooke, QC **Location**: Maurice-O'Bready (B3)
6:15 PM – 7:15 PM	**Plenary Lecture** **#396** **The CRISPR craze: functions and applications of Cas-based molecular machines in bacteria** *Invited keynote speaker*: **Rodolphe Barrangou**, North Carolina State University, Raleigh, NC, USA **Location**: Maurice-O'Bready (B3)
7:15 PM – 8:45 PM	**Welcome Reception** **Location:** Hall d'entrée, Centre Culturel (B3)

Tuesday June 11, 2019		
8:00 AM – 8:30 AM	**Registration** / **Coffee and Tea** **Location**: Hall d'entrée, Centre Culturel (B3)	
8:30 AM – 10:00 AM	**Session 1** **One Health – Antibiotics and resistance, from animal production to public health I** *Chair*: **France Daigle**, Université de Montréal **Location:** Balcon du Foyer du Mont-Orford (B3)	**Session 2** **Industrial microbes and biotechnology** *Chair*: **Carole Beaulieu**, Université de Sherbrooke **Location:** Balcon du Foyer du Mont-Bellevue (B3)
	8:30 AM #400 *Invited keynote speaker*: **Michael R. Mulvey** Public Health Agency of Canada, Winnipeg, MB **One Health - Antibiotics and resistance, from animal production to public health**	**8:30 AM** #393 *Invited keynote speaker*: **Elizabeth Edwards** University of Toronto, Toronto, ON **Industrial microbes and biotechnology: some examples**
	9:00 AM Evelyne Lacasse #33 Université de Sherbrooke, Sherbrooke, QC In vitro activity of siderophore-β-lactam conjugates against Gram-negative and Gram-positive pathogens	**9:00 AM Xiafei Zhang** #265 McMaster University, Hamilton, ON Genetic control of antibiotic production in *Streptomyces*
	9:15 AM Branden S. J. Gregorchuk #133 University of Manitoba, Winnipeg, MB Examination of biocide cationic antimicrobial resistance in *Escherichia coli* to identify phenotypic and genotypic adaptations	**9:15 AM Daniel Giguere** #298 University of Western Ontario, London, ON Characterizing naphthenic acid degrading bacterial communities
	9:30 AM Hindra Hindra #192 McMaster University, Hamilton, ON Accessing novel antibiotics in diverse *Streptomyces* bacteria	**9:30 AM Kapil Tahlan** #359 Memorial University of Newfoundland, St. John's, NL In vivo functional analysis of a class A β-lactamase-related protein essential for clavulanic acid biosynthesis in *Streptomyces clavuligerus*
	9:45 AM Rahgavi Poopalarajah #107 University of Calgary, Calgary, AB Exploiting polymicrobial interactions to thwart antimicrobial resistance	**9:45 AM Adrian Van Dyk** #370 University of Waterloo, Waterloo, ON Identification of glyphosate degradation genes in *Pseudomonas* by Tn-seq
10:00 AM – 10:45 AM	**Coffee Break** **Location**: Hall d'entrée, Centre Culturel (B3)	

10:45 AM – 12:15 PM	**Session 3** **One Health – Antibiotics and resistance, from animal production to public health II** *Sponsored by Op+lait* *Chair*: **Mario Jacques**, Université de Montréal **Location:** Balcon du Foyer du Mont-Orford (B3)	**Session 4** **Systems and synthetic biology I** *Chair*: **Jean-Philippe Côté**, Université de Sherbrooke **Location:** Balcon du Foyer du Mont-Bellevue (B3)
	10:45 AM #398 *Invited keynote speaker*: **Herman W. Barkema** University of Calgary, Calgary, AB **Effect of restriction in the use of antibiotics in food animals on antibiotic resistance in food animals and humans**	**10:45 AM** #399 *Speaker*: **Sébastien Rodrigue** (*in replacement of John I. Glass*) Université de Sherbrooke, Sherbrooke, QC **Engineering of a probiotic bacterium for microbiome editing**
	11:15 AM Finlay Maguire #198 Dalhousie University, Halifax, NS Precise identification of antimicrobial resistance determinants from metagenomic data	**11:15 AM Shawn Lewenza** #302 Athabasca University, Athabasca, AB Bacterial biosensors as a novel technology for detecting naphthenic acids in oilsands wastewater
	11:30 AM Kezia R. Fourie #202 University of Saskatchewan, Saskatoon, SK Quantifying antimicrobial resistant *E. coli* - A study on better methodology for surveillance data	**11:30 AM Vincent Baby** #15 INRA, UMR 1332, Bordeaux, France Construction of a *Mycoplasma feriruminatoris* chassis for the development of a vaccine for contagious caprine pleuropneumonia
	11:45 AM Rahat Zaheer #281 Agriculture and Agri-Food Canada, Lethbridge, AB Environmental continuum profiling: microbiomes and resistomes of livestock production, agricultural soil and urban wastewater	**11:45 AM Georgina Cox** #156 University of Guelph, Guelph, ON The genetic determinants of *Staphylococcus aureus* host cell adhesion
	12:00 PM Dominic Poulin-Laprade #306 Agriculture and Agri-Food Canada, Sherbrooke, QC Plasmids carrying antibiotic resistance genes in swine production	**12:00 PM Carolyn Amundsen** #282 Agriculture and Agri-Food Canada Harnessing rare enzyme activities from an agarolytic bacterium to engineer artificial metabolic pathways and selective delivery of heterologous proteins to the intestine
12:15 PM – 1:30 PM	**Lunch** **Location**: Hall d'entrée, Centre Culturel (B3)	

12:45 PM – 1:15 PM	**DNA Genotek Workshop** A higher standard for microbiome studies: Optimizing your study design *Presenter*: **Heloise Breton** **Location**: Balcon du Foyer du Mont-Bellevue (B3)
1:30 PM – 3:00 PM	**Student Symposium I** *Chairs*: **Denice Bay**, University of Manitoba, Winnipeg, MB **Josh Neufeld**, University of Waterloo, Waterloo, ON **Location:** Maurice-O'Bready (B3) **1:30 PM** **Sarah Martinez**, Institut Armand Frappier, Laval, QC #117 Implementation of a directed evolution strategy for rhamnolipid biosurfactant overproduction by *Burkholderia thailandensis* **1:45 PM** **Kevin Neil**, Université de Sherbrooke, Sherbrooke, QC #71 Delivery of CRISPR-Cas9 by conjugative probiotics enables precise microbiome editing **2:00 PM** **Nikhil A. George**, University of Waterloo, Waterloo, ON #35 Viral populations and CRISPR-resolved virus-host interactions in a municipal landfill **2:15 PM** **Stephanie Tan**, University of Toronto, Toronto, ON #50 The lasso peptide antibiotic, siamycin-I, inhibits lipid II biosynthesis at the Gram-positive cell surface **2:30 PM** **Jason Tran**, McMaster University, Hamilton, ON #113 CRISPRs and competence: sexually transmitted immunity **2:45 PM** **Jackson Tsuji**, University of Waterloo, Waterloo, ON #128 Ecology of phototrophic iron-oxidizing bacteria in Canadian Boreal Shield lakes
3:00 PM – 5:00 PM	**Poster Session I and Exhibitors** Coffee Break and Cash Bar Refreshments will be served **Location**: Parterres du Foyer du Mont-Bellevue et du Mont-Orford, Salon Rouge (B3)
3:15 PM – 4:15 PM	**Op+lait Workshop** *Presenters*: • **Christopher Fernandez Prada**, Université de Montréal The new frontier in diagnostics: pathogens, milk and exosomes • **François Malouin**, Université de Sherbrooke Development of *Staphylococcus aureus* mastitis-specific vaccines and antibiotics • **Jennifer Ronholm**, McGill University Vitamin B12 in milk - defining the optimal microbiota for consistent and high levels of production **Location**: Balcon du Foyer du Mont-Bellevue (B3)

5:00 PM – 6:00 PM	**Thermo Fisher Award Lecture** **#394** *Sponsored by Thermo Fisher Scientific* **Bacterial thermosensory diguanylate cyclases behave like the heat-sensing proteins of neurons** **Joe Harrison**, University of Calgary, Calgary, AB **Location**: Maurice-O'Bready (B3)
6:00 PM – 9:00 PM	**Student Mixer & ASM Networking** *Sponsored by ASM and RECSUS* Social and networking event in a casual setting including a small buffet, beverages and activities. **Location**: Atrium Sciences de la Vie (1st Floor D7-D8)

Wednesday June 12, 2019		
8:00 AM – 8:30 AM	Registration / **Coffee and Tea** Hall d'entrée, Centre Culturel (B3)	
8:30 AM – 10:00 AM	**Session 5** **One Health – Climate change, vector-borne diseases and emerging pathogens** *Chair*: **Alex Carignan**, Université de Sherbrooke **Location:** Balcon du Foyer du Mont-Orford (B3)	**Session 6** **Environmental microbiomes** *Chair*: **Adnane Sellam**, Université Laval **Location:** Balcon du Foyer du Mont-Bellevue (B3)
	8:30 AM #404 *Invited keynote speaker*: **Nicholas Ogden** Public Health Agency of Canada, Université de Montréal, Montréal, QC **Emerging infectious diseases: causes, consequences and how we respond**	**8:30 AM** #407 *Invited keynote speaker*: **Alexander Culley** Institut de biologie intégrative et des systèmes (IBIS), Université Laval, Québec, QC **Wild viruses in the High Arctic**
	9:00 AM Arinjay Banerjee #4 McMaster University, Hamilton, ON The role of bats in the enzootic cycle of *Borrelia*	**9:00 AM Nicolas Fontaine** #8 Thompson Rivers University, Kamloops, BC Developing and testing the efficacy of a topical probiotic on captive bats to prevent White Nose Syndrome
	9:20 AM Eric Bottos #312 Thompson Rivers University, Kamloops, BC Predicting permafrost microbial community responses to thaw based on pre-thaw ecosystem characteristics	**9:15 AM Amanda Carolina Prado de Moraes** #5 Federal University of São Carlos, Brazil Biotechnological potential of endophytic and rhizospheric bacteria for plant growth promotion in *Paspalum* species
	9:40 AM Maarten J. Voordouw #343 University of Saskatchewan, Saskatoon, SK Maternal antibodies provide strain-specific protection against infection with the Lyme disease pathogen in a wild rodent	**9:30 AM Danielle VanMensel** #183 University of Windsor, Windsor, ON Metatranscriptomics to assess Great Lakes near shore sediment contribution to water quality and human pathogenicity
		9:45 AM Judith Mogouong #63 INRS-Institut Armand-Frappier, Laval, QC Dynamics of invasive insect gut microbiome when facing environmental stress
10:00 AM – 10:45 AM	**Coffee Break** **Location**: Hall d'entrée, Centre Culturel (B3)	

10:45 AM – 12:15 PM	**Session 7** **One Health – Microbiome, microbial interactions and molecular pathogenesis** *Sponsored by CIHR III* *Chair*: **Alfredo Menendez**, Université de Sherbrooke **Location:** Balcon du Foyer du Mont-Orford (B3)	**Session 8** **Food microbiology** *Chair*: **Steve Labrie**, Université Laval **Location:** Balcon du Foyer du Mont-Bellevue (B3)
	10:45 AM #408 *Invited keynote speaker*: **Corinne Maurice** McGill University, Montréal, QC **Interactions between bacteria and bacteriophages in the human gut**	**10:45 AM** #401 *Invited keynote speaker*: **Jacques Goulet** Université Laval, Québec, QC **La microbiologie des aliments : d'hier à demain**
	11:15 AM Mariia Taguer #37 McGill University, Montréal, QC Gut bacterial physiology and activity along the progression to dysbiosis	**11:15 AM Alice Perrault-Jolicoeur** #84 Université Laval, Québec, QC Evolution of virulent phages infecting an industrial *Lactococcus lactis* subsp. *cremoris* strain over 15 years in a cheese factory
	11:30 AM Simone Renwick #222 University of Guelph, Guelph, ON Investigating the gut microbial ecosystem of infants genetically predisposed to developing type 1 diabetes	**11:30 AM Devin B. Holman** #261 Agriculture and Agri-Food Canada, Lacombe, AB Comparative genomic analysis and antimicrobial resistance of *Enterococcus* spp. isolated from a beef processing plant and retail meat
	11:45 AM Carole Creuzenet #149 University of Western Ontario, London, ON Making a dent in campylobacteriosis by biting in *Campylobacter jejuni* sugar coat.	**11:45 AM Guillaume Nguyen** #276 Université Laval, Québec, QC Maple sap microbial community structure associated with maple syrup defects
	12:00 PM Anaïs Burgain #256 Université Laval, Québec, Canada A novel genetic circuitry governing hypoxia-induced metabolic reprogramming, commensalism and virulence in the fungal pathogen *Candida albicans*	**12:00 AM Jennifer Ronholm** #150 McGill University, Montréal, QC Correlations between the bovine microbiome and aspects of milk quality
12:15 PM – 1:30 PM	**Lunch** **Location**: Hall d'entrée, Centre Culturel (B3)	
12:45 PM – 1:15 PM	**Illumina Workshop** Recent advances in microbial genome sequencing and microbiome profiling using Illumina technology *Presenter*: **Mehdi Keddache** **Location**: Balcon du Foyer du Mont-Bellevue (B3)	illumina®

1:30 PM – 3:00 PM	**Student Symposium II** *Chairs*: **Denice Bay**, University of Manitoba, Winnipeg, MB **Josh Neufeld**, University of Waterloo, Waterloo, ON **Location:** Maurice-O'Bready (B3) **1:30 PM** **Alex Anderson**, Wilfrid Laurier University, Waterloo, ON #97 Characterizing the cellulose-modifying enzyme BcsG **1:45 PM** **Hicham Bessaiah**, INRS-Institut Armand-Frappier, Laval, QC #112 Inactivation of *yqhG* in uropathogenic *Escherichia coli* reduces expression of type 1 fimbriae and attenuates colonization of the mouse urinary tract **2:00 PM** **Philippe C Després**, Université Laval, Québec, QC #53 Systematic perturbation of yeast essential genes using base editing **2:15 PM** **Jolene Garber**, University of Georgia, Athens, GA, USA #118 Impacts of carbohydrate metabolism on *Campylobacter jejuni* infection of breastfed infants **2:30 PM** **Branden S. J. Gregorchuk**, University of Manitoba, Winnipeg, MB #83 A rapid fluorescent dye membrane integrity assay to detect biocide resistant and susceptible phenotypes of Gram-negative bacteria **2:45 PM** **Catherine Maggiori**, McGill University, Montréal, QC #56 Life detection with nanopore sequencing and microbial diversity in Mars analogue environments
3:00 PM – 5:00 PM	**Poster Session II and Exhibitors** Coffee Break and Cash Bar Refreshments will be served **Location:** Parterres du Foyer du Mont-Bellevue et du Mont-Orford, Salon Rouge (B3)
4:30 PM – 5:00 PM	**Exhibitors Door Prize Draw** **Location:** Maurice-O'Bready (B3)
5:00 PM – 6:00 PM	**Armand Frappier Outstanding Student Award Lecture** #402 **Characterization of Slam-mediated surface lipoprotein translocation across the bacterial outer membrane** **Yogesh Hooda**, University of Toronto, Toronto, ON **Location:** Maurice-O'Bready (B3)
6:00 PM – 6:45 PM	**CSM Annual General Meeting** **Location:** Maurice-O'Bready (B3)
9:00 PM – 10:30 PM	**Excursion to Foresta Lumina** Transportation will be provided to and from the Parc de la Gorge de Coaticook. *Bus pickup: 8:00 – 8:15 PM, in front of the George-Cabana building (B1-B2)* *Drop-off: 11:00 – 11:30 PM, Hotels and residences* *Bring your rain gear. Avoid open-toed shoes.*

Thursday June 13, 2019		
8:00 AM – 8:30 AM	**Registration** / **Coffee and Tea** **Location:** Hall d'entrée, Centre Culturel (B3)	
8:30 AM – 10:00 AM	**Session 9** **One Health – Alternatives to antibiotics** *Chair*: **François Malouin**, Université de Sherbrooke **Location:** Balcon du Foyer du Mont-Orford (B3)	**Session 10** **Plants and microbes** *Chair*: **Pascale Beauregard**, Université de Sherbrooke **Location:** Balcon du Foyer du Mont-Bellevue (B3)
	8:30 AM #397 *Invited keynote speaker*: **Thomas Louie** University of Calgary, Calgary, AB **Alternatives to antibiotics: opportunities to prevent microbiome destruction and to repair /replace healthy microbiomes**	**8:30 AM** #351 *Invited keynote speaker*: **Allyson M. MacLean** University of Ottawa, Ottawa, ON **Unraveling the molecular mechanisms of plant-microbe interactions**
	9:00 AM Yan D. Niu #160 University of Calgary, Calgary, AB Use of bacteriophages in the biocontrol of Shiga toxigenic *Escherichia coli*	**9:00 AM Jennifer N. Russell** #368 University of Regina, Regina, SK Pesticide-driven genetic enrichment in prairie biobed systems
	9:15 AM Brent Weber #199 McMaster University, Hamilton, ON Use of human serum as a growth medium to identify novel antibacterial compounds for treatment of bloodstream infections	**9:15 AM Ashley N. Williams** #209 University of Regina, Regina, SK Resistance to two vinylglycine antibiotic analogs is conferred by inactivation of two separate amino acid transporters in *Erwinia amylovora*
	9:30 AM Anissa Brahami #287 INRS-Université du Québec Discovery of an active peptide from hospital biofilm by functional metagenomic screening	**9:30 AM Edel Pérez-López** #21 University of Saskatchewan, Saskatoon, SK Molecular characterization of a novel cysteine protease inhibitor from the obligate biotrophic pathogen *Plasmodiophora brassicae* that targets papain-like cysteine proteases
	9:45 AM Samat Amat #310 Agriculture and Agri-Food Canada The effect of intra-nasal delivered novel bacterial therapeutics on the respiratory microbiota of beef cattle entering the feedlot	**9:45 AM Jason V.S. Kearsley** #377 McMaster University, Hamilton, ON Isolation of a preliminary minimal N_2-fixing symbiotic gene set of the pSymB megaplasmid in the host legume symbiont *Sinorhizobium meliloti*
10:00 AM – 10:45 AM	**Coffee Break** **Location**: Hall d'entrée, Centre Culturel (B3)	

10:45 AM – 12:15 PM	**Session 11** **Microbial chemosphere** *Chair*: **Jean-Philippe Bellenger**, Université de Sherbrooke **Location:** Balcon du Foyer du Mont-Orford (B3)	**Session 12** **Systems and synthetic biology II** *Chair*: **Sébastien Rodrigue**, Université de Sherbrooke **Location:** Balcon du Foyer du Mont-Bellevue (B3)
	10:45 AM #406 *Invited keynote speaker*: **Clarissa S. Sit** St-Mary's University, Halifax, NS **A happy accident: microbial volatiles for plant defense**	**10:45 AM** #395 *Invited keynote speaker*: **Carol A. Gross** University of California San Francisco, CA, USA **The role of extracytoplasmic function (ECF) Sigma factors across the bacterial domain**
	11:15 AM Nabiha MF Mehina #273 University of Calgary, Calgary, AB Novel insights into the role of pyocins in interstrain competition	**11:15 AM George diCenzo** #11 Queen's University, Kingston, ON A systems biology approach to interrogating the metabolism of symbiotic nitrogen fixation
	11:30 AM Steven Sutcliffe #36 McGill University, Montréal, QC Induction of prophages of human gut bacteria by xenobiotics	**11:30 AM Alexander W. Ensminger** #72 University of Toronto, Toronto, ON "Effect" the unexpected: metaeffectors in *Legionella pneumophila* and beyond
	11:45 AM Julie Paulssen #77 University of Calgary, Calgary, AB Biodegradation of 1-adamantanecarboxylic acid by microorganisms originating from Alberta's oil sands surface mining operations	**11:45 AM Jean-Christophe Lachance** #98 Université de Sherbrooke, Sherbrooke, QC Genome-scale metabolic modelling reveals key features of a minimal gene set for *Mesoplasma florum*
	12:00 PM Erin Anderson #293 University of Guelph, Guelph, ON Peptidoglycomics: Using metabolomic techniques to examine the composition of peptidoglycan of *Pseudomonas aeruginosa* biofilms	**12:00 PM Marie-Laurence Lemay** #17 Université Laval, Québec, QC A phage protein impedes bacterial resistance to phage infection
12:15 PM – 1:30 PM	**Lunch** **Location**: Hall d'entrée, Centre Culturel (B3)	

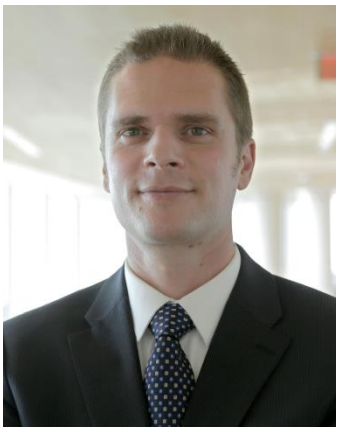
1:30 PM – 3:00 PM	**Infection & Immunity Section Symposium** *Chair*: **Sébastien Faucher**, McGill *Vice-Chair*: **Georgina Cox**, Guelph **Location:** Balcon du Foyer du Mont-Orford (B3)	**Applied & Environmental Microbiology Section Symposium** *Chair*: **Steven Short**, Toronto Mississauga *Vice-Chair*: **Laura Hug**, Waterloo **Location:** Maurice-O'Bready (B3)	**Molecular Genetics & Cellular Microbiology Section Symposium** *Chair*: **Tracy Raivio**, Alberta *Vice-Chair*: **Alexander Hynes**, McMaster **Location:** Balcon du Foyer du Mont-Bellevue (B3)
	1:30 PM #22 Dynamic proteomic profiling of the *Salmonella*-host interplay reveals new modes of action for known and novel virulence factors **Jennifer Geddes-McAlister**, University of Guelph, Guelph, ON	**1:30 PM** #266 Relationship between methane emission and *Methanoculleus* spp. concentration of treated swine manure **Guylaine Talbot** Agriculture and Agri-Food Canada, Sherbrooke, QC	**1:30 PM** #3 CRISPR-based functional genomic platforms for gene deletions and modulating gene expression in *Candida* pathogens **Rebecca Shapiro,** University of Guelph, Guelph, ON
	1:45 PM #29 Despite antagonistic activities in vitro, *Pseudomonas aeruginosa* enhances *Staphylococcus aureus* colonization in a murine lung infection model **Guillaume Millette,** Université de Sherbrooke, Sherbrooke, QC	**1:45 PM** #173 Tracking functional bacterial biomarkers in response to a gradient of contaminant exposure within river continuums **Thomas Reid,** University of Windsor, Windsor, ON	**1:45 PM** #18 The Bet/Exo recombination system allows IncC conjugative plasmids and STX/R391 ICEs to evade host defense mechanisms during conjugation **David Roy**, Université de Sherbrooke, Sherbrooke, QC
	2:00 PM #34 Using the intestinal microbiome in the prediction of cancerous colonic lesions **Thierry Chénard,** Université de Sherbrooke, Sherbrooke, QC	**2:00 PM** #204 Toxic rocks: characterization of fungal/algal communities on peridotite **Steven D. Harris**, University of Manitoba, Winnipeg, MB	**2:00 PM** #122 Induction of specialized metabolites in *Streptomyces* by heterologous expression of the engineered response regulator AfsQ1* **Martin Daniel-Ivad**, University of Toronto, Toronto, ON
	2:15 PM #65 Inhibition of Cagα, a T4SS ATPase required for *Helicobacter pylori* virulence **Claire Morin,** Université de Montréal, Montréal, QC	**2:15 PM** #59 Metagenomic analysis of virus diversity and relative abundance in a freshwater harbor **Christine N. Palermo,** University of Toronto Mississauga, Mississauga, ON	**2:15 PM** #243 Site-specific excision of pneumococcal prophages from genes essential for natural transformation **Geneviève Garriss**, Karolinska Institutet, Stockholm, Sweden

	2:30 PM #171 Bacteriophages modulate ulcerative colitis-derived bacterial communities **Anshul Sinha,** McGill University, Montréal, QC	**2:30 PM** #286 Single cell Raman-SIP to study the carbon flow in contaminated aquifers **Ali Akbari,** University of Duisburg-Essen, Essen, Germany	**2:30 PM** #342 Exploiting synthetic interactions to explore outer membrane biology in *E. coli* **Kristina Klobucar**, McMaster University, Hamilton, ON
	2:45 PM #175 New exosome-based biomarkers for the rapid diagnosis of drug-resistant parasites **Noélie Douanne,** Université de Montréal, Montréal, QC	**2:45 PM** #392 Application of MinION sequencing in food safety **Dele Ogunremi,** Canadian Food Inspection Agency	**2:45 PM** #382 Forged in iron: Conflict enhances the survival of all members in a defined bacterial community **Nicole A. Lerminiaux**, University of Regina, Regina, SK
3:00 PM – 3:30 PM	**Coffee Break** **Location**: Hall d'entrée, Centre Culturel (B3)		
3:30 PM – 5:00 PM	**Postdoctoral Research Symposium** *Chair*: **Louise Nelson**, University of British Columbia Okanagan, Kelowna, BC **Location**: Maurice-O'Bready (B3) **3:30 PM** **April Gislason**, University of Manitoba, Winnipeg, MB #70 Prey versus Predator: *Pseudomonas brassicacearum* DF41 biofilm blocks *Caenorhabditis elegans* feeding **3:45 PM** **Marcus M. Dillon**, University of Toronto, Toronto, ON #61 The pan-genome effector-triggered immunity landscape of a host-pathogen interaction **4:00 PM** **Fiona Jane Whelan**, McMaster University, Hamilton, ON #62 The co-occurrence and co-exclusion of genes in prokaryote genomes **4:15 PM** **Wael Elhenawy**, McMaster University, Hamilton, ON #88 Host-specific adaptive diversification of Crohn's disease-associated adherent-invasive *Escherichia coli* **4:30 PM** **Alison Besse**, INRS-Institut Armand-Frappier, Laval, QC #93 Phenotypic adaptation of *Pseudomonas aeruginosa* into biofilms **4:45 PM** **Omar M. El-Halfawy**, McMaster University, Hamilton, ON #106 Discovery of an anti-virulence compound targeting the bacterial envelope stress response that reverses β-lactam resistance in MRSA		
5:00 PM – 6:00 PM	**CSM Murray Award for Career Achievement** #403 *Sponsored by: Canadian Science Publishing* **A multidisciplinary study of the bacterial cell** **Yves Brun**, Université de Montréal, Montréal, QC **Location**: Maurice-O'Bready (B3) Canadian Journal of Microbiology		

6:00 PM – 6:15 PM	**Invitation to next year's meeting from the CSM 2020 Chairs Kari Dunfield & Cezar M. Khursigara**, University of Guelph, Guelph, ON **Location**: Maurice-O'Bready (B3)
7:00 PM	**President's Banquet & Awards Presentations** **Théâtre Granada** 53 Wellington Nord, Sherbrooke, QC, J1H 5A9 Transportation will be provided to and from the banquet *Bus pickup: 6:30 – 6:45 PM, STS bus stop behind the Multifunctional building (B5)* *Drop-off: 10:30 PM – 12:00 AM, Hotels and residences*

Plenary lecture

Dr. Rodolphe Barrangou, North Caroline State University, North Carolina, USA



Dr. Barrangou is the T. R. Klaenhammer Distinguished Professor in Probiotics Research in the Department of Food, Bioprocessing and Nutrition Sciences at North Carolina State University, focusing on the evolution and functions of CRISPR-Cas systems, and their applications in bacteria used in food manufacturing. Dr. Barrangou earned a BS in Biological Sciences from Rene Descartes University in Paris, France, a MS in Biological Engineering from the University of Technology in Compiegne, France, a MS in Food Science from NC State, a PhD in Genomics from NC State and a MBA from the University of Wisconsin-Madison. He spent 9 years in R&D and M&A at Danisco and DuPont in the Food Industry. Rodolphe is also an associate member of the Microbiology graduate program, the Biotechnology graduate program, the Functional Genomics graduate program, the Genetics program, the Genome Engineering and Society Center, and the Comparative Medicine Institute. Recently, for his work establishing the biological function of CRISPR, Rodolphe received the 2016 Warren Alpert Prize, the 2016 Canada Gairdner International Award, the 2017 NAS Award in Molecular Biology and the 2018 NAS Prize in Food and Agriculture Sciences. Dr. Barrangou was elected into the US National Academy of Sciences in 2018 and the US National Academy of Engineering in 2019. Dr. Barrangou is also the former Chairman of the Board of Directors of Caribou Biosciences, a co-founder and member of the Scientific Advisory Board of Intellia Therapeutics, a co-founder of Locus Biosciences, an advisor to Inari Ag, and the Editor in Chief of the CRISPR Journal.

The CRISPR craze: functions and applications of Cas-based molecular machines in bacteria

Rodolphe Barrangou, NC State University, Raleigh, North Caroline, USA

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR), together with associated sequences (*cas*) constitute the CRISPR-Cas system, which provides adaptive immunity against invasive elements in many bacteria and most archaea. In prokaryotes, CRISPR-Cas systems afford DNA-encoded, RNA mediated, nucleic acid targeting, using a diverse set of Cas nucleases that yield various cleavage outcomes, and carry out various biological functions. Recently, the CRISPR machinery has been repurposed to fuel the Cas9-enabled genome editing craze. Actually, the democratization of CRISPR-based technologies in the past six years hinges on the portability and functionalities of these molecular machines, and has revolutionized biology. I will look back on the historical milestones that have paved the way for the CRISPR era, and discuss the diverse applications that have impacted and continue to shape the fields of Medicine, Ag, Food and Biotechnology. Specifically, I will highlight numerous CRISPR-based molecular machines that can be readily repurposed in a diversity of microbial applications, including genotyping, phage resistance, immunization against invasive nucleic acids, screening and antimicrobials. I will highlight how diverse endogenous CRISPR-Cas systems can be used in native bacteria for the engineering of industrial workhorses, such as probiotics. Once the occurrence and diversity of CRISPR-Cas systems is established in bacteria of interest, the functional attributes encompassing Cas effector nucleases, guide crRNAs and target PAM sequences can be determined and repurposed for programmable targeting and generating various outcomes such as death, or edits including insertions, deletions, knock outs and specific mutations. Lastly, I will discuss the impacts this transformative field has had on science and society, and address business implications of this disruptive technology for microbiology and beyond.

Themo Fisher Award Recipient

Dr. Joe Harrison, University of Calgary, Calgary, AB

Dr. Joe J. Harrison is an Associate Professor in the Department of Biological Sciences at the University of Calgary (U of C). Harrison is passionate about microbiology, genetics and biochemistry, and seeks to better understand chronic infectious diseases and to devise new ways to defeat them. Harrison holds a Tier II Canada Research Chair in Biofilm Microbiology and Genomics. He is a member of the Snyder Institute for Chronic Diseases and is the chair of the Biofilm Research Group at the U of C. He is a co-lead for Integrated Microbiome Platforms for Advancing Causation Testing and Translation (IMPACTT), which is the CIHR Canadian Microbiome Core. During his PhD, Harrison had a lead role in developing and commercializing the MBECTM assay, which is used for biofilm antimicrobial susceptibility testing. This technology was commercialized to create a spinoff company acquired in 2006 by Innovotech Incorporated, which is now listed on the Toronto Stock Exchange.

Bacterial thermosensory diguanylate cyclases behave like the heat-sensing proteins of neurons

Joe J. Harrison, University of Calgary, Calgary, AB

Many bacteria use the second messenger cyclic diguanylate (c-di-GMP) to control motility, biofilm production and virulence. Although the biochemistry of c-di-GMP synthesis, degradation and effector function has been studied in diverse model organisms for three decades, little is known about how natural stimuli are perceived by c-di-GMP networks. Here I report my lab group's discovery of the thermosensory diguanylate cyclase (TdcA). This *Pseudomonas aeruginosa* protein orchestrates temperature-dependent biofilm development and virulence in nematodes, insects and mice. We show that TdcA synthesizes c-di-GMP with catalytic rates that increase more than a hundred-fold over a ten degree Celsius change. This thermosensitive biomolecular behavior is analogous to the high enzymatic rate-temperature dependencies exhibited by the hot- and cold-sensing proteins of neurons. Analyses using engineered protein chimeras indicate that temperature-sensing is mediated by a cofactorless, thermosensitive Per-Arnt-SIM domain, which is a previously unreported function for this widespread family of protein domains. TdcA orthologues and proteins like it are widespread in genome and protein databases. I propose, therefore, that thermotransduction is a prevalent function of bacterial c-di-GMP signaling networks, and that thermosensitive catalysis of a second messenger constitutes a fundamental mechanism for thermal sensation in bacteria.

Armand-Frappier Outstanding Student Award Recipient

Mr. Yogesh Hooda, University of Toronto, Toronto, ON

Yogesh Hooda did his bachelors from the Birla Institute of Technology and Science, Pilani, India. He then went on to do his masters and PhD at the University of Toronto, Canada. During his PhD, he worked on developing biochemical approaches to investigate the molecular mechanisms of protein transport in bacteria. He is currently working as a postdoctoral scientist at the MRC Laboratory of Molecular Biology, Cambridge, UK researching the assembly of membrane protein complexes.

Characterization of Slam-mediated surface lipoprotein translocation across the bacterial outer membrane

Yogesh Hooda, University of Toronto, Toronto, ON

Surfaces of many Gram-negative bacteria are decorated by peripheral membrane proteins referred to as surface lipoproteins or SLPs. SLPs contain a covalently attached lipid molecule that anchors to them to the outer membrane. They play key roles in nutrient acquisition, immune evasion and have been proposed as excellent vaccine antigens. Previously our lab had used genetic screens to an outer membrane protein called Slam that is necessary for transport of a transferrin-binding SLP named TbpB in *Neisseria meningitidis*. The aim of the current study was to investigate the molecular role played by Slam proteins in SLP biogenesis.

Using bioinformatic analysis, I showed that genes encoding Slam-like proteins are present in a number of Gram-negative bacteria and these are often found adjacent to genes encoding putative SLP substrates. In *N. meningitidis*, two Slam paralogs were identified - Slam1 and Slam2 that are specific for SLPs TbpB and HpuA respectively. All putative Slam-dependent SLPs contain a C-terminal 8-stranded soluble barrel domain. The last two strands of the SLP were found to be essential for TbpB translocation and the C-terminal 8-stranded barrel domain mediated Slam specificity. Using co-immunoprecipitation experiments, I showed that the Slam-dependent translocation occurs from the C-terminus to the N-terminus.

To investigate Slam mechanism further, I also developed an in vitro translocation system to study Slam function. Upon the addition of the periplasmic chaperone LolA, SLPs can be released from spheroplasts into the supernatant. Slam containing proteoliposomes could successfully translocate spheroplast released SLPs into the liposomal lumen. Addition of other outer membrane factors such as the Bam complex did not increase the efficiency of SLP insertion and Slam1&2 retained their specificity in the assay. Slam1 proteoliposomes were also sufficient in their ability uptake purified and unfolded TbpB into the lumen. Collectively, these findings show that Slams are both necessary and sufficient for the translocation of SLPs across the outer membrane, indicating that they act as outer membrane translocons.

CSM Murray Award for Career Achievement Recipient

Dr. Yves Brun, Université de Montréal, Montréal, QC

Dr. Brun did his B.Sc. (1983) and M.Sc. (1985) studies at the Université de Moncton, Canada where he performed research on the mechanisms of protein synthesis with Alan Fraser, funded by undergraduate and graduate fellowships from the National Science and Engineering Research Council of Canada (NSERC). He received the Governor General of Canada Gold Medal for graduate work. He obtained his Ph.D. from the Université Laval, Canada (1990) for work on the evolution and regulation of tRNA and aminoacyl tRNA synthetase genes with Jacques Lapointe and with fellowship support from NSERC. He conducted postdoctoral research (1990-93) at Stanford University, where he performed research on the regulation of bacterial cell differentiation with Lucy Shapiro, funded by fellowships from NSERC and from the Medical Research Council of Canada. He moved to a faculty position at Indiana University in 1993 (until 2019) where he was the Clyde Culbertson and Distinguished Professor of Biology, served as Director Microbiology Graduate Program and as Associate Chair for Microbiology, and received many teaching awards. He received an Academic Scientific Achievement Award from the American Society for Microbiology (2005), was elected to the American Academy of Microbiology (2010) and to the American Association for the Advancement of Science (2012), and received a Fulbright US Research Scholar Award (2014). He has served as Editor of the Journal of Bacteriology (2003-07 and 2018-pres.) and mBio (2013-16). In January 2019, he moved to the Université de Montréal in the Département de microbiologie, infectiologie et immunologie where he is the Canada 150 Research Chair in Bacterial Cell Biology. His research spans a wide area in microbiology and is characterized by the use of multidisciplinary approaches. He has made important contribution to our understanding of the regulation of cell division, cell cycle control, development and cell differentiation, protein localization, the function, determination, and evolution of cell shape, and the mechanisms, regulation, and biophysics of surface adhesion and biofilm formation in bacteria.

A multidisciplinary study of the bacterial cell

Yves Brun, Université de Montréal, Montréal, QC

In 1994, the evolutionary biologist Stephen Jay Gould, who had never done research on bacteria, wrote in Scientific American “This is truly the ‘age of bacteria’ – as it was in the beginning, is now, and ever shall be.” Indeed, with our recent realization of the benefits of the microbiome for the health of our body and our environment, it is even more critical to gain a detailed understanding of the function of bacterial cells. I will present examples from our multidisciplinary studies of bacteria combining evolutionary biology, genetics, cell biology, biophysics, and high throughput and single cell studies. In the first example, I will show how bacteria use dynamic fimbrial structures called pili to sense their contact with a surface and trigger the synthesis of permanent adhesins to initiate the process of biofilm formation. In the second example, I will show how bacteria direct the synthesis of peptidoglycan to specific sites for growth and morphogenesis and how changing the location of these sites leads to the evolution of both processes.

Dr. Louise Nelson

Recipient of CSM Honorary Membership

Dr. Nelson received her BSc (Hons) in Bacteriology and Immunology from the University of Western Ontario in 1972 and her PhD in Microbial Ecology from the University of Calgary in 1976. She held a Killam Postdoctorate Scholarship from the Canada Council in Roger Knowles' laboratory, MacDonald Campus of McGill University (1976-78) and a Rhodes Visiting Fellowship at St. Anne's College, University of Oxford (1978-79) before joining the National Research Council of Canada, Plant Biotechnology Institute (1978-1995) where her research centred on symbiotic nitrogen fixation, nitrogen cycling soil bacteria and plant growth-promoting rhizobacteria. She served as Manager, Ag Biologicals Research, Agrium Inc. (1995-99) before joining the University of Saskatchewan as Professor and Agri-Food Innovation Fund Chair in Agricultural Biotechnology (1999-2003). Since moving to British Columbia she has held several administrative positions: Vice President Research and Internationalization, Okanagan University College (2004-05); Head, Biology and Physical Geography, University of British Columbia, Okanagan Campus (2005-08); Associate Dean, Research, Irving K. Barber School of Arts and Sciences, UBC Okanagan Campus (2008-13); and Acting Associate Dean, Faculty and Academic Planning (2013-15). She was recently appointed Honorary Professor and Professor Emerita at UBC (2019). Her research has continued to focus on plant-microbe interactions, biological control, soil health and nitrogen cycling soil populations.

Dr. Nelson has been an active member of the Canadian Society of Microbiologists for 47 years, serving as Vice-Chair and Chair of the Environmental Microbiology section (1982-86), on the Executive (1986-1990), as Secretary-Treasurer (2006-2009), and on several Award Committees. She became the second female President of the Society in 1988-89. She is also a member of the American Society for Microbiology, the International Society for Microbial Ecology, the International Society for Molecular Plant-Microbe Interactions, and the Canadian Phytopathological Society. She was a member of the Editorial Board of Applied and Environmental Microbiology (1984-95), a co-editor of the Canadian Journal of Microbiology (1993-98) and several NSERC committees. She served on the Boards of the United Way of Saskatoon, of the Central and South Okanagan/Similkameen and of the United Way of Canada, receiving the Queen's Golden Jubilee Medal for Service to Canada in 2003 for her contributions to United Way.

Dr. Joseph S. Lam

Recipient of a CSM Honorary Membership

Dr. Lam has a long-standing record of loyalty and services to the CSM, of which he has been a member for 40 years. He has held the positions of vice-chair and chair, respectively, of the Morphology and Structure section of the CSM (1986-1990). In 1989, he received the Fisher Scientific Award and in 2006, the Roche Diagnostic CSM Award. As testimony to his ability as a mentor, many of Dr. Lam's students have received awards for oral or poster presentations at the CSM student competitions; in 2013, one of his PhD students, S. Islam, received the prestigious Armand-Frappier Outstanding Student Award.

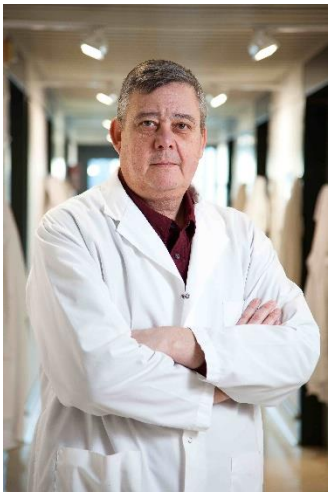
In addition to the CSM, Dr. Lam is a member of the American Society for Microbiology, the American Society for Biochemistry and Molecular Biology, the Biophysical Society, and he is a founding member of the International Endotoxin Society (currently the International Endotoxin and Innate Immunity Society).

Over the course of his career, Dr. Lam has received numerous prestigious awards. He is the recipient of the Queen Elizabeth II Diamond Jubilee Medal for contributions to Cystic Fibrosis research (2013), a former Tier I Canada Research Chair (2003-2017), a Fellow of the American Academy of Microbiology (since 2016), a Marsha Morton Scholar and a Canadian Cystic Fibrosis Foundation Scholarship recipient, and a University of Guelph President's Scholar. At the 2018 University of Guelph Spring Convocation, he was honored with the title, University Professor Emeritus, a senate approved recognition.

Dr. Lam's highly successful research program on *Pseudomonas aeruginosa* lipopolysaccharide and Microbial Glycobiology has resulted in 166 journal publications, 18 book chapters, over 200 conference abstracts, 64 invited research talks and 13 patent applications (4 of which were granted US patent approval). Citations of his work has reached 9987 and earned him an h-index of 57.

Invited Keynote Speakers and Chairs

Session 1 – One Health – Antibiotics and resistance, from animal production to public health I June 11th 8:30 AM



Keynote speaker: **Dr. Michael R. Mulvey**, Public Health Agency of Canada (PHAC), University of Manitoba, Winnipeg, MB

One Health - Antibiotics and resistance, from animal production to public health

Dr. Mulvey obtained his Ph.D. from the University of Manitoba in 1990 where he discovered *rpoS*, the gene responsible for the stationary phase regulon in *E. coli*. After a post-doc studying picornavirus infection at the University of Alberta, he moved to Health Canada in 1996, now the Public Health Agency of Canada, where he established the Antimicrobial Resistance and Nosocomial Infections (ARNI) Laboratory.

Dr. Mulvey's research interests include the molecular mechanisms and epidemiology of antimicrobial resistance and the translation of resistance data into interventions to limit the spread of resistant organisms. He has co-authored over 250 peer-reviewed publications.

Dr. Mulvey is the Co-Chair of the Canadian Public Health Laboratory Network Working Group on AMR, sits on the Executive Board of the Canadian Committee on Antimicrobial Susceptibility Testing (CANCAST), is a member of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) subcommittee on the role of whole genome sequencing in antimicrobial susceptibility testing, and has sat on the CIHR Institute of Infection and Immunity Institute Advisory Board for the last seven years. Dr. Mulvey has received numerous awards including the 2015 Honorary Membership in the Association of Medical Microbiology and Infectious Diseases (AMMI Canada) and the 2016 University of Manitoba Faculty of Science Honoured Alumni of the Year.

Chair: **Dr. France Daigle**, France Daigle, Université de Montréal, Montréal, QC

Dr. Daigle, Ph.D. (Microbiology, Université de Montréal, 1996); post-doctoral studies at INRA, Toulouse (1996-1997) and Washington University in St-Louis (1998-2001) is professor in the Microbiology, Infectiology, and Immunology Department at Université de Montréal since 2001. She is a molecular microbiologist with expertise in bacterial pathogenesis, genetics and genomics. She uses an arsenal of genetics, genomics, and cellular tools to understand how Salmonella strains develop host-adaptive traits and virulence factors. She has a very strong expertise in host-pathogen interactions, using host cells as a model to study bacterial adherence, uptake, and survival. She also developed a unique approach to study bacterial gene expression in vivo. Her current projects involve the study of molecular adaptation of Salmonella to the host, with focus on adherence mechanisms and biofilm formation and on high throughput screening of *Salmonella* serovars in cell culture to estimate virulence.

Session 2 – Industrial microbes and biotechnology June 11th 8:30 AM

Keynote speaker: **Dr. Elizabeth Edwards**, University of Toronto, Toronto, ON

Industrial microbes and biotechnology: some examples

Elizabeth A. Edwards, PhD, P. Eng., Professor, Department of Chemical Engineering and Applied Chemistry, and Cell and Systems Biology (Status only), University of Toronto. Dr. Elizabeth Edwards holds Bachelor's and Master's degrees in Chemical Engineering from McGill University, Montreal, and a PhD degree (1993) in Civil and Environmental Engineering from Stanford University. Over the two decades, Dr. Edwards' research team has discovered and characterized novel microbial cultures such as the now commercial KB-1® consortium that metabolize pollutants previously thought to be recalcitrant. This discovery led to the founding of SiREM Laboratories (www.siremlab.com) in Guelph in 2002 that recently celebrated 15 years in business. She has received many awards including most notably an NSERC Synergy Award for Innovation with Geosyntec Consultants (2009), and the Killam Prize in Engineering (2016). She is also the founding director of BioZone, a Centre for Applied Bioscience and Bioengineering Research and a Tier 1 Canada Research Chair in Anaerobic Biotechnology.

Chair: **Dr. Carole Beaulieu**, Université de Sherbrooke, Sherbrooke, QC

Carole Beaulieu, dean of the Faculty of sciences at the Université de Sherbrooke, is a plant pathologist. She received a Ph.D. in plant biology from Université Laval in 1988. Professor at the Université de Sherbrooke since 1990, her laboratory is focusing its research on the interactions between actinobacteria and plants. Dr. Beaulieu has published over 100 peer-reviewed papers. From 2004 to 2018, she had led Centre SÈVE, a strategic network funded by FQRNT that brings together about sixty researchers working in plant and agriculture sciences. She has supervised the work of 55 graduate students and she leads AgroPhytoSciences, a NSERC CREATE training program designed to address the technological, environmental and socio-economic challenges of crop production.

Session 3 – Antibiotics and resistance, from animal production to public health II June 11th 10:45 AM

Keynote speaker: **Dr. Herman Barkema**, University of Calgary, Calgary, AB

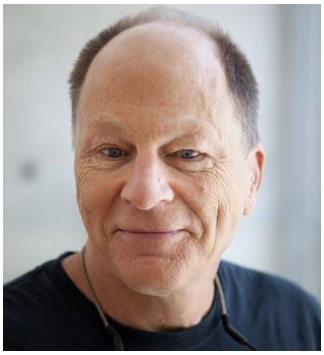
Effect of restriction in the use of antibiotics in food animals on antibiotic resistance in food animals and humans

Dr. Herman Barkema completed his DVM degree at Utrecht Univ., The Netherlands, in 1988. He spent 2½ years as manager of a large dairy and beef herd in Costa Rica, then taught bovine herd health at Utrecht Univ. and worked as an epidemiologist for the Dutch Animal Health Service while completing his PhD. In 2001, Dr. Barkema moved to the Univ. of PEI. He subsequently moved to the Univ. of Calgary, where he founded the Univ. of Calgary Faculty of Veterinary Medicine's (UCVM) Dept. of Production Animal Health. He currently is Professor in Epidemiology of Infectious Diseases at UCVM and NSERC Industrial Research in Infectious Diseases of Dairy Cattle, with a joint appointment in the Dept. of Community Health Sciences of the Cumming School of Medicine. He is also a Guest Professor at Ghent Univ. (Belgium) and Foreign Expert at the China Agricultural Univ. in Beijing. Dr. Barkema's research program focuses on prevention and control of diseases in cattle herds, including antimicrobial resistance. He has published > 300 scientific manuscripts and has lectured all over the world. He leads the University of Calgary Biostatistics Centre, the Environment research theme in the Canadian Bovine Mastitis Research Network, the Alberta Johne's Disease Initiative, the Antimicrobial Resistance – One Health Consortium, and University of Calgary's Office of One Health and One Health Emerging Cross-cutting Research Theme.

Chair: **Dr. Mario Jacques**, Université de Montréal, Montréal, QC

After completing his doctoral thesis in microbiology at the Université de Montréal, Mario Jacques did his post-doctoral training with J.W. Costerton at the University of Calgary. He then joined the Department of Pathology and Microbiology of the Faculty of Veterinary Medicine of Université de Montréal in 1985. He is currently Professor in this department and Director of the Regroupement de recherche pour un lait de qualité optimale (Op+lait) as well as co-PI of the NSERC-CREATE in Milk Quality. His research interests focus on virulence factors of bacterial pathogens with an emphasis on biofilm formation. He has published close to 200 scientific papers. He received the Fisher Award from the Canadian Society of Microbiologists and the Pfizer and Vétoquinol research awards for his outstanding contributions in animal health.

Session 4 – Systems and synthetic biology I June 11th 10:45 AM



Keynote speaker: **Dr. John Glass**, J. Craig Venter Institute, Rockville, MD, USA

Design, construction, and analysis of a synthetic minimal bacterial cell

Dr. John Glass is a Professor and leader of the J. Craig Venter Institute (JCVI) Synthetic Biology Group. His expertise is in molecular biology, microbial pathogenesis, RNA virology, and microbial genomics. Glass is part of the JCVI team that created the first bacterial cell with a chemically synthesized genome and a bacterial cell with a synthetic genome encoding only the essential gene set needed for life. To do this, JCVI scientists developed the fundamental techniques of the new field of synthetic genomics including genome transplantation and assembly. Glass led the JCVI project that rapidly made synthetic influenza virus vaccine strains. Glass and his JCVI colleagues are now using synthetic biology and synthetic genomics approaches developed at the JCVI to build fully synthetic human artificial chromosomes and viruses, prokaryotic cells, and eukaryotic cells with redesigned genomes capable of expanding biological knowledge and solving human problems in medicine, industry and the environment

Prior to joining the JCVI, Glass spent five years in the Infectious Diseases Research Division of the pharmaceutical Eli Lilly. There Glass was part of the Lilly team that developed Incivek, one of the first drugs to cure hepatitis C virus. Glass earned his undergraduate (Biology) and graduate degrees (Genetics) from the University of North Carolina at Chapel Hill. His Ph.D. work was on RNA virus genetics in the laboratory of Gail Wertz.

Chair: **Dr. Jean-Philippe Côté**, Université de Sherbrooke, Sherbrooke, QC

Jean-Philippe receive his B.S. in biochemistry at the Université de Sherbrooke in 2007. He then completed his PhD under the supervision of Dr. Michael Mourez at the Université de Montréal in 2014, characterizing the structure-function relationship of a family of autotransporters of Gram-negative bacteria. After his PhD, he joined the laboratory of Dr Eric Brown at McMaster University to explore genetic interaction networks in *Escherichia coli* and discover novel molecules with activity against Gram-negative bacteria. He joined the department of biology at the Université de Sherbrooke in 2019.

Session 5 – One Health – Climate Change, Vector-borne diseases and emerging pathogens June 12th 8:30 AM

Keynote speaker: **Dr. Nicholas Ogden**, Public Health Agency of Canada (PHAC)

Emerging infectious diseases: causes, consequences and how we respond

Dr. Nick Ogden is a UK-trained veterinarian (University of Liverpool, 1983). After 10 years of mixed clinical practice, he then completed a doctorate in Lyme disease ecology at the Department of Zoology, University of Oxford in 1996. During the six years he spent as a professor at the Faculty of Veterinary Science, University of Liverpool, he continued his research into the ecology and epidemiology of tick-borne diseases of public health importance in Europe and those of importance to livestock production in Africa. In 2002, he moved to Canada, where he continued research on the ecology of Lyme disease and other zoonoses and climate change as a research scientist at the Public Health Agency of Canada (PHAC). As interim Director of the Environmental Issues Division of PHAC he directed a program on climate change and vector- and water-borne disease risks, and community adaptation to these risks. As Director of the Zoonoses Division he directed programs on national coordination, surveillance and prevention of zoonoses including Lyme disease and West Nile virus. He is now a senior research scientist and Director of Public Health Risk Sciences division within the National Microbiology Laboratory of PHAC focusing on assessing risk by study of the ecology, epidemiology and genetic diversity of vectors and zoonotic and vector-borne microorganisms, assessing impacts of climate change on zoonoses and vector-borne diseases, and developing tools for public health adaptation.

Chair: **Dr. Alex Carignan**, Université de Sherbrooke, Sherbrooke, QC

Dr. Carignan is an Associate Professor at the Faculté de Médecine et des Sciences de la Santé de l'Université de Sherbrooke. He is a medical microbiologist and infectious disease consultant at the Centre hospitalier universitaire de Sherbrooke and has also obtained a Master's degree in epidemiology from the London School of Hygiene and Tropical Medicine. Dr. Carignan is a clinical research scholar from the Fonds de Recherche en Santé du Québec, and his work includes, among others, emerging infections.

Session 6 – Environmental microbiomes June 12th 8:30 AM

Keynote speaker: **Dr. Alexander Culley**, IBIS, Université Laval, Québec, QC

Wild viruses in the High Arctic

Dr. Alexander Culley is an Associate Professor in the Department of Biochemistry, Microbiology and Bioinformatics at Université Laval. I have been active in the field of Viral Ecology for more than 15 years, starting with my graduate work at the University of British Columbia, as a postdoctoral fellow in the Center for Microbial Oceanography: Research and Education at the University of Hawaii at Mānoa, and now as an Associate Professor at Université Laval. The overarching goal of my research program is to gain a deeper understanding of the impact, diversity and dynamics of the wild virus communities in aquatic habitats, and how viral ecology is affected by the rapid and evolving changes due to climate change. The Arctic provides a model environment to pursue these two themes because the region is experiencing some of the most dramatic changes on Earth due to rising temperatures and it harbours a wide diversity of aquatic habitats whose viral communities are largely uncharacterized. My laboratory, the Viral Discovery and Ecology Laboratory (ViDEL) has been working in two locations in the subarctic and High Arctic that exemplify the dynamism of the region attributable to climate change to answer some important first order ecological questions about viruses.

Chair: **Dr. Adnane Sellam**, Université Laval, Québec, QC

Dr. Adnane Sellam is an associate professor at Université Laval in Quebec City. He is also a member of the CHU de Québec research center and Big Data Research Centre (BDRC-UL). Dr. Sellam held a Ph.D. from University of Angers (France) and undertook a postdoctoral training at University McGill/NRC Canada. Dr. Sellam is an expert on systems biology and genomics, applied to fungal pathogens. His research has been funded by different recognized provincial (CQDM, FRQS) and federal (CIHR, NSERC, CFI) funding organisms. The main objective Dr. Sellam' research is to decipher regulatory circuits that control the sensing and the metabolic flexibility of *Candida albicans* to hypoxic environment, a condition predominantly encountered inside the host, but neglected so far. Additionally, mechanisms that coordinate growth and division in *C. albicans*, and thus controlling cell size homeostasis, are investigated. These biological processes are intimately linked to *C. albicans* virulence and fitness inside the host. Dr. Sellam' team is also exploiting forward chemogenomic to uncover small molecules that inhibit key fungal traits associated with virulence including the invasive filamentous growth, biofilm formation and adaptation to acidic environment such as the one encountered in the human vagina. His group has recently identified novel antifungal chemical scaffolds that are currently under clinical validation.

Session 7 – One Health – Microbiome, microbial interactions and molecular pathogenesis June 12th 10:45 AM

Keynote speaker: **Dr. Corinne F. Maurice**, McGill University, Montréal, QC

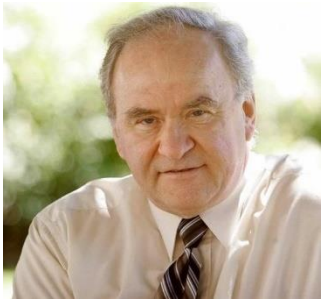
Interactions between bacteria and bacteriophages in the human gut

Dr. Maurice is a Tier 1 Canada Research Chair in Gut Microbial Physiology and a CIFAR Azrieli Global Scholar. She has been working with a systems biology approach on microbial communities for over 10 years, in aquatic systems and in the human gut. She pioneered the use of single cell approaches to understand how gut bacteria respond to perturbations and interact with their viral predators (bacteriophages). She demonstrated that common therapeutic drugs can rapidly alter bacterial activity – a possible explanation for different side effects to the same therapeutic treatments. Her collaborative work determined how diet and infection can alter bacterial activity and diversity. At McGill University, her lab is currently taking these approaches further to characterize gut bacterial metabolism, and how to regulate this metabolism with bacteriophages to promote health.

Chair: **Dr. Alfredo Menendez**, Université de Sherbrooke, Sherbrooke, QC

Dr. Menendez is currently an Associate Professor at the department of Microbiology and Infectious Diseases at the Université de Sherbrooke. He graduated with a BSc in Biology from Havana University, Cuba, in 1985. He obtained his PhD in 2005 under the supervision of Dr. Jamie K. Scott at Simon Fraser University, Vancouver, in the field of anti-HIV-1 vaccine development and phage-displayed peptide libraries. His PhD was funded by a Canada Graduate Scholarship from NSERC and a graduate scholarship from the Michael Smith Foundation for Health Research. After completing his PhD, Dr. Menendez joined the group of Dr. Brett Finlay at Michael Smith Laboratories in the University of British Columbia as a post-doctoral fellow. He worked on the molecular mechanisms driving the innate immune response against enterohepatic bacterial pathogens and obtained postdoctoral fellowships from NSERC and the Michael Smith Foundation for Health Research. His postdoctoral research was focused on the immunopathogenesis of *Salmonella enterica* infections in the hepatobiliary system and the study of the molecular mechanisms for the regulation of intestinal antimicrobial peptides synthesis and function. In 2011, Dr. Menendez was recruited as an Assistant Professor to the Université de Sherbrooke. As an independent researcher, he aims to understand the role of bacteria (both commensal and pathogens) in the molecular crosstalk between the gut and the hepatobiliary system, as these interactions are a fundamental aspect of enterohepatic function and homeostasis. The two general research areas of his group are (i) the involvement of hepatobiliary function in the regulation of the intestinal immunological homeostasis and immune response, and (ii) the molecular mechanisms underlying bacterial control of the intestinal enteroendocrine function, in health and disease.

Session 8 – Food microbiology June 12th 10:45 AM



Keynote speaker: **Dr. Jacques Goulet**, Université Laval, Québec, QC

La microbiologie des aliments : d'hier à demain

Détenteur d'un doctorat en microbiologie appliquée de l'Université McGill, M. Goulet a été actif en enseignement et en recherche à l'Université Laval pendant 38 ans. Auteur de nombreux articles scientifiques et rapports techniques touchant divers aspects de la transformation des aliments, il a œuvré et continue d'œuvrer comme conseiller scientifique et technique auprès de plusieurs entreprises bio-alimentaires, particulièrement en transformation laitière et en production de levures.

Ses recherches et ses compétences en microbiologie appliquée et en technologie alimentaire lui ont permis d'apporter une contribution significative au développement de nouveaux procédés de fermentations industrielles, de valorisation de sous-produits laitiers et d'assainissement des usines de transformation alimentaire.

Chair: **Dr. Steve Labrie**, Université Laval, Québec, QC

Le Professeur Steve Labrie a obtenu son doctorat en microbiologie à l'Université Laval. Il est professeur titulaire au Département des Sciences des aliments depuis 2006. Il est directeur du Centre de recherche en sciences et technologie du lait (STELA) et menbre de l'Institut sur la nutrition et les aliments fonctionnels (INAF). Ses activités de recherche sont surtout ciblées sur la microbiologie des fromages de spécialité. Plus précisément, son équipe travaille à la caractérisation des levures et des moisissures d'affinage des fromages à croûte fleurie et des fromages à croûte lavée. Il collabore depuis plusieurs années avec des fromageries de toutes tailles dans différents projets de recherche. Depuis 2009, il travaille activement sur un projet de caractérisation des laits du terroir québécois. Son équipe utilise les outils modernes de la biologie moléculaire et de la génomique qui permettent une meilleure compréhension de la dynamique des populations de microorganismes et aident à décrire leurs activités en cours d'affinage. Le Dr Labrie est également coéditeur d'un ouvrage de référence de l'International Dairy Federation portant sur le rôle et le contrôle du sel dans le fromage. En plus, des recherches sur les cultures d'affinage, son équipe a développé plusieurs outils d'identification des contaminants fongiques en environnement laitier, ce qui permet des interventions ciblées en hygiène industrielle.

Session 9 – One Health –Alternatives to antibiotics June 13th 8:30 AM

Keynote speaker: **Dr. Thomas Louie**, University of Calgary, Calgary, AB

Alternatives to antibiotics: opportunities to prevent microbiome destruction and to repair /replace healthy microbiomes

Dr. Thomas Louie MD FRCPC is Clinical Professor of Medicine (Emeritus) at the University of Calgary. After completing internal medicine and infectious diseases at UCLA and Tufts University / New England Medical Center 1973-76, he has been an attending infectious diseases clinician over 4 decades in Boston, Winnipeg and Calgary. He currently is medical director of the Microbial Health Clinic at the Foothills Medical Center, Calgary specializing in RCTs of narrow spectrum agents for *Clostridium difficile* infection, RCTs for microbiome therapies. Interest has focused on hospital infection control program development, clinical trials of new and novel antimicrobial agents which spare the normal microbiota and pioneering in fecal transplantation since 1996 to replace or augment the microbiome to arrest recurrent *C. difficile* infection. Development of oral capsules of fecal microbiota in 2010-2013 and introduction into clinical practice has facilitated an expanding role of restoring or modifying the microbiome in clinical medicine. RCTs to evaluate components of microbiome replacement in CDI, autism, clinical depression and clearance of intestinal carriage of multi-drug resistant gram- negative bacilli are current activities.

Chair: **Dr. François Malouin**, Université de Sherbrooke, Sherbrooke, QC

François Malouin is professor (microbiology) at the Department of biology at the Faculty of sciences of Université de Sherbrooke since 2000. He is a microbiologist that has more than 30 years of academic and industrial experience in drug discovery for use in humans and food-producing animals. Prof. Malouin obtained a doctoral degree (Ph.D.) in Medical Sciences (Medical Microbiology) at the University of Calgary (1988) and did postdoctoral training in the anti-infective research group at Lilly Research Laboratories (Eli Lilly & Co., Indianapolis, USA) from 1988 to 1990. Prof. Malouin was also Assistant professor of microbiology at the Faculty of medicine of Université Laval in Quebec City where he had a Fellowship from the Medical Research Council of Canada (1990-1994). He was then recruited by biotechnology companies, first Microcide Pharmaceuticals, Inc., then a sister company, Iconix Pharmaceuticals, Inc. (Silicon Valley, California, USA) from 1994 to 2000, where he was Associate Director of technology development for the discovery of new therapeutic targets and antibiotics. He was also a co-founder of Ulysses Pharmaceuticals (Sherbrooke, QC). Currently at U. de Sherbrooke, his research projects aim at exploiting virulence genes for the development of new antibiotics, vaccines and non-antibiotic alternatives for applications in human and animal health. He is co-author of >100 scientific publications and co-inventor of more than 20 patents (delivered or pending).

Session 10 – Plants and microbes June 13th 8:30 AM

Keynote speaker: **Dr. Allyson M. MacLean**, University of Ottawa, Ottawa, ON

Unraveling the molecular mechanisms of plant-microbe interactions

Dr. Allyson MacLean recently joined the University of Ottawa as an Assistant Professor to study the molecular mechanisms that underpin the beneficial symbiotic relationship that occurs between land plants and arbuscular mycorrhizal fungi. In 2009, Dr. MacLean received her doctoral degree from McMaster University, under the mentorship of Dr. Turlough Finan. As a recipient of a Marie Curie International fellowship in 2010, Dr. MacLean led a postdoctoral research project at the John Innes Centre in Norwich, England, to examine the role of effector proteins in the plant pathogen phytoplasma. Intrigued by the idea of identifying microbial effector proteins in a beneficial symbiosis between plants and microbes, Dr. MacLean joined the Harrison lab at the Boyce Thompson Institute at Cornell University in 2014, before accepting a position as Assistant Professor at the University of Ottawa in 2017. Her long-term research goal is to improve our understanding of the roles of both plant and fungal proteins in modulating arbuscular mycorrhizal symbiosis.

Chair: **Dr. Pascale Beauregard**, Université de Sherbrooke, Sherbrooke, QC

Dr. Pascale Beauregard obtained her B.Sc. in biochemistry from Université de Montréal (2002), and completed her PhD thesis on a yeast prion-like phenomenon in 2009 (Université de Montréal). She then became a postdoctoral fellow at Harvard Medical School (2010-2014), where she started exploring the relations between bacteria, biofilms and plants under the mentorship of Dr Roberto Kolter. She joined the biology department at Université de Sherbrooke in 2014, and was awarded tenure in 2019. Her laboratory explores diverse topics related to biofilm formation and interactions between bacteria and their environment, such as biofilm's role in Alzheimer's disease, sporulation of *Bacillus* spp. on plants, micro- and macro-nutrient acquisition, and bacterial conjugation in biofilms. Her most recent work was published in MPMI, mSphere, mBio and Applied and Environmental Microbiology. She is also a member of the local organizing committee for the current CSM meeting.

Session 11 – Microbial chemosphere June 13th 10:45 AM

Keynote speaker: **Dr. Clarissa S. Sit,** St-Mary's University, Halifax, NS

A happy accident: microbial volatiles for plant defense

Born and raised in Edmonton, Clarissa completed a BSc Honours in Pharmacology and a PhD in Chemistry at the University of Alberta. She then moved to Boston to work as an NSERC Banting and Alberta Innovates Health Solutions Postdoctoral Fellow at Harvard Medical School. In 2016, she started her independent career at Saint Mary's University, where she is now an Associate Professor in the Department of Chemistry. Clarissa runs a large group (12 undergraduate and graduate students) that investigates microbial warfare and other interactions between environmental fungi and bacteria. The team uses an applied science approach to tackle human health challenges, such as antibiotic-resistant bacteria, and ecological pests, such as White Nose Syndrome in bats and American Foulbrood Disease in honey bees. Her group has also started a partnership with local vineyards to study the impact of environmental microbes on the productivity of grape vines and the flavour of the resulting wines. All of this research is possible with the support of NSERC, CFI, NSHRF, Springboard Atlantic, NS Productivity Innovation Voucher Program, and Saint Mary's University's internal grants. While Clarissa enjoys nearly every aspect of her job, she is most passionate about working with budding student scientists, all of whom inspire hope for an exciting future.

Chair: **Dr. Jean-Philippe Bellenger**, Université de Sherbrooke, Sherbrooke, QC

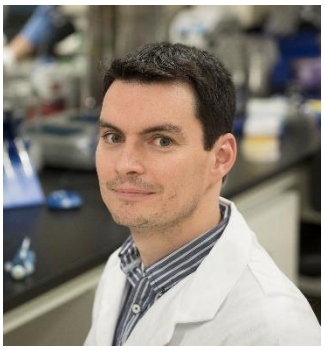
Jean-Philippe Bellenger is a trace metal biogeochemist. He received his PhD from Strasbourg University (France) in 2005, and worked as a postdoc fellow at the Princeton Environmental Institute (USA) for 5 years before joining Université de Sherbrooke in 2010. He is the chair holder of the Canadian research chair in boreal biogeochemistry (2010-2020). His research investigates how trace metal availability affects key biological processes controlling the global biogeochemical cycling of carbon and nitrogen. His research contributed to identify molybdenum limitation of biological nitrogen fixation in ecosystems ranging from the tropical forest to the arctic tundra. His recent research aimed at characterizing the importance of nitrogenase enzymatic diversity on biological nitrogen fixation in cold ecosystems. He also investigates the role of metallophores, small organic ligands, on metal acquisition and homeostasis in model microorganisms. His research group also studies the fate and environmental impact of organic contaminants in various ecosystems and biological models.

Session 12 – Systems and Synthetic Biology II June 13th 10:45 AM

Keynote speaker: **Dr. Carol A. Gross**, University of California, San Francisco, CA, USA

The role of extracytoplasmic function (ECF) Sigma factors across the bacterial domain

Carol Gross is a Professor at UCSF. Her long-term goal is to understand how bacterial circuits and networks are integrated into cellular function. Her current studies are geared in three directions—to understand translational control in prokaryotes; to utilize chemical genomic and genetic interaction analyses at a global level to uncover networks in the cell, particularly those involving the cell envelope; and to develop global genetic interrogation methods that function across a large swath of bacteria, including pathogenic and microbiome-associated bacteria. Her work not only addresses the "phenotype gap" currently impeding effective use of genomic information, but also critically demonstrates the joint power of systems-level phenotyping and mechanistic studies to establish gene function and higher-order connections between processes. She is a member of the National Academy of Science, a Fellow of the American Association for the Advancement of Science, and a recipient of the NAS Waksman Prize(2011), and the ASM Lifetime achievement Award (2019) Her other passion is increasing diversity in the sciences. She has received the UCSF Martin Luther King Award for spearheading the efforts that have increased graduate student diversity at UCSF, and the ASM EMD Millipore Alice C. Evans Award for contributions to the advancement of women in science. Most recently, Carol has been working to increase faculty diversity at UCSF as part of the Differences Matter Initiative.



Chair: **Dr. Sébastien Rodrigue**, Université de Sherbrooke, Sherbrooke, QC

Prof. Sébastien Rodrigue earned a Ph.D in Biology (Université de Sherbrooke, Canada) for his work on transcription regulation and gene expression regulatory mechanisms in the human pathogen Mycobacterium tuberculosis. He next joined the laboratory of Sally W. Chisholm at the Massachusetts Institute of technology were where he studied the cyanobacterium *Prochlorococcus*, the most abundant photosynthetic organism on Earth. He acquired a solid background in genomics and transcriptomics, established robust protocols for whole-genome sequencing from individual cells, and developed new methods for high-throughput nucleic acid sequencing technologies. During this period, Prof. Rodrigue also initiated a collaboration with Dr. Tom Knight Jr to study the near-minimal bacterium *Mespolasma florum* with the objective of creating a safe and convenient prototyping platform for systems and synthetic biology. He then returned to the Université de Sherbrooke to establish his own research group on microbial systems and synthetic biology. His group was able to clone the entire genome of *M. florum* in yeast, before transplantation into a recipient bacterium that undergoes a species conversion. The Rodrigue laboratory is also engineering probiotic bacteria as living therapeutics that could be used to treat several diseases such as infections caused by antibiotic-resistant pathogens. Prof. Rodrigue collaborates with many top scientists internationally and with innovating Canadian life science companies.

CSM Awards

CSM Murray Award for Career Achievement

Microbiology in Canada received national attention when Professor Emeritus R.G.E. Murray, University of Western Ontario, was appointed an officer of the Order of Canada. He was honoured for his lifetime contributions to the development of microbiology in Canada. The Canadian Society of Microbiologists owes a special debt of gratitude to Dr. Murray. He was chairman of the Founding Committee for the Society (1950-1951) and Founding President (1951-1952). The Society, he said, was "a much-needed catalyst and unifier for the diverse applied and basic microbiologists of the country". He has remained an active member of the Society throughout his scientific career and was elected an honorary member in 1985. Societies and their journals, particularly the Canadian and American Societies, continued to be important to Dr. Murray over the years. He persuaded the National Research Council of Canada that a microbiology journal was a justifiable publication and was appointed the founding editor of the Canadian Journal of Microbiology (1954-1960). Dr. Murray's research in bacterial cytology, structure and function, and systematics and taxonomy has brought numerous honours and awards: the Harrison Prize, Royal Society of Canada (shared with C.F. Robinow), 1957; Fellowship in the Royal Society of Canada, 1958; the Canadian Society of Microbiologists Award, 1963; election to the American Academy of Microbiology, 1973; the Flavelle Medal, Royal Society of Canada, 1984; and the J. Roger Porter Award, American Society for Microbiology (U.S. Federation of Culture Collections), 1987. He also holds honorary degrees from the University of Western Ontario and the University of Guelph and was granted the degree Doctor of Science honoris causa by McGill University in 2007. And, many of his students and postdoctoral fellows continue the fine road in microbiology he has forged for them.

Canadian Journal of Microbiology

Thermo Fisher Scientific Award

This award is generously supported by Fisher Scientific and is intended to stimulate and recognize new researchers in the microbiological sciences. Specifically, this prestigious award recognizes outstanding contributions to microbiology as a discipline by early career researchers who are within a decade of receiving their PhD degrees. The award comprises a complementary registration to the annual conference, a cheque for $1,500, and a framed certificate.

ThermoFisher SCIENTIFIC

Armand-Frappier Outstanding Student Award

Armand Frappier was a founding member of the Canadian Society of Microbiologists and its third president (1954-55). He was inducted into the Canadian Medical Hall of Fame in 2012. A full accounting of his accomplishments and links to a vignette are available at Canadian Medical Hall of Fame website (http://cdnmedhall.org/inductees/dr-armand-frappier). In order to honor Armand Frappier, the Society has renamed one of its major awards. The CSM Gold award is now Armand-Frappier Outstanding Student Award/La bourse Armand-Frappier pour l'étudiant exceptionnel. The award with its new name was presented for the first time at the CSM 2012 Annual Conference in Vancouver. University nominations are reviewed and ranked by a 5-member International Scientific Committee. The award consists of a cheque for $500, a framed certificate, travel, accommodation, and complementary registration to the annual conference. The Armand Frappier Award is made possible by the generous financial support of CSM.

CSM Knowles Postdoctoral Research Prize

Professor Roger Knowles, Professor Emeritus, Department of Natural Resource Sciences, Faculty of Agricultural and Environmental Sciences, Macdonald College, McGill University. Roger Knowles was an internationally recognized leader

in nitrogen and carbon cycling in terrestrial and aquatic ecosystems. His contributions to advancing our knowledge of denitrification, nitrification, nonsymbiotic nitrogen fixation and methane cycling is documented in over 160 peer-reviewed articles and book chapters. Roger's lab provided training to over 50 graduate students and 20 postdoctoral fellows. Roger was a past president of the Association des Microbiologistes du Quebec and the Canadian Society of Microbiologists. He served on several committees as a member of the American Society for Microbiology and Soil Science Society of America. Roger was made a Fellow of the Royal Society of Canada in 1987, awarded a D.Sc. from the University of London in 1986 and was a member of the Society of Sigma Xi.

Terry Beveridge Poster Competition Award

Terry Beveridge was a Professor of Microbiology at the University of Guelph for 29 years where he developed many approaches using electron microscopy to understand bacterial structure. Terry published extensively during his career, producing over 260 peer-reviewed journal articles, more than 60 books, book chapters and reviews, and gave numerous guest lectures, travelling worldwide. Dr. Terry Beveridge was a strong supporter of the CSM, always an advocate for excellence in student research and in communication of their work. He participated as a reviewer of poster and oral presentations and was enthusiastically supportive of recognizing outstanding achievements. Following his death in 2007, his colleagues in the Department of Molecular and Cellular Biology established a scholarship fund to help graduate students expand their knowledge in the field of microbiology. Terry's family and his colleagues in the Department of Molecular and Cellular Biology at the University of Guelph have worked with the CSM to transfer the Terry Beveridge Memorial Scholarship Fund for administration by the CSM in support of graduate student activities. The Terry Beveridge Memorial Scholarship Fund is now sponsoring awards of $500 each for three meritorious poster presentations at the annual CSM conference. As of the 2013 CSM Conference, the poster competition will be named the Beveridge Poster competition and three awards will be presented to the best student, first/senior authored posters at the CSM Annual conference.

CSM Students Awards

Student Awards Symposium

1:30-3:30 PM | Tuesday, June 11th and Wednesday, June 12th

The selection of students for this symposium was made based on submitted extended abstracts by the CSM Education Committee. The student applying for the award must be a member of the CSM.

Poster Competition

3:00-5:00 PM | Wednesday, June 12th

The selection of students for this symposium was made based on submitted extended abstracts by the CSM Education Committee. The student applying for the award must be a member of the CSM. Students presenting as part of the competition are to be by their posters to respond to judges' questions from 3:00 – 5:00 PM, Wednesday, June 12th.

The Terry Beveridge Poster Awards

The Terry Beveridge Memorial Scholarship Fund since 2013 has been sponsoring three awards of $500 each for best poster presentations.

CSM Knowles Postdoctoral Research Prize

3:30-5:00 PM | Thursday, June 13th

Rogers Knowles Lab Alumni Fund since 2017 has been sponsoring one award for $500 for best Oral Presentation at the Postdoctoral Research Symposium.

Canadian Science Publishing (CSP) Student Symposium Award

The Award is sponsored by CSP in the amount of $500 and a certificate. It will be presented to one of the best student presentations as part of the Student Awards Symposium.

Cedarlane Student Symposium Award

One award of $500 and a plaque. The Award is sponsored by Cedarlane and will be presented to the best student presentation as part of the Student Awards Symposium.

CCM Student Symposium Award

One award of $500 and a certificate. The award will be presented to one of the best student presentations as part of the Student Awards Symposium.

ISME-8 Student Symposium Award

One award of $500 and a certificate presented by the Atlantic Canada Society for Microbial Ecology. The award will be presented for the best student oral presentation in microbial ecology.

ASM Best Poster Layout Award

The ASM will provide one award consisting of a $200 value of a complementary ASM student membership and ASM book or journal. The award will be offered for the best poster layout.

CSM Undergraduate Award

Up to six awards valued at $500 each. The award is accompanied by a certificate of merit. To recognize outstanding promise in an undergraduate student for early career contributions to microbiology and to encourage continued interest in microbiology and student engagement with the CSM.

CSM Graduate Ambassador Award

Up to two awards valued up to $1,500 each. The award includes a $200 honorarium and up to $1,300 to cover travel-associated expenses. To enable outstanding graduate students or early career postdocs (within 2 years of graduating) to present research at (at least) two Canadian academic institutions (typically a regional location and national location).

CSM Student / Postdoctoral Travel Awards

A maximum of twenty travel awards to the CSM Annual Conference in the amount of $250 each will be given. The CSM Meetings Secretary will select the awardees on a lottery basis and advise the award winners before the conference

ISME-8 Student Travel Awards

A maximum of five travel awards in the amount of $400 each are available to cover travel to the CSM Annual Conference. Awards will be given to students from outside the province where the conference is being held.

Poster Titles

Poster Competition

Wednesday, June 12th, from 3:00 - 5:00 PM

Presenting students are to be by their posters from 3:00 – 5:00 PM to respond to judges' questions.
Note: **AEM**: Applied Environmental Microbiology
II: Infection and Immunity | **MGCM**: Molecular Genetic Cellular Microbiology

II-PC01

Reovirus modulates host cell alternative splicing landscape during infection through the µ2 protein

Simon Boudreault[1], Guy Lemay[2], Martin Bisaillon[1]

[1]Université de Sherbrooke, [2]Université de Montréal

II-PC02

Changes in host cell gene expression levels and alternative splicing during *Flavivirus* infections

Carolin Brand[1], Kristen Bullard[2], Brian Geiss[2], Martin Bisaillon[1]

[1]Université de Sherbrooke, [2]Colorado State University

II-PC03

Heterogeneity in two-component signalling systems within different strains of inflammatory bowel disease associated *Escherichia coli*

Adam Khan[1], Youn Hee Cho[1], Michael Renouf[1], Joseph McPhee[1]

[1]Ryerson University

II-PC04

Characterization of three new SPATE autotransporters and cumulative role of SPATEs in pathogenesis of Extra-intestinal pathogenic *Escherichia coli*

Pravil Pokharel[1,2], Hajer Habouria[1,2], Segolène Maris[1], Charline Herscher[1], Noëmie Fessy[1], Sebastien Houle[1,2], Charles M. Dozois[1,2]

[1]INRS-Institut Armand-Frappier, Laval, Quebec, Canada, [2]CRIPA-Centre de recherche en infectiologie porcine et avicole, Saint-Hyacinthe, Quebec, Canada

AEM-PC01

Reevaluation of biological nitrogen fixation (BNF) by deadwood of boreal forest

Apolline Benoist[1], Daniel Houle[2,3], Robert L. Bradley[4], Jean-Philippe Bellenger[1]

[1]Centre Sève, Département de Chimie, Université de Sherbrooke, J1K 2R1, QC, Canada, [2]Direction de la Recherche Forestière, Ministère des Forêts, de la Faune et des Parcs, G1P 3W8, QC, Canada, [3]Ouranos, H3A 1B9, QC, Canada, [4]Centre Sève, Département de Biologie, Université de Sherbrooke, J1K 2R1, QC, Canada

AEM-PC02

Metagenomic analysis of virus diversity and relative abundance in a freshwater harbour

Christine N. Palermo[1], Roberta R. Fulthorpe[2], Rosemary Saati[2], Steven M. Short[1]

[1]University of Toronto Mississauga, [2]University of Toronto Scarborough

AEM-PC03

A model cooling tower harboring *L. pneumophila* and *V. vermiformis*: Is there Legionella in the biofilm?

Adriana Torres Paniagua[1], Kiran Paranjape[1], Sebastien Faucher[1]

[1]McGill University

AEM-PC04

How an Antarctica *Rhodotorula* novel strain adapts to cold environments - a combination of Phenotypic MicroArray, mRNA transcriptomic and small RNA analysis

David Touchette[1], Jacqueline Goordial[2], Cene Gostinčar[3], Nina Gunde-Cimerman[3], Lyle G. Whyte[1]

[1]McGill University, [2]Bigelow Laboratory for Ocean Sciences, Maine, USA, [3]University of Ljubljana, Ljubljana, Slovenia

MGCM-PC01

Genome-scale metabolic modelling reveals key features of a minimal gene set for *Mesoplasma florum*

Jean-Christophe Lachance[1], Dominick Matteau[1], Joelle Brodeur[1], Nathan Mih[2], Zachary A. King[2], Colton Lloyd[2], Pierre-Étienne Jacques[1], Adam Feist[2], Bernhard O. Palsson[2], Jonathan M. Monk[2], Sébastien Rodrigue[1]

[1]Université de Sherbrooke, [2]University of California San Diego

MGCM-PC02

Single-stranded DNA annealing and ATP-dependent DNA unwinding are conserved activities of hairpin telomere resolvases

Siobhan McGrath[1], Shu Hui Huang[1], Kienna Mills[1], Kerri Kobryn[1]

[1]Department of Biochemistry, Microbiology and Immunology, College of Medicine, University of Saskatchewan

MGCM-PC03

Antimicrobial resistance dissemination in *Vibrio cholerae*: mechanistic insights into the insidious role of IncC plasmids

Nicolas Rivard[1], Vincent Burrus[1]

[1]Département de biologie, Université de Sherbrooke

MGCM-PC04

Unique synergistic interaction in *Bacteroides* reveals unexpected hemolytic phenotypes

H. Shareefdeen[1], A.P. Hynes[1,2]

[1]Department of Medicine, McMaster University, [2]Department of Biochemistry and Biomedical Sciences, McMaster University

Poster Session I

Tuesday, June 11th, from 3:00 - 5:00 PM

Presenters to be by their posters:
Odd number posters from 3:00 - 4:00PM & **Even number** posters from 4:00 - 5:00 PM

Note: **AEM**: Applied Environmental Microbiology
II: Infection and Immunity | **MGCM**: Molecular Genetic Cellular Microbiology

Infection & Immunity

II0

SalmoSeq, an AmpliSeq-based molecular tool for rapid identification and virulence prediction of *Salmonella*

Jean-Guillaume Emond-Rheault[1], Alanna Cruse[2], Maude Kerhoas3[3], Jérémie Hamel1[1], Irena-Kukavica-Ibrul [1], Samantha Gruenheid[2], Danielle Malo[2], France Daigle[3], Lawrence D. Goodridge[4], Brian Boyle[1], Roger C. Levesque[1]

[1]Institut de Biologie Intégrative et des Systèmes, [2]McGill University Research Centre on Complex Traits, [3]Département de microbiologie et Immunologie, Université de Montréal, [4]Department of Food Science, University of Guelph.

II1

A novel plasmid-encoded fimbriae from extra-intestinal pathogenic Escherichia coli (ExPEC) contains a distinct type of P fimbrial adhesin

Hajer Habouria[1,2], Amélie Garénaux[1,2], Sébastien Houle[1,2], Charles Dozois[1,2]

[1]INRS-Institut Armand-Frappier, Laval, Quebec, Canada, [2]CRIPA-Centre de recherche en infectiologie porcine et avicole, Saint-Hyacinthe, Quebec, Canada

II2

A siderophore analog of fimsbactin from Acinetobacter hinders growth of the phytopathogen *Pseudomonas syringae* and induces immunity in *Arabidopsis thaliana*

Fabrice Betoudji[1], Kamal Bouarab[1], Marvin J. Miller;[2], Manuka Ghosh;[3], Mario Jacques;[4], François Malouin[1]

[1]Université de Sherbrooke, [2]University of Notre Dame, [3]Hsiri Therapeutics, [4]Université de Montréal

II3

Analyse structurale de la protéine TraE impliquée dans la conjugaison bactérienne chez *E. coli*

Bastien Casu[1], Aleksandr Sverzhinsky[2], Aurélien Fouillen[3], Zakaria Jemouai[4], Christian Baron[5]

[1]Université de Montréal, [2]Université de Montréal, [3]Université de Montréal, [4]Université de Montréal, [5]Université de Montréal

II4

Bacterial biofilms and their role in Beta-amyloid agregation and Alzheimer's disease

David Dumoulin[1], Pascale Beauregard[1]

[1]Université de Sherbrooke

II5

Characterization of a bacterial feeding strategy based on the manipulation of the host sugar export machinery

Gaële Lajeunesse[1], Charles Roussin-Léveillée[1], Peter Moffett[1]

[1]Centre SÈVE, Département de Biologie, Université de Sherbrooke

II6

Characterization of the host-mediated modification of bacterial effector NleA and its role in virulence

Lindsay Burns[1], Natalia Giannakopoulou[1], Rufaida Hassan Khan[2], Sadjia Bekal[2], Samantha Gruenheid[1]

[1]McGill University, [2]Institut national de sante publique du Quebec

II7

Characterizing *Staphylococcus aureus* endovascular infection during vancomycin treatment

Thiago Fauerharmel Nunes[1], Ronald S. Flannagan[1], Mariya Goncheva[1], David E. Heinrichs[1]

[1]The University of Western Ontario

II8

Characterizing the inflammatory response following interaction between Ebola virus and host cells

Andrea Vucetic[1,2], Marceline Côté[3], Darwyn Kobasa[4], Martin Olivier[1,2]

[1]Department of Microbiology and Immunology, McGill University, [2]McGill University Health Centre, [3]University of Ottawa, [4]National Microbiology Laboratory, Public Health Agency of Canada

II9

Comparative therapeutic analysis of treatment A (Sofosbuvir + Daclatasvir) and treatment B (Sofosbuvir + Ribavirin) in chronic hepatitis C patients from Pakistan

Kashif Rauf[1], Bashir Ahmad[2], Safdar Ali[1], Zahoor ul Hasan[1]

[1]Sargodha Medical College, University of Saroghda, Sargodha Pakistan, [2]International Islamic Univeristy, Islamabad

II10

Competitive interaction between bovine enterohemorrhagic and commensal *Escherichia coli* and the impact of physiological stress on the host-bacteria interaction in a gnotobiotic murine model

Lange M.E.[1,2], Clarke S.T.[1,2], Boras V.F.[3], Metz G.A.[4], Kalmokoff M.L[5], Laing C.R.[6], Gannon V.P.J.[6], Church J.S.[7], Uwiera R.R.E.[2], Inglis G.D.[1]

[1]Lethbridge Research and Development Centre, Agriculture and Agri-Food Canada, Lethbridge AB, Canada, [2]Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton AB, Canada, [3]Alberta Health Services, Lethbridge AB, Canada, [4]Canadian Centre for Behavioural Neuroscience, Department of Neuroscience, University of

Lethbridge, Lethbridge AB, Canada, [5]Kentville Research and Development Centre, Agriculture and Agri-Food Canada, Kentville NS, Canada, [6]National Microbiology Laboratory, Public Health Agency of Canada, Lethbridge, AB, Canada, [7]Thompson Rivers University, Kamloops, British Columbia, Canada

II11

Construction of a multivalent peptide vaccine for dairy cows

Diana Vanessa Bran-Barrera[1], Eric Brouillette[1], Céline Ster[2], François Malouin[1]

[1]Université de Sherbrooke, [2]Agriculture and Agri-Food Canada

Bovine mastitis, an inflammation of the mammary gland mainly caused by a bacterial infection, is the most

II12

Current status of hepatitis E virus among blood donors in Qatar

Enas S. Al Absi[4], Sara Taleb[4], Laila Hedaya[4], Fatima Ali[4], Mariam Huwaidy[4], Rula Ghandour[3], Abdullatif Husseini[2], Gheyath K. Nasrallah[1,4], Nadima H. Ali[1]

[1]Biomedical Research Center, Qatar University, Doha, , [2]Department of Public Health, College of Health Sciences, [3]Institute of Community and Public Health, Birzeit University, Birzeit, Palestine, [4]Biomedical Science Department, College of Health Sciences, Doha, Qatar

II13

Deciphering the regulation mechanisms of the Std fimbrial cluster of *Salmonella enterica* serovar Typhi

Karine Dufresne[1], France Daigle[1]

[1]Université de Montréal

II14

Design of a multivalent vaccine for control and prevention of Mycobacterium avium subsp. paratuberculosis infections in dairy cattle

Alexis Dubé-Duquette[1], Céline Ster[2], François Malouin[1]

[1]Université de Sherbrooke, [2]Agriculture and Agri-Food Canada

II15

Development of a virology lab on a chip for the isolation and study of giant viruses infecting amoebae

Cynthia Gagné-Thivierge[1,2,3], Guillaume Blanc[4], Bernard La Scola[5], Denis Boudreau[6,7], Alexander I. Culley[2,3], Jesse Greener[6,8], Steve J. Charette[1,2,3]

[1]Centre de recherche de l'Institut universitaire de cardiologie et de pneumologie de Québec, Quebec City, QC, Canada, [2]Institut de Biologie Intégrative et des Systèmes, Université Laval, Quebec City, QC, Canada, [3]Département de biochimie, de microbiologie et de bio-informatique, Faculté des sciences et de génie, Université Laval, Quebec City, QC, Canada, [4]Mediterranean Institute of Oceanography, Aix-Marseille Université, Marseille, France, [5]Unité de Recherche Microbes Evolution Phylogeny and Infection (MEPHI), Aix-Marseille Université, Marseille, France, [6]Département de chimie, Faculté des sciences et de génie, Université Laval, Quebec City, QC, Canada, [7]Centre d'optique photonique et laser, Université Laval, Quebec City, QC, Canada, [8]Centre de recherche du CHU de Québec, Université Laval, Quebec City, QC, Canada

II16

Effect of vaccination against *Staphylococcus aureus* on milk quality

Julie Beaulieu[1], François Malouin[1], Rachel Gervais[2], Céline Ster[3]

[1]Université de Sherbrooke, [2]Université Laval, [3]Agriculture and Agri-Food Canada

II17

EHEC utilizes two-component systems to modulate expression of the major flagellar subunit protein, FliC, in response to host intestinal cues

Sarah Birstonas[1], Debora Barnett Foster[1,2]

[1]Department of Chemistry and Biology, Ryerson University, Toronto, Ontario, Canada, [2]Faculty of Dentistry, University of Toronto

II18

Enhanced methodologies for the isolation of *Mycoplasma bovis* from beef cattle

Sara Andres Lasheras[1], Rahat Zaheer[1], Reuben Ha[1], Catrione Lee[1], Murray Jelinski[2], Trevor Alexander[1], Tim A. McAllister[1]

[1]Lethbridge Research and Development Centre, Agriculture and Agri-Food Canada, [2]Western College of Veterinary Medicine, University of Saskatchewan

II19

EnvY as a possible negative regulator of the omptin protease OmpT in *Escherichia coli* BW25113

Youn Hee Cho[1], Riasad Monir Fadle Aziz[1], Joseph B. McPhee[1], Tanuja Sutradhar[1], Jasika Bashal[1], Veronica Cojocari[1]

[1]Ryerson University

II20

Evaluating the role of GraS in promoting *Staphylococcus aureus* adaptation to combined antimicrobial conditions of human skin

Robert Kuiack[1], Martin McGavin[1]

[1]University of Western Ontario

II21

Evidence of slipped-strand mispairing in a putative sialidase gene of *Gardnerella* sp. W11

Shakya P Kurukulasuriya[1], Janet E Hill[1]

[1]Department of Veterinary Microbiology, Western College of Veterinary Medicine, University of Saskatchewan

II22

Examination of biocide cationic antimicrobial resistance in *Escherichia coli* to identify phenotypic and genotypic adaptations

Nicola H. Cartwright[1], Kari Green[1], George G. Zhanel[1], Denice C. Bay[1]

[1]University of Manitoba

II23

Examination of iron acquisition strategies employed by *Staphylococcus aureus* small colony variants

Izabela Batko[1], Ronald S. Flannagan[1], David E. Heinrichs[1]

[1]University of Western Ontario

II24

Examining the role of zinc on the proteome, growth and morphology of *Klebsiella pneumoniae*

Arjun Sukumaran[1], Jennifer Geddes-McAlister[1]

[1]University of Guelph

II25

Functional role of Resistance-Nodulation-Division efflux pump AdeIJK and its TetR transcriptional regulator AdeN in resistance and virulence of *Acinetobacter baumannii*

Rakesh Patidar[1], Mark Unger[2], Patrick Chong[2], Garrett Westmacott[2], Ayush Kumar[1]

[1]Department of Microbiology, University of Manitoba, Winnipeg, MB, Canada, [2]National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, MB, Canada

II26

Genetic background influences mean and variability of responses to prolonged antifungal drug exposure in *Candida albicans*

Aleeza Gerstein[1,2], Judith Berman[2]

[1]University of Manitoba, [2]Tel Aviv University

II27

HcpE is a new pro-inflammatory virulence factor of *Helicobacter pylori*

Lester [1], Salloum [1], Kichler [1], McDonald [1], Roach [2], Khursigara [2], Atanassov [3], Bodet [3], Burucoa [3], Creuzenet [1]

[1]University of Western Ontario, [2]University of Guelph, [3]Université de Poitiers

II29

Identification of 2 novel T3SA substrates in *Shigella flexneri*

Navoun Silue[1], F-X Campbell Valois[1]

[1]University of Ottawa

II30

Identification of Canid herpesvirus 1 entry pathways in canine epithelial cells

Mohamed Eisa[1], Angela Pearson[2]

[1]INRS-Institut Armand-Frappier, [2]INRS-Institut Armand-Frappier

II31

Identification of IcsB-like acyltransferase family

Waad Bajunaid[1], François-Xavier Campbell-Valois[1]

[1]University of Ottawa

II32

Identification of regulators involved in class IIb bacteriocin expression in *Streptococcus pyogenes*

Amanda Marple[1], Lana Estafanos[1], Brent Armstrong[1], John McCormick[1,2]

[1]University of Western Ontario, [2]Lawson Health Research Institute

II33

Impact de l'infection par le virus de l'herpès simplex 1 sur la voie d'immunité innée médié par oligoadenylate synthetase-like (OASL)

Fernando Sanchez Quete[1], Angela Pearson[2]

[1]INRS-Institut Armand-Frappier, [2]INRS-Institut Armand-Frappier

II34

A genomic epidemiology approach to investigating a persistent hospital-associated outbreak of vancomycin-resistant *Enterococcus faecium*

Keith D MacKenzie[1,2], Morgan WB Kirzinger[1,2], Meredith Faires[3], Emilie Wellman[1,2], Benjamin J Perry[1,2], Jessica Minion[4], Andrew DS Cameron[1,2]

[1]Institute for Microbial Systems and Society, University of Regina, [2]Department of Biology, University of Regina, [3]Regina Qu'Appelle Health Region, [4]Saskatchewan Health Authority

Applied and Environmental Microbiology

AEM1

Bacteriocinogenic Enterococcus: a potential probiotic as an alternative to antibiotics in poultry

Ayesha Lone[1], Riadh Hamammi[1], Walid Mottawea[1]

[1]University of Ottawa

AEM2

"Green" ZnO-interlinked chitosan nanoparticles for the efficient inhibition of sulfate-reducing bacteria in inject seawater

Gheyath Nasrallah[1,2], Nadine Younes[1], Kashif Rassol3[3]

[1]1Department of Biomedical Science, College of Health Sciences, Qatar University, Doha, Qatar, [2]Biomedical Research Center, Qatar University, Doha, Qatar, [3]Qatar Environment and Energy Research Institute (QEERI), Hamad Bin Khalifa University (HBKU), Qatar Foundation, P.O. Box 34110, Doha, Qatar

AEM3

A comparative genomic analysis of two avirulent *Pseudomonas syringae* strains belonging to phylogroup 2c

J.S.H. Lorv[1], B.J. McConkey[1]

[1]University of Waterloo

AEM4

A new diagnostic assay for food safety: detection and characterization of *Salmonella* contamination of lettuce by means of a whole genome-culture independent diagnostic test (WG-CIDT)

Dele Ogunremi[1], Andrée Ann Dupras[1], Ruimin Gao[1,2], Sohail Naushad[1], Katayoun Omidi[1], Imelda Galván Márquez[1], Hongsheng Huang[1], Marc-Olivier Duceppe[1], Lawrence Goodridge[2], Rogers C. Levesque[3], Nur A. Hasan[4], Manoj Dadlani[4], Brent Dixon[5], Sebastian Magierowski[6]

[1]Ottawa Laboratory Fallowfield, Canadian Food Inspection Agency, Ottawa, ON, Canada, [2]Department of Food Science and Agricultural Chemistry, McGill University, Ste-Anne-de- Bellevue, QC, Canada, [3]Institut de Biologie Intégrative et des Systèmes, Université Laval, Québec City, QC, Canada, [4]CosmosID Inc., Rockville, MD, United States, [5]Bureau of Microbial Hazards, Food Directorate, Health Canada, Ottawa, ON, Canada, [6]Department of Electrical Engineering and Computer Science, York University, Toronto, ON,

AEM5

A One Health approach for monitoring antibiotic resistance development and dissemination in a developing country

Teddie Rahube[1], Kesego Tapela[1], Lindiwe Tamocha[1], Monamodi Kesamang[1]

[1]Department of Biological Sciences and Biotechnology, Botswana International University of Science and Technology

AEM6

A soft direct DNA extraction method more effectively extracts the γ-proteobacterial community in soil than Mobio lysis

Patrick Hill[1], Mathieu F Dextraze[2], David Kroetsch[3], Christopher N Boddy[4]

[1]Department of Biology, University of Ottawa, Ottawa, ON K1N 6N5, Canada, [2]Department of Chemistry and Biomolecular Sciences, Centre for Chemical and Synthetic Biology, University of Ottawa, 10 Marie-Curie, Ottawa, K1N 6N5, Canada., [3]School of Environmental Science, University of Guelph, Guelph, N1G 2W1 Canada, [4]Department of Chemistry and Biomolecular Sciences, Centre for Catalysis Research and Innovation, University of Ottawa, Ottawa, K1N 6N5 Canada

AEM7

Adaptability of the common scab-inducing species to the hydroxycinnamates content of potato periderm

Mario Khalil[1], Carole Beaulieu[1]

[1]Université de Sherbrooke

AEM8

Alder bacterial endophytes and their interactions: a symbiotic symphony

Louis Garneau[1], Sophie Lalonde[1], Pascale B. Beauregard[1], Sébastien Roy[1]

[1]Université de Sherbrooke

AEM9

Anti-*Pseudogymnoascus destructans* (Pd) activities from western Canadian bat wings

Brandon Hayashi[1], Julianna Bissonnette[1], Aaron Wong[1], Nick Fontaine[1], Gabrielle Kam[1], Monique Nijjer[1], Adrian Forsythe[2], Dr. Jianping Xu[2], Dr. Cori Lausen[3], Dr. Naowarat Cheeptham[1]

[1]Department of Biological Sciences, Faculty of Science, Thompson Rivers University, Kamloops, BC, V2C 08C, Canada, [2]McMaster University, Canada, [3]Wildlife Conservation Society Canada, P.O. Box 606, Kaslo, BC, V0G 1M0, Canada

AEM10

Antimicrobial activity and antibiotic susceptibility of some probiotic bacteria isolated from Egyptian traditional fermented dairy products

Gharieb Salwa A.H.[1,2], Ali F. S.[1], Zayed G.[1], Saad O.A.O.[1], Fliss I.[2]

[1]Department of Agricultural Microbiology - Faculty of Agriculture, Minia University, Minia, Egypt., [2]Institute of Nutrition and Functional Foods, Université Laval, Québec, Canada.

AEM11

Antimicrobial activity of Thai cave bacteria

Chadabhorn Insuk[1,2], Sada Yoosathaporn[2], Wasu Pathom-aree[2], Naowarat Cheeptham[1]

[1]Thompson River University, [2]Chiang Mai University

AEM12

Antimicrobial susceptibility of intestinal bacterial strains to antidepressant drugs

Yasmina Ait Chait1[1], Walid Mottawea[1], Riadh Hammami[1]

[1]1NuGut Research Platform, School of Nutrition Sciences, Faculty of Health Sciences, University of Ottawa

AEM13

***Bacillus velenzensis* and *Paenibacillus peoriae* isolates as promising biopesticides and biofertilizers in sustainable agriculture**

Kanak Bala[1], Eric Déziel[1]

[1]INRS-Institut Armand-Frappier

AEM14

Biomineralization by bacteria isolated from popcorn and soda straw speleothem samples of the Iron Curtain Cave in Chilliwack, BC, Canada

Keegan Koning[1], Lynnea Carr[1], Richenda McFarlane[2], Patrick Hill[2], Chris Boddy[2], Nancy van Wagoner[1], Kingsley Donkor[1], Naowarat Cheeptham[1]

[1]Thompson Rivers University, [2]University of Ottawa

AEM15

Bioremediation of seafood waste using Paenibacillus sp. AD and concomitant production of chitin oligosaccharides having application as biotherapeutic agent

Rahul Warmoota[1], Dr Aditya Kumar[2], Dr. Naveen Gupta[3]

[1]Research Scholar Department of Microbiology Panjab University Chandigarh, India, [2]Research Scholar Department of Microbiology Panjab University Chandigarh, India, [3]Assistant Professor, Department of Microbiology, Panjab University, Chandigarh, India

AEM16

Bovine mammary gland commensal culture collection

Tiffany Feng[1], Emily Kretschmann[1], Chloe Duvernay[1], Jennifer Ronholm[1]

[1]McGill University

AEM17

Bugs from slugs: exploring the bacterial diversity of nudibranchs by metagenomics

Sheila Pimentel-Elardo[1], Karen Cheney[2], Mary Garson[3], Justin Nodwell[1]

[1]Department of Biochemistry, University of Toronto, Toronto ON M5G 1M1, Canada, [2]School of Biological Sciences, University of Queensland, Brisbane 4072, Queensland, Australia, [3]School of Chemistry and Molecular Biosciences, University of Queensland, Brisbane 4072, Queensland, Australia

AEM18

Characterization of a carbon source dependent, phytohormone responsive free methionine-(R)-sulfoxide reductase from *Komagataeibacter hansenii*

Jarret W. Arbing[1], Dr. Janice L. Strap[2]

[1]University of Ontario Institute of Technology, [2]University of Ontario Institute of Technology

AEM19

Characterization of antibiotic resistant bacteria from imported plant-based food products in Canada

Dongyun Jung[1], Joseph E. Rubin[1]

[1]Department of Veterinary Microbiology, University of Saskatchewan

AEM20

Characterization of antimicrobial resistance in the respiratory microbiota of beef calves from farm to feedlot

Yongmei Guo[1,2], Christopher McMullen[3], Edouard Timsit[3], Karin Orsel[3], Frank van der Meer[4], Sumei Yan[2], Trevor W. Alexander*[1]

[1]Agriculture and Agri-Food Canada, Lethbridge, AB, Canada, [2]College of Animal Science, Inner Mongolia Agricultural University, Hohhot, Inner Mongolia, China, [3]Department of Production Animal Health, Faculty of Veterinary Medicine, University of Calgary, Calgary, AB, Canada, [4]Department of Ecosystem and Public Health, Faculty of Veterinary Medicine, University of Calgary, Calgary, AB, Canada

AEM21

Characterizing soil microbial diversity through freeze-thaw cycles and the winter transition

Grant Jensen[1], Konrad Krogstad[1], Fereidoun Rezanezhad[1], Laura Hug[1]

[1]University of Waterloo

AEM22

***Clostridium perfringens* and other gram positive spore formers persists for many years in soil following an application of swine manure to an agricultural field**

Scott Wushke[1,2], Edward Topp[1,2]

[1]Agriculture and Agri-Food Canada, London Research and Development Centre, London, ON, Canada., [2]Department of Biology, University of Western Ontario, London, ON, Canada.

AEM23

Comparing methods to assess the biodiversity of soil microbes

Andrew Blakney[1,2], Simon Morvan[1,2], Marc St-Arnaud[2], Mohamed Hijri[1,2]

[1]Université de Montréal, [2]Center for Biodiversity, Institute de Recherche en Biologie Végétale

AEM24

Competitive interactions are common in mixed communities of *Gardnerella* spp.

Salahuddin Khan[1], Maarten J. Voordouw[1], Janet Hill[1]

[1]University of Saskatchewan

AEM25

Contaminant organisms and antibiotic resistance in holy water

Anni Moore[1], Lynsey Matulka[1], Sidney Hildahl[1], Matthew Hermelbracht[1]

[1]Morningside College

AEM27

Creating synthetic wheat holobionts for increased drought resistance

Asmaâ Agoussar[1], Hamed Azarbad[1], Etienne Yergeau[1]

[1]INRS-Institut Armand-Frappier

AEM28

Defining and improving the dairy cattle microbiota to assure consistent and high vitamin B12 concentrations in milk

Julian Lopez-Franco[1], William Poisson[2], Coralie Reymond[1], An Bui[1], Lya Blais[3], Rachel Gervias[2], Yvan Chouinard[2], Christiane Girard[4], Melissa Duplessis[4], Jennifer Ronholm[1]

[1]McGill University, [2]Université Laval, [3]Université de Sherbrooke, [4]Agriculture and Agri-Food Canada

AEM29

Detection of individual viable microbial components in a unique microbial consortia-based plant biostimulant using ddPCR

Chih-Ying Lay[1], David Sanchez[1]

[1]Concentric Agriculture Inc.

AEM30

Developing a synthetic disinfectant for use on chicken eggs

Jones Kalyniuk[1], John Stavrinides[1]

[1]University of Regina

AEM32

Development of a mobile genome sequencing tool for the detection and characterization of biothreats in food

Sohail Naushad[1], Ruimin Gao[1,2], Nooshin Fattahi Ghazi[1], Hongsheng Huang[1], Marc-Olivier Duceppe[1], Brent Dixon[3], Sarah J. Reiling[3], Harriet Merks[3], Nur A. Hasan[4], Manoj Dadlani[4], Sebastian Magierowski[5], Lawrence Goodridge[2], Sudhakar Ganapati Bhandare[2], Jérémie Hamel[6], Roger C. Lévesque[6], Dele Ogunremi[1]

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AEM33

Development of bacteriophage cocktails targeting the *Burkholderia cepacia* complex

Philip Lauman[1], Jonathan Dennis[1]

[1]University of Alberta

AEM34

Differential contributions of *Saccharomyces cerevisiae* and *Brettanomyces claussenii* to a Belgian strong beer

Breanne McAmmond[1], Eric Bottos[1], Tzu-Chiao Chao[2], Kelsey Dodds[3], Jonathan Van Hamme[1]

[1]Thompson Rivers University, [2]University of Regina, [3]Parallel 49 Brewing

AEM35

Differential gene expression of specific metabolic pathways in a marine, methylotrophic, denitrifying biofilm cultured in different conditions

Richard Villemur[1]

[1]INRS-Institut Armand-Frappier

AEM36

Discovery of two aptamers that bind to *Legionella pneumophila*

Mariam Saad[1], Maryam Tabrizian[1], Sebastien Faucher[1]

[1]McGill University

AEM37

Diversity and physiological capabilities of aerobic anoxygenic phototrophs in Lake Winnipeg

Steven Kuzyk[1], Kaitlyn Wiens[1], Xiao Ma[1], Vladimir Yurkov[1]

[1]Faculty of Science - Microbiology Dept. 422 Buller, 45 Chancellor's Circle, The University of Manitoba, R3T 2N2, Winnipeg, MB, Canada

AEM38

Does human exposure to antibiotics through food consumption select for resistance in the digestive tract?

Jessica Subirats Medina[1,2], Ashley Dominguez[1,2], Edward Topp[1,2]

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AEM39

Dynamics of denitrification in saline environments of species *Methylophaga nitratireducenticrescens* and *Hyphomicrobium nitrativorans* in co-cultures

Alexandra Cucaita Vasquez[1]

[1]INRS- Armand frappier

AEM40

Dynamics of *Pseudomonas aeruginosa* biofilm formation in a microfluidic system

William Harvey[1,2,3], Cynthia Gagné-Thivierge[1,2,3], Jean Barbeau[4], Jesse Greener[5], Steve Charette[1,2,3]

[1]Centre de recherche de l'Institut universitaire de cardiologie et de pneumologie de Québec, Quebec City, QC, Canada., [2]Institut de Biologie Intégrative et des Systèmes, Université Laval, Quebec City, QC, Canada., [3]Département de biochimie, de microbiologie et de bio-informatique, Faculté des sciences et de génie, Université Laval, Quebec City, QC, Canada., [4]Faculté de médecine dentaire, Université de Montréal, Montreal, QC, Canada., [5]Département de Chimie, Faculté des sciences et de génie, Université Laval, Quebec City, QC, Canada.

AEM41

Ecological interactions among aquatic microbial communities using metabarcoding in Southern Ontario lakes

Javad Sadeghi[1], Subba Rao Chaganti[1], Abdolrazagh Hashemi Shahraki[1], Daniel D Heath[1]

[1]Great Lakes Institute for Environmental Research, University of Windsor, Windsor, Canada

AEM42

Effect of biodiversity manipulation on generalist and specialist function of a forest soil

Julien Saavedra-Lavoie[1], Philippe Constant[2]

[1]INRS-IAF, [2]INRS-IAF

AEM43

Effects of crude secondary metabolites extracted from Solanum mauritianum and its bacterial endophytes on pathogenic bacteria and resistant human cancer cells

Nkemdinma Uche-Okereafor[1], Tendani Sebola[1], Kudzanai Tapfuma[1], Lukhanyo Mekuto[2], Ezekiel Green[1], Vuyo Mavumengwana[3]

[1]Department of Biotechnology and Food Technology, Faculty of Science, University of Johannesburg, PO Box 17011, Doornfontein, Johannesburg, South Africa, 2028, [2]Department of Chemical Engineering, Faculty of Engineering and the Built Environment, University of Johannesburg, PO Box 17011, Doornfontein, Johannesburg, South Africa, 2028, [3]South African Medical Research Council Centre for Tuberculosis Research, Division of Molecular Biology and Human Genetics, Department of Medicine and Health Sciences, Stellenbosch University, Tygerberg 7505, South Africa

AEM44

Effects of insecticide resistance on *Aedes aegypti* microbiota in French Guiana

Josiann Normandeau-Guimond[1], Claude Guertin[1], Philippe Constant[2], Isabelle Dusfour[3]

[1]INRS-Institut Armand-Frappier, [2]INRS-IAF, [3]Institut Pasteur

AEM45

Enumeration of probiotic strain *Lactobacillus gasseri* Lg-36 using viability real-time PCR

Hanan R. Shehata[1,2], Anthony Kiefer[3], Steven G. Newmaster[1]

[1]NHP Research Alliance, College of Biological Sciences, University of Guelph, Guelph, Ontario, Canada, N1G 2W1, [2]Department of Microbiology, Faculty of Pharmacy, Mansoura University, Mansoura, Egypt, [3]DuPont Nutrition & Health, Madison, Wisconsin, USA

AEM46

Evaluating the potential for transmission of enteric bacteria and antibiotic resistance genes from livestock manure to harvested hay

Andrew Scott[1], Yuan-Ching Tien[1], Edward Topp[1,2]

[1]Agriculture & Agri-Food Canada, [2]University of Western Ontario

AEM47

Evaluation of microbiological contamination of fresh salads and vegetables from farms, processing plants and retail markets in Korea

You Jin Kim[1], Ju Yeon Park[1], Soo Hwan Seo[1], Mi-Gyeong Kim[1], Hyo-Sun Kwak[1], Eun Jeong Heo[1]

[1]National Institute of Food and Drug Safety Evaluation

AEM48

Exploration of the hidden side of the bacterium *Leptospira*

Antony T. Vincent[1], Olivier Schiettekatte[2], Pascale Bourhy[2], Mathieu Picardeau[2], Frédéric J. Veyrier[1]

[1]INRS-Institut Armand-Frappier, Bacterial Symbionts Evolution, Laval, Québec, Canada., [2]Institut Pasteur, Unité Biologie des Spirochètes, Paris, France.

AEM49

Exploring aromatic compound metabolism in the foodborne pathogen *Listeria monocytogenes* by elucidating the protocatechuate pathway

Kevin Xue[1], Dinesh Christendat[1]

[1]University of Toronto

AEM50

Exploring microbiomes in traditionally important wild Arctic salmonids

Virginia K. Walker[1], Geraint Element[1], Erin Hamilton[1], Kristy Moniz[1], Peter van Coeverden de Groot[1], Vishal Shah[2], Charles W. Greer[3], Katja Engel[4], Josh D. Neufeld[4], James Qitsualik[5]

[1]Dept. of Biology, Queen's University, Kingston ON, [2]College of the Sciences and Mathematics, West Chester University of Pennsylvania, [3]Natural Research Council of Canada, Montreal PQ, [4]Dept. of Biology, University of Waterloo, Waterloo ON, [5]Hunters and Trappers Association, Gjoa Haven, NU

AEM51

Exploring the efficiency of microcins J25, B17 and L against *Salmonella* clinical isolates

Soufiane TELHIG[1,2], Laila BEN SAID [1], Isabelle Iachella [1]

[1]Laval Univerzsity, [2]Muséum National de l'Histoire Naturelle

AEM52

Exposure to sub-lethal 2, 4-dichlorophenoxyacetic acid arrests cell division and alters cell surface properties in *Escherichia coli*

Supriya V. Bhat[1], Sean C. Booth[2], Belma C. Kamencic[3], Tanya E. S. Dahms[4]

[1]University of Regina, [2]University of Calgary, [3]University of Regina, [4]University of Regina

AEM53

Fecal source tracking: The decay dynamics of mitochondrial DNA markers in water

Rose Ragot[1], Richard Villemur[1]

[1]INRS-Institut Armand-Frappier

AEM54

Free living Amoeba: a potential reservoir and transport vessel for Human Norovirus GII.4

Alyssa Wiedemeyer[1], Rafik Dey[1], Nicholas Ashbolt[1]

[1]University of Alberta

AEM55

Heat resistance in Shiga-Toxin producing *Escherichia coli* from cattle

Eduardo Figueiredo[1,2,3], Kim Stanford[2], Tim Reuter[2], Tim McAllister[3], Peipei Zhang[4], Xiaqin Yang[4]

[1]Federal Univeristy of Mato Grosso, Cuiaba MT, Brazil, [2]Alberta Agriculture and Forestry, Lethbridge AB, [3]Agriculture and Agri-Food Canada, Lethbridge AB., [4]Agriculture and Agri-Food Canada, Lacombe, AB

AEM56

How is the activated sludge community assembled? Experimental decoupling of wastewater substrates and microbial compositions

Claire Gibson[1], Shameem Jauffur[1], Bing Guo[1], Nouha Klai[1], Dominic Frigon[1]

[1]McGill University

AEM57

How to incorporate genetic information into models for pesticide degradation in soils

Luciana Chavez Rodriguez[1,2], Holger Pagel[1], Brian Ingalls[2], Thilo Streck[1]

[1]University of Hohenheim, [2]University of Waterloo

AEM58

Hybrid sequencing and genome analysis of four *Bacillus thuringeinsis* strains

M.G. Lubberts[1], S. Chaudhary[1], J.S.H. Lorv[1], S. Jain[2], S.D.A. de Souza Jr[3], B.J. McConkey[1]

[1]University of Waterloo, [2]Universidade Federal de Sergipe, [3]Embrapa Genetic Resources and Biotechnology

AEM59

Identification and characterization of an extracellular amylase conserved in *Gardnerella* spp

Pashupati Bhandari[1], Janet Hill[1]

[1]Department of Veterinary Microbiology, University of Saskatchewan

AEM60

Identification of novel Type VI Secretion Systems in *Salmonella enterica*

Zhiwei Li[1]

[1]McGill University

AEM62

Identifying the target of LP2006, an antibacterial lasso peptide

Gaelen Moore[1], Justin Nodwell[1]

[1]University of Toronto

AEM63

Immune enhancing effects of Queso Blanco cheese supplemented with *Lactobacilli* and *Bifidobacteria* in healthy Beagle dogs

Ho-Eun Park[1], Kyung-Hyo Do[1], Jun-Sang Ham[2], Wan-Kyu Lee[1]

[1]College of Veterinary Medicine, Chungbuk National University, Cheongju, 28644, Korea, [2]Animal Products Development and Utilization Division, National Institute of Animal Science, Wanju-gun, 55365, Republic of Korea

AEM64

Impact of water distribution systems on microbiological quality of drinking water in First Nation communities in Manitoba, Canada

Anita Murdock[1], Sabrin Bashar[1], Rakesh Patidar[1], Ayush Kumar[1], Annemieke Farenhorst[1]

[1]University of Manitoba

AEM65

Impacts of the phi027 prophage on the biology and virulence of the epidemic strain *Clostridioides difficile* ribotype 027

Garneau R. Julian[1,2,3], Peltier Johann[2,3], Hamiot Audrey[2,3], Tremblay Yannick[2,3], Turcotte Annie[1], Dupuy Bruno[2,3], Valiquette Louis[1], Monot Marc[1,2,3], Fortier Louis-Charles[1]

[1]Département de Microbiologie et d'infectiologie, Faculté de Médecine et des Sciences de la Santé, Université de Sherbrooke, Sherbrooke, QC, Canada, [2]Laboratoire Pathogenèse des Bactéries Anaérobies, Institut Pasteur, Paris, France, [3]Université Paris Diderot, Sorbonne Paris Cité, Paris, France

AEM66

Inclusion of qualitative data to improve predictive capability of MLR model for recreational beach microbial water quality

Mohammad Madani[1], Rajesh Seth[1], Luis F. Leon[2]

[1]Department of Civil and Environmental Engineering, University of Windsor, Windsor, ON, Canada, [2]Environment and Climate Change Canada, Water Science and Technology, Canada Centre for Inland Waters, Burlington, ON, L7S 1A1, Canada

AEM67

Intra- and inter-species horizontal transfer potential of integrative and conjugative elements carrying antimicrobial resistance genes

Reuben Ha[1], Rahat Zaheer[1], Cheyenne Sargeant[1], Cassidy Klima[2], Tim McAllister[1]

[1]Agriculture and Agri-Food Canada, [2]Iowa State University College of Veterinary Medicine

AEM101

Study of conservation and diversity of mobile genomic islands in *Vibrionaceae*: detection, identification and classification

Audrey Bioteau[1], Pierre-Étienne Jacques[1], Vincent Burrus[1]

[1]Université de Sherbrooke

Molecular Genetics & Cellular Microbiology

MGCM1

A bifunctional ATPase drives tad pilus extension and retraction

Courtney K. Ellison[1], Yves V Brun[2]

[1]Indiana University, Bloomington, IN, USA, [2]Université de Montréal, Montréal, Qc, Canada

MGCM2

A distinctive new class of conjugation system widely distributed in the rhizobia

N. Dulmini Wathugala[1], Kasuni M. Hemananda[1], Hao Ding[2], Cynthia B. Yip[1], Michael F. Hynes[1]

[1]Biological Sciences, University of Calgary, [2]Renaissance BioScience, Vancouver BC

MGCM3

A DNA junction superstructure regulates Type III Secretion System gene expression and virulence in *Vibrio parahaemolyticus*

Landon J. Getz[1], Justin Brown[1], Nikhil Thomas[1]

[1]Dalhousie University

MGCM4

A locus on the *Sinorhizobium meliloti* pSymA megaplasmid that restricts phage growth

Leah Sather[1], Zahed Muhammed[1], Maryam Zamani[1], Kathryn M. Jones[2], Turlough M. Finan[1]

[1]McMaster University, [2]Florida State University

MGCM5

A novel computational approach for the investigation of human associated microbiota

Emily Haidl[1], Cory Butz[1], John Stavrinides[1]

[1]University of Regina

MGCM6

A pH-regulated integral membrane protein inhibits an unusual transcription module

Maria C. Davis[1,2], Eukene Bensig[2], Shawn R. MacLellan[2]

[1]University of Regina, [2]University of New Brunswick

MGCM7

Acetone and isopropanol production by the hyperthermophilic archaeon *Hyperthermus butylicus*

Sarah D. Kim[1], Ching Tse[2], Farhad Yusifov[3], and Kesen Ma[4]

[1]University of Waterloo, [2]University of Waterloo, [3]University of Waterloo, [4]University of Waterloo

MGCM8

Adaptation of a reduced-genome *Escherichia coli* strain to different growth conditions

Antoine Champie[1], Sébastien rodrigue[1]

[1]Département de biologie, Faculté des Sciences, Université de Sherbrooke

MGCM9

An alternative toxic model of *Streptomyces*-Insect interactions in nature

Louis Ho[1], Craig Smibert[1], Justin Nodwell[1]

[1]Department of Biochemistry, University of Toronto

MGCM10

Anti-*Neisseria* drugs screening start revealing the Achilles' heel of *N. meningitdis* and *N. gonorrhoeae*

Marthe Lebughe[1], Eve BERNET[1], Golara Golbaghi[1], Medhi Haghdoost[1], Robin Vidal[1], Annie Castonguay[1], Frederic veyrier[1]

[1]INRS-Institut Armand-Frappier

MGCM11

Armeniaspiroles are pan-proteosome inhibitors targeting ClpYQ and ClpXP in Gram-positive bacteria

Puneet Labana[1], Mark H. Dornan[1], Matthew Lafrenière[1], Tomasz L. Czarny[2], Eric D. Brown[2], John P. Pezacki[1], Christopher N. Boddy[1]

[1]University of Ottawa, [2]McMaster University

MGCM12

Arrested Development: investigating DNA damage in the filamentous microorganism *Streptomyces*

Jan Falguera[1], Charul Jani[2], Scott McAuley[1], Justin R. Nodwell[1]

[1]Dept. of Biochemistry, University of Toronto, [2]Dept. of Biochemistry & Biomedical Sciences, McMaster University

MGCM13

Assembly and export of the serotype O4 polysaccharide antigen from *Klebsiella pneumoniae* O4

Danielle Williams[1], Olga G. Ovchinnikova[1], Chris Whitfield[1]

[1]1University of Guelph, Department of Molecular and Cellular Biology

MGCM14

Assembly of the *Klebsiella pneumoniae* O1 and O2ac polysaccharide antigens

Steven D. Kelly[1], Bradley R. Clarke[1], Olga G. Ovchinnikova[1], Monica L. Williamson[1], Ryan Sweeney[2], Todd L. Lowary[2], Chris Whitfield[1]

[1]Department of Molecular and Cellular Biology, University of Guelph, Ontario Canada N1G 2W1, [2]University of Alberta, Edmonton, Alberta, Canada T6G 2G2

MGCM15

Biosynthesis of a conserved glycolipid anchor for Gram-negative bacterial capsules

Liam Doyle[1], Olga G Ovchinnikova[1], Katharine Myler[1], Evan Mallette[1], Bo-Shun Huang[2], Todd L Lowary[2], Matthew S Kimber[1], Chris Whitfield[1]

[1]University of Guelph, [2]University of Alberta

MGCM16

***Burkholderia cepacia* complex's clinical strains are able to produce 4-hydroxy-3-methyl-2-alkylquinolines and their Hmq system could be negatively and positively regulated**

Pauline Coulon[1], James Zlosnik[2], Eric Déziel[1]

[1]INRS-Institut Armand-Frappier, [2]University of British Columbia

MGCM17

Characterization and quantification of *Pseudomonas aeruginosa* biofilms

Alyssa Banaag[1], Cezar M. Khursigara[1]

[1]University of Guelph

MGCM18

Characterization of exclusion mechanisms in the conjugative plasmid TP114

Sébastien Rodrigue[1], Kevin Neil[1], David Jordan[1], Patricia Roy[1]

[1]Université de Sherbrooke

MCGM19

Characterization of the replicating gut bacterial community in a mouse model of colitis

Eve Beauchemin[1], Corinne Maurice[1]

[1]McGill University

MGCM20

Characterization of the role of the Cpx stress response pathway in the regulation of proteolysis at the inner membrane of *Escherichia coli*

Valeria Tsviklist[1], Randi Lynn Guest[1], Tracy Raivio[1]

[1]University of Alberta

MGCM21

Characterization of the small regulatory RNA lpr0050 in *Legionella pneumophila*

Malak Sadek[1], Sebastien Faucher[1]

[1]McGill University

MGCM22

Characterizing the mouse gut microbiota and developing an in vitro model of the mouse colon

Caroline Ganobis[1], Erin Wong[2], William Navare[2], Emma Allen-Vercoe[1]

[1]University of Guelph, [2]University of Toronto

MGCM24

Chemically perturbing the regulatory network for secondary metabolism in *Streptomyces coelicolor*

Vanessa Yoon Calvelo[1], Arryn Craney[2], Justin Nodwell[1]

[1]University of Toronto, [2]University of Nebraska Medical Center

MGCM25

Come out, come out, wherever you are! Characterizing colonic microbiome metabolites as triggers of prophage induction

Jacob Wilde[1], Hedieh Attai[2], David Pride[2], Emma Allen-Vercoe[1]

[1]University of Guelph, [2]University of California San Diego

MGCM26

Coupling between bacterial physiology and mutation rate

Christian Henderson Barna[1], Matthew Scott[1]

[1]Department of Applied Mathematics, University of Waterloo

MGCM27

Covariance analysis reveals important residue-residue contacts in the *Escherichia coli* division protein FtsK

Laura Seidel[1], Matthew S. Kimber[1], Cezar M. Khursigara[1]

[1]University of Guelph

MGCM28

Development and application of tunable CRISPRi gene silencing in *Burkholderia*

Andrew Hogan[1], ASM Zisanur Rahman[1], Kevin R Jeffers[1], Silvia Cardona[1,2]

[1]Department of Microbiology, University of Manitoba, Winnipeg, Manitoba, Canada, [2]Department of Medical Microbiology and Infectious Disease, University of Manitoba, Winnipeg, Manitoba, Canada

MGCM29

Development of a high-efficiency genomic integration platform for near-minimal bacteria

Catherine Chamberland[1], Dominick Matteau[1], Sébastien Rodrigue[1]

[1]Université de Sherbrooke

MGCM30

Development of CRISPR interference system for investigation of the role of the natural antisense transcript in *Toxoplasma gondii*

Grace Wen[1], Ambreen Fahim[1], Sirinart Ananvoranich[1]

[1]University of Windsor

MGCM31

Effects of alignment on contact based cell-cell interactions

Julien Smith-Roberge[1], Ayana Kawe[1], Brian Ingalls[1]

[1]University of Waterloo

MGCM32

Efficient inter-species conjugative transfer of a CRISPR nuclease for targeted bacterial elimination

Thomas A. Hamilton[1], Gregory M. Pellegrino[1], Jasmine A. Therrien[1], Dalton T. Ham[1], Peter C. Bartlett[1], Bogumil J. Karas[1], Gregory B. Gloor[1], David R. Edgell[1]

[1]Department of Biochemistry, Schulich School of Medicine and Dentistry, London, ON, N6A5C1, Canada

MGCM33

Elucidating the molecular basis of AmpC-mediated ceftazidime/avibactam and ceftolozane/tazobactam resistance in *Pseudomonas aeruginosa*

Cole Slater[1], Judith Winogrodzki[1], Antonio Oliver[2], Brian Mark[1]

[1]University of Manitoba, [2]Instituto de Investigación Sanitaria IllesBalears

MGCM34

Entry exclusion of conjugative plasmids of the IncA, IncC and related untyped incompatibility groups

Malika Humbert[1], Kevin Huguet[1], Frédéric Coulombe[1], Vincent Burrus[1]

[1]Université de Sherbrooke

MGCM35

Evading antimicrobial resistance: Using synthetic organophosphorus compounds to slow evolution

Noah Choboter[1], John Stavrinides[1]

[1]University of Regina

MGCM36

Examining the intraspecific interactions in mixed-strain biofilms containing the prairie epidemic strain and unique clinical isolates of *Pseudomonas aeruginosa*

Nicole E Garnier[1], Dr. Amber J Park[1], Dr. Michael D Parkins[2], Dr. Cezar M Khursigara[1]

[1]University of Guelph, [2]University of Calgary

MGCM37

Generation of a large set of small plasmids for synthetic biology

France Manigat[1], Adam Shuhendler[1,2], Corrie daCosta[1,2], Francois-Xavier Campbell-Valois[1,2]

[1]University of Ottawa, [2]Chemistry and Biomolecular Sciences

MGCM38

Generation of a *Streptomyces venezuelae*-derived chassis strain and investigation of global repressor Lsr2-deficient *Streptomyces* species

Meghan Pepler[1], Hindra Hindra[1], Marie Elliot[1]

[1]McMaster University

MGCM39

Genetic regulation of the locus of heat resistance in *Escherichia coli*

Oanh Nguyen[1], Lynn McMullen[1], Michael Gänzle[1]

[1]University of Alberta

MGCM40

HcpE and DsbK effects on the pro-inflammatory immune response

Noor Salloum[1], Andrew MacDonald[1], Elyse Roach[2], Cezar Khursigara[2], Carole Creuzenet[1]

[1]University of Western Ontario, [2]University of Guelph

MGCM41

Hide and seek with bifidobacteria: Improving microbial profiling techniques in the infant gut microbiome

Bhavya Singh[1,2], Sara Dizzell[3], Luna ElDakiky[2,5], Eileen Hutton[3], Katherine Morrison[4], Jennifer C. Stearns[2,5]

[1]Department of Chemical Biology, McMaster University, [2]. Farncombe Family Digestive Health Research Institute, McMaster University, [3]Department of Obstetrics and Gynecology, McMaster University, [4]Department of Pediatrics, McMaster University, [5]Department of Medicine, McMaster University

MGCM42

Host-plasmid interactions in antibiotic resistant *Escherichia coli*

Amanda Carroll[1], Alex Wong[1]

[1]Department of Biology, Carleton University

MGCM43

How Streptomyces explorers move and what they need to do it

Matthew P. Zambri[1,2], Stephanie E. Jones[1,2], Marie A. Elliot[1,2]

[1]McMaster University, [2]Institute for Infectious Disease Research

MGCM44

Identification of a novel mechanism of surface-induced expression of RsmZ in *Pseudomonas aeruginosa*

Charles Morin[1], Eric Déziel[1]

[1]INRS-Institut Armand-Frappier

MGCM45

Identification of genes involved in autoagglutination and biofilm formation in *Escherichia coli* strains O157: H7

Yaindrys Rodriguez Olivera[1], Philippe Vogeleer[1], Frédérique White[2], Antony T. Vincent[3], Steve J. Charette[4], Josée Harel[1]

[1]Faculté de médecine vétérinaire, Université de Montréal, [2]Université de Sherbrooke, [3]INRS-Institut Armand-Frappier, [4]Institute of Integrative Biology and Systems, Université de Laval

MGCM46

Identifying prophage-mediated responses to bioactive compounds

Anisha Nandy[1,3], Alexander Hynes[1,2,3]

[1]Department of Biochemistry and Biomedical Sciences, Faculty of Health Science, McMaster University, Ontario, Canada, [2]Department of Medicine, Faculty of Health Science, McMaster University, Ontario, Canada, [3]Farncombe Family Digestive Health Research Institute, McMaster University, Ontario, Canada

MGCM47

In the pits with pap: Translational coupling of a bacterial phosphate uptake system

Lauren Tiller[1], Daniel Hsieh[1], Rahat Zaheer[1], Turlough M. Finan[1]

[1]McMaster University

MGCM48

Integrative and systems biology of the *Borrelia* species and Lyme disease complex in a One Health perspective

Jérémie Hamel[1], Jean-Guillaume Emond-Rheault[1], Iain Mainprize[2], Elyse Roach[2], Victoria Sanderson[2], Jordan Ko[2], Marian Golovchenko[3], Nataliia Rudenko[3], Irena-Kukavica-Ibrulj [1], Brian Boyle[1], Vett Lloyd[4], Cezar Kursigara[2], Melanie Wills[2], Roger C. Levesque[1]

[1]Institut de Biologie Intégrative et des Systèmes, Université Laval, [2]University of Guelph, [3]Biology Center CAS, Institute of Parasitology, Branisovska, Ceske Budejovice, [4]Mount Allison University

MGCM49

Investigating changes to the *Pseudomonas aeruginosa* proteome upon exposure to antibiotics

Mara Goodyear[1], Nicole Garnier[1], Jonathan Krieger[2], Jennifer Geddes-McAlister[1], Cezar Khursigara[1]

[1]University of Guelph, [2]SPARC BioCentre, The Hospital for Sick Children

MGCM77

Transient replication of a mobilizable resistance island in the presence of its helper plasmid

Kevin Huguet[1], Jason Palanee[1], Daniel Garneau[1], Vincent Burrus.[1]

[1]Université de Sherbrooke

Poster Session II

Wednesday, June 12th, from 3:00 - 5:00 PM

Presenters to be by their posters:
Odd number posters from 3:00 - 4:00PM & **Even number** posters from 4:00 - 5:00 PM

Note: **AEM**: Applied Environmental Microbiology
II: Infection and Immunity | **MGCM**: Molecular Genetic Cellular Microbiology

Infection & Immunity

II35

Impact of Canid herpesvirus 1 infection on the cellular miRNA profile of canine epithelial cells

Maha Ben Hamouda[1], Angela Pearson[2]

[1]INRS-IAF, [2]INRS-Institut Armand-Frappier

II36

Infection with microsporidia induces an immune response in the offspring of *Caenorhabditis elegans*

Alexandra Willis[1], Winnie Zhao[1], Lina Wadi[1], Aaron Reinke[1]

[1]University of Toronto

II37

Integrated Rapid Infectious Disease Analysis: A comprehensive platform for public health bioinformatics and AMR surveillance using genomic data

Finlay Maguire[1], Brian Alcock[2], Amogelang R. Raphenya[2], Baofeng Jia[3], Emma J Griffiths[5], Thomas C Matthews[4], Josh Adam[4], Geoffrey L Winsor[3], IRIDA Consortium[4], Robert G. Beiko[1], Fiona S.L. Brinkman[3], Aaron Petkau[4], William WL Hsiao[5], Gary Van Domselaar[4], Andrew G. McArthur[2]

[1]Faculty of Computer Science, Dalhousie University, NS, Canada, [2]Department of Biochemistry and Biomedical Sciences, McMaster University, Hamilton, ON, Canada, [3]Department of Molecular Biology and Biochemistry, Simon Fraser University, Burnaby, BC, Canada, [4]National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, MB, Canada., [5]British Columbia Centre for Disease Control Public Health Laboratory, Vancouver, BC, Canada

II38

Interaction of the *Escherichia coli* heat-stable enterotoxin b (STb) with the NCI-H716 human intestinal cell line

Eyad Kinkar[1], Shahn Butt[1], Mazen Saleh[1]

[1]Department of Biology, Laurentian University. Sudbury, ON. Pe# 2C6

II39

Investigating microbial engraftment via comprehensive culture-enriched and culture-independent metagenomics in patients with ulcerative colitis

Shahrokh Shekarriz[1], Jennifer Lau[1], Fiona Jane Whelan[2], Paul Moayyedi[1], Michael Surette[1]

[1]McMaster University, [2]University of Nottingham

II40

Investigation of a staphylococcal secreted product with activity against methicillin-resistant *Staphylococcus aureus*

Denny Chin[1], David W. Watson[1], David E. Heinrichs[1]

[1]The University of Western Ontario

II41

Lighting-up *Pseudomonas aeruginosa*: reporter system for anaerobic tracking

Caetanie Tchagang[1], Li Zhang[1], Dr. François-Xavier Campbell-Valoi[1], Dr. Thien-Fah Mah[1]

[1]University of Ottawa

II42

Mechanism of action of the synergistic combination tomatidine-aminoglycoside against *Staphylococcus aureus* virulent and persistent phenotypes

Jean-Philippe Langlois[1], Isabelle Guay[1], Viktor Steimle[1], Kamal Bouarab[1], Éric Marsault[1], François Malouin[1]

[1]Université de Sherbrooke

II43

Molecular epidemiology of Rotavirus in children with gastroenteritis in Qatar

Shilu Mathew 1[1], Maria Smatti 1[1], Asmaa AlThani 1[1], Khalid AlAnsari 2[2], Hadi Yassine 1[1]

[1]Biomedical Research Center, Qatar University, Doha, Qatar 2713, [2]Pediatric Emergency Center, Hamad Medical Corporation, Doha, Qatar 3050

II45

Optimizing transmission electron microscopy-based visualization of *Pseudomonas aeruginosa* PAO1 within bronchial epithelial cells

Madison Wright[1], Emmanuel Faure[2], Amber Park[1], Simon Rousseau[2], Dao Nguyen[2], Cezar Khursigara[1]

[1]University of Guelph, [2]McGill University

II46

Producing a porcine epidemic diarrhea virus virus-like particle in plants for oral administration to pregnant sows and protection of neonate piglets

Zayn Khamis[1,2], Hong Zhu[1], Rima Menassa[1,2]

[1]London Research and Development Centre, Agriculture and Agri-Food Canada, [2]Biology Department, Western University

II47

Purification and characterization of two new antimicrobial molecules produced by an endophytic strain of *Paenibacillus polymyxa*

Nadja Fernanda Gonzaga Serrano1*[1], Douglas Ferreira2[2], Edson Ro[3], José António Teixeira4[4], Jean Daniel Dubreuil5[5], Cristina Paiva[1]

[1]1Laboratory of Microbiology and Biomolecules, Department of Morphology and Pathology, Federal University of Sao Carlos, Brazil (UFSCar), , [2]2Department of Chemistry, UFSCar., [3]3LaBioMMi, Department of Chemistry, [4].4Laboratory of Fermentations, University of Minho, Braga, Portugal., [5]5Université de Montréal, Dép. Pathologie et microbiologie, Saint-Hyacinthe, Canada.

II48

Reduction of biofilm formation by sub-inhibitory concentration of polymyxin B in *Vibrio cholerae*

Sean Giacomucci[1], Candice Cros[1], Xavier Perron[1], Marylise Duperthuy[1]

[1]Université de Montréal

II49

Resolution and co-occurrence patterns of *Gardnerella leopoldii*, *Gardnerella swidsinskii*, *Gardnerella piotii* and *Gardnerella vaginalis* within the vaginal microbiome

Janet Hill[1], Arianne Albert[2]

[1]University of Saskatchewan, [2]University of British Columbia

II50

Role of the Streptococcus pyogenes Adhesion Locus in Nasopharyngeal Infection

Akshay Sule[1,2], Delfina M Siroen[4], John McCormick[1,2,3]

[1]University of Western Ontario, [2]Department of Microbiology & Immunology, , [3]Lawson Health Research Institue, [4]Sernova

II51

***Salmonella enterica* serovar Typhimurium induces inflammation to overcome the mechanisms of colonization resistance in a swine model**

Bescucci D[1,2], Moote PE[1,2], Abbott DW[1], Uwiera RRE[2], Inglis GD.[1]

[1]Lethbridge Research and Development Centre, Agriculture and Agri-Food Canada, Lethbridge AB, Canada, [2]Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton AB, Canada

II52

Single-strand HIV-1 RNA induces CCR7-dependent monocytes transmigration through the blood-brain barrier via T LR7 and 8 activation

Marie-Yolande Borget[1], Alexandre Paradis[1], Vanessa Leblond-Drol[1], Nancy Dumais[1]

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II53

Species-level molecular typing of clinical *Pantoea* strains with cpn60

Craig Soutar[1], John Stavrinides[1]

[1]University of Regina

II54

Structural genomics of proteins mediating Gram-negative cell envelope biogenesis

P.J. Stogios[1,3], Z. Eltsova[2], N. Watanabe[2,3], Evdokimova E[1,3], Skarina T[1,3], Di Leo R[1,3], Savchenko A[2,3]

[1]University of Toronto, [2]University of Calgary, [3]Center for Structural Genomics of Infectious Diseases (CSGID)

II55

Structural insights on the mechanism of acquired sulfonamide antibiotic resistance

Meenakshi Venkatesan[1,3], Tatiana Skarina[1,3], Alexei Savchenko[1,2,3], Peter Stogios[1,3]

[1]Department of Chemical Engineering and Applied Chemistry, University of Toronto, [2]Department of Microbiology, Immunology and Infectious Diseases, University of Calgary, [3]Center for Structural Genomics of Infectious Diseases (CSGID)

II56

Study of the interaction between the bacterium *Streptococcus suis* and protozoa of the genus *Tetrahymena*

Stéphanie Brodeur[1,2,3], Valérie Paquet[1,2,3], Daniel Grenier[4], Steve Charette[1,2,3]

[1]Université Laval, IBIS, [2]Centre de recherche de l'Institut universitaire de cardiologie et de pneumologie de Québec, [3]Département de biochimie, de microbiologie et de bio-informatique, Faculté des sciences et de génie, Université Laval, [4]Faculté de médecine dentaire, Université Laval

II57

Temperate phage-antibiotic synergy

Amany Al-Anany[1], Alexander Hynes[1,2]

[1]Department of Biochemistry and Biomedical Sciences, McMaster University., [2]Department of Medicine, McMaster University.

II58

The effect of unfolded protein response on *Pseudomonas aeruginosa* infection

Toka Omar[1], Zhenyu Cheng[1], Brent Johnston[1]

[1]Dalhousie University

II59

The impacts of urban environments on the pathogenic bacteria of Mountain Chickadees (*Poecile gambeli*)

Colton Stephens[1], Jonathan Van Hamme[1], Eric Bottos[1], Matt Reudink[1]

[1]Thompson Rivers University

II60

The involvement of two small-RNA degrading nucleases in the set up of optimal immune responses

R. Ajmi[1], M.B.D. Diam[1], T. Abd El Rahman and K. Bouarab[1]

[1]Université de Sherbrooke

II61

The pathogenicity of commensals: Immunogenic and genetic comparison between clinical isolates of the *Streptococcus milleri/anginosus* group

Daphnée Lamarche[1], Rachelle Szymkiewicz[1], Dessi Loukov[1], Louis-Patrick Schenck[1], Dawn Bowdish[1], Michael Surette[1]

[1]McMaster University

II62

The role of Streptococcus pyogenes surface virulence factors in colonization and autoimmune disease development

Jacklyn Hurst[1], John K. McCormick[1,2]

[1]Department of Microbiology and Immunology, Schulich School of Medicine and Dentistry, London, ON, Canada, [2]Lawson Health Research Institute, Western University, London, ON, Canada

II63

The role of streptolysin O and streptolysin S in Streptococcus pyogenes nasopharyngeal infection

Blake A. Shannon[1], Jacklyn R. Hurst[1], Katherine J. Kasper[1], John K. McCormick[1,2]

[1]University of Western Ontario, [2]Lawson Health Research Institute

II64

The tail-specific protease of *Legionella pneumophila* is necessary to survive shift in temperature

Saoud J[1], Mani T[1], Faucher SP[1]

[1]Department of Natural Resource Sciences, Faculty of Agricultural and Environmental Sciences, McGill University

II65

Vaccination with a live-attenuated small-colony variant improves humoral and cellular responses against Staphylococcus aureus, an important pathogen in Canadian dairy herds

J. Côté-Gravel[1], E. Brouillette[1], F. Malouin[1]

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II66

Versatile use of ubiquitin protein ligase activity by attaching and effacing (A/E) pathogens

Georgy Popov[1], Aline Fiebig[2], Dustin J. Little[2], Dylan Valleau[3], Brian K. Coombes[2], Alexei Savchenko[1]

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II67

Developing a mucosal vaccine delivery system to mitigate the bovine respiratory pathogen *Mannheimia haemolytica*

Muhammed Salah Uddin[1,2], Le Luo Guan[1], Jose Ortiz Guluarte[2], Trevor W. Alexander[2]

[1]Department of Agricultural, Food and Nutritional Science, University of Alberta, [2]Lethbridge Research and Development Centre, Agriculture and Agri-Food Canada

II68

Environmental and nutritional regulation of the toxic shock syndrome toxin-1 (TSST-1) in *Staphylococcus aureus* MN8

Stephen W. Tuffs[1], Vladyslav A. Podskalniy[1], Christine A. Herfst[1], Erica N. DeJong[1], G. Reid[1], John K. McCormick[1]

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Applied and Environmental Microbiology

AEM31

Development of a biosensor for *Bacillus* species using recombinant antibodies and aptamers

Houman Moteshareie[1], Jonathan Vermette[1], Walid M. Hassen[1], Jan J. Dubowski[1], Azam F. Tayabali[2]

[1]Université de Sherbrooke, [2]Environmental Health Canada

AEM68

Investigating marine diesel microbiome using culture dependent and independent techniques

Margaux Durand[1,2], Ianina Altshuler[1,2], Charles W. Greer[1,2]

[1]McGill University, [2]National Research Council Canada

AEM69

Investigating seasonal impact on microbial load and antibiotic resistance genes in drinking water from a First Nation community, Manitoba, Canada

Sabrin Bashar[1], Anita Murdock[1], Rakesh Patidar[1], Annemieke Farenhorst[1], Ayush Kumar[1]

[1]University of Manitoba

AEM70

Investigation of microbial quality of various health functional food categories for reevaluation of microbial criteria

Eun Jeong Heo[1], You Jin Kim[1], Ju Yeon Park[1], Soo Hwan Seo[1], Mi-Gyeong Kim[1], Hyo-Sun Kwak[1]

[1]National Institute of Food and Drug Safety Evaluation

AEM71

Investigation of prevalence and diversity of *Acinetobacter species* in environmental aquatic sources

Thomas Benoit[1,2], Michel Cloutier[1], Graham Wilkes[1], Emilia Craiova[1], David R. Lapen[1], Edward Topp[3], Izhar U.H. Khan[1]

[1]Agriculture and Agri-Food Canada, Ottawa Research and Development Centre, Ottawa, ON K1A 0C6, Canada, [2]Department of Chemistry and Biomolecular Sciences, University of Ottawa, Ottawa, ON K1N 6N5, Canada, [3]Agriculture and Agri-Food Canada, London Research and Development Centre, London, ON N5V 4T3, Canada

AEM72

Investigation of *Pseudomonas syringae* as an organic biocontrol for Canada thistle

Alexander Cameron[1], John Stavrinides[1]

[1]University of Regina

AEM73

Isolation and characterization of lactic acid bacteria from starter cultures with high production capacity of γ-aminobutyric acid

Rojaalsadat Mousavi[1], Walid Mottawea[1], Marie-Claude Audet[1], Riadh Hammami[1]

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AEM74

Log transformation and its Impact on the interpretation of linear regression in the assessment of quantitative microbiological methods

Anli Gao[1], Perry Martos[1]

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AEM75

Microbial communities of wastewaters in the Canadian Arctic

Gromala M[1], Neufeld JD[1], McConkey BJ[1]

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AEM76

Microbial community composition and evolution in a CSTR digesting municipal sludge subjected to an acid-crash

Guillaume Bruant[1], Fred Ngoundjo[2], Marie-Josée Lévesque[1], Christine Maynard[1], Julien Tremblay[1], Jessica Wasserscheid[1], Jean-Claude Frigon[2]

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AEM77

Microbial community dynamics in wastewater treatment bioreactors

Nancy A. Elnaker[1], Shadi W. Hasan[2], Ahmed F. Yousef[1]

[1]Deaprtment of Chemistry, Khalifa University, Abu Dhabi, UAE, [2]Department of Chemical Engineering, Khalifa University, Abu Dhabi, UAE

AEM78

Microbial community responses to mine site reclamation strategies

Eric Bottos[1], Breanne McAmmond[1], Ashley Fischer[1], Jordann Foster[1], Lauchlan Fraser[1], Jonathan Van Hamme[1]

[1]Thompson Rivers University

AEM79

Microbial interactions and metabolisms across high Arctic ice-wedge permafrost cores and their consequences on carbon cycling and global warming potential

Isabelle Raymond-Bouchard[1], Ianina Altshuler[1], Lyle G Whyte[1]

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AEM80

Microbial source tracking of waterborne pathogens by new designed nanofluidic qRT-PCR chip

Abdolrazagh Hashemi Shahraki[1], Daniel Heath[1], Suba Rao Chaganti[1]

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AEM81

Nesting dolls: The viruses in the bacteria in us

Felix R Croteau[1], Alexander P Hynes[1]

[1]McMaster University

AEM82

Novel hybrid-network analysis approach for determining microbial species interactions while controlling for abiotic niche filtering

Ianina Altshuler[1], Shaun Turney[1], Charles Greer[1], Lyle Whyte[1]

[1]McGill University

AEM83

Novel Loop-mediated Isothermal Amplification (LAMP) assay development for rapid and quantitative detection of Arcobacter species

Izhar U.H. Khan[1], Amir Abdulmawjood[2], André Becker[2], Michel Clou[1], Maria Mahmood[1], David R. Lapen[1], Graham Wilkes[1,3], Emilia Craiovan[1], Edward Topp[4]

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AEM84

Occurrence of *Campylobacter* species, *Escherichia coli* and total coliforms in Canadian First Nation drinking water distribution systems

Maria Mahmud[1], Michel Cloutier[1], Sabrin Bashar[2], Anita Murdock[2], Rakesh Patider[2], Annemieke Farenhorst[3], Ayush Kumar[2], Izhar U.H. Khan[1]

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AEM85

Optimization of the denitrifying activity of a continuous methanol-fed biofilm bioreactor

Livie Lestin[1], Richard Villemur[1]

[1]INRS-IAF

AEM86

Pan-genome analysis of *Devosia* reveals role for PQQ biosynthesis locus in deoxynivalenol detoxification

Dion Lepp[1], Yousef I. Hassan[1], Ting Zhou[1]

[1]Guelph Research and Development Centre, Agriculture and Agri-Food Canada, Guelph, Ontario

AEM87

Phylogenetic affiliations and functional properties of alginate lyase extracted from actinomycetes associated with Sargassum seaweed wastes

Bidyut Mohapatra[1]

[1]Department of Biological and Chemical Sciences, The University of the West Indies, Cave Hill Campus, Barbados

AEM88

Plant genotype and soil type influence fungal and bacterial assemblages in the rhizosphere of balsam poplar

Karelle Rheault[1], Denis Lachance[1], Christine Martineau[1], Marie-Josée Morency[1], Gervais Pelletier[1], Évelyne Thiffault[2], Armand Séguin[1]

[1]Ressources Naturelles Canada, Service Canadien des Forêts, Centre de Foresterie des Laurentides, Québec, Qc, Canada, [2]Université Laval

AEM90

Potato suberin increases iron metabolism in *Streptomyces scabies*, a common-scab inducing pathogen

Lauriane Giroux[1], Carole Beaulieu[1]

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AEM91

Prevalence and antimicrobial resistance profiles of extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* recovered from natural and conventional feedlots

Krysty D. Munns[1], Rahat Zaheer[1], Edward Topp[2], Tim A. McAllister[1]

[1]Agriculture and Agri-Food Canada - Lethbridge Research and Development Centre, Lethbridge, Alberta, [2]Agriculture and Agri-Food Canada - London Research and Development Centre London, Ontario

AEM92

Prevalence and characterization of plasmid-mediated quinolone resistance in various aquatic sources

Farhan Yusuf[1], Kimberley Gilbride[1]

[1]Ryerson University

AEM93

Primer mixture design for increased diversity detection and quantification of anaerobic hydrocarbon biodegradation gene biomarkers

Gurpreet S. Kharey[1], Lisa M. Gieg[1]

[1]University of Calgary

AEM94

Protective efficacy of recombinant heavy chain fragment of *Clostridium botulinum* D/C

Byeong Yeal Jung[1], Mi-Hye Hwang[1], Young Min Son[1], Kichan Lee[1], Bang-Hun Hyun[1]

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AEM95

Recovery of nutrients from *Fusarium*-damaged grain using solid-state fermentation and insect culture combinations

Zehra Gülsünoğlu[1], Smitha Aravind[1], Yuchen Bai[1], Lipu Wang[2], H. Randy Kutcher[2], Takuji Tanaka[1]

[1]Food and Bioproduct Sciences, University of Saskatchewan, [2]Plant Sciences, University of Saskatchewan

AEM96

Souches microbiennes bio-protectrices comme alternatives aux antibiotiques dans la filière porcine

Eveno Mégane[1,2,3], Frédérique Gancel[1,3], Djamel Drider[1,3], Laurent Bazinet[2,3], Ismail Fliss[2,3]

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AEM97

Stability of microbial community profiles associated with compacted bentonite from the Grimsel Underground Research Laboratory

Katja Engel[1], Sian E. Ford[2], Sara Coyotzi[1], Jennifer McKelvie[3], Greg Slater[2], Josh D. Neufeld[1]

[1]Department of Biology, University of Waterloo, Waterloo, Ontario, Canada, [2]School of Geography & Earth Sciences, McMaster University, Hamilton, Ontario, Canada, [3]Nuclear Waste Management Organization, Toronto, Ontario, Canada

AM98

Stability of nitrogen and phosphorus cycling genes in activated sludge revealed by quantitative genomics

Claire Freeman[1], Chris Yost[1]

[1]University of Regina

AEM99

Structural and functional study of the bacterial acetylcholinesterase ChoE from *Pseudomonas aeruginosa*

Van Dung Pham[1], Tuan Anh To[1], Cynthia Gagné-Thivierge[1], Deqiang Yao[3], Marie-Ève [1], Manon Couture[1], Roger Levesque[2], Steve Charette[1], Rong Shi[1]

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AEM100

Structural and mechanistic insights into phenolic acid decarboxylases

Marie-Ève Picard[1,2], Rong Shi[1,2]

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AEM102

Systems and evolutionary biology of the *Pseudomonas aeruginosa* population structure

Luca Freschi[1], Antony T. Vincent[1,2,3], Julie Jeukens[1], Jean-Guillaume Emond-Rheault[1], Irena Kukavica-Ibrulj[1], Marie-Josée Dupont[1], Steve J. Charette[1,2,3], Brian Boyle[1], Roger C. Levesque[1]

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AEM103

The effects of moisture and nitrogen content on nitrous oxide emissions and nitrifying and denitrifying gene abundances in orchard soil from the Okanagan Valley

Melissa Larrabee[1], Louise Nelson[1]

[1]The University of British Columbia

AEM104

The milk storage effect on milk microbiome and an optimized bacterial DNA extraction protocol for *Staphylococcus aureus* mastitic milk

Soyoun Park[1], Jennifer Ronholm[1]

[1]McGill University

AEM105

The production of pyrrolnitrin and phenazine by *Pseudomonas chlororaphis* PA23 is involved in the amoebicidal activity against *Acanthamoeba castellanii*

Akrm Ghergab[1], Ann Karen Brassinga[1], Teresa Dekievit[1]

[1]University of Manitoba

AEM106

The spore formation of the PGPR *Bacillus subtilis* is directly influenced by the plant

Vincent Charron-Lamoureux[1], Pascale Beauregard[1]

[1]Université de Sherbrooke

AEM107

Understanding the probiotic potential of GABA producing bacteria using an ex-vivo continuous fermentation model

Walid Mottawea[1], Roja Mousavi[1], Yasmina Ait Chait[1], Marie-Claude [1], Riadh Hammami[1]

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AEM108

Using the microbiome to detect geographic food fraud in seafood

Maria Lee Ai Lan[1], Saurabh Ner[1], Kassandra Ma[2], Nicholas Petronella[2], Januana S. Teixeira[2], Swapan Banerjee[2], Jennifer Ronholm[1]

[1]McGill University, [2]Health Canada

AEM109

Vancomycin polymeric nanoparticles for the destruction of methicillin-resistant *Staphylococcus aureus* biofilms

Livia Maria do Amorim Costa Gaspar [1], Afonso Celso Silva Dorea [1], Daniela Droppa Almeida[1,2], Maria Lucila Hernández Macedo[1], Francine Ferreira Padilha[1]

[1]Tiradentes University, [2]Université de Sherbrooke

AEM110

Wild blueberry rhizophere microbiome

Simon Morvan[1], Mohamed Hijri[1]

[1]Université de Montréal

AEM111

Worldwide limitation of biological nitrogen fixation by molybdenum availability: implications for trace metal and nitrogen biogeochemistry in natural and anthropogenic ecosystems

Bellenger JP[1]

[1]Department of Chemistry, Université de Sherbrooke, QC, Canada

AEM112

Antimicrobial activity of bactofencin, nisin, pediocin and reuterin against multi-drug resistant mastitis associated Staphylococcus and Streptococcus isolates

Samantha Bennett[1], Laila Ben Said[2], François Maloin[1], Pierre Lacasse[3], Ismail Fliss[2]

[1]Université de Sherbrooke, [2]University Laval, [3]Agriculture and Agri-food Canada

AEM113

Association of individual prey specialization with the gut microbiome of an Arctic seabird

Esteban Góngora[1], Lyle Whyte[1], Kyle Elliott[1]

[1]McGill University

AEM114

Characterizing hydrocarbon biodegradation along the shorelines of Canada's Northwest Passage

Madison Ellis[1], Mira Okshevsky[1], Ianina Altshuler[1], Esteban Gongora[1], Charles Greer[1], Lyle Whyte[1]

[1]McGill University

AEM115

Culture-dependent bioprospecting of antibiotic producing bacteria isolated from the Canadian high Arctic

Evan Marcolefas[1], Mira Okshevsky[1], Tiffany Leung[2], Geoffrey McKay[2], Emma Hignett[2], Jérémie Hamel[3], Gabriela Aguirre[1], Olivia Blenner-Hassett[1], Brian Boyle[3], Roger C. Lévesque[3], Dao Nguyen[2], Samantha Gruenheid[2], Lyle Whyte[1]

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AEM116

Developing enzyme biotechnology to remove filter cakes during the hydraulic fracturing process

Gabrielle Scheffer[1], Carolina Berdugo-Clavijo[1], Seyyed M.S.N. Abbad[2], Arindom Sen[2], Lisa M. Gieg[1]

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AEM117

Évolution de la qualité microbienne de l'air circulant dans les centrales de traitement de l'air (CTA) d'un centre hospitalier nouvellement construit

Pamela Morissette[1,2], Nathalie Turgeon[1], Caroline Duchaine[1,2]

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AEM118

Growth phase-dependent physiology of bacterial isolates from the Human gut microbiota

William Jogia[1], Corinne Maurice[1]

[1]McGill University

AEM119

Investigating the occurrence of *Micromonospora* in nodules and rhizosphere of actinorhizal plants growing on mine sites in Québec

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AEM120

Is hybridization an adaptive force in response to UV conditions?

Carla Bautista Rodríguez[1,2,3,4], Souhir Marsit[1,2,3,4], Christian R Landry[1,2,3,4]

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AEM121

Isolating and characterizing novel Arctic microorganisms

Olivia Blenner-Hassett[1], Evangelos Marcolefas[1], Charles Greer[2], Lyle G Whyte[1]

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AEM122

Isolation and characterization of marine Myxobacteria clade for the identification of novel antibiotics

Bahar Pakseresht[1], Susan McLatchie[2], Yves Gélinas[1], David Walsh[2], Brandon Findlay[1]

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AEM123

***Legionella pneumophila* and the microbiome of cooling towers**

Kiran Paranjape[1], Émilie Bédard[2], Lyle G. Whyte[1], Jennifer Ronholm[1], Michèle Prévost[2], Sébastien P. Faucher[1]

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AEM124

Leveraging macroecological concepts for microbiological processes: modelling global co-occurrence of methane cyclers

Angus S. Hilts[1], Laura A. Hug[1]

[1]University of Waterloo

AEM125

Microbiological investigation of lava tube ice for determining its habitability on Mars

Brady O'Connor[1], Richard Léveillé[2], Lyle Whyte[1]

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AEM126

Potential of pre-harvest UV treatment as an ecological approach for the control of *Xanthomonas campestris* pv. *vitians* in lettuce

Amadou sidibé[1,2], Marie Thérèse Charles[1,2], Olbert Nicolas[1,2], Carole Beaulieu[1]

[1]Université de Sherbrooke, [2]Agriculture and AgriFood Canada

AEM127

Predicting the bacterial hosts of the gut viral dark matter with CRISPR spacers

Moira Dion[1,2], Pier-Luc Plante[3], Shiraz A. Shah[4], Ling Deng[5], Josue Leonardo Castro Mejia[5], Jakob Stokholm[4], Marie-Agnès Petit[6], Dennis S. Nielsen[5], Hans Bisgaard[4], Jacques Corbeil[3], Sylvain Moineau[1,2]

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AEM128

Quantification of moss-associated cyanobacteria using phycocyanin pigment extraction

Marie Renaudin[1], Romain Darnajoux[2], Jean-Philippe Bellenger[1]

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AEM129

Social motility selects for quorum sensing mutants in the opportunistic pathogen *Pseudomonas aeruginosa*

Sophie Robitaille[1], Eric Déziel[1]

[1]INRS-Institut Armand-Frappier

AEM130

Use of an adsorptive matrix to assess microbial biodegradation of aromatic hydrocarbons

Nicole Taylor[1], Courtney Toth[1,2], Victoria Collins[3], Paolo Mussone[3], Lisa Gieg[1]

[1]University of Calgary, [2]University of Toronto, [3]Northern Alberta Institute of Technology

AEM132

Response of temperate forest soil microbial communities to drought and precipitation events

Sarah Piché-Choquette[1], Petr Baldrian[1]

[1]The Czech Academy of Sciences

AEM133

Effect of bio-protective culture on the surface microbial flora of pork loins

Menel BEN TAHER[1,2,3], David Rigal[1,2,3], Pierre Antoine Jegu[1,2], Ismail Fliss[1,2], Marie Filteau[1,2,3]

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AEM134

Transcriptional response of *Rhizobium leguminosarum* bv. *viciae* 3841 to exposure of 2,4-dichlorophenoxyacetic acid (2-4-D)

Aditi Sharma[1], Alexa Pierce[1,2], Tanya Dahms[2], Christopher Yost[1]

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Molecular Genetics & Cellular Microbiology

MGCM50

Investigating several putative mitochondrial pumps in *Saccharomyces cerevisiae*

Md Deen Islam[1], Deborah A. Court[1]

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MGCM51

Investigating the genetic determinants for *Escherichia coli* group 2 capsule assembly

Caitlin Sande*[1], JP Côté[2], Victoria Wilson[1], Bradley Clarke[1], Eric Brown[2], Chris Whitfield[1]

[1]University of Guelph, [2]McMaster University

MGCM52

Investigating the potential use of diketopiperazines as anti-virulent agents in combination with antibiotics to treat *Burkholderia cepacia* complex infections

Nicole Jervis[1], Joe J Harrison[1], Douglas G Storey[1]

[1]University of Calgary

MGCM53

Investigating the predator-prey relationship between *Bdellovibrio* and virulent *Fusobacterium nucleatum*

Avery Robinson[1], Wonsik Mun[2], Robert J. Mitchell[2], Emma Allen-Vercoe[1]

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MGCM54

Keep your enemies close: Secretion of a *Pseudomonas* anti-bacterial effector protein

Brianne Burkinshaw[1], Tao Dong[1]

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MGCM55

Lack of a functional Csa quorum-sensing (QS) system in *Pseudomonas chlororaphis* strain PA23 does not directly affect biocontrol activity

Grace Saliga[1], Nidhi Shah[1], Kelly Duke[1], Teresa Dekievit[1]

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MGCM56

Light inducible secondary metabolite from the fungus *Hyphodiscus hymeniophilus*

Glenna Kramer[1], Justin Nodwell[1]

[1]University of Toronto

MGCM57

Metabolism of D-arabinose and L-fucose in *Sinorhizobium meliloti* 1021

Kohlmeier MacLean G[1], Kim Derek D[1], Oresnik Ivan J[1]

[1]University of Manitoba

MGCM58

Modulating the microbiome through CRISPR interference

Gregory Pellegrino[1], Thomas Hamilton[1], David Edgell[1], Gregory Gloor[1], Bogumil Karas[1]

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MGCM59

New S-adenosylmethionine riboswitch variants reveal a surprising versatility of their structures and mechanisms

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MGCM60

One Health genomic investigation of the rise in gentamicin resistance in *Salmonella* from human and chicken sources in Canada, 2014-2017

Graham W. Cox[1,2], Amrita Bharat[1,2], E. Jane Parmley[3], Brent P. Avery[3], Rebecca J. Irwin[3], Richard J. Reid-Smith[3], Anne E. Deckert[3], Rita L. Finley[3], Danielle Daignault[4], Linda Hoang[5], Marie Louie[6], Jessica Minion[7], David C. Alexander[8], Vanessa Allen[9], Sadjia Bekal[10], Sameh El Bailey[11], David Haldane[12], Greg J. German[13], George Zahariadis[14], Michael R. Mulvey[1,2]

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MGCM61

Optimal characterization of physiologically-aware gene regulatory models

Nathan Braniff[1], Brian Ingalls[1]

[1]University of Waterloo

MGCM62

Oxygen-dependent role of copper metabolism in the modulation of drug resistance of the opportunistic yeast *Candida albicans*

Inès Khemiri[1]

[1]Université Laval

MGCM63

Prevalence and characterization of antimicrobial resistance among *E. coli* causing canine urinary tract infections: Passive surveillance of laboratory isolates in Saskatoon, Canada 2013-2018

R Courtice[1], M Sniatynski[1], JE Rubin[1]

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MGCM64

Probing *E. coli* growth in different carbon environments

Madeline Tong[1], Shawn French[1], Eric Brown[1]

[1]McMaster University

MGCM65

Production and identification of the unknown *Burkholderia cenocepacia* biosurfactant

Xavier Perron[1], Marie-Christine Groleau[1], Donat Mokono[1], Eric Déziel[1]

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MGCM66

Random mutagenesis of self-conjugating plasmid to identify super-conjugative variants

Dalton Ham[1]

[1]University of Western Ontario

MGCM67

Regulation of fatty acid efflux pump FarE by TetR family transcriptional regulator FarR in *Staphylococcus aureus*

Katherine Ferguson[1], Robert Kuiack[1], Heba Alnaseri[1], Martin McGavin[1]

[1]University of Western Ontario

MGCM68

Regulation of *rpoS* expression by the small regulatory RNA Lpr10 impacts survival of *Legionella pneumophila* in water

Joseph Saoud[1], Sebastien Faucher[1]

[1]McGill University

MGCM69

Ribonuclease interacting partners and behaviour in *Streptomyces venezuelae*

Emma L. Mulholland[1], Marie A. Elliot[1]

[1]McMaster University

MGCM70

Role of a DEAD-box RNA helicase, named TgHoDI, in translational repression and the life cycle of *Toxoplasma gondii*

Scott Roscoe[1], Nadya Morrow[1], and Sirinart Ananvoranich[1]

[1]University of Windsor

MGCM71

***Streptococcus milleri* group in cystic fibrosis airways: geographic variability and contribution to disease progression**

Michael Surette[1,2,3], laura rossi[1,3], Gayatri Nair[1,3]

[1]McMaster University, [2]Biochemistry and Biomedical Sciences, [3]Medicine

MGCM72

Structural and biochemical studies of ribosomal frameshifting in Porcine reproductive and respiratory syndrome virus (PRRSV)

Ankoor Patel[1], Emmely Treffers[2], Ying Fang[3], Eric Snijder[2], Brian L. Mark[1]

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MGCM73

Study of *thiC* riboswitch transcriptional pauses

Elsa Hien[1], Jean-François Nadon[1], Daniel Lafontaine[1]

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MGCM74

The minimal pSymA symbiotic nitrogen fixation gene region of *Sinorhizobium meliloti*

Jiarui Huang[1], Barney Geddes[1], Zahed Muhammed[1], George C. diCenzo[1], Maryam Zaman[1], Turlough M. Finan[1]

[1]McMaster University

MGCM75

The role of scaffold protein Cst5 in the *Candida albicans* pheromone response pathway

Anna Carolina Borges Pereira Costa[1], Yuan Sun[1], Adithi Sundarakrishnam[1], Malcolm Whiteway[1]

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MGCM76

The Type IVb Pilus of conjugative plasmid TP114 is required for conjugation in the mouse gut microbiota

Nancy Allard[1], Kevin Neil[1], Sébastien Rodrigue[1]

[1]Université de Sherbrooke

MGCM78

TxtH is a key component of the thaxtomin biosynthetic pathway in the plant pathogen *Streptomyces scabies*

Yuting Li[1], Jingyu Liu[1], Damilola Adekunle[1], Luke Bown[1], Kapil Tahlan[1], Dawn R.D. Bignell[1]

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MGCM79

Understanding the role of glycerol in the exploration of *Streptomyces venezuelae*

Evan Shepherdson[1], Marie Elliot[1]

[1]McMaster University

MGCM80

Using the fluoride riboswitch as a molecular tool to offset reporter gene toxicity while screening for potent bacterial promoters

Vesta Korniakova[1], Jonathan Perreault[1]

[1]INRS-Institut Armand-Frappier

MGCM81

When genetics fails to identify your antibiotic cluster, try genomics

Naveen Sorout[1], John Stavrinides[1]

[1]Department of Biology, University of Regina, Regina, SK, Canada

MGCM82

Characterization of *tktB* locus associated with second site suppressors of *tktA* mutation in *Sinorhizobium meliloti*

Sabhjeet Kaur[1], Justin P. Hawkins[1], Ivan J. Oresnik[1]

[1]University of Manitoba

MGCM83

Cold-directed knockdown of a bipartite antipathogenic protein in a model cereal

Collin Juurakko[1]

[1]Queen's University

MGCM84

Designer *Sinorhizobium meliloti* strains and multi-functional vectors for direct inter-kingdom transfer of DNA

Stephanie L. Brumwell[1], Michael R. MacLeod[2], Tony Huang[1], Ryan Cochrane[1], Rebecca S. Meaney[3], Maryam Zamani[2], Ola Matysiakiewicz[4], Preetam Janakirama[3], David R. Edgell[1], Trevor C. Charles[4], Turlough M. Finan[2], Bogumil J. Karas[1,3]

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MGCM85

Exploiting polymicrobial interactions to thwart antimicrobial resistance

Rahgavi Poopalarajah[1], Andrew J. Flewelling[2], Michael D. Parkins[3], John M. Conly[3], Douglas J. Mahoney[3], Darren J. Derksen[2], Joe J. Harrison[1]

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MGCM86

Inter-kingdom transfer of conjugative plasmid from *Escherichia coli* to *Saccharomyces cerevisiae* within solid media

Maximillian P.M. Soltysiak[1], Rebecca S. Meaney[2], Samir Hamadache[1], Preetam Janakirama[2], Bogumil J. Karas[2,3]

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MGCM87

Investigating the biofilm stimulation response to sub-MIC antibiotics

Luke N. Yaeger[1], Michael R. Ranieri[1], Jessica Chee[1], Derek C.K. Chan[1], Hanjeong Harvey[1], Lori L. Burrows[1]

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MGCM88

Modulation of bacterial multicellularity via differential polysaccharide secretion

Fares Saidi[1,2], Israel Vergara[3], Emilia M. F. Mauriello[3], Salim T. Islam[1,2]

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MGCM89

Natural antisense transcripts of *Toxoplasma gondii*

Ambreen Fahim[1], Sirinart Ananvoranich[1]

[1]University of Windsor

MGCM90

Old drug, new trick: thiostrepton inhibits growth of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* in low-iron conditions

Michael R. Ranieri[1], Derek C. K. Chan[1], Luke Yaeger[1], Madeline Rudolph[1], Sawyer Karabelas-Pittman[1], Hamdi Abdo[1], Jessica Chee[1], Hanjeong Harvey[1], Uyen Nguyen[1], Lori Burrows[1]

[1]McMaster University

MGCM91

Phage moron JBD30-4 mediates twitching and swimming motility in *Pseudomonas aeruginosa*

Zafrin Islam[1], Véronique L. Taylor[1], Yu-Fan Tsao[1], Karen L. Maxw[1]

[1]University of Toronto

MGCM92

Physical, physiological, and molecular characterization of the near-minimal bacterium *Mesoplasma florum*

Dominick Matteau[1], Jean-Christophe Lachance[1], Frédéric Grenier[1], Daniel Garneau[1], Pierre-Étienne Jacques[1], Sébastien Rodrigue[1]

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MGCM93

Plasmid-based tools for Cas9 genome editing and stable maintenance of synthetic pathways in *Phaeodactylum tricornutum*

Samuel S. Slattery[1], Andrew Diamond[2], Helen Wang[1], Jasmine A. Therrien[1,3], Jeremy T. Lant[1], Teah Jazey[3], Kyle Lee[3], Zachary Klassen[3], Isabel Desgagné -Penix[2,4], Bogumil J. Karas[1,3], David R. Edgell[1]

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MGCM94

Rapid multicellular cell shape evolution: lesson form *Neisseriaceae* family

Sammy Nyongesa[1], Antony T. Vincent[1], Juan Guerra[1], Frédéric J. Veyrier[1]

[1]INRS-Institut Armand-Frappier, Bacterial Symbionts Evolution, Laval, QC, Canada

MGCM95

The fidelity of DNA polymerases in microgravity

Aaron Rosenstein[1]

[1]Queen's University

MGCM96

Towards a mechanistic understanding of bacterial social networking via outer-membrane extrusion

Eric Ramirez[1,2], Charles Gauthier[1], Salim Timo Islam[1,2]

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Abstracts

Invited Keynote Speakers & Session Speakers Abstracts

Session 1 – One Health: Antibiotics and resistance, from animal production to public health I

Tuesday, June 11th, from 8:30 - 10:00 AM

IS1-400

One Health - Antibiotics and resistance, from animal production to public health

M. R. Mulvey[1,2,4], A. Bharat[1,2], G. Cox[2], C. Mangat[1], E.J. Parmley[3,4], B. Avery[3], C. Carson[3], R. Reid-Smith[3], R. Irwin[3], G.G. Zhanel[2]

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Antimicrobial resistance (AMR) is a growing concern globally as it limits the ability to treat serious invasive infections in both humans and animals, and to a limited degree in food producing crops. Over the past 30 years, we have observed the emergence of multidrug resistant *Escherichia coli* and *Salmonella* serotypes that are approaching extreme drug resistance levels in which invasive infections can be very difficult to treat. AMR can emerge in pathogens by many different pathways, although the use of antimicrobials is one of the major risk factors driving AMR. To understand the emergence of resistance, one must take a One Health approach to investigate the multitude of factors that could drive AMR. Understanding the drivers can be extremely complicated and although reservoirs such as humans, animals and ecosystems are important to consider, a One Health approach should also consider the contribution of economics, cultural differences, and growing interconnectivity in our increasingly globalized world.

In this presentation, I will be giving examples of One Health approaches to AMR using data collected as part of the Public Health Agency of Canada's the Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) which integrates a number of surveillance activities including on-farm, abattoir, retail food, and human surveillance activities to form a farm-to-fork-to-flush continuum of AMR in *Salmonella* in Canada and AMR in agri-food *Escherichia coli*. In addition, we will also be examining data collected from the human hospital-based infection surveillance program called CANWARD (www.can-r.com) for *E. coli* causing human infections.

I will describe findings from recent studies involving the rise and fall of cephalosporin-resistant *Salmonella* Heidelberg in human and agri-food isolates over a 12-year period, and the recent increase in gentamicin-resistant Salmonella and *Escherichia coli* in agri-food poultry isolates. I will include data about antimicrobial use in chickens which may be driving some of the rise and fall of this resistance. I will also highlight a recent study of multidrug resistance in *Salmonella* Dublin from animal and human cases in Canada and compare it to the global picture using whole genome sequencing. Finally, I will describe a growing issue of extended-spectrum beta-lactamase (ESBL) producers causing human infections in our hospitals and the potential linkage to agri-food isolates.

SS1-33

In vitro activity of Siderophore-β-lactam conjugates against Gram-negative and Gram-positive pathogens

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Background: Bacteria have developed many ways to insure assimilation of essential iron. One of them is the secretion of small iron chelators called siderophores. For some years now, attempts have been made to conjugate β-lactam antibiotics to synthetic siderophores to create Trojan horse molecules that enhance the biological activity of the antibiotic by improving Gram-negative outer membrane transport through siderophore-specific receptors. The aim of this study was to evaluate the *in vitro* activity of four different siderophore-antibiotic (SID-ATB) conjugates: bis-catechol-ampicillin, bis-catechol-loracarbef, bis-catechol-cefaclor and mixed bis-catechol-mono-hydroxamate-cefaclor. Their activity against five different bacterial species was tested: *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Staphylococcus. aureus*.

Methods: First, the siderophore and SID-ATB conjugates were evaluated in their capacity to chelate iron in a chrome azurol-S (CAS) agar assay. Then, minimal inhibitory concentrations (MICs) of the antibiotics and SID-ATB conjugates were measured using a broth microdilution method. Chelex was used to remove iron from the cation-adjusted MHB medium for antibiotic susceptibility testing. MICs in combination with a β-lactamase inhibitor (either tazobactam or clavulanate) were also determined to evaluate the impact of this resistance determinant on antibiotic susceptibility. The capacity of the SID-ATB conjugates to covalently bind the bacterial penicillin-binding proteins (PBPs) was confirmed using a competition assay with a fluorescent penicillin (Bocillin FL). The concentration of the conjugates needed to block 50% of the binding of Bocillin FL to each PBP (IC_{50}) was estimated by quantification of fluorescence after SDS-PAGE.

Results: The CAS assay demonstrated that each SID-ATB conjugate could chelate iron and that the mixed siderophore was the best chelator. For the Gram-negative bacteria, the MICs demonstrated that conjugating a siderophore to a β-lactam mainly improved or did not affect the inhibitory activity of the drug and when improved, this effect was amplified in the iron-depleted medium. This positive effect was not observed with *S. aureus*, which does not possess an outer membrane barrier. All SID-ATB conjugates tested were active against *E. coli* in the iron-depleted medium (MIC 0.02-3.1 µM). The addition of tazobactam in susceptibility tests for *P. aeruginosa* increased the activity of the bis-catechol-cefaclor/loracarbef conjugates while the bis-catechol-ampicillin was very effective by itself (MIC 0.1 µM). Clavulanate increased the activity of the bis-catechol-ampicillin against *K. pneumoniae*. All the SID-ATB conjugates tested inhibited *A. baumannii* even if the bacterium was resistant to the antibiotic alone. The mixed SID-ATB conjugate showed the best activity against *A. baumannii* (MIC 0.2 µM). Finally, the PBP binding assay demonstrated that the SID-ATB conjugates, compared to the unconjugated antibiotic, could still bind the PBP targets quite efficiently.

Summary: The conjugation of a siderophore to a β-lactam can enhance the inhibitory activity even if the antibiotic alone is inactive against Gram-negative bacteria. Iron depletion increases the inhibitory effect and shows that the activity of the SID-ATB conjugates depends on the expression of bacterial iron-transport systems.

SS1-107

Exploiting polymicrobial interactions to thwart antimicrobial resistance

Rahgavi Poopalarajah[1], Andrew J. Flewelling[2], Michael D. Parkins[3], John M. Conly[3], Douglas J. Mahoney[3], Darren J. Derksen[2], Joe J. Harrison[1]

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Microbes rarely exist in solitude but rather live in multispecies communities that are rich with ecological interactions. Although mutualistic interactions between diverse groups of microorganisms can enable co-habitation of certain niches, competition is the dominant form of interspecies interaction. Competition originates from nutrient limitation and space restriction. During interference competition, microbes can use antimicrobial compounds to damage adjacent cells, and therefore, natural antimicrobial resistance may have a role in allowing some bacteria to co-colonize the same spaces as antibiotic producers. Unsurprisingly, some antibiotic-producing organisms have evolved the capacity to subvert the antimicrobial resistance of competitors via the secretion of small molecules that act as antibiotic potentiators. For example, *Streptomyces clavuligerus* co-regulates production of cephamycin C, which is a beta-lactam antibiotic, with clavulanic acid, which is a beta-lactamase inhibitor. Nevertheless, the prevalence of these chemical interactions among microbes is unknown. We propose that polymicrobial interactions may be a source for natural antibiotic adjuvants with the capacity to thwart antimicrobial resistance of pathogens that top the WHO global priority list of antibiotic resistant pathogens to guide research and development of new antibiotics. I have worked as part of team to develop a natural product pipeline for the discovery of microbial metabolites that can potentiate the activity of meropenem against carbapenem-resistant *Pseudomonas aeruginosa*. Carbapenems are antibiotics of last resort. This work was enabled by first helping to build the Alberta Microbiota Repository, which is a collection of bacteria and fungi cultivated from a diverse range of clinical, animal and environmental sources. Using a high-throughput method, the spent media from 400 of the repository isolates were assayed in the presence and absence of meropenem. A well-defined mathematical criterion was used to classify organisms producing soluble molecules that alter antibiotic resistance. In collaboration with natural product chemists, we used activity-guided fractionation, mass spectrometry and nuclear magnetic resonance (NMR) spectroscopy to isolate and identify the active component from the spent medium. Our initial efforts revealed that spent media from 11 strains reduced growth of meropenem-resistant *P. aeruginosa* by 2.5- to 40-fold. Here, I will describe the progress we've made with one of our top candidates from the meropenem adjuvant screens. The bacteria that produces our compound of interest, called strain 171, comes from the oropharyngeal tract of a healthy human male adult volunteer. Spent medium from strain 171, when combined with meropenem, increased killing of *P. aeruginosa* by up to 1000-fold. Extraction into hexanes, fractionation, and NMR led to the identification of a derivative of a human essential dietary fatty acid (which we term B2D-1A) that can alter carbapenem sensitivity of *P. aeruginosa*. We found that B2D-1A can work synergistically with meropenem to reduce the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) by 4-fold. Taken together, our results not only identify a group of fatty acid derivatives that affect antimicrobial sensitivity, but also suggest that microbe-microbe interactions determining antimicrobial sensitivity have been grossly underestimated.

SS1-133

Examination of biocide cationic antimicrobial resistance in *Escherichia coli* to identify phenotypic and genotypic adaptations

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Background: Cationic antimicrobial (CA) agents are defined as antimicrobials with cationic charge at neutral pH and commonly include a wide range of disinfectants such as quaternary ammonium compounds (QAC) and bisbiguanides (BG) that we rely on to sterile and disinfect skin and surfaces. CAs also include therapeutic antibiotics such as polymyxins (PMX) used as a last resort therapy for drug resistant infections. In general, CAs kill bacteria by disrupting the cell membrane causing cells to leak to death. The overuse of CAs can place selective pressure on bacteria to adapt to these compounds and as a result, potentially increase antimicrobial cross-resistance to therapeutically relevant extended-spectrum beta-lactamase (ESBL) and carbapenemase producers. CA adaptation is known to alter bacterial cell membrane compositions (protein and lipid alterations), such as increased efflux pump activity, lipid A modifications, and reduced porin expression, however it is unclear if all CAs impact phenotype and genotype similarly.

Aim: We have adapted an *E. coli* K-12 BW25113 strain over 20-40 subcultures to one of four CAs: QACs benzalkonium and cetrimide, the BG chlorhexidine and PMX colistin in an effort to characterize the genotypic and phenotypic alterations caused by prolonged increasing bacterial exposure to commonly used CAs.

Hypothesis: We hypothesize that *E. coli* adapted to different CA will result in reduced growth fitness, unstable antimicrobial resistance phenotypes, cross-resistance to biocide and therapeutics and genetic alteration of efflux pump, porin and lipid modifying membrane proteins as compared to the unadapted wildtype strain.

Results: As compared to the unadapted *E.coli* strain the final minimal inhibitory concentration of each CA adapted strain after 40 broth subcultures revealed a 2-4-fold increase in all CA adapted strains, except for the 200-fold increase by the colistin adapted strain. Unexpectedly, only colistin and chlorhexidine adapted cultures could stably maintain their respective antimicrobial resistance after 10 subcultures without CA selection, indicating that only QAC adaption was unstably inherited. Growth curve experiments of CA adapted strains in rich and minimal media revealed that only growth in minimal media but not rich media significantly reduced cell growth. Antimicrobial susceptibility testing to determine cross-resistance to other antimicrobials revealed that both QAC adapted strains were cross-resistant to different QACs but not to other classes of antimicrobials we tested suggesting CA adaption may be antimicrobial class specific. Chlorhexidine and colistin adapted strains did not identify significant cross-resistance to other antimicrobials and were much more susceptible to Gram-negative antibiotics (vancomycin). Illumina Mi-Seq whole genome sequencing analysis identified few commonly identified single nucleotide polymorphisms (SNPs) in genes and non-coding regions shared between different CAs but SNPs were identified in different outer membrane porin, lipid A modifying genes and efflux pumps systems by each CA adapted strain. QAC adapted strains shared the greatest number of genes and non-coding regions with SNPs as compared to BG and PMX adapted strains.

Significance: This study suggests CA adaption is dependent on how each CA specifically disrupts the cell membrane, since QACs, BG and PMX all have different mechanisms of action. It also reveals new insights into mechanisms of CA resistance.

SS1-192

Accessing novel antibiotics in diverse *Streptomyces* bacteria

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Streptomyces bacteria are a tremendous source of antibiotics. Bioinformatic analysis of the accumulating genomic data for these bacteria, however, is revealing that these organisms have even greater biosynthetic potential than previously appreciated. A challenge in accessing novel molecules is that in many cases, the corresponding biosynthetic machinery is often silent or lowly expressed under common laboratory conditions. Our lab has revealed that the conserved nucleoid-associated protein Lsr2 has potent antibiotic regulatory functions. We have found that manipulating Lsr2 activity can effectively 'break the metabolic silence' in diverse *Streptomyces* isolates, and can stimulate the production of novel compounds.

The **objective** of my current work is to mine the metabolic reservoir activated upon modulation of Lsr2 activity in a well-studied strain (*S. venezuelae*) and a 'wild' *Streptomyces* isolate (named BIM8). We **hypothesize** that *lsr2* deletion (knockout) or disruption of Lsr2 binding to DNA (knockdown) is a robust bioengineering approach to discovering novel antibiotics. Disruption of Lsr2 DNA-binding activities was achieved by overexpressing dominant negative mutant variants of Lsr2. **Results**: (i) RNA-Seq analysis comparing transcription profiles of wild-type and *lsr2* knockout/knockdown strains revealed the upregulation of the biosynthetic genes encoding putative non-ribosomal peptide and ladderane molecules in *S. venezuelae*, and ribosomally synthesized and post-translationally modified peptides in *Streptomyces* sp. BIM8. (ii) HPLC and LC-MS analyses of these different strains revealed remarkable metabolic changes in both *lsr2* knockout and knockdown strains relative to their wild-type parents. (iii) Antibiotic bioassays using crude and partially-purified extracts from these knockout/knockdown strains are revealing novel antibiotic activities. In future work, these compounds will be purified and analysed for structural novelty. **Conclusion**: Modulating Lsr2 levels is an outstanding strategy to stimulate antibiotic synthesis and discover new metabolites in streptomycetes. **Significance**: This work establishes Lsr2 modulation as a novel approach to accessing new reservoir of antibiotics, and has the potential to serve as a powerful tool in future drug discovery pipelines.

IS2-393

Industrial microbes and biotechnology: some examples

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Anaerobic fermentation reactions produce all kinds of useful products, from ethanol to chocolate, and contribute to valuable ecosystem services in engineered and natural environments. For example, anaerobic digestion producing methane is a useful treatment option for many different municipal, agricultural and industrial waste streams. Anaerobic processes in sediments, swamps and wetlands contribute significantly to element cycling and pollutant stabilization. Anaerobic processes in groundwater help to remediate spills of petroleum hydrocarbons, industrial solvents, pesticides, herbicides and metals. Microbes have evolved and adapted in multiple ways to deal with contaminants in the environment. Many new groups of microbes that remarkably obtain energy for growth from metabolizing pollutants are being used in engineered systems for waste treatment and bioremediation. Through enrichment cultures and metagenome investigations, we are learning more about interspecies interactions in these remarkable, ubiquitous, yet complex microbial communities, and their specialized enzymes that catalyze extraordinary reactions. Examples of such microbial communities that perform valuable ecosystem services will be highlighted.

SS2-265

Genetic control of antibiotic production in *Streptomyces*

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Streptomyces bacteria are noted for their ability to produce a wide range of bioactive compounds, including antifungal and anticancer drugs, as well as over 70% of the antibiotics in clinical use today. Genome sequences of diverse *Streptomyces* species have revealed that they have the potential to produce far greater numbers of secondary metabolites than have been detected in the lab, and the products of many of these 'cryptic' biosynthetic gene clusters remain unknown. Notably, many of these 'cryptic' biosynthetic clusters are transcribed at low levels under laboratory conditions. Activating the expression of these clusters is therefore critical to identifying and characterizing these potentially novel bioactive molecules. In *Streptomyces venezuelae*, we have discovered that a nucleoid-associated protein termed Lsr2, represses the expression of many secondary metabolic clusters. Nucleoid associated proteins play roles not only in organizing chromosome structure but also in regulating global gene expression. To understand how Lsr2 is exerting its gene silencing effects, we have focused our attention on the well-studied, but transcriptionally silent, chloramphenicol cluster in *S. venezuelae*. We found that deleting *lsr2* led to significant upregulation of cluster expression and production of chloramphenicol. Using a range of molecular biology (chromatin-immunoprecipitation, RNA-sequencing) and biophysical (atomic force microscopy) techniques, we have established that Lsr2 represses transcription of the chloramphenicol cluster by binding DNA both within the chloramphenicol biosynthetic cluster, and at more

distantly positioned sites. CmlR is a known activator of the chloramphenicol cluster, but unexpectedly, expression of its associated gene was not upregulated in an *lsr2* mutant strain. This suggested that CmlR may function to 'counter-silence' Lsr2, alleviating its repressive effects and permitting production of chloramphenicol. We found there was an inverse correlation between CmlR levels, and the extent to which Lsr2 could bind within the chloramphenicol cluster. We have discovered that CmlR exerts its effects on Lsr2 indirectly, by facilitating the action of RNA polymerase, which effectively clears bound Lsr2 from the chloramphenicol cluster DNA. These findings provide insight into the networks governing antibiotic gene regulation in *Streptomyces,* and have the potential to guide novel strategies aimed at stimulating the production of new antibiotics in these bacteria.

SS2-298

Characterizing naphthenic acid degrading bacterial communities

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Oil production generates wastewater containing toxic naphthenic acids (NAs), which cannot be released into the environment. To prevent environmental discharge, oil-refineries currently store wastewater indefinitely in tailings ponds, and at smaller sites, they remove naphthenic acids through a combination of adsorption and biodegradation by granular activated charcoal (GAC) filters. This process is effective though the filters must be replaced every six weeks because of bacterial biofilm formation. An efficient and cost-effective reclamation strategy needs to be developed. Anaerobic biodegradation of single surrogate naphthenic acids has revealed alkane-degrading pathways potentially involved in NA degradation. More recently, anaerobic biodegradation has revealed additional pathways. Recent literature describes the composition of the bacterial communities by 16S rRNA gene sequencing of samples from tailings ponds, typically revealing unique communities at each location.

How these bacterial communities degrade complex mixtures of naphthenic acids has not been studied at a functional level, and the mechanisms of naphthenic acid biodegradation *in situ* are unknown.

We developed DNA and RNA extraction protocols to obtain material directly from GAC filters suitable for Oxford Nanopore and Illumina sequencing. Preliminary results show a dynamic microbiome in GAC filters that expresses known genes related to degrading complex hydrocarbons. Using a combination of Oxford Nanopore and Illumina sequencing, we generated metagenomically-assembled genomes for several spent GAC filters. Taxonomic prediction suggests the microbiome changes significantly over time and in different conditions. Reads were mapped to open reading frames predicted from the assemblies, and we identified novel open reading frames that were highly associated with hydrocarbon-degrading functions, potentially representing novel genes involved in naphthenic acid degradation. Our results suggest novel genes highly-associated to hydrocarbon degradation remain to be discovered.

SS2-359

In vivo functional analysis of a class A β-lactamase-related protein essential for clavulanic acid biosynthesis in *Streptomyces clavuligerus*

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In *Streptomyces clavuligerus,* the gene cluster involved in the biosynthesis of the clinically used β-lactamase inhibitor clavulanic acid contains a gene (*orf12* or *cpe*) encoding a protein with a C-terminal class A β-lactamase-

like domain. The *cpe* gene is essential for clavulanic acid production, and the recent crystal structure of its product (Cpe) was shown to also contain an N-terminal isomerase/cyclase-like domain, but the function of the protein remains unknown. In the current study, we show that Cpe is a cytoplasmic protein and that both its N- and C-terminal domains are required for *in vivo* clavulanic acid production in *S. clavuligerus*. Our results along with those from previous studies allude towards a biosynthetic role for Cpe during the later stages of clavulanic acid production in *S. clavuligerus*. Amino acids from Cpe essential for biosynthesis were also identified, including one (Lys_{89}) from the recently described N-terminal isomerase-like domain of unknown function. Homologues of Cpe from other clavulanic acid-producing *Streptomyces* spp. were shown to be functionally equivalent to the *S. clavuligerus* protein, whereas those from non-producers containing clavulanic acid-like gene clusters were not. The suggested *in vivo* involvement of an isomerase-like domain recruited by an ancestral β-lactamase related protein supports a previous hypothesis that Cpe could be involved in a step requiring the opening and modification of the clavulanic acid core during its biosynthesis from 5*S* precursors.

SS2-370

Identification of glyphosate degradation genes in *Pseudomonas* by Tn-seq

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Glyphosate is the most widely used herbicide on Earth, and while it is generally considered safe it has the potential to affect the soil microbiome if it is not degraded sufficiently rapidly. Thankfully, many microorganisms can break down and inactivate glyphosate, including members of *Pseudomonas*. Glyphosate can be inactivated by breaking a carbon-phosphorus bond within it, usually performed by enzymes in the CP-lyase pathway. *Pseudomonas* glyphosate degraders are of particular interest because little is known about the molecular mechanism of glyphosate utilization and degradation, and because some research suggests a different gene arrangement and possibly different gene interactions than the more widely studied mechanism found in *E. coli* and in members of the Rhizobiales such as *Ochrobactrum* and *Agrobacterium*. Some *Pseudomonas* species such as *Pseudomonas stutzeri*, contain two CP-lyase operons *phn* and *htx*, with the *htx* operon likely derived from horizontal gene transfer from Rhizobiales. We would like to know more about the structure and arrangement of glyphosate degrading genes within *Pseudomonas*. To do this, novel glyphosate degrading strains were isolated from environmental samples using enrichment culture in which glyphosate supplies the only source of phosphorus. The genomes of the isolates were then sequenced using PacBio Single-Molecule Real-Time technology, which facilitated complete genome assembly. Genome annotation was performed using RAST, as well as manually searching known CP-lyase proteins with BLAST to identify potential genes involved in glyphosate degradation. Neither RAST nor BLAST were able to predict sufficient CP-lyase members for glyphosate degradation within glyphosate degrading strains of *Pseudomonas*. This suggests that our strains may use an alternate pathway to CP-lyase or they may have novel CP-lyase component sequences that avoid detection by current annotation methods. Ongoing work includes the use of genetic and biochemical methods to identify the nature of glyphosate degradation employed by these strains, including Tn-Seq experiments.

IS3-398

Effect of restriction in the use of antibiotics in food animals on antibiotic resistance in food animals and humans

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Antimicrobial resistance is considered one of the greatest threats to global and public health today. The World Health Organization has called for urgent action. Antibiotic use in human medicine, veterinary medicine, and agriculture has been linked to the rise of antibiotic resistance globally.

In a systematic review of 181 studies, interventions that restrict antibiotic use in food-producing animals were associated with a reduction in the presence of antibiotic-resistant bacteria in these animals. The pooled absolute risk reduction of the prevalence of antibiotic resistance in animals with interventions that restrict antibiotic use commonly ranged between 10 and 15%, depending on the antibiotic class, sample type, and bacteria under assessment. Similarly, in the human studies, the pooled prevalence of antibiotic resistance reported was 24% lower in the intervention groups compared with control groups, with a stronger association seen for humans with direct contact with food-producing animals.

In a sub-analysis, we analyzed whether different types of restriction are associated with differential effectiveness in reducing resistance. We created a classification scheme of different approaches to antibiotic restriction: 1) complete restriction; 2) single antibiotic-class restriction; 3) single antibiotic restriction; 4) all non-therapeutic use restriction; 5) growth promoter and prophylaxis restriction; 6) growth promoter restriction; and 7) other/undetermined. A total of 114 studies were included. The most frequently studied intervention type was complete restriction (n=43), followed by restriction of non-therapeutic (n=29) and growth promoter (n=17) indications. None examined growth promoter and prophylaxis restrictions together. Though complete restrictions were associated with a 17% reduction in antibiotic resistance, less prohibitive approaches also demonstrated reduction in antibiotic resistance of 9 to 29%. Broad interventions that restricted global antibiotic use appeared to be more effective in reducing antibiotic resistance compared to restrictions that narrowly target one specific antibiotic or antibiotic class. Importantly, interventions that allowed for therapeutic antibiotic use appeared similarly effective compared to those that restricted all uses of antibiotics, suggesting that complete bans are not necessary.

Finally, we undertook a sub-analysis of the original review to determine whether (and what) unintended consequences may arise from such interventions. A total of 47 studies described potential consequences of antibiotic restrictions. There were no consistent trends to suggest clear harm. There may be increased bacterial

contamination of food products, the clinical significance of which remains unclear. There is a need for rigorous evaluation of the unintended consequences of antibiotic restrictions in human health, food availability, and economics, given their possible widespread implications. These findings directly inform the creation of specific policies to restrict antibiotic use in food-producing animals.

SS3-198

Precise identification of antimicrobial resistance determinants from metagenomic data

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Metagenomics, the direct sequencing of the mixture of genomes present in a sample, is an increasingly common workflow within the life sciences. It is frequently used to investigate previously intractable problems such as the functional characterisation of entire microbial environments. One such use-case of global and national public-health importance is analysing the nature and transmission dynamics of antimicrobial resistance (AMR) determinants in human, agri-food and environmental samples. While recently some tools have been developed to profile AMR from metagenomes, these are generally limited to profiling at the level of arbitrarily identity-clustered genes. By exploiting the expertly curated ontological structure of the Comprehensive Antibiotic Resistance Database (CARD) and new CARD Resistomes and Variants data-sets, we have developed a 3 level hierarchical machine learning classifier for the rapid identification of 2386 specific protein AMR determinants from metagenomic data-sets. Firstly, pairwise heuristically accelerated homology searches are used to filter non-AMR related metagenomic reads using DIAMOND. This filtering has been optimised to prioritise minimisation of false negatives over minimising false positives. Features generated from these homology searches, as well as sequence composition information, are used to train a random forest classifier to classify filtered reads into one of 227 curated CARD AMR gene families (e.g. MCR phosphoethanolamine transferases). For each gene family an additional random forest classifier is then trained to classify reads into one of the specific AMR determinants belonging to that family (e.g. MCR-1, MCR-2, MCR-3, etc.). On a fully held out test-set of MiSeq reads simulated from the CARD canonical gene sequences (i.e. sequences associated with peer-reviewed, published evidence of resistance), the hierarchical model resulted in an average precision and recall of 0.993 and 0.987 at the AMR gene family level. Within the 227 AMR families, 70% (158) had an average F1-score > 0.99 for classification to specific AMR determinants. A further 10% (24) averaged F1-scores between 0.8 and 0.99. In comparative analyses on the same dataset this outperformed both read mapping and variation graph based methods in terms of average overall recall and precision. Work is on-going incorporate variant based AMR models (e.g. regulatory mutations, gyrase mutations, etc.) as well as meta-models (e.g. multi-component efflux pump systems). This precision and these variant model features make AMRtime the only comprehensive means of AMR gene detection from metagenomic data.

SS3-202

Quantifying antimicrobial resistant *E. coli* - A study on better methodology for surveillance data

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Antimicrobial resistance is one of the greatest challenges to medical science; the emergence of multidrug resistance bacteria is increasingly threatening the ability of physicians and veterinarians to treat their patients. Antimicrobial resistant bacteria have been identified throughout the food chain, including farms, abattoirs, and retail meat. This provides numerous opportunities for antimicrobial resistance bacteria or their resistance genes to come into contact with or colonize in humans. The Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) is responsible for collecting data on resistant *E. coli, Salmonella* and *Campylobacter* spp. from beef, pork and poultry on farms, slaughter and from retail meat across the country. The current methodology used by CIPARS to recover *E. coli* does not include media for the selective isolation of antibiotic resistant *E. coli*. The objective of this study was therefore to compare the recovery rate of *E. coli* resistant to 3rd generation cephalosporins using the conventional CIPARS approach with a selective media (CHROMagar ESBL).

In this study, an aliquot of all fecal samples (n=56) collected as part of CIPARS chicken on farm surveillance in Saskatchewan were tested. Samples were quantitatively cultured on CHROMagar orientation (to identify all *E. coli)* and CHROMagar ESBL (for the identification of 3rd generation cephalosporin resistance). Samples were diluted 1:20 and 1:4000 prior to plating using a spiral plater, allowing 10^3 – 10^9 CFU/g to be accurately quantified. *Escherichia coli* was found in all samples while 18 (32%) contained 3rd generation cephalosporin resistant isolates. On average we identified $1.28x10^7$CFU/g of *E. coli* while 3rd generation cephalosporin resistant isolates were present at a density of $9.33x10^4$CFU/g. For each sample, the antimicrobial susceptibility of two isolates was determined. In cases where growth on CHROMagar ESBL was observed, the susceptibility of two of those isolates and one grown on non-selective media was determined.

Resistance to cephalosporins such as ceftriaxone was the most common in samples from ESBL media, isolates resistant to ceftriaxone were obtained from 100% of samples. Other cephalosporins which shared this were cefazolin and cefotaxime. Next common was resistance to cefuroxime (94%), tetracycline (94%) and ampicillin (89%). Multi-drug resistant isolates were isolated from 22.2% of samples, including two isolates resistant to β-lactams, tetracycline, gentamicin, and sulfonamides. In the case of samples from non-selective media, resistance to tetracycline was the most common (23%). Next common was resistance to ampicillin (12%), cefazolin (9%) and gentamicin (9%). One isolate from non-selective media was identified as being multidrug resistant, which included resistance to β-lactams, tetracycline, and gentamicin.

ESBL positive isolates were identified form 18 samples including CTX-M (n=17) and SHV (n=7). All were isolated from CHROMagar ESBL. The results of this study indicate that chickens in Saskatchewan are frequently carriers of ESBL producing *E.coli*. We also found that the colonization intensity with ESBL producers was several orders of magnitude lower than with non-ESBL producers.

SS3-281

Environmental continuum profiling: microbiomes and resistomes of livestock production, agricultural soil and urban wastewater

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Antimicrobial use in beef cattle may contribute to increased prevalence of antimicrobial resistance (AMR) in bacteria within beef production systems and surrounding environments. Comparative knowledge of microbiomes and resistomes across environmental interfaces between animal production and urban settings is lacking. In this study, we executed a comparative analysis of the microbiomes and resistomes of metagenomic samples (n=35) isolated from conventional (cattle raised with antibiotics) and natural (raised without antibiotics) feedlot cattle feces (FC), runoff catch basins adjacent to feedlots (CB), manured agricultural soil from fields in the vicinity of feedlots, and urban sewage influent (SI). Metagenomic DNA samples were subjected to Illumina sequencing, generating on average ~54 million paired-end reads per sample. Firmicutes exhibited the highest prevalence (40%) in FC, whereas Proteobacteria were most abundant in CB (64%), soil (60%) and SI (83%). Among sample types, SI had the highest diversity of AMR and metal/biocide resistance (MBR) classes (13 & 15) followed by FC (10 & 8), CB (8 & 4), and soil (6 & 1). Highest AMR gene abundance was harboured by FC whereas soil samples had a very small, but unique resistome which did not overlap with FC & CB resistomes. Consistent with antimicrobial use patterns in beef, tetracycline resistance predominated followed by macrolide resistance. Although natural FC samples exhibited a microbial ecology that was similar to conventional, their resistome was less complex. Similarly, the SI resistome was indicative of drug classes used in humans, including β-lactam, macrolide, tetracycline, aminoglycoside fluoroquinolone and fosfomycin determinants. Metal and biocide resistance accounted for 26% of the SI resistome with a predominance of mercury resistance that may be associated with contamination of municipal water with household and industrial products. This study demonstrates an increasing divergence of the microbiome and resistome as distance from the feedlot increases.

SS3-306

Plasmids carrying antibiotic resistance genes in swine production

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Antibiotic resistance is a recognized menace which will most likely surpass cancer as the leading cause of death by 2050. In Canada, approximately 80% of all the antibiotics sold are administered to livestock animals, promoting the development and spread of antibiotic resistance genes in food-borne pathogens. Among them are the *Enterobacteriaceae* producing extended spectrum β-lactamases (ESBLs), which are secreted enzymes conferring resistance to most of the available antibiotics targeting the bacterial cell wall. When localized on mobile elements, these ESBLs can propagate throughout populations and other species, thus increasing the risk to public health. The aim of this study was to assess and characterize antibiotic resistant bacteria in feces of pigs

reared in commercial facilities with conventional or antibiotic-free husbandry practices, and to identify plasmids carrying multidrug resistance in these samples.

Feces were sampled from four groups of 14 sows during lactation, and for each sow, feces were sampled from 5 piglets at four time points spanning their lifetime. The first group were animals reared under conventional practices while the animals in the three other groups were reared in antibiotic-free settings. The total and resistant bacteria were quantified by plating serial dilutions of feces onto MacConkey II medium supplemented or not with cefotaxime or tetracycline and resistant isolates were picked. The minimal inhibitory concentrations of 24 antibiotics were determined for a subset of 300 multidrug resistant isolates using the Sensititer automated system. The genetic context of the resistance determinants was defined in 195 isolates by Nanopore and Miseq sequencing followed by MOB-suite analyses of the assembled genomes and identification of the resistance genes in the chromosomes and plasmids using the Resistance Gene Identifier of the Comprehensive Antibiotic Resistance Database.

The frequency of cefotaxime resistance decreased as the pigs grew older for two antibiotic-free groups, whereas it remained low but consistent in the conventional system. ESBL-producing *Enterobacteriaceae* were isolated from all husbandries, but the ESBL phenotype was more prevalent in antibiotic-free than in conventional settings. Conventional isolates were on average resistant to a higher number of antibiotics than isolates originating from antibiotic-free animals. Numerous plasmids carrying resistance genes were found, including IncI1 plasmids coding for CTX-M-1, CMY-2 and TEM-1 ESBLs. These plasmids also contained genes conferring resistance to antibiotics commonly used in swine production such as co-trimoxazole, spectinomycin and tetracycline, suggesting co-selection of the resistance genes. As contamination of carcasses with *Enterobacteriaceae* is common, there is a potential for these resistant bacteria to contaminate fresh meat products. Moreover, pig manure is used as a fertilizer on arable land leading to contamination of vegetables and the environment with antibiotic resistance genes and resistant bacteria. It is especially worrying as ESBLs provide resistance against antibiotics of very high importance in human medicine with limited availability of alternative treatments.

IS4-399

Engineering of a probiotic bacterium for precise microbiome editing

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Developing a bacterium that could selectively target microbes to prevent or treat infectious diseases and dysbiosis would have an extraordinary impact on medicine. This could for example constitute a powerful alternative to antibiotics and an accurate tool to study the microbiota. My laboratory has recently initiate efforts to engineer a probiotic strain that exploits the most potent bacterial conjugation machineries for high-efficiency delivery of CRISPR-Cas9 systems directly into the mouse microbiota. The CRISPR-Cas9 systems can be programmed to specifically cleave DNA simply by providing custom guide RNA (gRNA) spacer sequences that match the desired loci. Targeting DNA sequences located in a bacterial chromosome leads to cell death by compromising genome integrity. For instance, a single oral dose of our probiotic was sufficient to specifically eliminate ~98% of a targeted *E. coli* strain carrying an antibiotic resistance gene relative to an otherwise isogenic strain. The CRISPR-Cas9 system can also cure plasmids carrying antibiotic resistance or virulence genes, thereby "disarming" potentially dangerous bacteria by restoring susceptibility to antibiotics.

SS4-15

Construction of a *Mycoplasma feriruminatoris* chassis for the development of a vaccine for contagious caprine pleuropneumonia

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Background: Contagious caprine pleuropneumonia caused by *Mycoplasma mycoides* subsp. *capripneumoniae* (*Mccp*), is a disease of utmost importance in Africa and Asia. The use of vaccine is currently the most cost-effective method to control this pathogen in those regions. By expressing *Mccp* antigens on the surface of the fast-growing *Mycoplasma feriruminatoris* (*Mferi*) we will produce such vaccine at a lesser cost, making it more accessible for farmers with lower income.

Methods: The *Mferi* genome was cloned in *Saccharomyces cerevisiae* and engineered using CRISPR/Cas9. Modified *Mferi* genomes were then back transplanted from yeast into a recipient cell to produce the recombinant strains. A base avirulent chassis was made by performing seamless deletions of genes for glycerol transport and H_2O_2 production as well as the immunoglobulin cleavage MIB-MIP system. Functional analysis of the deletion mutant was made by measuring H_2O_2 production and IgG cleavage assays. Potential *Mccp* specific antigens

were identified by comparative genomic analysis of the complete genomes of six *Mccp* strains, *Mferi* and other representatives of the mycoides cluster.

Results: The *Mferi* chassis was proven to no longer produce H_2O_2 and lost its ability to cleave IgG. Albeit the deleted strain has a slightly longer generation time, it remains among the fastest growing *Mycoplasmas*. Thirteen *Mccp* specific genes grouped in four clusters were selected as potential antigens and their grafting in *Mferi* has debuted.

Conclusion: *Mferi* offers new possibilities in term of vaccine production. Its rapid growth and the available genome engineering techniques are assets to further improve its usefulness.

SS4-156

The genetic determinants of *Staphylococcus aureus* host cell adhesion

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Staphylococcus aureus is a colonizing opportunistic pathogen causing infections when the skin is breached and/or the host immune system is weakened. For example, ~80% of aggressive hospital infections are caused by isolates ordinarily found living on our skin. *S. aureus* harbors a wide range of covalently attached cell wall-anchored (CWA) proteins (also referred to as adhesins) that are implicated in adhesion to host cells. Despite adhesion being an integral component of skin colonization and the infectious process, we only have a superficial understanding of the genetic underpinnings of *S. aureus* adhesion. Importantly, *S. aureus* uses different virulence factors and adhesins to cause different types of infection in distinct sites within the body. Therefore, we sought to identify the genetic determinants of adhesion in different clinically relevant contexts. Specifically, we investigated keratin-mediated skin colonization; fibronectin-mediated host cell invasion; and fibrinogen-mediated immune evasion. A genome-wide study of the genetic determinants of *S. aureus* adhesion has not previously been described. To enable such studies, we developed the first sensitive, robust, and high-throughput *S. aureus* adhesion assay. We used this assay to profile an ordered and annotated methicillin-resistant *S. aureus* (MRSA) USA300 transposon library (n= 1,952) to identify mutants that were deficient in adhesion in the three aforementioned clinical contexts.

Overall, we identified 27 gene disruptions that reduced the affinity of MRSA for keratin, 14 for fibronectin and 22 for fibrinogen. Performing three different screens enabled comparative analysis of the genetic determinants of adhesion in these different contexts. The screen was validated by the identification of known adhesion-related proteins such as the sortase (*srtA*) that anchors adhesins to the cell wall. Furthermore, a number of known global regulators of virulence such as SarA and the alternative sigma factor B were identified. We also identified genes encoding proteins that have never before been associated with adhesion. Here we focus on the peptidoglycan hydrolase autolysin; disruption of the gene encoding this protein reduced the affinity of MRSA for both keratin and fibronectin. Our findings support the hypothesis that autolysin is indirectly associated with adhesion, which is reliant upon the proteins peptidoglycan degradative/remodeling function. CWA proteins that contain a YSIRK/R N-terminal signal peptide motif are secreted and incorporated into the peptidoglycan at the division septum. They can then be detected across the cell surface in a ring-like/hemispherical distribution following peptidoglycan splitting and cell separation. Our immunofluorescence studies suggest that loss of autolysin, which also localizes to the septum, causes sequestration of CWA adhesins at the septum of these division impaired cells, which prevents CWA adhesin presentation/distribution upon the surface of the cell.

This study describes the first genome-wide assessment of the genetic determinants underlying *S. aureus* host cell adhesion. Targeting bacterial host cell adhesion is a promising anti-virulence strategy that in contrast to antibiotics, will be less likely to favor drug resistance and cause dysbiosis of our microbiota. Importantly, we

reveal that the disruption of single genes is sufficient to inhibit adhesion, which opens the way for the development of strategies to inhibit these elements.

SS4-282

Harnessing rare enzyme activities from an agarolytic bacterium to engineer artificial metabolic pathways and selective delivery of heterologous proteins to the intestine

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Bioconversion of agarose is predominantly catalyzed by bacteria that live in marine ecosystems, and correspondingly, the genomes of these microorganisms are enriched in carbohydrate active enzyme (CAZyme) genes belonging to hallmark agarase families (e.g. GH50, GH86, and GH117). Recently, a polysaccharide utilization locus (PUL) specific for agarose metabolism (AG-PUL) was identified in the genome of the human gut bacterium, *Bacteroides uniformis* NP1 (BuNP1). In addition to its unique collection of CAZymes and other metabolic enzymes, the AG-PUL is found in a range of geographically distinct human microbiomes, suggesting that it has a complex evolutionary origin. Here, we present the comprehensive structure-function analysis of the AG-PUL from *B. uniformis* NP1. Using recombinant enzymes from the AG-PUL, we demonstrate that metabolism and bacterial growth can be conferred to a non-agarolytic strain of *B. uniformis*. This relationship underscores that rare nutrient utilization by intestinal bacteria is facilitated by the acquisition of genes encoding specialized enzymes that serve to unlock inaccessible carbohydrate resources contained within unusual and polysaccharide substrates. Based upon these observations, we designed a rapid and reproducible chromosomal integration system called 'pINT' (pExchange-INTegrate) to facilitate the insertion of transgenic agarolytic machinery into the genome of the model intestinal bacterium, *Bacteroides thetaiotaomicron* (*Bt*-AG+). The successful engineering of *Bt*-AG+ strains underpins that selective metabolism can be transferred between species of *Bacteroides*. Significantly, these findings establish that the pINT system can be harnessed to deliver heterologous proteins to the intestine, with applications in designer therapeutics for a wide range of intestinal ailments.

SS4-302

Bacterial biosensors as a novel technology for detecting naphthenic acids in oilsands wastewater

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Bitumen is extracted from the Athabasca surface sands with hot water, ultimately extracting numerous other organic compounds from the soil. Ponds that occupy hundreds of square kilometres are used to store the tailings and wastewater, referred to as oil sands process-affected water (OSPW). The long-term requirement is to treat the water, remove pollutants, and return the water to the Athabasca river. The microbes living in OSPW are a tremendous resource to exploit and produce new biotechnologies that can support the long-term water treatment

goals. This study addresses a bacterial biosensor approach to environmental monitoring and bioremediation of a primary OSPW component, naphthenic acids (NA).

There is a dynamic microbial community living in tailings ponds, which includes many microbes that can degrade naphthenic acids and other organic compounds in OSPW. We isolated *Pseudomonas* species from OSPW samples and sequenced their genomes. Using resident OSPW bacterial species, we employed a genomic and synthetic biology approach to construct biosensors that detect naphthenic acids. A NA-induced operon was initially identified by screening a library of mini-Tn5-*lux* mutants for transcriptional *lux* fusions that responded to naphthenic acids. To expand the scope of potential promoters, RNA-seq was performed to identify all the bacterial genes induced by exposure to various simple and complex preparations of naphthenic acids. The promoter sequences were extracted from the bacterial genome, synthesized and cloned as plasmid-encoded, transcriptional *lux* fusions using Gibson assembly. Sixty-four potential NA biosensor circuits were constructed and screened for their sensitivity and limits of detection of NA mixtures, as well as their specificity to a small panel of individual NA compounds that are commercially available.

Bioremediation is one treatment approach that is effective, low cost and scalable for use in the oilsands industry. Bioremediation studies have demonstrated naphthenic acids degradation by many bacteria, yet some NA compounds are recalcitrant to breakdown and the degradation mechanisms are poorly understood. We also employed our NA biosensor in high throughput screens to identify novel isolates from metagenomic libraries that are of capable of degrading NA mixtures. Confirmation of NA degradation using GC-MS is underway. Biosensor technology is specific, sensitive, low cost, high throughput, and can compete with analytical detection methods. These microbiological tools are also useful for identifying new strains and bioremediation pathways that are required to degrade naphthenic acids.

IS5-404

Emerging infectious diseases: causes, consequences and how we respond

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Emergence and re-emergence of infectious diseases are an increasing threat to human health, and an increasing challenge to public health. In this presentation, mechanisms of infectious disease emergence and drivers of emergence are discussed, with a focus on zoonoses, vector-borne diseases and climate change. The consequences for Canada of infectious disease emergence are explored and our public health responses of outbreak management, risk assessment, surveillance and control are detailed, with an emphasis on One Health approaches.

SS5-4

The role of bats in the enzootic cycle of *Borrelia*

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Bats are reservoirs of several emerging viruses such as Ebola and Marburg filoviruses, SARS and MERS coronaviruses, and Nipah and Hendra paramyxoviruses. These viruses often cause lethal disease in spill over mammals such as humans and agricultural animals but bats that are naturally or experimentally infected do not demonstrate classical signs of disease. There are also speculations about the role of bats in the enzootic cycle of relapsing fever group of spirochetes, including *Borrelia*. Lyme borreliosis is an emerging disease in Ontario, Canada. Because Lyme disease-associated *Borrelia* have previously been detected in ectoparasites collected from bats in other parts of the world, we hypothesized that bats in Ontario may have been exposed to *Borrelia burgdorferi*. To determine if hibernating big brown bats (*Eptesicus fuscus*) in Ontario have been exposed to *Borrelia*, we tested serum from 31 *E. fuscus* for the presence of anti-*B. burgdorferi* antibodies by enzyme-linked immunosorbent assay (ELISA). By using anti-bat secondary antibodies, we adapted a human diagnostic kit to detect bat antibodies to *B. burgdorferi*. We observed that 45.16% of the sampled bats were seropositive, including 36.36% of the bats that were born in captivity. To date, we have been unable to culture *B. burgdorferi* from bat blood. We have also been unable to detect spirochetes by gram staining and immunofluorescence staining, although these attempts are ongoing. Our preliminary data support other published reports suggesting that bats have been exposed to spirochetes that may be causative agents of relapsing fever. Future studies to establish the role of bats in the enzootic cycle of *Borrelia* are being developed using *in vivo* infection and vector-mediated transmission models. Results from our study will inform public health

policies for Lyme borreliosis and further elucidate the possible role of other small mammals in the enzootic life-cycle of *Borrelia*.

SS5-312

Predicting permafrost microbial community responses to thaw based on pre-thaw ecosystem characteristics

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Permafrost environments contain vast stocks of frozen organic matter, which are being mobilized as climate change increases the rate and extent of permafrost thaw. The conversion of this organic matter to CO_2 and CH_4 through microbial metabolic processes represents one of the greatest potential future transfers of greenhouse gasses to the atmosphere. Improved understanding of the processes governing permafrost microbial community structure and function and post-thaw dynamics are required to improve how carbon fluxes from permafrost landscapes are represented in current climate models. We hypothesized that 1) microbial community distributions in permafrost soils will be governed most strongly by homogenous selection across a landscape, reflecting shared constraints on communities imposed by prolonged freezing; and 2) patterns in community structure and function that emerge post-thaw will be governed most strongly by variable selection across a landscape, reflecting responses to emergent environmental conditions that are predictable from initial pre-thaw conditions. To test these hypotheses, we examined bacterial and fungal community composition in 59 permafrost cores taken across a boreal forest landscape near Fairbanks, Alaska, and monitored emergent environmental conditions and shifts in microbial community structure and function that developed post-thaw after 100 days of incubation at 4 °C. We employed 16S rRNA and ITS gene sequencing analysis to characterize microbial community structure, Fourier transform ion cyclotron resonance mass spectrometry (FTICR-MS) and gas flux analysis to monitor metabolomic shifts in the soil organic matter pool, and metatranscriptomics to monitor changes in community function. Proportionally, patterns of permafrost community composition across the landscape were most strongly influenced by dispersal limitation (0.36) and homogenous selection (0.21), and less so to variable selection (0.16) and homogenizing dispersal (0.05), supporting our hypothesis that shared constraints of the permafrost environment govern microbial distributions in permafrost. Contrary to our second hypothesis, post-thaw communities did not shift strongly from pre-thaw compositions, reflecting the importance of historical contingencies on post-thaw community composition and suggesting that characteristics of the pre-thaw communities may be important for dictating post-thaw responses. These results are being combined with those from the metabolomic and metatranscriptomic analyses to develop a landscape scale model aimed at predicting gas flux and community dynamics following permafrost thaw.

SS5-343

Maternal antibodies provide strain-specific protection against infection with the Lyme disease pathogen in a wild rodent

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The vertebrate immune system can produce antibodies that protect the host against re-infection with the same pathogen. Females can transmit these antibodies to their offspring, which provides them with short-term protection against infection. *Borrelia afzelii* is a tick-borne spirochete bacterium that causes Lyme disease in Europe. Local populations of *B. afzelii* often consist of multiple strains that cycle between *Ixodes ricinus* ticks and rodents such as the bank vole *Myodes glareolus*. We used an experimental infection approach to show that infected female bank voles transmit *B. afzelii*-specific antibodies to their offspring. Bank vole offspring were challenged via tick bite at 5 weeks post-weaning with a strain that was either identical or different compared to the maternal strain. Offspring from infected mothers were either 100% protected or 100% susceptible depending on whether the challenge strain was the same as or different from the maternal strain. Our study shows that maternal antibodies provide strong but highly strain-specific protection against *B. afzelii* in an important rodent reservoir host. The transfer of strain-specific protection between generations of hosts can have important consequences for the ecology and evolution of this multiple-strain pathogen in nature.

IS6-407

Wild viruses in the High Arctic

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The most abundant and diverse group of biological entities in aquatic environments are the protists, prokaryotes, and viruses that comprise the microbial community. Microbes play a critical role in the cycling of nutrients and energy, and therefore understanding the dynamics and interactions of this group is vital to understanding the ecology of aquatic ecosystems as a whole. An important, but relatively understudied, component of the microbial community is the viruses. As well as being the most abundant and diverse biological entities on Earth, viruses influence the community structure and evolution of their hosts, and ultimately the productivity of the entire biota. The overarching theme of my research is the study of aquatic viral ecology in a changing climate. The Arctic is a model environment to pursue this theme because the region is experiencing some of the most dramatic changes due to climate change on Earth and harbors a wide diversity of aquatic habitats whose viral communities are largely uncharacterized. As an example of the ongoing research in our laboratory, I will focus on the characterization of the wild viruses from Lake A, a highly stratified, ice-dependent lake located on the northern coast of Ellesmere Island, that embodies the dramatic perturbations that are occurring in this region.

SS6-5

Biotechnological potential of endophytic and rhizospheric bacteria for plant growth promotion in *Paspalum* species

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Paspalum is a large grass genus of the Poaceae family, comprising several important forage species which occur in the Tropics and warm temperate regions, mainly in the American continent, especially abundant in Brazil. The forage-based production has been intensified due to a greater demand for livestock production in the past decades and thus increasing the use of chemical fertilizers and pesticides, causing serious negative environmental impacts. Therefore, there is an urgent need to create new solutions for sustainable agricultural practices. Endophytic and rhizospheric microorganisms associated with these plants play a fundamental role in this process since they are able to promote plant growth and protection against pathogens, so they may be employed as biofertilizers and biopesticides. Bacterial endophytes colonize the internal tissues of plants while rhizospheric bacteria are typically found around plant roots. They can benefit host plants directly by facilitating the acquisition of essential nutrients such as nitrogen and phosphorus or by modulating phytohormones levels like auxin, cytokinin, and gibberellin. The indirect plant health promotion occurs when the bacteria inhibit phytopathogens with antibiotics, hydrolytic enzymes, nutrient limitation, and by priming plant defenses. Thus,

the aim of this study was to isolate and analyze the plant growth-promoting bacteria potential associated with *Paspalum rojasii* (BGP 272), *P. lenticulare* (BGP 281) and *P. compressifolium* (BGP 380), which are used as bridge species in forage plant breeding programs. The samples were collected from the Germplasm Bank (GB) of *Paspalum*, maintained by Embrapa Pecuária Sudeste - São Carlos, São Paulo, Brazil. The culturable bacteria from leaves, roots, and rhizospheres were isolated in Tryptic Soy Agar + Benomyl medium and their potential for three functional traits were evaluated: phosphate solubilization (PS), synthesis of indole 3-acetic acid (IAA) and biological nitrogen fixation (BNF). The PS assay was performed in a solid medium containing $CaHPO_4$, IAA synthesis was performed in Tryptic Soy Broth + L-tryptophan, and the BNF assay was carried out in semi-solid medium without nitrogen. Out of 107 bacterial isolates obtained, 40 isolates were from *P. lenticulare* (11 rhizospheric and 29 endophytes), 35 were from *P. rojasii* (7 rhizospheric and 28 endophytes), and 32 were from *P. compressifolium* (14 rhizospheric and 18 endophytes). Among all the 107 isolates tested, 53% solubilized phosphorus, 35% fixed nitrogen and 47% produced more than 100 ug/ul of indole 3-acetic acid. A total of 20 bacterial isolates showed potential to promote plant growth (11 endophytic and 9 rhizospheric). The next steps of this study will be to identify these bacterial isolates by sequencing the 16S rDNA gene, perform antibiosis test against *Paspalum* phytopathogens, and verify *in vivo* the bacterial potential for plant growth promotion in *Paspalum* species.

Keywords: Biofertilization. Plant growth-promoting bacteria. Indole 3-acetic acid. Nitrogen fixation. Phosphate solubilization. Forage grasses.

SS6-8

Developing and testing the efficacy of a topical probiotic on captive bats to prevent White Nose Syndrome

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Wildlife diseases can have drastic consequences for species at an unprecedented rate. Without proper intervention, diseases can threaten population viability and result in species extinction. The delicate balance of an ecosystem is associated with individual species' niche's and can quickly become unstable upon the introduction of an invasive disease. Managing these diseases is often quite challenging and typically requires immediate action to prevent further ecological loss. The psychrophilic fungus, *Pseudogymnoascus destructans*, *Pd*, is known to cause devastation to several North American bat species throughout hibernation. White Nose Syndrome (WNS) is a deadly disease that indirectly causes hibernating bat mortality by growing on the cutaneous surfaces of the bat and causing more frequent arousal periods. In addition to physiological imbalances caused by the degradation of wing tissue, the decreased torpor length and associated increased metabolic rate will burn precious fat stores, cause rapid dehydration, and lead to bat mortality. Reducing the severity of the Pd infection could result in lower mortality rates. In this study, we are conducting captive bat experiments to alter native wing microflora found on *Myotis yumanensis*, using a prophylactic topical probiotic cocktail. The probiotic contains *Pseudomonas* bacterial species that were isolated from wings of healthy British Columbia bats and were found to inhibit *Pd*. The three main objectives of this study are, (1) to develop a technique of applying the anti-*Pd* microbes to high risk Myotis bats; (2) to test whether an altered microbiome can be sustained on their wings with persistent anti-*Pd* microbes during and after probiotic applications; (3) and to test whether the altered wing skin can better resist the growth of *Pd* than wings that do not receive the prophylaxis. A preliminary captive bat trial was conducted at the B.C. Wildlife Park and the results suggest that application of a concentrated prophylactic aerosol to bat wings successfully shifted the wing microflora. We will continue to work on the above objectives with a second captive trial in spring 2019; pending outcomes of these tests, our next step will be

working with collaborators who would apply the probiotic to wild bats at summer roosts. Our goal is to reduce the spread, or at minimum the severity, of WNS in western North America.

Keywords**:** bats, conservation, *Pseudogymnoascus destructans, Myotis yumanensis, Pseudomonas,* white-nose syndrome, disease management, captive trial, microbiome, probiotic.

SS6-63

Dynamics of invasive insect gut microbiome when facing environmental stress

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The emerald ash borer (EAB) is an exotic wood borer beetle causing important environmental and economic damages in North America. Beetles are important vectors of microbial communities to which adaptive capacities are related. It is established that environmental stress factors may induce phytochemical changes on the host trees, which may trigger changes in the structure of a herbivorous insect's microbial community. As a biotic stress factor, the EAB density on an ash tree can be correlated to the level of damage induced by insects. The research aim was to explore the relationship that may exist between the structure of the EAB gut microbiome and the density of insect found on an ash tree. Insect densities of EAB populations were set on randomly selected ash trees located in a 75 km North-East transect from Montréal (QC) along the St. Lawrence River. Using Lindgren green traps, three levels of insect densities (low, intermediate, and high) were established. Collected EAB adults were dissected, and the gut used for DNA extraction. The microbial community associated with the insect's gut was determined by 16S rRNA and ITS2 sequencing for bacteria and fungi respectively. Results indicated that the bacterial community was mainly composed of three phyla: Proteobacteria (73%), Actinobacteria (9%), and Firmicutes (9%), whereas two phyla dominated the fungal community: Ascomycota (58%) and Basidiomycota (40%). The composition of microbial communities among the samples appeared largely diversified with the Chao1 and Simpson index significantly different across the three distinct levels in the bacterial community. Principal coordinate analysis based on Bray Curtis distances of 16S rRNA sequences showed a significant variation between the structure of the bacterial communities depending on host tree infestation level. Moreover, the analysis showed that some species indicators were strongly correlated with newly infested host trees. Further investigations will help to identify if the taxonomical structure of the gut microbiome may be driven by some metabolites related to tree defense mechanisms. This study is the first to present a complete overview of the bacterial and fungal communities associated with the gut of EAB exclusively based on culture-independent methods and to suggest correlations between the taxonomical structure of the microbiota and stress induced by insects on host trees.

SS6-183

Metatranscriptomics to assess Great Lakes near shore sediment contribution to water quality and human pathogenicity

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Recreational water usage is a major tourist attraction within the Laurentian Great Lakes, where pathogenic bacterial levels have increased substantially compared to historic trends. Pathogens associated with freshwater ecosystems can pose significant health risks and research regarding this concern has gained much attention, specifically in locations where recreational water use is popular. Common water quality assessments involve

quantifying broad or ambiguous taxa (i.e. enterococci) within the water column, but neglect to consider physical and geochemical factors. Further, sampling protocols do not take into account the energy dynamics of the system (waves, currents, swimmer density) and often rely on assessments of planktonic populations sampled during calm low activity periods. It has been shown that sediment resuspension, transport, and deposition influence both the temporal and spatial variation in microbial communities within both the sediment and water compartments. In this study, we used genomics and metatranscriptomic approaches to investigate the active communities within the beach sediment at four freshwater public beaches along Lake St. Clair and Lake Erie in southern Ontario, Canada. We analyzed bacterial community structure, function, and pathogenic activity with relation to physicochemical properties. Presented results will explain the expression of transcripts in bed sediment that encode for microbial metabolism, nitric oxide detoxification and several genes associated with infectious diseases. Signatures of active pathogenicity include expression of *Salmonella* virulence factors and genes involved in pertussis, as well as evidence of antimicrobial peptide (AMP) resistance at these locations. Finally, with consideration of horizontal gene transfer among microbes in highly populated environments (e.g. sediments), we propose a universal bacterial pathogen in which to consider the combined mechanisms these microbes use to survive and function. To our knowledge, this is the first study of its kind to analyze the expression of pathogenic transcripts within bed sediment at freshwater public beaches, and will help to further current understanding and management of health risks in these environments.

IS7-408

Interactions between bacteria and bacteriophages in the human gut

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The human gut is home to complex communities of microbial cells, bacteriophages, fungi, and eukaryotes; referred to as the gut microbiota. The gut microbiota is key to human health: it is central to digestion, synthesizes essential vitamins, metabolizes therapeutic drugs, and shapes host immunity; yet we have no clear understanding of the metabolic activities performed by individual members of this community, or how they interact. These gaps in our functional understanding of the gut microbiota hinder our attempts to manipulate this community for health. Here, I provide evidence for the potential active role of bacteriophages in child stunting. Stunting, a severe growth impairment, affects 22% of children under the age of five worldwide, with multi-generational consequences. This delayed and reduced growth results from a combination of nutritional deficiencies, genetics, and altered gut bacterial communities. Despite the links between an altered gut microbiota and stunting, the role of bacteriophages is unknown. In collaboration with the International Center for Diarrhoeal Diseases Research, Bangladesh (icddr,b), we show that stunted children younger than 38 months harbor distinct gut bacteriophages relative to their healthy counterparts. These gut bacteriophages are infectious and can regulate the bacterial composition and abundance in an age-specific manner, highlighting their possible role in the pathophysiology of child stunting. Collectively, our projects aim to increase our understanding of the ecological processes and interactions between bacteria and phages in the gut, to modulate them for health purposes.

SS7-37

Gut bacterial physiology and activity along the progression to dysbiosis

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During homeostasis, the gut microbiota provides many critical functions to the human host. Conversely, when the gut microbiota is in a state of imbalance, termed dysbiosis, it is correlated to a wide range of diseases. Dysbiosis is currently defined as a disease-specific shift in bacterial community diversity. A specific, functional-based description of dysbiosis is lacking, as well as the understanding of how the gut microbiota reaches this dysbiotic state. We hypothesize that there is a bacterial succession to dysbiosis where changes in bacterial physiology and activity occur before the onset of dysbiosis, specifically in the active bacterial population. These changes in bacterial physiology and activity are what lead to the change in community composition that is currently the endpoint of dysbiosis characterization. Using single-cell techniques such as fluorescently activated cell sorting and 16S sequencing (FACS-Seq), and bioorthogonal non-canonical amino acid tagging (BONCAT), we follow the dynamics of bacterial physiology and activity during the progression to dysbiosis. Specifically, we look at relative nucleic acid content, membrane damage, and translation. Our preliminary data show that upon

dextran sodium sulphate (DSS) induced colitis in mice, changes in bacterial physiology occur on the same day as the onset of symptoms, which both precede the onset of dysbiosis. The active population of the gut microbiota is specifically enriched for *Akkermansia muciniphila*, highlighting the importance of low-abundant bacteria in promoting the disease state. Characterizing changes in bacterial physiology and activity during the progression of intestinal dysbiosis will allow for the identification of windows for successful therapeutic intervention.

SS7-149

Making a dent in campylobacteriosis by biting in Campylobacter jejuni sugar coat

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Campylobacter jejuni (CJ) is a commensal bacterium in animals raised for food production, including poultry but it is an important pathogen in humans. It is a predominant cause of bacterial enteritis worldwide and, in developed countries, campylobacteriosis is associated with consumption of undercooked poultry meat that had been contaminated by CJ during slaughter. CJ's capsule is an external polysaccharide important for colonization and virulence that comprises modified heptoses. We investigate their biological role and their biosynthesis with a view to inhibit their synthesis in poultry before slaughter, which would decrease the CJ load in poultry meat and prevent harmful transmission to humans.

Our knockout mutagenesis studies of heptose modifying genes showed that heptose modification is not necessary for capsule synthesis but is essential for capsular function as a virulence factor. Namely, it affects bacterial resistance to serum and bile salts, biofilm formation, and adhesion to human intestinal epithelial cells and their invasion. The mutants also showed slightly decreased phagocytosis by macrophages. Most importantly, we also demonstrate that heptose modifying genes are important for colonization and persistence of CJ in chicken. These findings suggest that fine-tuning the capsule composition via heptose modification contributes to host pathogen interactions and likely to host specificity. We also elucidated the enzymatic pathways for heptose synthesis in 2 CJ strains that produce slightly different heptoses and identified conserved C3, C5 epimerases and C4 reductases that could be targeted for inactivation. We hypothesize that inhibitors of these enzymes could reduce chicken colonization, thus decreasing meat contamination and downstream human campylobacteriosis. This requires understanding their mechanism of action and substrate specificity. We initially modeled the enzymes' structures (Swiss-Prot), identified residues potentially involved in substrate binding or catalysis and determined their role in catalysis and substrate and product specificity using site directed mutagenesis and capillary electrophoresis. Key catalytic residues were identified, some differing from predictions based on well-studied hexose modifying epimerases and reductases. Several residues that govern heptose vs hexose substrate specificity were identified, which is important to enable development of inhibitors specific for heptose modifying enzymes. Also, residues that govern C3 vs C5 heptose epimerization specificity were identified, which explains how highly similar enzymes perform different epimerization reactions. Finally, we crystallized the enzymes to determine their structure with and without substrate and interprete our mutagenesis data in light of these structural data.

This work provides new enzymes that can be targeted for inhibition to decrease Campylobacteriosis by application to chickens pre-slaughter. Identified features of the substrate binding site and its specificity can be used to design inhibitors or to narrow down the types of molecules to screen for inhibitory capacity. This work also provides new tools to synthesize carbohydrate antigens useful for chicken vaccination and provides grounds for the elucidation of similar pathways of other pathogens.

SS7-222

Investigating the gut microbial ecosystem of infants genetically predisposed to developing type 1 diabetes

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Type 1 Diabetes (T1D) is a genetic autoimmune disease in which T lymphocytes destroy the insulin-producing β-cells of the pancreas. In recent decades, the incidence of T1D has drastically increased worldwide at a rate that cannot solely be explained by genetic susceptibility. Evidence arising from both human and animal studies indicate that an altered gut microbiome is strongly associated with development of the disease. However, we lack an understanding of the underlying mechanisms that drive these changes and how the gut microbiome impacts disease progression. In general, more research emphasis has been placed on the presence or absence of pathogenic species during diseased states, rather than more holistic investigations of the impact of the metabolic output of a given gut microbial community on the host. This study aims to characterize the microbial abundance and metabolic output of fecal communities derived from infants that are genetically predisposed to developing T1D. Through a collaboration with Dr. Jayne Danska at the Hospital for Sick Children, Toronto, stool samples were obtained from infants with predisposing genetic risk factors who participated in the DIABIMMUNE Microbiome Project. Metadata accompanying these samples indicates which infants have tested positive for autoantibodies – termed “T1D progressors” – and which have remained healthy to date. The fecal microbiota contained within these samples were cultured in the unique ‘Robogut’ platform, designed to the mimic the conditions of the human distal colon. Subsequently, a combination of 16S rRNA gene sequencing and metabonomic analyses were applied to comprehensively characterize the composition and behaviour of the fecal ecosystems. The functional disparities between the gut microbiomes of healthy infants and T1D progressors highlighted by this study will help to define the role of the gut microbiome in the pathogenesis of T1D and identify which microbial metabolites are associated with development or exacerbation of autoimmunity. With this knowledge, it may become possible to predict which of the genetically predisposed infants are at a greater risk of T1D based on their microbiome characteristics, and possibly intervene to prevent development of autoimmunity and disease.

SS7-256

A novel genetic circuitry governing hypoxia-induced metabolic reprogramming, commensalism and virulence in the fungal pathogen *Candida albicans*

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Candida albicans is an ascomycete yeast that is an important commensal and opportunistic pathogen in humans. Inside the human host, *C. albicans* colonize predominantly oxygen-poor niches such as the gastrointestinal (GI) and vaginal tracts but also oxygen-rich environments such as cutaneous epithelial cells and oral mucosa. This suppleness requires an effective mechanism to reprogram reversibly its metabolism in response to oxygen variation. So far, studies on *C. albicans* metabolism have been mainly made under normoxic conditions and

undervalued the contribution of hypoxia. To identify regulator that communicate oxygen level status to the metabolic machinery in *C. albicans*, we screened a collection of transcriptional regulators mutant libraries for their ability to grow and utilize different fermentable and non-fermentable carbon sources under both normoxia and hypoxia. This survey identified *snf5* as a mutant with a severe growth defect exclusively under hypoxia and in the presence of different carbon sources. Snf5 is a part of SWI/SNF complex that is an ATP-dependent chromatin-remodeling machinery, which is required for the regulation of gene expression in eukaryotes.

Genome-wide transcriptional profiling showed that Snf5 was required for the activation of transcripts related to carbohydrate utilization and host-pathogen interaction under hypoxia. Genomic occupancy using ChIP coupled to high resolution tiling arrays showed that SWI/SNF complex binds directly to the promoters of genes related to carbohydrate metabolism to modulate their expression. Genes associated with carbohydrate metabolism such as sucrose utilization, glucose and galactose metabolism and TCA cycle were among the direct target of SWI/SNF complex. This suggest that Snf5 is a direct regulator of aforementioned carbohydrate genes. Quantitative analysis of *snf5* metabolome uncovers a major defect of TCA cycle, beta-oxidation and Coenzyme A biosynthesis. The alteration of *snf5* metabolome imply impaired metabolic flexibility and suggests that Snf5 is required for metabolic homeostasis to accommodate *C. albicans* to the metabolic demand accompanying oxygen depletion. Snf5 was also essential for the colonization of the mouse GI tract including stomach, cecum and colon where oxygen levels are significantly depleted. *snf5* mutant was avirulent as it was unable to invade *Galleria* larvae or to cause damage *in vitro* to human enterocytes and macrophages. Interestingly, we also uncovered a differential role of hypoxia in sensitizing *snf5* mutant ROS and cell wall perturbing agents.

The concept of complex haploinsufficiency was used to establish the Snf5 regulatory network that governs hypoxic metabolic flexibility. Our data revealed that adenylate cyclase Cyr1, a key component of the Ras1-cAMP pathway, interacted genetically with Snf5. Other regulators of hypoxic adaptation will be discussed and a comprehensive genetic connectivity with signaling pathways that control fungal metabolism will be shown.

This study uncovered, for the first time in a fungal pathogen, that Snf5, a subunit of SWI/SNF chromatin remodeling complex, behaves as an oxygen sensor that modulates metabolism flexibility in response to hypoxia. Snf5 was also essential for stress resistance and host colonization which make it an attractive target for antifungal therapy.

IS8-401

La microbiologie des aliments : d'hier à demain

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Bien avant la colonisation de l'Amérique par les européens, certaines notions relatives à la salubrité des aliments étaient utilisées par les peuples autochtones.

Après une brève incursion historique dans nos traditions alimentaires, nous dégagerons les grandes étapes qui ont marqué le développement de la microbiologie des aliments en Amérique du Nord et plus particulièrement au Canada. Nous prendrons comme exemples divers aliments fermentés et diverses industries impliquées dans la production de biomasses microbiennes.

Nous présenterons 3 exemples de procédés de fermentation qui ont été développés pour la valorisation de lactosérums résultant de la production de fromages et de yaourts.

En conclusion, nous présenterons quelques pistes prometteuses et considérations pertinentes pour le développement de nouvelles connaissances et de procédés innovateurs.

SS8-84

Evolution of virulent phages infecting an industrial *Lactococcus lactis* subsp. *cremoris* strain over 15 years in a cheese factory

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Since the 1930's, bacteriophages of lactic acid bacteria have been extensively studied because they can slow down the milk fermentation process which can lead to low quality fermented dairy products. Although complete failed productions due to virulent phages are now rare, their populations are still monitored in large factories to avoid production delays and to ensure cheese uniformity. For over two decades, we have been studying the lactococcal virulent phage population within a cheddar cheese plant in the Province of Québec. This university-industry collaboration led to the isolation of several novel phages infecting various industrial strains of *Lactococcus lactis* subsp. *cremoris*. The analysis of these virulent phages shed light on a peculiar phage-host relationship. Indeed, a frequently used strain of *L. lactis subsp. cremoris* was found to be sensitive to a panel of more than 50 distinct lytic phages belonging to the *Skunavirus* genus (*Siphoviridae* family, Caudovirales order). Yet, this industrial strain remains efficient during manufacture, always leading to cheeses with excellent

organoleptic qualities. The aim of this project is to better characterize the interactions between this industrial *L. lactis* strain and its virulent phages.

The first objective was to characterize the genetic diversity of these phages through a host range analysis and comparative genomics. In order to easily differentiate each bacterial virus isolated over these years and as a safeguard against cross-contamination, a multi-locus sequence typing (MLST) profile was setup targeting five late-expressed genes. The five loci, namely genes coding for the terminase large subunit, the major capsid protein, the major tail protein, the tape measure protein and the endolysin, hold different functions: initiation of DNA packaging by cleavage of the *cos* site, major protein constituent of the capsid, principal protein component of the noncontractile tail, overseeing of the tail length and facilitation of DNA transit, and lysis of the host cell, respectively. Then, a host range on 123 strains of *L. lactis subsp. cremoris* showed that these phages are almost host-specific and do not infect any other strains used in the same cheese plant. Short reads sequencing with Illumina MiSeq, allowed us to assemble more than 50 genomes of this phage group. These phage genomes are made of 30,201 bp to 31,846 bp coding from 48 to 56 open reading frames, with a GC content averaging 34.3%. In the late expressed genes of known function, the two subunits of the terminase, the major capsid protein, the portal protein, the holin, and the lysin are the most conserved among these phages. The tape measure protein, the receptor binding protein and a putative neck passage structure display the widest variety in sequence and in length. The middle and early expressed genes were less conserved among these genomes and further investigation is in progress. The bacterial genome sequencing is also underway.

SS8-150

Correlations between the bovine microbiome and aspects of milk quality

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Several different features such as somatic cell count (SCC), protein, lactose, and fat content, as well as vitamin B12 concentration help to measure and define the overall quality bovine milk. Each of these measures are economically important to dairy farmers and to the industry as a whole. Over the past decade a great deal of work has been done to identify the correlations between the feed content and milk quality, but less focus has been placed on understanding the role of the microbiota in different aspects of milk quality. In this work we attempted to identify correlations between certain bacterial phyla and each of the various measures of milk quality. To identify these correlations, we collected rumen, fecal, blood, and milk samples from 55 Holstein dairy cows, housed at the AAFC Sherbrooke Research and Development Center, at two different time points. Each sample was analyzed for vitamin B12 concentration, and the milk samples were additionally analyzed for fat, protein, SSC, urea, and lactose content. Extraction and purification of microbial DNA was performed on each rumen, fecal, and milk sample and the microbial community present in each sample was characterized via 16S rRNA targeted amplicon sequencing. Sequencing data was processed using Mothur. The each of the extensive meta-data sets were individually correlated with the microbial community data using either LEfSe or MaAsLin and resulted in finding several correlations between microbial phyla and milk quality parameters. For example, high-levels of *Proteobacteria,* specifically *Succinivibrionaceae*, in the rumen are correlated with high-vitamin B12 production levels in the rumen. However, somewhat surprisingly, high levels of vitamin B12 production in the rumen did not directly predict high levels of vitamin B12 in milk. To be absorbed by the cow, and ultimately be excreted in the milk, vitamin B12 must be transported from the rumen and into the lower intestine. The data produced in this study showed that in the lower intestine, vitamin B12 concentration were rapidly depleted if the bacterial population is rich in *Rhodospirillales*, *Alphaproteobacteria*, or *Desulfovibrionaceae*. Alternatively, if the lower intestine houses a microbial population richein *Lactobacillaceae*, *Tannerellaceae*, *Gammaproteobacteria*, *Bacteroidales*, and *Succinivibrionaceae* the

vitamin B12 levels remain high. The cellular density of microbiota in the mammary gland is several-fold less dense than the microbiota in the rumen and lower-intestine, and although we attempted to identify correlations between this population and the vitamin B12 concentration none were identified. The correlations between specific bacterial populations and important measures of milk quality that have been identified in this investigation will be used in future work that aims to modify the bovine microbiota for the improvement of milk quality.

SS8-261

Comparative genomic analysis and antimicrobial resistance of *Enterococcus* spp. isolated from a beef processing plant and retail meat

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Aspects of antimicrobial use and resistance (AMR) in livestock production could pose a threat to veterinary and public health. Potentially, AMR bacteria and genes in food-producing animals may be transferred to humans in meat production facilities and retail meat products. Surveillance of AMR in indicator microorganisms such as *Enterococcus* spp. can provide important information on the dynamics and prevalence of AMR in these environments. We collected swabs or samples from along the production line in a commercial beef packing operation: after hide removal, after final washing of the carcass, the conveyor belt, and from ground product (n = 150). We also sampled from ground beef collected from several retail stores (n = 60). All samples/swabs were enriched for *Enterococcus* spp. and suspected enterococci isolates were identified using species-specific PCR primers. Of the 774 *Enterococcus* spp. isolates cultured, most were identified as *Enterococcus faecalis* (n = 572). Not surprisingly, the majority of enterococci were isolated at hide removal, from ground product, and retail samples (n = 696). This finding suggests that control measures reduce the prevalence of enterococci during carcass processing, but that it once again increases as a result of post-dressing contamination. A number of *Enterococcus hirae* isolates were also recovered (n = 112), but these were mostly isolated from the carcass immediately after hide removal (n = 94). We selected 136 *Enterococcus faecium* and *E. faecalis* isolates for screening of antimicrobial resistant genes and sensitivity testing to 16 different antimicrobials. These isolates had 28 unique AMR profiles and 90% were resistant to at least two antimicrobials, including six that were resistant to vancomycin. The resistance genes *tetM* (28%), *tetL* (1.4%), *ermB* (8.9%) were detected in the isolates via PCR, although *vanA* and *vanB* were not identified. Representative *E. faecium* and *E. faecalis* isolates from each AMR profile were selected for whole genome sequencing and analysis. Enterococci isolated from retail meat tended to cluster together as did many of the isolates from the hides. These results show that AMR is widely distributed among *E. faecalis* and *E. faecium* isolates from ground product and retail ground beef and that contamination of these products with enterococci appears to occur downstream of beef processing facilities.

SS8-276

Maple sap microbial community structure associated with maple syrup defects

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As for many other transformed food products, microorganisms are known to be associated with maple syrup quality. Maple sap predominant contaminants have been correlated with maple syrup flavors. However, little is known about the relationship between microbial communities and syrup defects. We recently showed that along with the tree metabolic changes occurring during Spring due to dormancy release, microbial activity may alters maple sap composition leading to an increase in chemical compound diversity over the harvest period and the appearance of flavor defects. Here, we investigate this relationship by comparing microbial community present in saps producing syrup of standard quality and various defects, including the “buddy defect” typically which appears at the end of the harvest period. Bacterial and fungal microbial communities were analyzed by metabarcoding the 16S rDNA gene and ITS regions, respectively. We then constructed a co-occurrence network to investigate microbial community dynamics and identify groups associated with defects. We also correlated taxa with previously identified biomarkers for the buddy defect. We observed exclusion and co-occurrence patterns between some fungi and bacteria, suggesting strong interactions between them. Our results show a generally high prevalence of the *Pseudomonas* or *Rahnella* genus in standard syrups, and the specific presence of *Leuconostoc*, *Serratia* or *Fusicolla* in syrups with defects. Thus, the development of strategies to favor or avoid the development of specific maple sap contaminants may result in improved syrup quality.

IS9-397

Alternatives to antibiotics: opportunities to prevent microbiome destruction and to repair /replace healthy microbiomes

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We don't really have alternatives to antibiotics in terms of treatment of established infection, but we do have alternative strategies to prevent infection, and possibly to prevent transmission of antibiotic resistant organisms. Eight decades of antimicrobial usage and six decades of rising rates of antibiotic resistance has created the crisis of untreatable antibiotic resistant organism (ARO) infections. Antibiotic over prescribing, poor appreciation of the adverse impacts of antimicrobials on the microbiome, and limited effectiveness of antibiotic stewardship and infection control allow the current situation to persist. The prompt arrest of multiply recurrent *Clostridioides difficile* infection (CDI) by fecal microbiota transplantation (FMT) demonstrates the power of a restored healthy microbiome. Current FMT clinical trials explore variables that could alter FMT outcomes (fresh, frozen, lyophilized, inoculum, microbial subcomponents, fecal components without microbes, durations of FMT and routes of administration) and provide some insights into mechanisms of protection. Based on clearance of AROs during FMT for CDI, exploration of FMT to suppress or eradicate intestinal carriage of AROs is being explored. Application of FMT in conditions associated with alterations in the microbiome including intestinal pathobiont dominance and host invasion as seen in bone marrow transplantation programs, inflammatory bowel diseases, Autism Spectrum Disorder and NAFLD are being considered. FMT is also being considered in situations where there is little appreciation of a dysbiotic microbiome, such as depression, schizophrenia, MS and Parkinson's disease. For CDI, in addition to antimicrobial treatment of a standard duration of 10-14 days and a follow up period to assess recurrence of CDI, early FMT at resolution of diarrhea, or even as an alternative to starting antibiotics is being considered since repair of the microbiome should prevent subsequent recurrences. Prevention of death from severe cdi in ICU patients is also being explored.

In addition to the use of current antibiotics that are primarily not eliminated via the gastrointestinal tract thereby reducing damage to the intestinal microbiome, enzymes that selectively destroy beta lactams or devices that sequester antimicrobials in the colon are undergoing clinical trials. Repairing microbial ecologic damage in a preventative fashion during or after antimicrobial therapy is hypothesized to prevent ARO transmission and fungal overgrowth. Achievement of a healthy microbiome, either by prevention of ecologic destruction, or repair by replacement appears to be a critical new approach to solving some of the current problems in modern medicine. As final additional benefit, should these strategies prove to be effective, the duration of clinical usefulness of antibiotics would be extend. To make this microbiome theme successful, collaboration between basic scientists, clinicians, industry, and health outcomes administrators is needed.

SS9-160

Use of bacteriophages in the biocontrol of Shiga toxigenic *Escherichia coli*

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Shiga toxigenic *Escherichia coli* (STEC) remains a leading cause of foodborne illnesses in Canada and elsewhere. Previously, we found that disease-causing STEC (i.e. serogroups O26, O45, O103, O111, O121 and O145, "top 6") readily produced biofilms on abiotic surfaces. Moreover, biofilm-forming STEC are tolerant to common sanitizers, providing an impetus to develop novel disinfection strategies. Bacteriophages (phages) have been recognized as alternative antimicrobial agents for controlling bacterial infections and decontaminating food. To evaluate the effectiveness of phage against the "top 6 STEC" in planktonic culture, we selected 12 phages with activity against O103 (P11), 2 phages against O26 (P3, P5), O45 (P13, P14), O111 (P9, P10) and O121 (P1, P2) and 3 phages against O145 (P7, P34, P36). Four strains of STEC per serogroup were treated with their respective phages at multiplicity of infection (0.1−10,000) at 37°C for 10 h in 96 well microplates. Optical density at 600 nm (OD_{600}) was measured at 2, 4, 6, 8 and 10 h. Overall, phages were effective ($P < 0.001$) at inhibiting STEC with an average OD_{600} value of 0.05 ± 0.07 across STEC strains, MOIs and times. To further evaluate phage effectiveness against biofilm, 6 intermediate or strong biofilm formers representing the "top 6" were selected to form biofilm on polystyrene at 22°C for 72 h. The 72-h biofilms were exposed to individual phages as described above at MOI of 0.1, 1, 10 at 37°C or 22°C for 3, 5, 10 and 24 h. Overall, phages were more effective ($P < 0.001$) in removing biofilm at 37°C than at 22°C. At 37°C, 60% of phage-treated biofilms were either eliminated or of weak strength ($OD_{590} < 0.2$) and only 14% remained of strong strength ($OD_{590} \geq 0.4$). In addition, phages were capable of initiating biofilm removal after 3 h and most biofilm mass was removed by 10 h ($P < 0.001$). Across STEC, phages as well as exposure temperature and time, phages at MOI = 10 were most efficacious. Among the phages used, highest virulence ($P < 0.001$) was found for O121 phages P1 and P2, O45 phage P14 and O111 phage P10. These phages either eliminated biofilms or reduced 64−88% of biofilms to a weak level. Taxonomically, P1 and P10 belong to *rV5virus,* P2 belong to *T4virus* and P14 belong to *T5virus.* Comparative genomic and proteomic analysis is now in progress to identify gene features that determine their lytic activity against the "top 6" STEC.

SS9-199

Use of human serum as a growth medium to identify novel antibacterial compounds for treatment of bloodstream infections

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Hospital-acquired infections caused by opportunistic bacterial pathogens are a significant contributor to patient death and a major healthcare burden. Coupled with an alarming increase in antibiotic resistance, and few new antibiotics in development, drug-resistant bacterial pathogens have quickly established themselves as a global threat to healthcare security. Bloodstream infections (BSI) caused by antibiotic resistant Gram-negative bacteria are a leading cause of mortality and can be challenging to treat. The bloodstream is a hostile environment for bacteria, containing a multitude of factors that can kill bacteria. Blood serum contains soluble components, such as complement, that can efficiently lyse susceptible bacteria, and bacteria must adapt to varied levels of nutrients and sequestered micronutrients in serum by synthesizing important metabolites *de novo* or evolving systems to acquire them from the host. We hypothesized that the mechanisms used by bacteria to survive in serum

represent novel targets for the development of new antibacterial drugs to treat BSI. Importantly, these targets are largely inaccessible to traditional antibacterial drug discovery programs because they are not essential for growth *in vitro*. We utilized a sequence-defined transposon library of *Klebsiella pneumoniae*, an important cause of BSI, to identify the factors required by this organism to grow in human serum. Using this data as a target list, we undertook a high-throughput screening campaign aimed at finding chemical inhibitors of these pathways that are essential for serum growth. This screen yielded several compounds that show enhanced activity in human serum, and significantly reduce the bacterial burden in a rat bloodstream infection model. Our data suggest this is a promising approach for developing new therapeutics against bacterial BSI.

SS9-287

Discovery of an active peptide from hospital biofilm by functional metagenomic screening

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Bacterial resistance to antibiotics is spreading faster than the introduction of new drugs into clinical practice, causing a public health crisis. We are now facing a rise in untreatable infections caused by multidrug-resistant bacteria, emphasizing the need to discover novel antibiotics. As soil microorganisms still constitute the most important source of antibiotics, and considering that the vast majority remains so far uncultured, functional metagenomic screenings provide access to genetic material representing a promising source of new molecules. In this study, total genomic DNA extracted from biofilms sampled in wastewater drainage pipes from hospitals were used to construct and express metagenomic BAC (Bacterial Artificial Chromosome) vector libraries in an *Escherichia coli* host. It was hypothesized that hostile environments favour competition between drug-resistant bacteria with the need to develop new ways to defend themselves against each other. A functional screening of 150,000 clones allowed the identification of ten clones exhibiting an inhibitory activity against a methicillin-resistant *Staphylococcus aureus* (MRSA) strain. Interestingly, among these actives clones, one *E. coli* clone presented a very slow growth phenotype. Analysis of its eDNA insert revealed a short 99 bp sequence identical to a conserved portion of a *Mycobacterium* sp. gene. A blast ORF finder analysis predicted two different peptides from this metagenomic DNA insert. Structure modeling, circular dichroism and nuclear magnetic resonance analyses confirmed that peptide-1 (derived from ORF1) likely adopts an α-helical conformation, a common structure among antibacterial peptides, whereas peptide-2 (derived from ORF2) is unstructured. Unexpectedly, that unstructured peptide-2 could inhibit the growth of MRSA, whereas no activity was observed for peptide-1. Peptide-2 could be classified as a novel member of the proline/arginine-rich group of AMPs because of its high content in these amino acids (nearly 20% each) and based on its low similarity with other known AMPs.

These results suggest that using eDNA isolated from an environment with high antimicrobial selective pressure to perform a functional metagenomic screenings may reveal new metabolites active against antibiotic-resistant bacteria.

SS9-310

The effect of intra-nasal delivered novel bacterial therapeutics on the respiratory microbiota of beef cattle entering the feedlot

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Control of bovine respiratory disease (BRD) primarily occurs through parenteral mass medication of cattle with antimicrobials upon arrival at the feedlot (i.e. metaphylaxis). However, a recent increase in antimicrobial-resistant BRD bacterial pathogens highlights the need for developing novel BRD control strategies such as intra-nasal administered bacterial therapeutics (BT). This study aimed to evaluate the effects of intra-nasal administered BT on the nasopharyngeal (NP) microbiota in calves entering a feedlot, and compared the efficacy of BT with a commonly used metaphylactic antimicrobial, tulathromycin. Sixty beef heifers (Initial BW=266 ± 13 kg) derived from a local auction market were randomly assigned to one of three treatment groups (n = 20) on d 0 (24h after feedlot arrival) and received either an intranasal cocktail of six *Lactobacillus* strains in equal concentrations (3×10^9 CFU; BT group) or intranasal saline (CTRL group), or a subcutaneous injection of tulathromycin (MP group). Nasopharyngeal swabs were collected (days -1, 0, 1, 2, 4, 7, 14, 28 and 42), and were processed for the assessment of NP microbiota using 16S rRNA gene sequencing and genus-specific quantification of *Lactobacillus* by RT-qPCR. Inoculation of BT increased both DNA copies and relative abundance of *Lactobacillus* on d 1 ($P < 0.01$). The structure of NP microbiota changed significantly following BT and MP administration with a significant difference in Beta diversity observed between treatment groups during post treatment period ($P < 0.05$). For BT heifers, phylogenic diversity and richness were lower compared to MP heifers but did not differ from CTRL heifers except for d 42. The genera *Psychrobacter* was significantly enriched within the 14 days post BT administration, while *Bifidobacterium* and *Histophilus* (except for d 14) remained lower in BT heifers during the course of study compared to MP and CTRL groups. MP heifers experienced an enrichment of *Mycoplasma* within the first 7 days post tulathromycin administration ($P < 0.05$). However, a similar increase in *Mycoplasma* was not detected in BT heifers. In contrast, *Pasteurella* and *Mannheimia* were lower in MP group, compared to both CTRL and BT groups which experienced an increase in these genera in the first 7 d. In conclusion, intranasal administration of BT upon arrival at the feedlot altered the composition of NP microbiota during the first 42 days on feed and could prevent the proliferation of BRD-associated *Histophilus* and *Mycoplasma* genera compared to CTRL and MP.

IS10-351

Unraveling the molecular mechanisms of plant-microbe interactions

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The interactions that take place between plants and microbes define a spectrum that spans beneficial to pathogenic symbioses, with plant-associated microorganisms having a unique potential to affect the growth and development of their hosts. In this talk, I will focus on two microorganisms that occupy opposite ends of this spectrum: phytoplasma, bacterial pathogens that employ an arsenal of secreted proteins to transform infected plants into better hosts for their insect vectors; and arbuscular mycorrhizal fungi, ubiquitous root-colonizing symbionts that form long-term and intimate partnerships with approximately 80% of all known terrestrial plants. Remarkably, arbuscular mycorrhizal fungi are themselves hosts to endosymbiotic bacteria, referred to as Mollicutes/Mycoplasma-related endobacteria (MRE), representing a fascinating case of an endosymbiosis within an endosymbiosis.

SS10-21

Molecular characterization of a novel cysteine protease inhibitor from the obligate biotrophic pathogen *Plasmodiophora brassicae* that targets papain-like cysteine proteases

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Clubroot caused by *Plasmodiophora brassicae*, is a serious disease of cultivated cruciferous crops worldwide, however, the pathogenicity of *P. brassicae* is not well understood. Through RNA seq and expression analysis an array of putative, small secreted *P. brassicae* proteins (SSPbPs) has been identified. SSPbP53, is a cystatin-like protein highly expressed by the pathogen in Arabidopsis roots 14, 21 and 28 days post inoculation (dpi) with resting spores, suggesting a potential role of SSPbP53 during secondary infection of a host plant required for clubroot characteristic root galls formation. Recombinant SSPbP53 inhibits activity of papain-like cysteine proteases (PLCPs), a group of proteases that control key processes of plant defense such as induction of cell death. In plants PLCPs are normally induced by increased levels of salicylic acid (SA) upon pathogen infection, however, during *P. brassicae* secondary infection, SA is converted to inactive methyl SA and we found that the expression of several Arabidopsis PLCPs is down regulated. We suggest that SSPbP53 is a key player in the still to be determined machinery used by *P. brassicae* to suppress and/or escape from plant defence mechanisms.

SS10-209

Resistance to two vinylglycine antibiotic analogs is conferred by inactivation of two separate amino acid transporters in *Erwinia amylovora*

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Fire blight is a destructive disease of apple and pear trees caused by the bacterial pathogen *Erwinia amylovora*. Several natural product antibiotics demonstrate antimicrobial activity against *E. amylovora*, including dapdiamides, herbicolins, and pantocins, as well as, the vinylgycine antibiotics 4-formylaminooxyvinylgycine (FVG) and aminoethoxyvinylglycine (AVG). *Pantoea* Natural Product 1, later demonstrated to be FVG, is an antibiotic effective against *E. amylovora* that is produced by the bacterium *Pantoea ananatis* BRT175. It was shown previously that FVG exposure results in the development of spontaneously resistant *E. amylovora* mutants within the zone of inhibition of BRT175. To determine the mechanism of resistance, genome variant analysis was conducted on spontaneously resistant mutants, which identified mutations in *ansP*, a gene encoding an L-asparagine permease. Heterologous *ansP* expression in *Escherichia coli* that are normally resistant to FVG resulted in susceptibility, suggesting that AnsP imports FVG. Interestingly, *ansP* mutants were not susceptible to AVG, which is structurally similar to FVG. Exposure of *E. amylovora* to AVG also results in spontaneously resistant mutant development. To determine the mechanism of resistance, mutants were subjected to genome variant analysis, and mutations in the arginine ABC transporter permease subunit gene, *artQ*, were found to be responsible for resistance development. Encoded within the BRT175 FVG biosynthetic gene cluster is a *lysE*-like transporter, which is suggested to export FVG from the cell. Heterologous expression of the BRT175 *lysE*-like transporter in *E. amylovora* conferred resistance to both AVG and FVG. Overall, this work highlights amino acid transporters and their role in antibiotic transport and suggests modified amino acids as potential sources of designer antibiotics.

SS10-368

Pesticide-driven genetic enrichment in prairie biobed systems

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Biobeds are used to contain and microbially degrade on-farm pesticide waste and runoff to reduce environmental pesticide pollution. While these systems show great efficiency, how the community and genetic profiles shift after extensive pesticide exposure remains largely unknown; a greater understanding of the microbial community dynamics in biobed systems could aid researchers in proper biobed management and maintenance. This study implemented metagenomic and metatranscriptomic techniques to characterize the microbial community in a Canadian prairie biobed system before and after pesticide application. The subsequent data analysis identified an enriched community of xenobiotic degrading microbes, such as *Sphingopyxis* and *Pseudomonas*, as well as genes involved in aromatic xenobiotic degradation. However, results also revealed an enrichment for genes that encode resistance to quinolones, aminoglycosides, and beta-lactams, indicating that biobed systems may create environments that select for particular classes of antibiotic resistance genes.

SS10-377

Isolation of a preliminary minimal N2-fixing symbiotic gene set of the pSymB megaplasmid in the host legume symbiont Sinorhizobium meliloti

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Sinorhizobium meliloti is an important model organism for the understanding of the ecologically and agriculturally important N2-fixing symbiosis it forms with legumes. Its second megaplasmid, pSymB, contains several clusters of genes thought to necessary for symbiotic nitrogen fixation (SNF). The recent creation of an appropriate ΔpSymB background strain enabled a gain-of-function approach to examine the sufficiency of three previously identified critical regions on pSymB (representing 12% of the megaplasmid) for restoring SNF capacity. This work entailed the construction of plasmids that enabled Cre-loxP-mediated deletion of a large 325 kb region on pSymB that brought the three indispensable SNF regions on pSymB adjacent to one another. Subsequent work utilized plasmids harbouring FRT sites that were employed in concert with Flp recombinase to excise the three SNF regions as a large 205 kb plasmid that was capable of capture by *Escherichia coli*. The ΦC31 integrase-*attP*/*attB* site-specific recombination system was then harnessed to transfer the three captured pSymB SNF regions back into the ΔpSymB background strain. This strain represented a preliminary minimal SNF gene set for pSymB in *S. meliloti,* and the structure of the recombinant was confirmed with whole genome sequencing. It displayed an observable reduction in the production of the symbiotically relevant exopolysaccharide succinoglycan. This occurred despite the three regions encompassing the characterized *exo* cluster of succinoglycan biosynthesis genes. The symbiotic phenotype of the strain with *Medicago sativa* (alfalfa) was also severely impacted, manifesting as a sharp decrease in shoot dry weight and a reduction in nitrogenase activity within the root nodule. Methods for elucidating the absent genes responsible for these observed phenotypes are explored in conjunction with strategies for ascertaining and constructing a minimal N2-fixing pSymB.

IS11-406

A happy accident: microbial volatiles for plant defense

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A parasitic fungal contaminant that was inhibiting soybean germination was isolated in the laboratory. To search for an effective biocontrol against this fungus, we used pairwise assays to screen microbial isolates from grape vines for activity against the parasite. We identified multiple strains that demonstrated contact-dependent inhibition (i.e. inhibition through secondary metabolites released into the agar). We also identified several strains that demonstrated contact-independent or gas phase inhibition. This presentation will describe our findings and our characterization of the gas phase active inhibitors using gas chromatography. Future directions regarding further plant pathogen testing and application of these active strains as biocontrols will be discussed.

SS11-36

Induction of prophages of human gut bacteria by xenobiotics

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Bacteriophages are major contributors to the human gut microbiota: they are found in similar abundance to their bacterial hosts and contribute up to 17% of the human fecal metagenome. In the gut, bacteriophages are commonly found in bacterial genomes as prophages. Prophages are ecologically important as they can modulate their bacterial host's phenotype. Prophages are not an evolutionary dead-end for bacteriophages, as they can switch back to lytic replication through induction. Induction is typically caused by a stress response of the bacterial host to either environmental or cellular factors.

It is currently unknown what gut-specific environmental conditions might trigger prophage induction. We are investigating the inducing potential of xenobiotics in the human gut, as our previous work found that they could up-regulate prophage induction genes in this system. **Hypothesis:** Xenobiotics induce prophages leading to detectable changes in bacterial and phage communities in the gut.

We are tested several xenobiotics distributed over different drug classes: antibiotics, anticancer, and non-steroidal anti-inflammatory drugs. We screened these xenobiotics against multiple human gut bacterial isolates belonging to the major phyla of the human gut (Actinobacteria, Bacteroidetes, Firmicutes, and Proteobacteria) and show that induction is xenobiotic and strain-dependent. We identify many xenobiotics capable of inducing prophages, including non-steroidal anti-inflammatory drugs (Tolmetin and Diclofenac). As regulators of bacterial communities, bacteriophages are likely being influenced by regular xenobiotic exposure in the human gut.

SS11-77

Biodegradation of 1-adamantanecarboxylic acid by microorganisms originating from Alberta's oil sands surface mining operations

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Millions of liters of tailings water are produced from oil sands mining operations each year. Reclamation of tailings ponds is now a legislated, required process within 10 years after the end of an oil sands mining operation. To reclaim these waste ponds back to a natural landscape, toxic compounds must first be removed, including naphthenic acids (NAs), which are the primary toxic components. Biodegradation offers a cheap and potentially effective solution when treating millions of liters of tailings. Previous biodegradation research has used simple model NAs which while insightful, do not always accurately reflect the complexity and variability of NAs found in tailings ponds. Thus, there is great value in assessing the degradability of more structurally complex NAs, so a study was conducted that focused on the diamondoid NA 1-adamantanecarboxylic acid (ADCA). This NA has rarely been observed to be degradable, is extremely recalcitrant and represents NAs actually found in tailings. Both indigenous algae and bacteria have previously been investigated for their biodegradation abilities. Herein, we investigated the degradation ability of aerobic bacteria alone and compared it to algal-bacterial communities to determine if the addition of algae enhances the biodegradation of ADCA. The algal-bacterial communities used in this study originated from Alberta's tailings ponds, and the bacteria were isolated from within these enrichments. Over 90 days, the communities were able to degrade ADCA to varying degrees. Two algal groups that were found to be highly abundant in these communities were from the order Chlorellales and the genus *Acutodesmus*. These taxa have previously been used to degrade components of crude oil and therefore may enhance the degradation of ADCA. The ability of these tailings-derived organisms to degrade this complex model NA suggests they may be capable of degrading compounds of similar complexity and molecular weight, making them excellent candidates to help bioremediate oil sands wastewater.

SS11-273

Novel insights into the role of pyocins in interstrain competition

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Pseudomonas aeruginosa is a bacterium that is responsible for up to 40% of respiratory infections in Canadian cystic fibrosis patients and significantly impairs their lung function. Repeated bacterial infections eventually cause the patients to require a lung transplant or succumb to respiratory failure. *P. aeruginosa* strain replacement has been associated with worsening lung function. However, even though many mechanisms of bacterial competition have been described their role in cystic fibrosis lung infections are unknown. Our hypothesis is that pyocins may play a role in these interstrain and interspecies interactions. Further, that both killing by pyocins and regulatory interactions may play a role in these competitive events.

Pyocins are toxic proteins released by *P. aeruginosa* to target closely related strains. There are three types, R-type, F-type, and S-type. Our lab has reason to believe pyocins may play an important role in interstrain competition. Through PCR screening, we recently found that isolates of the prairie epidemic strain, a transmissible strain capable of displacing a patient's colonizing strain, lacks R-type pyocins. This suggests that the presence of pyocins may play a role in a strain's ability to displace another. Furthermore, previous research in our lab looked at co-infections of *P. aeruginosa* isolates and found an isolate that was able to attenuate the

virulence of other isolates. In the presence of this isolate, the other isolates showed decreased swarming, protease production, and siderophore production. The compound responsible for this interaction is believed to be an S-type pyocin. The decrease in virulence factor production is thought to be due to the inhibitory abilities of the secreted pyocin. However, given the results of another lab who found that subinhibitory levels of pyocin increased biofilm formation, we would like to examine if pyocins may have effects on other virulence factor production in addition to biofilm formation. It may be that subinhibitory concentrations of the pyocin are having an effect on the virulence factor production of the other isolates. Our lab has cloned and purified three S-type pyocins, S2, S4, and S5 from the genome of the laboratory strain of *P. aeruginosa* PAO1. To date, we have used an overlay inhibition assay to determine the inhibitory ability of pyocin S2 against 27 isolates from cystic fibrosis patients. Out of these isolates, 8 showed to be inhibited by pyocin S2. Interestingly, in the overlay inhibition assay, 2 of the 8 inhibited strains appear to show increased growth at subinhibitory concentrations of pyocin S2. We are currently working to determine the effect of the subinhibitory concentrations of pyocin S2 on the virulence factor production of these 8 strains. Strain replacement leads to increased deterioration of cystic fibrosis lungs and the results of our lab show evidence that pyocins may play a key role in interstrain interactions.

SS11-293

Peptidoglycomics: Using metabolomic techniques to examine the composition of peptidoglycan of *Pseudomonas aeruginosa* biofilms

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Pseudomonas aeruginosa is an important Gram-negative pathogen that causes significant morbidity and mortality in immune compromised individuals. The transition of *P. aeruginosa* infections from acute to chronic is associated with a significant decline in prognosis for infected patients. Chronic infections are often associated with the ability of *P. aeruginosa* to grow as an aggregation of bacteria, referred to as a biofilm. Treating chronic infections caused by biofilms is challenging due to their enhanced resistance to antibiotics. β-lactam antibiotics, which are often used to treat *P. aeruginosa*, are bacteriocidal and disrupt the production of the structural component of the bacterial cell wall known as peptidoglycan (PG). In Gram-negative bacteria the PG is located as a thin layer in the periplasm between the inner and outer membranes. PG is composed of a heteropolymer of β-1,4-linked *N*-acetylglycosamine (NAG) and *N*-acetylmuramic acid (NAM) that are crosslinked via short peptides. Variations to either the glycan strand or the peptide crosslinks can differ between different phases of growth or growth conditions and differ considerably between species. Also, modifications to the PG structure have been shown to provide resistance to antimicrobial treatment. To investigate changes in PG composition we applied mass spectrometry and bioinformatic techniques to examine the peptidoglycan composition in *P. aeruginosa* PAO1 biofilms. In total, we identified 157 component muropeptides, which is nearly double the muropeptides previously identified for Gram-negative bacteria. The PG from biofilm-cultured bacteria was shown to be compositionally distinct from motile (planktonic)-derived PG. The modifications showing the greatest difference between biofilm and planktonic-derived PG consisted of amino acid substitutions to the glycan-linked peptide, de-*N*-acetylation of the glycan chain and amidase activity. To our knowledge, this is the most detailed examination of PG composition and the changes that occur in *P. aeruginosa* biofilms. Results from this study sheds light on putative mechanisms behind the heightened antimicrobial resistance of bacterial biofilms.

IS12-395

The role of extracytoplasmic function (ECF) sigma factors across the bacterial domain

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Bacteria use sigma factors to recognize different promoters and partition the transcriptional space. Extracytoplasmic function (ECF) sigma factors are the largest and most diverse family of sigma factors, with up to 100 ECF sigma factors in a single bacterial genome. While many ECF sigma factors carry out stress responses, they have also been implicated in virulence, differentiation, and other processes. Unlike housekeeping sigma factors, ECF sigma factors show high promoter specificity and recognize relatively smaller regulons. Although the promoter specificity and regulons of a few ECF sigma factors, mostly in model organisms, have been determined, the vast majority of ECF sigma factors remain uncharacterized.

Taking advantage of the autoregulatory nature of many ECF sigma factors as a means of promoter discovery, we generated an aligned collection of ~50,000 ECF sigma factor sequences and their putative auto-regulatory promoter motifs. We used this collection to discover specific amino acid – nucleotide interactions that are important for promoter recognition in ECF sigma factors. Based on this data, we individually predicted the promoter specificity of ~100,000 ECF sigma factors across >20,000 bacterial species. We scanned these generated motifs across the relevant genome, and determined statistically significant targets (i.e., regulons) for a majority of ECF sigma factors using a newly developed phylogenetic footprinting method. Analyzing this vast collection of ECF sigma factor regulons, we find that unlike paradigmatic ECFs such as RpoE, most ECFs regulate only a few promoters located in close genomic proximity to the ECF itself. We show that these small regulons are often associated with genes of unknown function, and, in certain bacterial clades, are frequently horizontally acquired. Additionally, we identify ECFs with large regulons as candidates for further study. This work is the first comprehensive look at the function of a transcriptional regulator family in bacteria, and reveals important organizing principles of bacterial regulatory network evolution.

SS12-11

A systems biology approach to interrogating the metabolism of symbiotic nitrogen fixation

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The rhizobia are a polyphyletic group of soil-dwelling proteobacteria notable for their ability to enter into endosymbiotic relationships with leguminous plants. During this interaction, the rhizobia are located within a plant organ known as a nodule, where they convert atmospheric dinitrogen gas into ammonium for use by the plant in exchange for carbon. This process is known as symbiotic nitrogen fixation, and it is well-studied due to its tremendous ecological and agricultural significance. As a model system, we work with *Sinorhizobium meliloti*, a symbiont of legumes such as *Medicago sativa* (alfalfa) and *Medicago truncatula* (barrel medic). We are

particularly interested in developing a systems-biology perspective of its metabolism in the free-living and symbiotic states.

Here, transposon-sequencing was used to uncover all *S. meliloti* genes required for growth in free-living, laboratory conditions. This approach identified nearly 500 genes necessary for biomass production independent of growth condition or genetic background. These data were integrated with our previously constructed *S. meliloti* genome-scale metabolic network, facilitating significant refinements of the reaction content and gene-reaction pairings. This process simultaneously facilitated the functional annotation of previously uncharacterized genes. The updated model encompasses 1348 genes, 1408 reactions, and 1160 metabolites. This model correctly predicts biomass production when individually provided with 76 of 85 carbon substrates known to support the growth of S. meliloti, and it provides highly accurate predictions of essential metabolic genes.

To study the metabolism underlying symbiotic nitrogen fixation, we developed a custom workflow to integrate our *S. meliloti* metabolic model with an existing *M. truncatula* metabolic model, thereby producing a holistic representation of the metabolism of a nodulated legume. This Virtual Nodule Environment (ViNE) contains plant shoot and root tissues together with a nodule subdivided into five distinct developmental zones. In total, ViNE embeds 7285 compartmentalized reactions and 2136 genes. A combination of flux balance analysis and dynamic flux balance analysis was used to interrogate the metabolic properties of the *in silico* nodule. We observed clear differences in the metabolism of each nodule zone, as well as zone-specific patterns of gene essentiality. We have also examined the use of dicarboxylates as a carbon source by *S. meliloti* during nitrogen fixation, the effects of oxygen concentration on the rate of nitrogen fixation, and the trade-offs between nitrogen fixation and plant growth.

We expect ViNE will serve as a powerful platform for hypothesis generation and guiding attempts at engineering symbiotic nitrogen fixation. In future work, we intend to use experimental genetic approaches to begin validating the *in silico* predictions. We also hope to understand the regulatory mechanisms modulating *S. meliloti* metabolism in free-living and symbiotic states using a variety of experimental and computational methods.

SS12-17

A phage protein impedes bacterial resistance to phage infection

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The unequalled abundance and diversity of bacterial viruses (phages) partly explain why so many of the deduced proteins encoded by phage genomes have no known function and no homologue in public databases. While structural proteins are found in the virion particles, non-structural phage proteins are produced inside the bacterial host where it is presumed that they play a role in hijacking the cellular machinery for viral production. Virulent lactococcal phages belonging to the *Skunavirus* genus (*Siphoviridae* family) are by far the most endemic and problematic in the dairy industry worldwide. Phage p2 is a model for this viral genus and it infects *Lactococcus lactis* MG1363, the international reference strain for lactococcal research. Phage p2 structural proteins have been analyzed in great details, but most of its non-structural proteins are still uncharacterized. To study these proteins, a multidisciplinary approach is required.

Here, we made use of structural biology, genomics, physiology, and proteomics to provide insights into the function of phage p2 protein ORF47, the most conserved non-structural protein of unknown function among members of the *Skunavirus* genus. We solved the protein structure through circular dichroism and nuclear magnetic resonance. We knocked out *orf47* from phage p2 genome and confirmed gene disruption in a recombinant phage (p2Δ47) by whole genome sequencing. The lack of ORF47 did not affect the duration of the phage lytic cycle, but the number of infective particles released per infected bacterium was significantly lower

with p2Δ47 yielding a burst size of 80 ± 7 in comparison to 129 ± 17 for p2. Moreover, we made use of label-free quantitative proteomics to compare the proteotypes of *L. lactis* MG1363 infected by phage p2Δ47 or phage p2. Infection by p2 resulted in an increase of most metabolic pathways while a global decrease was observed during the infection by p2Δ47. Our data showed that ORF47 is inducing the expression of many genes that are not functionally related and that without this protein, the phage induces a dormancy-like state in the bacterial host. Most interestingly, we found that ORF47 hinders *L. lactis* MG1363 resistance to phage infection.

SS12-72

"Effect" the unexpected: metaeffectors in *Legionella pneumophila* and beyond

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A central pillar of molecular pathogenesis is that translocated bacterial effectors modulate host proteins to remodel the host cell and escape immunological defenses. We have recently uncovered a functionally heretical class of translocated proteins in *Legionella pneumophila* that adds a new dimension to our understanding of host-pathogen interactions. Through complementary systems biology approaches, we have identified several "metaeffectors" (or "effectors of effectors") that target other effectors rather than the host. This discovery reveals a critical gap in our understanding of how effector function is regulated during infection and opens up several exciting new avenues of study. We propose that, as built-in effector killswitches, metaeffectors will betray critical nodes of effector activity while also exposing ways to perturb them. Identifying metaeffectors is also of immediate practical importance as these proteins are a potential trap to the unsuspecting researcher using standard biochemical and molecular approaches in an attempt to identify host-pathgen interactions. In addition to our findings on *Legionella pneumophila* metaeffectors, we will describe how this pathogen, with its unmatched arsenal of over 300 Dot/Icm translocated proteins, serves as an excellent training ground for metaeffector discovery across a diverse set of intracellular pathogens.

SS12-98

Genome-scale metabolic modelling reveals key features of a minimal gene set for *Mesoplasma florum*

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Studying minimal cells could lead to the description of every molecular function required for autonomous cellular life. In turn, this exhaustive knowledge could facilitate the establishment of fundamental constraints to which cellular systems are submitted. Recently, a genome-scale model of metabolism (GEM) was reconstructed for the synthetic minimal organism JCVI-Syn3.0, a robust working approximation of a minimal cell. The parent of JCVI-Syn3.0, *Mycoplasma* mycoides, is phylogenetically close to the fast-growing, non-pathogenic and near minimal bacteria *Mesoplasma florum*. Here we reconstructed a GEM for *M. florum* and constrained it with experimental data. The set of molecular functions in *M. florum* was extracted using three different computational approaches. Firstly, the structure of 386 *M. florum* proteins were reconstructed. Secondly, enzyme commission numbers (EC) were compared between the sequence-based identification from DETECT-v2 and the structure-based identification from COFACTOR. 117 genes were associated to an EC number with a high-level of

confidence. Fianlly, the *M. florum* proteome was compared to that of 4 other species of mollicutes for which a GEM was available, including JCVI-Syn3.0. 411 proteins were conserved between *M. florum* and JCVI-Syn3.0, with ~90% (139/155) of the JCVI-Syn3.0 model proteins conserved in *M. florum*. The metabolic network of *M. florum* was then reconstructed that contains 209 genes. Dividing the network in 6 functional modules revealed that no JCVI-Syn3.0 homologs were found in the “Glycans” module, and the “Lipids” module only shared half of its gene content. The genes in these modules were also associated with fewer EC numbers and homology models. Integrating the experimental characterization of a semi-defined growth media with the use of flux-balance analysis allowed comparing growth phenotypes on 14 different carbon sources. While the model correctly predicted the growth phenotype on 11/14 sources, maltose, glycerol-3-phosphate and ribose were incorrectly predicted. Reviewing the discrepancies between observation and predictions will provide a basis to re-annotate the *M. florum* genome.

Section Symposium Abstracts
Thursday, June 13th, from 1:30 - 3:00 PM

Infection & Immunity

SSII22

Dynamic proteomic profiling of the Salmonella-host interplay reveals new modes of action for known and novel virulence factors

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Intracellular bacterial pathogens cause a diverse array of diseases in humans and represent a significant threat to global health. These pathogens have evolved sophisticated strategies including the secretion of virulence factors to interfere with host cell functions and to perturb immune responses. However, interplay between the host and pathogen at the protein level in the context of infection has not been systematically investigated. Our 'infectome' analysis aims to identify previously undescribed proteins involved in bacterial virulence and host immune defense, representing an opportunity to elucidate molecular mechanisms of host-pathogen interplay during disease. Here, we investigate the host-pathogen interplay between the pathogenic bacteria, *Salmonella enterica* serovar Typhimurium, and primary macrophages. We performed quantitative proteomics of the host cells infected with wild-type (SL1344) or the type 3 secretion system (T3SS) mutant strains (D*spi-1* and D*spi-2*) in single runs using high resolution mass spectrometry on a Quadrupole Orbitrap instrument. Our results provide a comprehensive and dynamic view of both pathogen and host proteins during infection. In the host cells, we observed the upregulation of proinflammatory and lysosomal proteins, representing host defense mechanisms to initiate immune responses and combat bacterial invasion. For *S.* Typhimurium, integration of proteome and infectome data identified eight proteins not encoded on SPI-1 or SPI-2 as being co-regulated with known virulence factors, suggesting a co-functional role in virulence and infection. Additionally, murine model competitive index assays revealed virulence-associated phenotypes of five proteins and defined their roles in bacterial cell regulation, as well as their impact on the host proteome. Overall, we provide an innovative strategy for profiling infection from dual perspectives in a single assay and characterizing novel virulence factors.

SSII29

Despite antagonistic activities in vitro, *Pseudomonas aeruginosa* enhances *Staphylococcus aureus* colonization in a murine lung infection model

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Background *S. aureus* (SA) and *P. aeruginosa* (PA) are the most prevalent lung pathogens in cystic fibrosis (CF) and are frequently co-isolated. Their co-infection is associated with a worse clinical outcome, as noted by

decreased lung functions and frequent pulmonary exacerbations. However, despite their co-occurrence, PA and SA prototypic strains exhibit antagonism *in vitro*: PA reduces SA growth and induces the small-colony variant (SCV) phenotype. Therefore, we attempted to better understand the apparent conflict between the *in vitro* observations and the high SA-PA co-occurrence in CF. We previously described clinical SA-PA co-isolates not displaying such an antagonism. The present study compares the colonization of various strains, including reference, clinical co-isolates or virulence-attenuated mutants, in a murine co-infection model.

Methods Growth kinetics were followed for co-cultures of five SA-PA clinical pairs isolated from adult CF patients. Selective plates were used to determine viable counts for each species. In other experiments, SA was spread over an agar plate where a PA spot was applied in the centre to visualize SA SCV formation around PA. *In vivo* interactions were characterized using a mouse lung infection model. Intra-tracheal inoculations of SA, PA or SA-PA pairs were performed, and infections developed for 24h. Lung homogenates were plated on selective media allowing CFU counts of either SA or PA. Inflammation was assessed by myeloperoxidase (MPO) quantification and expression of two known receptors for cellular adhesion of SA, ICAM-1 and ITGA-5, were measured by RT-qPCR.

Results Growth kinetics showed that some PA antagonized their SA co-isolate with a drop of ~3.6 $\log_{10}$ CFU/mL initiated after 8h of co-culture. However, other pairs did not interact negatively showing equivalent growth for SA in mono- and co-cultures. The agar co-culture model revealed the formation of SA SCVs in pairs where PA antagonized SA growth kinetics, while some non-antagonistic pairs did not result in the formation of SCVs. Paradoxically, in the infection model, SA colonization was significantly higher in SA-PA co-infections, even for pairs showing antagonism *in vitro*. In fact, SA colonization was most enhanced in co-infections with antagonistic PA. The SA colonization increased up to 2.00 $\log_{10}$ in co-infections compared to their respective SA mono-infections ($P < 0.05$). PA did not benefit from the co-infection showing equivalent colonization of lung tissues in presence or absence of SA ($P > 0.05$). Upon compiling all results from 200 co-infections (35 PA and 10 SA strains, including clinical, reference and mutant strains), SA colonization was found to be proportional to PA colonization. Different virulence-attenuated mutants for both species were evaluated in the co-infection model, but no virulence or regulatory genes could specifically be associated to this phenomenon. The level of inflammation was measured via MPO quantification but was not correlated to the promotion of SA colonization. However, RT-qPCR of ICAM-1 and ITGA-5 showed that PA significantly increased their expression, both in mono- or co-infections with SA.

Conclusion The observation that lung colonization by SA is improved in presence of PA may explain the frequent co-infections by these pathogens in CF and may contribute to their combined detrimental effect on patient health. The more effective SA colonization in presence of PA could involve an increase of its cell surface receptors by PA.

SSII34

Using the intestinal microbiome in the prediction of cancerous colonic lesions

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Colorectal cancer (CRC) is one of the main causes of cancer related mortality both in Canada and worldwide. CRC development starts with the formation of precancerous polyps in the colon that eventually develop into metastatic and invasive cancerous tumors, worsening the prognostic of patients. This is why early detection and removal of polyps are critical to patients' survival since they prevent the development of CRC. In Québec, a province-wide effort exists for early detection of CRC by using the Immunochemical Fecal Occult Blood Test (iFOBT). The detection of blood in the stool via the iFOBT is indicative of the presence of polyps and/or tumors

in the colon, which is confirmed by colonoscopy. However, in about 40% of blood-positive samples, no CRC-related pathology can be found (false-positive) by colonoscopy. This high level of false-positive results associated with iFOBT is worrisome since they strongly impact the patient's life with prolonged stress and discomfort as well as costly and invasive medical procedures. The Clinical Biochemistry Department at the CHUS is responsible for the iFOBT screening in Québec and tests more than 10 000 samples per week. The importance of the microbiome in the development of diseases such as CRC is undeniable but it remains mostly unused as a marker of disease progression. Having access to samples from the CRC screening provides a unique opportunity to study the microbiome composition linked to the development of CRC. Therefore, we investigated microbiome signatures in patients with various degrees of intestinal pathologies. To do so, bacterial DNA was isolated from stools remaining from the iFOBT. A PCR is performed to amplify the fourth variable region (V4) of the 16S ribosomal RNA's gene, which is then sequenced. This V4 region is flanked by regions highly conserved in all bacteria, which enables the amplification of the region using a single pair of oligonucleotides. We identified both bacterial species as well as their abundance using this sequencing in our samples. We sequenced and compared the microbiome of 720 patients (270 with negative iFOBT results, 171 with positive iFOBT results but negative colonoscopy and 279 with both a positive iFOBT and either precancerous polyps of CRC detected via a colonoscopy). These comparisons enabled us to identify various bacterial genera whose abundance vary between conditions. Furthermore, we created a supervised machine learning based predictive model using the microbiome data that is able to predict the presence of polyps or lesions in patients with positive iFOBT results. Our current predictions reach a accuracy of 82% which means we reduce the false-positive levels by about 50%, the equivalent of 3500 to 4000 patients annually in Quebec, without affecting the tests sensitivity. We will continue to refine our predictive model to show that the microbiome can be used to improve results from iFOBT and reduce the false-positive rate currently associated with it.

SSII65

Inhibition of Cagα, a T4SS ATPase required for *Helicobacter pylori* virulence

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Helicobacter pylori is a bacterium that colonizes half of the human population. It carries a pathogenicity island *cag* (cytotoxin-associated gene pathogenicity island : *cag*PAI) encoding 27-31 Cag proteins that assemble into a syringe-like apparatus, the T4SS (type 4 secretion system), consisting of a membrane-spanning secretion channel and an extracellular pilus. The T4SS allows the injection of CagA (cytotoxin-associated gene A), an oncoprotein, directly into gastric cells. CagA interacts with a large number of host cell proteins. This results in cytoskeleton rearrangements and in deregulation of several signal transduction pathways, highly increasing the risk of developing gastric disease like ulcer and gastric cancer. Stomach cancer is the sixth most common cancer (1.03 million cases) and the third most deadly one (WHO 2018). To reduce cancer risk, the eradication of pathogenic *H. pylori* with antibiotic treatment is indicated. However, *H. pylori* can easily be transformed and acquires antibiotic resistance. In 2017 the WHO published a report explaining the urgency of finding new antibiotics for several bacteria including *H. pylori*.

We study potential anti-virulence molecules that targeted specific proteins in the bacteria, such as inhibitors of the T4SS ATPase Caga. A fragment library was screened by differential scanning fluorimetry (DSF), and 16 stabilizing fragments were identified. A malachite green ATPase test and crosslinking assays were performed to assess the effects of these 16 fragments on Caga. Four of the sixteen fragments inhibited the ATPase activity and molecule 1G2 had the lowest inhibition constant. X-ray crystallography showed that 1G2 does not interact with Caga at the ATP binding site and it appears to disrupt the hexamer form of the protein. 1G2 and some of its derivates are not toxic for *H. pylori* and the molecules reduced IL-8 formation in AGS cells. 1G2 was also tested

on *H. pylori* clinicals strains. 1G2 decreased the induction of IL-8 in most of the strains and one of them is resistant. Our results show the efficiency of fragment-based screening to identified new chemical entities that bind Cagα and will enable the design of more potent small molecules to inhibit *H. pylori* virulence.

SSII171

Bacteriophages modulate ulcerative colitis-derived bacterial communities

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The human gut microbiota is a complex and diverse collection of microorganisms, comprised of viruses, bacteria, archaea, and eukarya. These organisms have a profound impact on human immune system function, pathogen protection, metabolism, and physiology. Inflammatory bowel diseases (IBD), which include Crohn's disease (CD) and ulcerative colitis (UC), are thought to arise from an inappropriate interaction between the immune system and the gut microbiota. It is well characterized that there is a shift in the gut bacterial community during IBD. However, this picture remains incomplete, as bacteriophages (phages), which occupy the gut at similar abundances to bacteria, are overlooked in most studies. Phages have been shown in several ecosystems to be powerful modulators of bacterial communities. In the context of IBD, the phage-bacteria interactions in the gut remain poorly understood. We propose that phages alter the abundance and diversity of UC-derived bacterial communities, similar to other systems. In order to address this hypothesis, we isolated bacteria from UC patient fecal samples and administered these to germ-free mice. Following colonization of bacteria, we administered phages derived from healthy or UC patient fecal samples in multiple doses and then induced colitis in these mice using dextran sodium-sulfate (DSS). We collected mouse fecal samples during the phage and DSS exposures to determine bacterial and viral abundances, as well as bacterial diversity with 16S rRNA gene sequencing. Our data indicate that, in comparison to the no phage control, the virus-to-bacteria ratio was elevated in mice given the phage treatments. These changes were maintained after the administration of DSS, suggesting that both healthy and UC-derived phages are infectious during the induced colitis period. In support of these findings, analysis of 16S sequencing revealed differences in compositional dissimilarity between bacterial communities given healthy or UC-derived phages relative to the no phage control. Furthermore, in mice given UC-phages, there was a 15% decrease in the phyla Bacteroidetes, which was maintained during the induced colitis period. Together, our data suggest that phages in the gastrointestinal tract can modulate whole communities of UC-derived bacteria. By understanding how phages modulate their host communities in the context of IBD, our data may guide therapeutic interventions focused on using phages to manipulate the gut microbiota.

SSII175

New exosome-based biomarkers for the rapid diagnosis of drug-resistant parasites

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Leishmaniasis is a complex of zoonotic vector-borne diseases transmitted by sand flies in more than 80 countries. Infected dogs develop the disease and serve as a major reservoir of transmission for humans. Leishmaniasis is a neglected disease so the resources available to eradicate it are limited. In the absence of an effective vaccine, leishmaniasis control is mainly based on chemotherapy, but with a limited number of licensed molecules available. Therefore, the same drugs are used to treat dogs and humans, which has stimulated the emergence

and propagation of drug-resistant (DR) strains, a global veterinary and public health problem. There is an urgent need for rapid and accurate methods to diagnose the disease, as well as the drug-resistant nature of certain populations in order to propose appropriate and more effective treatments. Recent studies in *Leishmania* have shown the occurrence of extracellular vesicles, so-called exosomes, and their diverse roles in intercellular communication. We have characterized leishmanial exosomes to identify signature patterns, which could be implemented as biomarkers for the rapid diagnosis of resistant parasite strains. To this end, we have analyzed different *L. infantum* strains resistant to either antimonial drugs or miltefosine. Exosomes were isolated and characterized in terms of size and morphology, and analyzed their protein content. Briefly, exosomes were isolated by ultracentrifugation and exclusion chromatography. Size, numbers and morphology were determined by nanoparticle-tracking analysis and TEM. Specific protein signatures were determined by LC-MS/MS analysis and some confirmed by Western blot. Our first results revealed the enrichment of unique proteins in the exosomes of DR parasites; a key point that could help to better understand antimicrobial resistance and their use in the development of new biomarkers for rapid diagnosis DR parasites. This project is supported by CIHR and NSERC grants to MO and CFP.

SSAEM59

Metagenomic analysis of virus diversity and relative abundance in a freshwater harbour

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Shotgun metagenomics has rapidly expanded DNA sequence databases and led to ground-breaking advancements in many areas of viral ecology. While marine viruses have been extensively studied over the past two decades, less research has been conducted on viruses in freshwater environments. The goal of our research was to characterize virus communities and capture fluctuations in the communities at nearshore and mid-harbour locations in a freshwater harbour over the mid-summer to early autumn. Water samples were filtered through 0.22 µm pore-size filters, and DNA was extracted and sequenced on an Illumina HiSeq 2500 platform. The resulting 250-bp reads were subjected to quality control and assembled into contigs. Contig alignment and annotation was achieved via BLASTx searches against the NCBI-nr database and relative abundances were obtained by mapping reads back to contigs. Assembled contigs were annotated as belonging to the virus groups *Caudovirales, Mimiviridae, Phycodnaviridae*, virophages (*Lavidaviridae*), unclassified bacteriophages, other dsDNA viruses, and ssDNA viruses. *Caudovirales* infect bacteria and typically comprise 10-40% of aquatic virus communities. *Mimiviridae* (including the so-called 'extended *Mimiviridae*') have been isolated primarily from aquatic environments and are a diverse group of dsDNA viruses that infect protists and eukaryotic algae. Until recently, *Phycodnaviridae* were considered the dominant group of algae-infecting viruses since all known dsDNA viruses of eukaryotic algae belonged to this family, but the recent expansion of the *Mimiviridae* has challenged that notion. Virophages are small viruses that co-infect eukaryotic hosts with some *Mimiviridae* and benefit their hosts by diminishing *Mimiviridae* propagation through the production of inactive *Mimiviridae* virions. The mid-harbour site displayed wide fluctuations in virus relative abundance, while the nearshore site was relatively stable. Contigs annotated as virophages were often the most abundant members of the community, especially at the nearshore site. At the mid-harbour site, virophages exhibited a wide range of abundance from >70% of the community to nearly undetectable. Virophages originally detected in Dishui Lake, China were consistently the most abundant virophage annotations. *Mimiviridae* were the second most abundant family, comprising an average of 21% of the virus community across all samples. Interestingly, though they are presumably intimately associated, *Mimiviridae* abundances did not fluctuate to the same extent as the virophages. Diverse *Mimiviridae* contigs were detected in the samples, and the nearshore and mid-harbour sites appeared to host distinct *Mimiviridae* communities. *Phycodnaviridae* were a minor component of the virus community regardless of site or sampling date, suggesting that *Mimiviridae* were the dominant algae-infecting viruses in this system. In contrast to other studies of eutrophic freshwater lakes, *Caudovirales* were highly abundant in only 2 of 10 samples. Our results provide insights into virus community composition in eutrophic freshwaters, highlighting the complexity of virus ecology. They also expand the diversity of documented communities and highlight the potential ecological importance of virophages and gaps in our understanding of how these presumably intimately associated viruses interact with their hosts.

SSAEM173

Tracking functional bacterial biomarkers in response to a gradient of contaminant exposure within river continuums

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Within all aquatic environments, aside from the *physical* dispersal of dissolved and/or particulate phase contaminants, alteration from both *biological* and *chemical* processes are shown to change the chemistry of the parent compounds. Often these alterations can lead to secondary influences as a result of cooperative microbial processes (i.e. coupled respiratory pathways and/or energy and biodegradation cycles), complicating our understanding of the biological impact that these mobile compounds impose on ecosystem health. The McMurray Formation (MF) (the formation constituting the minable bituminous oil sands) is a natural, ongoing source of hydrocarbon-bound sediments to river ecosystems in the region (via terrestrial and aquatic erosion), providing a natural “mesocosm” to track and characterize the effects of these compounds on regional aquatic primary productivity. Here we characterize the natural, *in-situ* microbial response to increasing hydrocarbon exposure down a river continuum. Using the Steepbank River (STB) as our oil sands representative mesocosm, suspended and bed sediment samples were collected at 3 sites from upstream to downstream, as the water flows into and through the MF. Samples were then analysed for the active, *in-situ* gene expression of the prokaryotic communities. Results from both suspended and bed sediments show clear and significant shifts in the microbial metabolic processes within each respective compartment, in response to elevated polycyclic aromatic compound (PAC) concentrations. Specific genes responsible for hydrocarbon breakdown (Alkane Monooxygenase, Benzoyl-CoA Reductase etc.) experience elevated expression levels, while certain energy metabolism genes (nitrogen, sulfur, methane) reveal fundamental shifts in their pathway specificity, indicating an adaptation response in their basic energy metabolism. Expression from suspended sediments reveal subtle yet delayed metabolic response further downstream compared to bed sediments, indicative of the erosion and transport dynamics within a lotic system. These results provide insight into the use of clusters of gene biomarkers to track the active, *in*-situ microbial response of both emerging and legacy contaminants. Such information will be important in determining the best management strategies for the monitoring and assessment of aquatic health in both natural and contaminated ecosystems worldwide.

SSAEM204

Toxic rocks: characterization of fungal/algal communities on peridotite

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Peridotite is a igneous rock that is dominant in the upper part of the Earth's mantle. It consists of olivine and pyroxene, and is considered toxic to most forms of plant and microbial life because of its high concentrations of magnesium and iron. The Tablelands of Gros Morne National Park in NL represent one of the most prominent exposures of peridotite at the Earth's surface. Because this exposure leads to the process of serpentinization, which also occurs on the Martian surface, the Tablelands is considered to be a Mars analog site. To date, there

have been no attempts to systematically investigate the diversity of fungi associated with peridotite in the Tablelands. We sought to address this using a combination of culture-based and metagenomic sequencing approaches. Our culture-based analysis particularly focused on fungi beloning to the Eurotiomycetes and Dothidiomycetes, which are known for their extremotolerance and ability to colonize rock surfaces. We also examined algal and bacterial diversity in our samples. In addition, all cultured fungi were subjected to detailed phenotypic analyses to identify traits potentially important for the ability to colonize peridotite. Preliminary results from our culture-based experiments have revealed that the fungal genera *Exophiala* and *Rhinocladiella* are abundant on the surface of peridotite. Our parallel metagenomic studies have identified 176 fungal taxa across four samples, as well as 670 bacterial taxa and 59 algal taxa. Strikingly, for three of the four samples, the most abundant fungal OTU is classified as "unidentified", whereas the lichen Porpidia is the most abundant on the fourth sample. Several other abundant (>5%) fungal OTUs in all four samples were also "unidentified". Our preliminary results point to a surprisingly complex fungal community on the surface of peridotite, though most members appear to be poorly characterized. Additional phenotypic analysis of our cultured isolates coupled with shotgun metagenomic sequencing should provide further insight into this intriguing fungal community.

SSAEM266

Relationship between methane emission and *Methanoculleus* spp. concentration of treated swine manure

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Under anaerobic conditions, the methanogenic microbial biomass remains fairly constant through time due to harsh conditions. However our previous studies on swine manure from storage tanks indicated an enrichment of Methanoculleus spp. that coincides with methane production. Manure treatments such as liquid separation from the solids have the potential to reduce the production of methane. Acidification reduces ammonia loss by gaseous emission. However studies aiming at identifying the impact of the treatments on methanogenic populations are scarce. In our study, we compared the concentration of *Methanoculleus* spp. and total methanogens in treated swine manure samples with untreated manure as control. Manure samples were incubated during 20 weeks at 20°C under anaerobic conditions similar to manure storage conditions in outside concrete tanks. The abundance of the *mcrA* gene encoding the alpha-subunit of the methyl coenzyme M reductase, a key enzyme of methanogenesis, was monitored by using two genus- and total methanogen-specific quantitative PCR assays. Biogas production and methane content were also monitored.

The *mcrA* gene copy number of *Methanoculleus* spp. was an order of magnitude lower than that of total methanogens in untreated manure. The dynamics of *Methanoculleus* spp. was more closely related to methane production than the level of total methanogens that remained constant through incubation time. The solid fraction contained lower levels of *Methanoculleus* when compared to the liquid fraction and the untreated manures. Acidification of the liquid fraction resulted in the abolishment of methane production and *Methanoculleus* levels remained constant during the entire incubation period, in contrast to a 1-log increase in untreated manure.

In conclusion, the study indicated that manure treatments have the potential to reduce the abundance of specific methanogen populations linked to methane production.

SSAEM286

Single Cell Raman-SIP to Study the Carbon Flow in Contaminated Aquifers

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Natural attenuation of the contaminants steadily occurs in contaminated subsurface environments at slow rates. The initial burst in microbial activity at hot-spots of biodegradation, however, is associated with a significant increase in the biomass concentration, a steep gradient of electron acceptors and exploits the background nutrients. Depletion of nutrients over time can lead to stalled degradation and higher cell death rate. We hypothesize that microbial communities adapt to the nutrient-limited conditions through recycling of nutrients which are locked up in the dead biomass (necromass), resulting in the slow and sustained attenuation of contaminants. However, the significance and potential of necromass degradation in contaminated aquifers is yet unknown. Here we provide quantitative evidence of the necromass degradation in an anaerobic hydrocarbon-degrading enrichment culture as a model for subsurface microbial communities. The culture was isolated from a contaminated aquifer in Düsseldorf, Germany, mainly consisting of *Desulfobacterium* N47 (hydrocarbon-degrading, sulfate-reducing) and the spirochaete *Rectinema cohabitans* (fermenting). Stable isotope probing and Raman microspectroscopy (Raman-SIP) demonstrated the assimilation of naphthalene as indicated by a shift in phenylalanine marker bands of the cells (ratio of 1003/ (991, 981, 968) cm^{-1}) in systems incubated with ^{13}C (0.37±0.1) compared to those incubated with ^{12}C naphthalene (1.4±0.5). Furthermore, isotope ratio infrared spectroscopy CO_2 measurement in the systems with and without labeled biomass added showed that the CO_2 produced from biomass degradation was 6-8% of the CO_2 from naphthalene degradation. Considering that a typical yield for anaerobic metabolism is around 5-10 %, this implies that the entire biomass produced from naphthalene biodegradation can be fermented by the spirochaete. These results demonstrate the enormous potential for necromass fermentation in the hydrocarbon-degrading enrichment culture. Therefore, the release of fermentation products will likely play a significant role in the recycling of electron, carbon, and nutrients. Our results provide quantitative insights on the significance of microbial loops in contaminated aquifers.

SSAEM392

Application of MinION sequencing in food safety

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The MinION sequencing platform (Oxford Nanopore Technologies, Oxford, UK) is a disruptive technology set to alter current genome analysis procedures in Food Microbiology. With ever increasing improvements to the hardware and software components of the technology, the MinION sequencing platform is showing a very strong advantage when compared to the other platforms, based on: rapid library preparation (10 – 90 minutes), easy set up that obviates the need for expensive equipment, possibility of re-starting an on-going run, repeat use of a flow cell, rapid base calling (with the introduction of the MinIT data analytics) and provision of raw reads in the fastq format which is compatible with many rapid, downstream data processing. The MinION sequencing procedure has proven very useful for analyzing microbial isolates, is amenable to the barcoding of multiple isolates in a single run, generates large amount of data (up to 150 GB on R9.4 flow cells over a 48-hour run), and can be used for metagenomics sequencing where DNA representing a large number of organisms are present in the sample. We have applied the MinION sequencing procedure at the pre-enrichment stage of bacterial culture by analyzing DNA extracts of peptone water cultured food samples and generated informative sequence data. Bioinformatic analysis of the data led to the rapid detection of contaminants. By detecting contaminants at the earliest step of a culture procedure, and avoiding a lengthy selective enrichment step(s),

quick regulatory intervention can be instituted to protect consumers from exposure to contaminated food. The results provide useful information for both the identification and characterization of the microbial contaminants. The main disadvantage of the MinION sequencing platform is the relatively high error rates of the raw sequence reads of up to 10%. This is viewed as a limitation if single nucleotide polymorphism analysis is the approach of choice to evaluate genetic distances among microbes as required when isolates from food samples are compared with human isolates to identify matches during food safety investigations. However, we showed that k mer analysis overcomes any limitation from base errors in MinION data by accurately matching identical isolates even when a different sequencing platform is used to generate one set of the sequence data. The MinION sequencing technology is highly adaptable and is expected to contribute to food safety in the immediate future.

SSMGCM3

CRISPR-based functional genomic platforms for gene deletions and modulating gene expression in *Candida* pathogens

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Opportunistic *Candida* pathogens are a leading cause of fungal infections, and new functional genomic tools enable our ability to better study the biology of these important pathogens. Here, we develop a CRISPR-based toolkit for functional genomic analysis in *Candida* species, using CRISPR-based deletions, and strategies for CRISPR-based regulation of gene expression. The first strategy is a CRISPR-Cas9-based 'gene drive' platform for rapid and precise genome editing in *C. albicans*, enabling applications for genetic interaction analysis of fungal pathogenesis. In our gene drive system, a modified DNA donor molecule acts as a selfish genetic element, replaces the targeted site, and propagates to replace any additional wild-type locus. Coupling this approach with mating-competent *C. albicans* haploids, we can rapidly create diploid *C. albicans* strains that are double homozygous deletion mutants, enabling us to create large-scale double-deletion libraries and analyze complex genetic interactions networks in *C. albicans*. In addition, we have developed two powerful technologies, never previously used in fungal pathogens: CRISPR interference (CRISPRi) and CRISPR activation (CRISPRa), for applications in *Candida* species. We demonstrate the ability of these systems to repress or induce gene expression in *Candida*. Since, unlike classic CRISPR systems, these platforms do not require a DNA repair construct, the simplicity of this system lends itself to high-throughput strain generation. Using a highly-efficient, high-throughput cloning strategy, we are able to efficiently and rapidly generate large numbers of fungal mutant strains that over- or under-express any gene of interest, providing a powerful new tool for functional genomic analyses in fungal pathogens.

SSMGCM18

The Bet/Exo recombination system allows IncC conjugative plasmids and STX/R391 elements to evade host defense mechanisms during conjugation

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The proliferation of antibiotic resistance is a serious public health problem that requires a better understanding of the mechanisms driving the dispersion of resistance genes. Resistance genes are mostly transmitted by horizontal gene transfer by mobile genetic elements, such as integrative and conjugative elements (ICEs) and conjugative plasmids. Conjugative plasmids of the incompatibility group C (IncC) and ICEs of the STX/R391 family found in pathogenic *Enterobacteriaceae* and *Vibrionaceae* are strongly associated with multidrug resistance (1).

IncC plasmids and SXT/R391 ICEs carry a large operon that encodes a RecA-independent homologous recombination system composed of the single-stranded DNA annealing protein Bet and 5' to 3' DNA exonuclease Exo (2). This recombination system is distantly related to the Lambda Red system encoded by bacteriophage Lambda. In IncC plasmids and SXT/R391 ICEs, expression of the operon containing *bet* and *exo* is concurrent

with the expression of the conjugative transfer genes. *bet* and *exo* were found to enhance the diversity of SXT/R391 ICEs by promoting inter-ICE recombination event during conjugation (3). While the Lambda Red system is widely used in bacterial mutagenesis, no clear biological role has been attributed to any of these systems to date.

Our results show that the Bet/Exo system encoded by IncC plasmids and SXT/R391 ICEs is a DNA repair system that protects these mobile genetic elements against defense mechanisms encoded by recipient cells. Using a *Vibrio cholerae* O1 strain carrying a native CRISPR/Cas system as a recipient strain and an SXT/R391 ICE or IncC plasmid engineered to be targeted by the Cas protein, we show that Bet and Exo are crucial to rescue these elements from double-stranded cleavage in the recipient after conjugative transfer. Sequencing of scars on a large sample of rescued plasmids and ICEs is ongoing to examine the impact of Bet/Exo-mediated repair on their genetic plasticity.

Besides bacteriophage Lambda and related phages, homologs of Bet and Exo are encoded by IncC, IncA, IncT and IncP-7 conjugative plasmids, SXT/R391 ICEs, as well as many other uncharacterized conjugative elements (2). Therefore this repair system likely plays a crucial role in the acquisition, persistence and dissemination of antibiotic resistance genes in bacterial populations by helping the conjugative elements that carry these genes to evade defense systems deployed by bacteria as barriers against infectious DNA molecules.

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SSMGCM122

Induction of specialized metabolites in *Streptomyces* by heterologous expression of the engineered response regulator AfsQ1*

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The *Streptomyces* genera of bacteria have a renowned ability to synthesize a vast array of specialized metabolites (SMs). These compounds have a variety of potent and selective bioactivities, most notably antimicrobial. Currently 60% of our antibiotics are derived from these bacteria and continued genome sequencing reveals that they contain genes to derive novel SMs. Many could pose as new antibiotic therapies used to treat the rise of multidrug resistant pathogens. SMs have remarkable chemical complexity; thus we rely on culture and fermentation to obtain them. However, during typical culturing the transcription of the proteins required to synthesize these metabolites are either extremely low or entirely silent. To address this challenge we developed a platform technology leveraging a highly conserved two-component system responsible for pleiotropic regulation of SM production. By introducing a phosphomimetic point mutation in the response regulator *afsQ1 (afsQ1*),* allowing for constitutive activity and heterologously expressing this allele in a library of *Streptomyces* strains, we remodeled their metabolism to increase or induce the expression of these difficult to obtain metabolites. Our technology was applied using two separate screening approaches: the first identified

strains where *afsQ1**induced antibacterial activity and the second utilized unbiased comparative metabolomics to identify novel masses via LC-MS.

Antibacterial screening led to the discovered of Siamycin-I, a lassopeptide previously identified as an antiviral compound. Siamycin-I has potent antibacterial activity across Gram-Positive bacteria including multidrug resistant strains and sequencing the producer organism allowed the identification of Siamycin's associated biosynthetic genes. To characterize its antibacterial target, we generated Siamycin-I resistant mutants in *Staphylococcus aureus*and compared cross-resistance profiles to antibiotics with known targets. This demonstrated that Siamycin-I likely targets the cell wall. We confirmed this hypothesis in *Bacillus subtilis,* where Siamycin-I can activate a cell wall stress reporter specific to lipid II inhibition and sub-MIC Siamycin-I treatment induces membrane blebbing.

Utilizing comparative metabolomics and cross-referencing masses to known SMs, we identified a novel metabolite with a mass of 781.3094Da. Using HPLC we isolated enough of the metabolite to elucidate the structure with NMR verified by MSMS. This new molecule shares a scaffold with Pepticinnamin E and via a retrosynthetic approach we elucidated the pepticinnamin biosynthetic genes. To validated our prediction we achieved production of pepticinnamin E and our new metabolite via overexpression of a cluster situated regulator. The analysis of the proteins within the cluster reveals some unprecedent nuances in NRPS synthesis. Pepticinnamins are known protein farnesyl transferase inhibitors (FTI) responsible for prenylating proteins such as Ras. This led to the early initiative of investigating FTI as anticancer therapies. These early endeavours offered no explanations of the role these compounds fill in nature when produced by *Streptomyces*. We propose that FTIs are inhibitors of morphological switching of fungal species, and show that FTIs can block filamentation in *Candida albicans*. This phenotype of *C. albicans*is key to its pathogenesis and FTI can be envisioned as possible new antivirulence molecules.

SSMGCM243

Site-specific excision of pneumococcal prophages from genes essential for natural transformation

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Background

Bacteriophages are important mediators of bacterial genome evolution. During their life cycle, temperate phages integrate into the bacterial chromosome through site-specific recombination between their attP site and a cognate attB site on the bacterial chromosome. They reside integrated until their excision and replication is triggered by conditions such as exposure to DNA-damaging agents. While most phages integrate into intergenic regions some integrate within open reading frames and regulate expression of bacterial genes by switching between integrated and excised states (1). The gram positive bacterium *Streptococcus pneumoniae* (*Spn*) is a major human pathogen that causes severe diseases such as pneumoniae, septicaemia and meningitis. *Spn* is competent for natural transformation, a process by which it acquires genetic material from its environment. Transformation confers an important evolutionary advantage to *Spn* as it promotes acquisition of antibiotic resistances and allows it to switch capsular serotypes, leading to vaccine evasion. The competent state is controlled by the auto-inducer CSP (competence stimulating peptide) and requires the production of a type IV pilus encoded by the *comG* operon. Interestingly, some *Spn* strains carry prophages integrated into *comGC*, the gene encoding the major pilin of the competence pilus. A full-length ComGC protein is required for transformation to occur. While determining the genome sequence of the *S. pneumoniae* clinical isolate BHN1198, which carries ϕ135B integrated into *comGC*, we found evidence of excised phage molecules in the sequenced cell population.

Using BHN1198 as a model, we investigated the genetic and transcriptional connections between phage excision and transformation.

Results

Using a quantitative PCR approach we confirmed that ca. 1% of the BHN1198 cell population has an empty attB site, indicating that ϕ135B has the ability to excise from the chromosome. ϕ135B was further shown to be a functional bacteriophage, capable of causing cell lysis and releasing phage particles upon induction with the DNA-damaging agent mitomycin C (MMC). As phage excision reconstitutes the normal sequence of *comGC* we hypothesized that the full-length *comG* operon would be transcribed in a fraction of the cell population. Using semi-quantitative PCR, we show that a transcript spanning the wild-type *comGC* sequence is detectable and induced by the addition of CSP, and further show that BHN1198 is capable of uptaking DNA by transformation. Using PCR and *in silico* screens, we identified other clinical isolates of *Spn* carrying prophages integrated into *comGC* and confirmed that the majority of these strains retain the ability to uptake DNA. Finally, using differential RNA sequencing and RT-qPCR, we determined the transcriptional response of ϕ135B to MMC and to CSP.

Conclusions

Taken together our results indicate that spontaneous site-specific excision of pneumococcal phages from *comGC* allows transcription of the full-length *comG* operon and DNA uptake.

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SSMGCM342

Exploiting synthetic interactions to explore outer membrane biology in Escherichia coli

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Gram-negative bacteria are intrinsically resistant to many antibiotics due to their outer membrane barrier. The outer membrane is an asymmetric bilayer with a phospholipid inner leaflet and lipopolysaccharide outer leaflet; it excludes molecules that are hydrophobic or large and hydrophilic from cell entry. Outer membrane permeability must be altered to allow these types of compounds into the cell. Although the Gram-negative outer membrane has been studied for decades, there is still much to learn about this complex barrier. Investigating synthetic genetic interactions can help uncover a great deal of information about genes. Here, we performed synthetic genetic arrays (SGAs) by crossing a subset of *E. coli* gene deletion strains that have been implicated in the outer membrane with the Keio non-essential gene deletion collection and Storz small RNA deletion library. The generated double deletion strains were grown on rich microbiological media with and without subinhibitory concentrations of two antibiotics precluded by outer membrane permeability, rifampicin and vancomycin, to probe both genetic interactions and outer membrane permeability. The interactions of particular interest are synthetic sick or lethal (SSL)—gene deletions that are detrimental to the cell in combination but result in viability alone. On average there are ~30, ~36, and ~40 SSL interactions per gene in the no drug, rifampicin, and vancomycin conditions, respectively. Strains in the collections which are SSL with at least 25% of query genes are deemed “frequent interactors”, several of which are common with those observed in our previous studies. We also see frequent interactors in rifampicin and vancomycin with deletion strains that are sensitized to either of these drugs. Our datasets have been compiled into a user friendly, interactive database called OM Explorer, where genetic interactions of these outer membrane implicated genes can be searched, visualized across the genome, compared between conditions, and enriched for gene ontology terms.

SSMGCM382

Forged in iron: Conflict enhances the survival of all members in a defined bacterial community

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Competition between bacterial species affects ecosystem stability and is a central topic in ecology. Bacterial communities are composed of diverse connections between member species, and it is challenging to characterize these interactions in a natural environment. To evaluate the impact that interspecies conflict has on community stability, we developed a defined community with model species *Escherichia coli* and *Salmonella enterica* in limiting nutrient conditions. We designed our community such that *E. coli* was the only species able to access a lactose carbon source, making *Salmonella* dependent on the presence of *E. coli* and its production of nutrient by-products. Using a *Salmonella* strain that produces an *E. coli*-killing colicin toxin resulted in a paradox whereby *Salmonella* required *E. coli* to produce food but simultaneously inhibited *E. coli* with a toxin. We found that this seemingly simple two-species community had multiple layers of competitive interactions that included cross-feeding, toxin antagonism and siderophore poaching. To intensify competition to a level where community structure collapses altogether, an iron chelator was added to enhance colicin production to bacteriocidal levels and increase the total competitive pressure between species. Under these highly competitive conditions, the community with colicin conflict grew better than *E. coli* alone or *E. coli* & *Salmonella* in the absence of colicins. In addition, we discovered a novel interaction wherein *E. coli* took advantage of *Salmonella*'s production of siderophores to further enable its growth in iron-limited media, which likely allowed the community to stabilize in these highly competitive environments. Altogether, our findings show that multiple antagonistic interactions within a bacterial community can contribute towards the community's stability and robustness.

Student Symposium Competition Abstracts

Student Symposium I - Tuesday, June 11th, from 1:30 - 5:00 PM

Note: AEM: Applied Environmental Microbiology
II: Infection and Immunity | MGCM: Molecular Genetic Cellular Microbiology

AEM-SSC35

Viral populations and CRISPR-resolved virus-host interactions in a municipal landfill

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Recent culture-independent approaches have described a previously-unknown diversity of viruses from a wide variety of environments. The comparatively poor representation of viruses in reference databases, despite their numerical abundance in ecosystems, illustrates how characterizing the virosphere has historically been a methods-limited endeavor. Understudied environments, like municipal waste sites, are likely to house novel, diverse viral populations, whose host preferences may impact nutrient cycling and contaminant degradation. Using metagenomics, we probed the viral diversity in a landfill and adjacent aquifer in Southern Ontario. Samples were collected from three leachate wells, a leachate collection cistern, and from an aquifer adjacent to the landfill. DNA was extracted from filtered biomass, and sequenced by the Joint Genome Institute. Using VirSorter and DeepVirFinder, computational, virus detection tools, we identified >60,000 viral scaffolds from six metagenomes. The different samples varied in their viral signatures, with the leachate samples showing a greater viral proportion of the microbial community relative to the aquifer sample.

To establish virus-prokaryotic host interactions in the landfill, we used CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) annotated in the landfill metagenomes. The spacers in the CRISPR arrays were extracted, and searched against Refseq and our VirSorter-identified viral scaffolds using BLASTn, in order identify the viruses from which the spacers originated. The vast majority of identified spacers had no hits in Refseq, suggesting that many of the viruses in our system are not present in this database. Spacer hits to our viral scaffolds allowed information on prior infections to be determined. From this information, we generated a virus-prokaryotic host network containing 71 hosts and 96 unique, virus-host links. We intend to use both this and future networks from temporal samples of the landfill as a framework to understand how virus-induced host mortality may be influencing landfill processes such as nutrient cycling and contaminant degradation.

II-SSC50

The lasso peptide antibiotic, siamycin-I, inhibits lipid II biosynthesis at the Gram-positive cell surface

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Siamycin-I is a 21-amino acid lasso peptide with potent antimicrobial activity against a broad spectrum of Gram-positive organisms, including priority pathogens methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococci* (VRE). This lasso peptide inhibits peptidoglycan biosynthesis by binding the key precursor molecule, lipid II, on the extracellular surface. It blocks polymerization of peptidoglycan, catalyzed

by the transglycosylation domain of PBP2, by occluding lipid II from incorporating into the growing glycan chain. By interfering with this essential process, the cell wall specific stress response, regulated by *liaRS*, is activated. The stress response however, is suppressed when lipid II or its polyprenol-coupled precursors are present.

Additionally, siamycin-I-resistant mutants in *Staphylococcus aureus* mapped mutations to the essential two-component system, WalKR. The histidine kinase, *walK*, and its cognate response regulator, *walR*, regulate cell wall metabolism, recycling of the cell wall and autolysis. These mutations can attribute to a thickened cell wall in the resistant mutants.

Uncovering siamycin-I's mechanism of action provides a new class of antimicrobials against Gram-positive pathogens. Interestingly, its mechanism of action is distinct from other known cell wall antagonists, such as the clinically relevant vancomycin, which also binds lipid II. This is the first report of a lasso peptide to bind lipid II and inhibit peptidoglycan biosynthesis.

MGCM-SSC71

Delivery of CRISPR-Cas9 by conjugative probiotics enables precise microbiome editing

Kevin Neil[1], Nancy Allard[1], Frédéric Grenier[1], Vincent Burrus[1], Sébastien Rodrigue[1]

[1]Université de Sherbrooke

Engineered probiotics that can target and eliminate specific microbes would represent a powerful microbiota manipulation tool and a potential alternative to antibiotics. An interesting strategy is to use a probiotic strain harbouring a conjugative plasmid for the delivery of a CRISPR-Cas9 system directly into the microbiota. The success of this approach depends on high *in situ* DNA transfer rates by the conjugative machinery, and on the presence of specific CRISPR-Cas9 targeted sequences in the recipient bacteria. Here we identify $IncI_2$ conjugative plasmid TP114 as a proficient DNA delivery system in the mouse gut microbiota, enabling the specific CRISPR-Cas9 mediated knock-down of a targeted bacterial strain. Unlike other conjugative plasmids from different groups, TP114 showed relatively modest transfer rates *in vitro* that increased >100 fold in the mouse gut microbiota, thereby reaching most target cells *in vivo*. A single oral dose of the *Escherichia coli* Nissle 1917 probiotic containing a TP114 mobilizable CRISPR-Cas9 system was sufficient to specifically eliminate ~99% of a targeted *E. coli* strain carrying an antibiotic resistance gene relative to an otherwise isogenic strain. Importantly, the strategy had no significant impact on the overall composition of the mouse intestinal microbiota. Taken together, these results constitute an important proof-of-concept for a new microbiota editing technology.

MGCM-SSC113

CRISPRs and competence: sexually transmitted immunity

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Clustered regularly interspaced short palindromic repeats (CRISPRs) and their associated (*cas)* genes behave as a microbial adaptive immune system to cleave invading genetic material such as bacteriophages (phages). Segments of DNA from phages that the host has previously encountered are stored in the CRISPR array 'memory' as spacers and serve as recognition tools against recurring phage threats. Continuous selective pressure from phage infections requires a host to 'update' its CRISPR spacer memory in a process known as adaptation. It is unclear how naïve CRISPR-Cas arrays can acquire a spacer derived from a novel phage in time,

before the phage inflicts irreversible damage. This study demonstrates that *Streptococcus thermophilus* exploits environmental DNA, acquired through natural competence, to prime the naïve CRISPR-Cas system. Natural competence is a form of bacterial 'sex', where a cell takes up environmental DNA, which can result in the acquisition of new genetic traits. Free-floating phage DNA internalized by the cell in this fashion is harmless but has the right, exploitable information for CRISPR to prepare itself for the infective phage. This finding supports an alternative theory for the existence of natural competence and provides insight into the basic mechanism of CRISPR adaptation. Remarkably, understanding how phages elicit competence provide novel methods in accessing and editing genomes that were previously difficult to manipulate, without the need for artificial genetic engineering.

AEM-SSC117

Implementation of a directed evolution strategy for rhamnolipid biosurfactant overproduction by *Burkholderia thailandensis*

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Introduction. Biosurfactants represent a valid and sustainable substitute for synthetic surfactants. Rhamnolipids are produced by only a few bacterial species, including *Burkholderia thailandensis.* Current bioprocesses for rhamnolipid production by this species are not efficient enough, due to low yields. However, regulation of rhamnolipid biosynthesis in *B. thailandensis* is poorly understood, and thus identifying regulatory factors could help increase the production of this biosurfactant.

Hypothesis. *B. thailandensis* is capable colonize environment using swarming motility, a social motility behaviour requiring both functional flagella and production of a surface-wetting agent. During swarming motility, *B. thailandensis* synthesizes and exports rhamnolipids, that reduce tension between the substrate and the bacterial cell, promoting spreading over surfaces. Under laboratory conditions, swarming is typically seen on agar concentrations above 0.3% and below 1.5%, depending on the bacteria and culture conditions. We hypothesized that this surfactant-dependant surface behaviour could be used to identify factors controlling rhamnolipid production. We took an adaptive evolution approach, a process by which natural evolution is applied through selective pressure to specifically improve a phenotype of choice, linked to a genetic modification. We proposed that if the agar concentration is increased, cells would have to produce more rhamnolipids to maintain their swarming phenotype.

Methodology. Three independent lineages initiated from a common ancestor strain, *B. thailandensis* E264, were submitted to experimental evolution through consecutive rounds of cultivation on swarming media at various concentrations of agar. After 10 transfers, resulting populations were cultured in liquid media during 5 days and then rhamnolipids were quantified. Results were compared to rhamnolipid production from the ancestral E264 strain and to the population from a similar tenth round but without agar in the medium. For each lineage, ten isolated clones were tested from the mixture of *evoluents* and rhamnolipid production was quantified. The genomes of the two best overproducer clones for each lineage were sequenced and compared to the wildtype genome.

Results. There is no difference in rhamnolipid production for populations evolved by transferring on culture medium without agar compared to the ancestral strain. In contrast, agar inclusion into the culture media increased rhamnolipid production from the evolved populations: the higher the agar concentration, the greater was the rhamnolipid production, until the swarming inhibition agar concentration was reached. These data support a model where the increase in surface tension *via* the agar concentration constrained the bacteria to synthetize more rhamnolipids to be able to achieve a swarming phenotype. Whole genome sequencing results

revealed a limited number of mutations in different genes. Interestingly, three of the six sequenced *evoluents* carry mutations in the *qsmR* gene, coding for an IclR-type transcriptional regulator. Preliminary results indicated that this gene product represses both rhamnolipid biosynthesis and an export system.

Conclusion. Using swarming motility to direct the evolution of a population of bacteria was a successful strategy for selection of spontaneous mutants of *B. thailandensis* overproducing rhamnolipids. The selective pressure converged towards a specific regulator.

AEM-SSC128

Ecology of phototrophic iron-oxidizing bacteria in Canadian Boreal Shield lakes

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Photosynthetic iron-oxidizing bacteria (photoferrotrophs) are thought to have been important contributors to primary production in Earth's early anoxic oceans, yet these bacteria have been presumed to play a minor role in modern environments. Here we probe the diversity and ecology of photoferrotrophy in seasonally anoxic and iron-rich Boreal Shield lakes at the IISD-Experimental Lakes Area (IISD-ELA) to test whether the capacity for photoferrotrophy is common within boreal lakes. Metagenomic sequencing of anoxic water column samples from nine lakes enabled the recovery of high-quality *Chlorobia* genome bins representing the dominant anoxygenic phototroph populations. Several of these genome bins contained the *cyc2* candidate gene marker for iron oxidation, whereas others contained the *dsrA* gene marker for sulfide oxidation, implying that photoferrotrophy and phototrophic sulfide oxidation co-occur in the water column. Additionally, incubation of anoxic lake water in iron(II)- or sulfide-containing media allowed for the enrichment of two new species of *Chlorobia*. These *Chlorobia* grew with sulfide as the electron donor, but their genomes contained both the *cyc2* and *dsrA* genes, implying photoferrotrophic potential testable once the species are in pure culture. *Chlorobia* containing *cyc2* were only detected in three of the nine lakes based on metagenomic data, whereas *Chlorobia* containing *dsrA* were detected in an additional four lakes, suggesting that ecological controls may affect the favourability of photoferrotrophy in Boreal Shield lakes more strongly than phototrophic sulfide oxidation. Metatranscriptomic data showed that *Chlorobia cyc2* was actively expressed in the water columns of at least two of the three lakes where *cyc2* was detected, along with co-expression of *Chlorobia dsrA*. Because Boreal Shield lakes number in the millions worldwide, these data suggest that photoferrotrophy is a globally relevant environmental process, alongside phototrophic sulfide oxidation, and that photoferrotrophy is controlled by ecological drivers relevant to understanding its contribution to ancient Earth ecosystems.

MGCM-SSC53

Systematic perturbation of yeast essential genes using base editing

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Investigating the link between genotype and phenotype is one of the greatest challenges in modern biology, and yeast is a powerful model in this field. Recent developments in genome editing technologies have allowed for the investigation of this question at high resolution using perturbation approaches. Methods based on the CRISPR-Cas9 genome editing technology have facilitated high throughput variant strain engineering but may not be appropriate for all organisms or experiments. Base editors, engineered from an inactivated Cas9 and a DNA editing enzyme such as cytidine deaminases offer an efficient alternative to double-stranded break based genome modification approaches, but have yet to be used at the genome scale. Here, we use the Target-AID base editor to systematically perturb yeast essential genes, allowing us to test the effect over 17 000 mutations simultaneously. We identify over 1100 guides that generate mutations with a significant impact on fitness. We then use a high throughput approach for essential gene variant strain construction to confirm these effects and investigate site-specific sensitivities. By using a modified protein fragment tag, we can then easily quantify the mutational effects on protein abundance and interactions. Interesting perturbations we investigate include a highly conserved residue in *GLN4* where substitutions have a high lethality rate. software. Using a set of stop codon generating guides, we find rules for effective base editing guide design based on sequence and target properties. This model can then be used to optimize library design in future experiments. Our results show that base editing is amenable to large-scale screening experiments and is a powerful alternative to Cas9-based approaches for residue-level precision mutagenesis.

AEM-SSC56

Life detection with nanopore sequencing and microbial diversity in Mars analogue environments

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The search for life in our solar system is among the highest priorities for space science. Studying relevant analogue environments and extremophiles greatly contributes to the elucidation of microbial diversity and detection of microbial biosignatures. The goal of this research is to further understand how microbial life can survive in analogue environments and reveal methods and techniques for biosignature detection. We used MinION sequencing to detect and characterize the microbial life in a set of paleochannels near Hanksville, Utah, USA, with supporting data from X-ray diffraction (XRD), reflectance spectroscopy, Raman spectroscopy, and Life Detector Chip (LDChip) microarray immunoassay analyses. The MinION-generated metagenomes reveal a

rich microbial community dominated by Bacteria and containing radioresistant, psychrophilic, and halophilic taxa. There are prominent organic and water-related features in the XRD data, reflectance spectra, and Raman spectra, though by themselves these biosignatures are not conclusive and an abiotic origin cannot be precluded. The LDChip detected signals corresponding only to the antibodies present on the chip and this introduces a strong bias towards specific protein groups and species. However, when these techniques are combined with the MinION, which can detect and sequence DNA from a wide variety of microorganisms in many types of samples and in very small amounts, the potential for biosignature identification and characterization is greatly increased. Here, we present a proof-of-concept utilization of the MinION for direct life detection and show how it can complement results from techniques analogous to current space mission instruments (e.g. XRD and spectroscopy). In addition, future work will focus on using the MinION's long reads to generate highly accurate, ultra-long contigs for efficient genome binning using samples from Canadian high Arctic cryoconites. This will contribute to the development of MinION sequencing as a tool for high accuracy metagenome studies and robust characterization of analogue microorganisms.

II-SSC83

A rapid fluorescent dye membrane integrity assay to detect biocide resistant and susceptible phenotypes of Gram-negative bacteria

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Background: We rely on biocidal quaternary antimicrobial compounds (QACs) on a daily basis in household products, clinical and food production facilities to disinfect and sterilize hard to treat surfaces/ materials. Due to QAC overuse, QAC resistance among bacteria is increasing among clinically relevant Gram-negative pathogens which, in turn, can enhance resistance to therapeutic antimicrobials such as last-line of defense antibiotics. Since QACs kill bacteria by disrupting cellular membranes, bacteria with resistant QAC phenotypes frequently exhibit alterations in membrane proteins and lipids that enhance membrane integrity and tolerance to QACs. Current screening methods to detect QAC resistant phenotypes are limited to traditional antimicrobial susceptibility testing (AST) techniques that are time-consuming (> 24 hrs) and hard to replicate due to a lack of QAC standards.

Objectives: To address this gap, we propose a rapid (30-minute) impermeant fluorescent dye-based membrane *i*ntegrity assay (RFDMIA), to discriminate and predict QAC susceptible and resistant phenotypes among Gram-negative bacterial species. We hypothesize that we can detect the loss of membrane integrity by cells exposed to increasing QAC concentrations by monitoring impermeant dye entry into cells; QAC resistant strains should exhibit lower levels of fluorescence as compared to susceptible strains at QAC concentrations at or above the minimal inhibitory concentration (MIC).

Methods: Using high-throughput 96-well fluorescent microplates, changes in fluorescence dye entering bacterial cells exposed to increasing QACs can be monitored over 30 minutes and compared to AST results for cells measured under similar concentrations. To confirm RFDMIA can predict membrane integrity and morphology, we performed scanning electron microscopy (SEM) with *Escherichia coli*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* strains.

Results: RFDMIA analysis of *E. coli* K-12 adapted to QACs cetrimide (CET) and benzalkonium (BZK) at >4 fold the MIC value of the unadapted *E. coli* strain, was able to differentiate QAC susceptible and adapted strains when exposed to increasing QAC concentrations and correlated with AST determined MIC values. SEM analysis verified that QAC adapted strains were susceptible at RFDMIA predicted concentrations and also revealed that

QAC adapted strains required the compound to maintain membrane integrity. RFDMIA was able to predict QAC susceptibility for *E. coli* and *A. baumannii* but had difficulty predicting *P. aeruginosa* due to dye overlaps with its intrinsic fluorophores.

Significance: We have developed a useful RFDMIA that can rapidly distinguish QAC susceptible and resistant species and strains validating the hypothesis and providing a new faster method for screening and detecting QAC resistance in Gram-negative species by clinical, research, and industrial labs. We are currently exploring other dyes to reduce intrinsic fluorescent overlaps in the assay.

MGCM-SSC97

Characterizing the cellulose-modifying enzyme BcsG

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Microbial biofilms are communities of microorganisms that exhibit co-operative behaviour, producing a matrix of exopolysaccharide that enmeshes the community. The well-studied human pathogens *Escherichia coli* and *Salmonella enterica* produce a biofilm matrix comprised chiefly of the biopolymer cellulose, along with amyloid protein fibers, termed curli. This biofilm matrix confers surface adherence and acts as a barrier to disinfectants, antimicrobials, environmental stressors, and host immune responses. Recent studies demonstrated that the *bcsEFG* operon encodes an inner-membrane spanning complex responsible for the addition of a phosphoethanolamine modification to microbial cellulose, essential for extracellular matrix assembly and biofilm architecture. We investigated the proposed phosphoethanolamine transferase BcsG to obtain its structure and the biochemical basis of its activity.

We solved the structure of BcsG, and a structural homology search revealed BcsG is a member of the phosphoethanolamine transferase family, including the mobile colistin resistance factors 1 and 2 (MCR-1 and MCR-2) which confer resistance to the antimicrobial drug colistin and related compounds. We then mapped the active site of BcsG using functional complementation studies on a nonpolar chromosomal mutant of BcsG in *E. coli* AR3110. In order to pursue *in vitro* studies of BcsG activity, we synthesized the substrate analogue paranitrophenyl phosphoethanolamine (*p*-NPPE) and found BcsG was capable of cleaving the phosphoethanolamine group *in vitro* with a catalytic efficiency and turnover number comparable to other glycan-decorating enzymes in the literature. Using cellooligosaccharides as artificial acceptors, we observed an acceptor-dependent increase in BcsG activity with a notable preference for saccharides with length > 5 residues. Further LC-MS analysis of the enzymatic product demonstrates BcsG is in fact capable of transfer to a pentamer *in vitro*. We have prepared co-crystals of BcsG in the presence of both *p*-NPPE and cellopentaose in hopes of observing the enzyme-substrate complexes *in crystallo* in order to fully elucidate the catalytic mechanism of BcsG.

A structural and functional model of the cellulose modifying complex offers opportunities in structure-based drug discovery and other efforts in inhibiting microbial biofilms and limiting the resilience of biofilm-forming pathogens. Additionally, an understanding of *bcsEFG*-directed phosphoethanolamine cellulose production may enable biosynthetic engineering of new cellulosic materials, or confer the advantages of phosphoethanolamine cellulose in new organisms.

II-SSC112

Inactivation of *yqhG* in uropathogenic *Escherichia coli* reduces expression of type 1 fimbriae and attenuates colonization of the mouse urinary tract

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Urinary tract infections (UTIs) are common bacterial infections and the vast majority of UTIs are caused by extraintestinal pathogenic *Escherichia coli* (ExPEC) strains referred to as uropathogenic *E. coli* (UPEC). For the successful colonization of the human urinary tract, UPEC employ a diverse collection of secreted or surface exposed virulence factors - toxins, iron acquisition systems, and adhesins. In order to identify factors involved in the expression of type 1 fimbriae, we constructed a chromosomal transcriptional reporter consisting of *lux* under the control of the fimbrial promoter region, *fimS* and this construct was inserted into the reference UPEC strain genome, CFT073, at the *att*Tn7 site. This *fimS* reporter strain was used to generate a Tn*10* transposon mutant library, coupled with high-throughput sequencing to identify genes that affect the expression of type 1 fimbriae.

Transposon insertion sites were localized to 29 genes and 3 intergenic regions. The insertions were linked to genes involved in protein fate and synthesis, energy metabolism, adherence, transcriptional regulation, and transport. We showed that YqhG is one of the important mediators that contribute to the decreased expression of type 1 fimbriae in UPEC strain CFT073. Our results demonstrated that CFT073 Δ*yqhG* has reduced expression of type 1 fimbriae and a decreased capacity to colonize the murine urinary tract. The Δ*yqhG* mutant was attenuated 100-fold in bladder and 10000-fold in kidneys ($P < 0.0001$). Reduced expression in type 1 fimbriae correlated with an increased bias for orientation of the *fim* switch in the OFF position. Interestingly, the Δ*yqhG* mutant was also more motile than the WT strain. Further, the *ΔyqhG* was also significantly more sensitive to hydrogen peroxide. Taken together, loss of YqhG may decrease virulence in the urinary tract due to a decrease in production of type 1 fimbriae and a greater sensitivity to oxidative stress.

II-SSC118

Impacts of carbohydrate metabolism on *Campylobacter jejuni* infection of breastfed infants

Jolene Garber[1], Xiaoming Bian[1], Harald Nothaft[2], Martin Stahl[3], Ben Pluvinage[4], Dilruba Nasrin[5], Jennifer Jones[5], Hua Huang[6], John A. Gerlt[6], Karen L. Kotloff[5], Sharon M. Tennant[5], Alisdair Boraston[4], Alain Stintzi[3], Christine M. Szymanski[1,2]

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More than 50% of *Campylobacter jejuni* isolates possess an operon for L-fucose catabolism that enhances pathogen colonization in the piglet diarrheal disease model. Here, we demonstrate that Cj0485 deletion within the *C. jejuni* NCTC11168 *fuc* operon prevents L-fucose chemotaxis while *cj0485* expression in 81-176 (*fuc*-) enables this strain to sense L-fucose. Crystallization of Cj0485 revealed a structure comparable to the FabG dehydrogenase of *Burkholderia multivorans*. CjFabG activity assays demonstrated that the dehydrogenase converts L-fucose to L-fuconolactone and also D-arabinose to D-arabinolactone while growth studies verified D-arabinose metabolism through the *C. jejuni fuc* pathway. However, *C. jejuni* prefers serine, glutamic and aspartic acids over fucose, indicating a metabolic hierarchy exists. Since *C. jejuni* lacks fucosidases it can only grow on fucose-containing gut oligosaccharides when co-incubated with *Bacteroides vulgatus,* suggesting a scavenging lifestyle within the intestine.

Previous reports indicate that fucosylated human milk oligosaccharides (HMOs) act as binding decoys to protect against *C. jejuni*-mediated diarrheal disease, yet exceedingly high rates of campylobacteriosis are reported among breastfed infants in low-to-middle income countries. Consistent with these reports, our studies comparing 16S rRNA profiles of fecal DNA isolated from infants in the Global Enteric Multicenter Study (GEMS) demonstrated significantly higher *Campylobacter* burdens in breastfed versus non-breastfed children leading us to evaluate the metabolic preferences among those isolates. Although non-breastfed infants were colonized with equal proportions of L-fucose metabolizing and non-metabolizing *C. jejuni* and *Campylobacter coli* strains, the proportion of strains unable to metabolize L-fucose increased significantly in breastfed children. This suggests that HMOs may indeed function as decoys for *Campylobacter fuc*+ strains, but also select for strains with metabolic properties better capable of thriving in the infant gut. Current studies focus on the interplay between *C. jejuni* chemotaxis, binding, and metabolism during diarrheal disease and the development of inexpensive novel intervention and treatment strategies.

Postdoctoral Research Symposium Abstracts
Thursday, June 13th, from 3:30 - 5:00 PM

PDR61

The pan-genome effector-triggered immunity landscape of a host-pathogen interaction

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Effector-triggered immunity (ETI) protects some plants against virulent pathogens but is generally assumed to be somewhat restricted due to a limited number of characterized interactions between pathogenic effectors and host immune receptors. In this study, we probed the ETI landscape of arguably the best studied plant pathosystem, the bacterium *Pseudomonas syringae* and the model plant *Arabidopsis thaliana*, using the *P. syringae* Type III Effector Compendium (PsyTEC). We generated PsyTEC to reduce the pan-genome complexity of effectors in *P. syringae* from more than 14,000 effectors to 529 representatives using comparative genomic approaches. We then chemically synthesized each of these representative effectors and moved them into a suitable *P. syringae* expression vector so that we could infect *Arabidopsis* with the entire PsyTEC library and identify effectors that elicit an ETI response. We found that more than 11% of effectors elicit *Arabidopsis* ETI and nearly all *P. syringae* strains harbor at least one immune eliciting effector. We then linked the effectors that elicit *Arabidopsis* immunity to a small number of plant immune receptors, showing that with only eight receptors, *Arabidopsis* can resist 96.6% of *P. syringae* strains. Even more remarkably, the two most dominant receptors (CAR1 and ZAR1) are able to protect *Arabidopsis* against 94.7% of *P. syringae* strains on their own. Finally, we used our results to predict the outcome between one particular strain of *P. syringae* and *Arabidopsis*, and we were able to reverse this outcome using a plant line that lacked the cognate immune receptor. Our results illustrate the major role played by ETI in determining the outcomes of host-pathogen interactions and provide exciting translational opportunities for engineering more durably resistant crop cultivars that will limit infectious disease outbreaks in critical crops.

PDR62

The co-occurrence and co-exclusion of genes in prokaryote genomes

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Since the sequencing of the first bacterial genomes, the fundamental boundaries and definitions of prokaryotic species have been questioned. The term pangenome has since been used to define the extensive genetic variability that exists between even closely related bacterial strains. Evolution is continuously acting on these genomes, forming new genes, proteins, and gene clusters. Horizontal gene transfer (HGT), in particular, has facilitated this combinatorial process meaning that genes/proteins that interact positively or synergistically with each other are expected to co-occur more often than by chance; conversely, genes may avoid co-occurrence, indicating an antagonistic or redundant functional relationship. In this work, we use methods adapted from networks and graph theory to understand patterns of co-occurrence and exclusion in bacterial and archaeal

species. We have implemented multi-level graph models in which each node (vertex) is a gene or species connected by an edge (relationship) to another node if-and-only-if there exists a coincidence relationship between them. Our method incorporates the phylogenetic distribution and synthenic distances of these genes, and we demonstrate how these concepts can be used to identify conserved clusters of vertical and horizontally inherited genes. We apply these multi-level graph models to a variety of datasets including prokaryotic pangenomes, and metagenomic sequencing datasets from human-associated microbial communities. We find evidence for genes that significantly co-occur with each other within each of these datasets; these results include genes from characterized biological pathways but also genes with unknown functions. Further, we identify genes that exclude each other, indicating evolving objects with antagonistic or redundant biological functions. This work represents a different approach to understanding the evolution of prokaryotes and allows us to draw novel hypotheses as to the potential role of genes with unknown function in prokaryote biology.

PDR70

Prey versus predator: *Pseudomonas brassicacearum* DF41 biofilm blocks *Caenorhabditis elegans* feeding

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Pseudomonas brassicacearum DF41 is a biocontrol agent that inhibits fungal plant pathogens.

The success of any biocontrol strain is dependent on its ability to persist in the rhizosphere where grazing predators represent a significant threat. DF41 is able to avoid predation by the bacterivorous nematode *Caenorhabditis elegans* through two distinct mechanisms: i) secretion of toxic metabolites and ii) formation of a biofilm on the nematode head, which blocks feeding. The ability to form biofilms on the *C. elegans* surface is a unique phenotype that has only been reported for *Yersinia pestis*, *Y. pseudotuberculosis*, and *Xenorhabdus nematophila*. This trait is dependent upon the exopolysaccharide Poly-N-Acetyl-Glucosamine (PNAG), the product of the *hmsHFRS*/*pgaABCD* operon. The objective of the current study was to determine the role of the *pgaABCD* operon in DF41 biofilm formation and to explore regulatory mechanisms overseeing this process. A DF41 *pgaABCD* insertional mutant was created that showed impairment in its ability to develop biofilms on *C. elegans,* suggesting PNAG is involved. Furthermore, L3, L4 and adult nematodes are more susceptible to biofilm formation, suggesting that outer cuticle development impacts bacterial binding. We have also discovered that in the presence of *C. elegans*, DF41 exhibits altered gene expression and secondary metabolite production in a Gac-dependent fashion. Consistent with this, WGA-binding increased when DF41 was propagated in the presence of nematodes, while a *gacS* mutation abolished WGA binding and biofilm formation. These results indicate that the presence of *C. elegans* induces the expression of PNAG in a GacS-dependent manner. As such, the Gac system appears to facilitate chemical communication between predator and prey. Activation of the GacS/GacA regulatory system induces the expression of small noncoding RNAs that bind to and inhibit global Regulator of Secondary Metabolites (RsmA) proteins. In other organisms, RsmA family proteins negatively regulate PNAG expression. The genome of DF41 encodes three putative RsmA proteins: RsmA, RsmE and RsmI. Currently, *rsmA*, *rsmE* and *rsmI* mutants along with translational reporters are being used to determine how PNAG production is regulated and the role of these proteins in DF41 biofilm formation on the *C. elegans* surface.

PDR88

Host-specific adaptive diversification of Crohn's disease-associated adherent-invasive *Escherichia coli*

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Inflammatory bowel diseases, such as Crohn's disease (CD), have reached alarming levels in Canada. Inflammation in CD is associated with drastic changes in the microbes that normally populate the gastrointestinal tract, including a marked increase in *Escherichia coli* at the expense of beneficial bacteria. Members of the adherent-invasive *E. coli* (AIEC) pathovar have been recognized as potential risk factors in CD development, where they are six times more likely to be isolated from CD patients compared to healthy subjects. Moreover, several AIEC strains have been found to invade the intestinal epithelial cells and induce a pro-inflammatory response, thus exacerbating CD symptoms. However, the mechanisms employed by AIEC to adapt to the gut environment and cause disease are unknown.

In this work, we tracked the adaptive evolution of AIEC in a murine model of chronic infection with natural host-to-host transmission for ~9 months (Elhenawy W et al., (2019) *Cell Host Microbe*). Whole-genome sequencing was used to capture the population diversity of AIEC lineages *in vivo*, and compare it to the founder population to define the adaptive mutations acquired by AIEC within the host. We detected several lineages that outcompeted the ancestral strain in the host through independent mechanisms. One lineage was hypermotile compared to the ancestral strain, which we traced to a mobile insertion sequence upstream of the master flagellar regulator, *flhDC*, that enhanced AIEC invasion, pro-inflammatory properties and establishment of a mucosal niche. A second lineage outcompeted the ancestral strain through improved use of acetate, a short-chain fatty acid found in the gut. Intriguingly, the presence of hypermotile and acetate-consuming lineages discriminated *E. coli* isolated from CD patients from healthy controls, suggesting an evolutionary trajectory that distinguishes AIEC from commensal *E. coli*. To our knowledge, this is the first study that compares the pathoadaptation of AIEC and commensals *in vivo*. We identified multiple genomic changes that disentangle the adaptive traits of commensals and CD-associated *E. coli*. In this regard, our results provide novel targets for therapeutic intervention against CD-associated pathobionts without disturbing the gut commensals.

PDR93

Phenotypic adaptation of *Pseudomonas aeruginosa* into biofilms

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The opportunistic pathogen *Pseudomonas aeruginosa* is a major cause of infections in immunocompromised individuals; it is the most prevalent pathogen in lungs of people with cystic fibrosis. This bacterium is versatile: in addition to its capacity to infect a wide variety of plant and animal hosts, it colonizes diverse environments such as soil, water, food, and built environments, such as hospital premise plumbing systems. This bacterium is resistant to host immunity, bactericidal and antibiotic treatments, causing significant concerns for public health. Persistence of *P. aeruginosa* is partly explained by its ability to form biofilms. Inside clinical biofilms, *P. aeruginosa* often adopts an alternative phenotype noticeable by the formation of Small Colony Variants (SCV). We are investigating the prevalence of SCVs in other environments beyond clinical samples. The SCV phenotype was observed in all the *P. aeruginosa* isolates from the various sampled environments (soil, meat, drains, etc.) when cultivated under appropriate biofilm conditions. This result demonstrates that the ability of *P. aeruginosa* to form SCVs is naturally widespread, and not restricted to clinical strains, e.g. from cystic fibrosis

individuals. As observed in the prototypical strain PA14, we found that emergence of SCVs is thermo-dependent, promoted at 37°C *versus* 30°C. Thus, temperature appears involved in the regulation of SCV phenotype emergence, triggered when a specific temperature is reached. Using fluorescence labelling, we confirmed that SCV bacteria form biofilms more readily compared to the wildtype. The prevalence of the SCV phenotype in diverse environments could be involved in bacterial persistence *via* the formation of biofilms, not exclusively in the host environment. The SCV phenotype is associated with high concentration of the second messenger cyclic-di-GMP. In order to identify the regulatory pathways involved in the emergence of SCVs, a screening of PA14 mutants in the genes coding for enzymes involved in synthesis and degradation of cyclic-di-GMP resulted in the identification of three mutants in which the SCV phenotype was not observed, even under favorable conditions. These genes could be involved in the regulation of the SCV phenotype emergence. This is the first report of high prevalence of SCV phenotype among *P. aeruginosa* strains, regardless of the origin of the isolates. *Pseudomonas aeruginosa* could use phenotypic diversification as a bet-hedging strategy to rapidly adapt and persist into diverse environmental conditions, accounting for its versatility and persistence.

Key words: *Pseudomonas aeruginosa*, Small colony variant, biofilm

PDR106

Discovery of an anti-virulence compound targeting the bacterial envelope stress response that reverses β-lactam resistance in MRSA

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Staphylococcus aureus is the leading cause of both hospital and community-associated infections worldwide and a major cause of morbidity and mortality due to the emergence of methicillin-resistant *S. aureus* (MRSA). The pipeline for innovative antibiotics is insufficient to overcome this healthcare threat and, thus new strategies are urgently needed. Using a cell-based screen of ~45,000 diverse synthetic compounds, we discovered a potent bioactive, MAC-545496, that reverses β-lactam resistance in the community-acquired MRSA USA300 strain. Further, MAC-545496 attenuates the MRSA virulence *in vivo*. With potency at the low nanomolar range against MRSA, MAC-545496 inhibits biofilm formation, and abrogates intracellular survival in macrophages. Mechanistic characterization through chemical-genomics and biochemical approaches revealed GraR to be the cellular target of MAC-545496. GraR (**g**lycopeptide **r**esistance **a**ssociated protein **R**), a regulator responding to insults to the bacterial cell envelope by antibiotics and host innate immune defences, is an important virulence factor and antibiotic resistance determinant. The small molecule bioactive discovered herein is the first inhibitor against GraR; it can serve as: (i) an antibiotic adjuvant reversing methicillin resistance; and (ii) an anti-virulence agent effective as a monotherapy *in vivo* against MRSA. Together, this work provides a novel antibacterial lead series of new mechanism to combat drug-resistant Staphylococcal infections.

Poster Competition Abstracts

Wednesday, June 12th, from 3:00 - 5:00 PM

Presenting students are to be by their posters from 3:00 – 5:00 PM to respond to judges' questions.
Note: **AEM**: Applied Environmental Microbiology
II: Infection and Immunity | **MGCM**: Molecular Genetic Cellular Microbiology

II-PC01

Reovirus modulates host cell alternative splicing landscape during infection through the µ2 protein

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Viruses have evolved to become experts in modulating various cellular pathways to gain advantage over their host cells. This tug of war will ultimately determine the fate of the infected cells. Alternative splicing (AS) is a mRNA maturation step allowing enhanced variability in mature mRNAs originating from the same gene. Recent evidence showed that numerous viruses alter cellular AS during infection although our understanding of the role of these changes remains limited. In our recent work, we wanted to investigate if reovirus, as a model cytoplasmic RNA virus, alters the host cell AS during infection, the mechanism of this modulation, and the possible impact on viral replication. To study the AS landscape of the host cell, reovirus-infected cells were analyzed by high-throughput RNA-sequencing. We identified 240 AS events that were significantly modified during infection ($Q<0.05$), often affecting genes involved in RNA processing and maturation. Cells grown in the presence of infected cells do not exhibit changes in AS, as determined by RT-PCR analysis, highlighting that cellular AS changes require reovirus presence. To gain further understanding of the mechanism involved, we examined another reovirus strain, and observed that it is a less potent modulator of AS, revealing a strain-dependent phenotype. Reassortant viruses, obtained by reverse genetics, allowed us to map the difference in AS between strains to the µ2 protein. This protein is involved in other differences between reovirus strains, such as differences in induction of interferon. We validated by mass-spectrometry and immunofluorescence experiments that µ2 enters the nucleus, both during infection and transient expression. In conclusion, reovirus modulates cellular AS during infection, and the µ2 protein appears as the primary determinant involved. Investigations are ongoing to explain how µ2 modulates AS and its role in viral replication. These studies should improve our knowledge on the role of AS in virus-host interactions.

II-PC02

Changes in host cell gene expression levels and alternative splicing during *Flavivirus* infections

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Recently, viral infections have been shown to largely alter the host cell transcriptome by modulating gene expression levels and alternative splicing. Here, we studied these alterations of the host cell transcriptome during infections with three related viruses, all belonging to the genus *Flavivirus*, namely Kunjin, Zika, and Yellow Fever.

These viruses are transmitted by infected mosquitoes in tropical areas, thus exposing a large part of the world's population to the risk of infection. Although they share high genome and protein sequence similarity, the symptoms associated with each virus are quite divergent. For example, only Yellow fever virus but not Kunjin virus or Zika virus may cause hemorrhagic fever, and Zika virus is the only Flavivirus that is associated with microcephaly and Guillain-Barré syndrome. To gain insights into the mechanisms of Flavivirus infections and pathogenesis, we used RNA sequencing of infected cells. More precisely, U87 cells were cultured and infected *in vitro* with any of the three viruses, whereas mock-infected cells served as control. RNA was extracted from the cells 24h post-infection and polyA-RNAs were purified prior to next-generation RNA sequencing. Data from infected cells was compared to data from mock-infected cells in order to identify changes in the host cell transcriptome that occurred during the viral infection. Differential gene expression analysis was performed using DESeq2 and alternative splicing was analyzed using rMATS. Several hundred genes were found to be differentially expressed or spliced upon viral infection, and there was little overlap between the genes whose expression levels changed and the genes whose alternative splicing was affected. Moreover, the variations in the host cell transcriptome prompted by the viral infections were compared with each other, and many modulations in gene expression and alternative splicing were found to be virus-specific. These findings highlight the complexity of virus-host interactions and show that even closely related viruses have very different effects on their host cells.

II-PC03

Heterogeneity in two-component signalling systems within different strains of inflammatory bowel disease associated *Escherichia coli*

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Inflammatory bowel disease (IBD) is characterized by chronic inflammation of the gastrointestinal tract. Patients with IBD possess distinct microbiota populations that differ from the gut microbiomes of healthy individuals. An example of this is a higher incidence rate of adherent-invasive *Escherichia coli* (AIEC) in diseased patients. AIEC elicit host immune responses such as inflammation which results in elevated production of small cationic antimicrobial peptides called host defence peptides (HDPs). Previous work in the McPhee lab has demonstrated that many clinical AIEC isolates are highly resistant to killing by HDPs. However, the magnitude of HDP-resistance varies greatly from strain-to-strain. As a result, we wanted to examine the underlying mechanisms behind the observed heterogeneity in these resistance phenotypes. The PhoPQ two-component system (TCS) is a well-characterized signalling pathway which responds to environmental conditions such as divalent cations and antimicrobial peptides. In response to such stimuli, several genes downstream are up-regulated ultimately leading to outer membrane modification resulting in resistance to antimicrobials. Here, we hypothesize that strains with different resistance profiles to HDPs have an altered capacity to signal through the PhoPQ system. To assess this, we created a promoter-GFP fusions of one of the PhoPQ regulated genes, *pmrD*, to monitor PhoPQ signalling in a panel of eight clinical AIEC isolates. Our data shows that strains have different baseline PhoPQ signalling potential, and also robust and significant differences in signalling when cultured under identical inducing and non-inducing conditions, supporting our hypothesis. Further, the altered signalling from our reporter screen matches resistance phenotypes from polymyxin B killing data collected using the same clinical isolates and conditions. Overall, our data strongly suggests that strains have an altered potential to respond to environmental signals, ultimately resulting in a broad level of resistance phenotypes. This novel finding provides mechanistic insight into how strains are able to differentially regulate resistance to HDPs.

II-PC04

Characterization of three new SPATE autotransporters and cumulative role of SPATEs in pathogenesis of Extra-intestinal pathogenic *Escherichia coli*

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Serine protease autotransporters of *Enterobacteriaceae* (SPATE) are associated with various pathogenic extraintestinal pathogenic *Escherichia coli* (ExPEC) including avian pathogenic *E. coli* (APEC). We have identified three new genes encoding SPATE proteins, two located adjacent to each other on a genomic island in an APEC strain and another in the plasmid (5 SPATES in total), and we refer to them as "tandem autotransporter genes, *tagB*, and *tagC* and serine-protease hemagglutinin autotransporter, *Sha*". Interestingly, these autotransporter genes are present in some APEC and also some human uropathogenic *E. coli* (UPEC). The possible function and roles of these new SPATES in the pathogenesis of ExPEC were investigated.

Clones of these proteins were tested for various phenotypes including adherence to human renal and bladder cell lines, biofilm formation, autoaggregation, cytotoxicity, and hemagglutination, which represent possible mechanisms of colonization of the host. Results showed that these SPATEs are autoaggregating, hemagglutinating and can promote adherence to the HEK 293 renal and 5637 bladder cell lines, but did not contribute significantly to biofilm production. TagB and TagC exhibited cytopathic effects on the bladder epithelial cell line. Following transurethral infection of CBA/J mice with a *tagBC* mutant or *sha* mutant, no significant difference in colonization was observed. However, the competitive fitness of a mutant derivative lacking all 5 SPATEs was significantly lower in the kidney. This underlines the potential cumulative role of SPATEs for survival and competitive fitness during extra-intestinal infection.

AEM-PC01

Reevaluation of biological nitrogen fixation (BNF) by deadwood of boreal forest

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The boreal forest represents ~50% of the world forest, and 30% is in Canada. It contributes to the storage of 0.5 Peta grams of C per year (10^{15} g/an). Primary production in boreal forests is strongly limited by nitrogen bioavailability. Although boreal soils are rich in organic nitrogen, its bioavailability is low due to slow decomposition at these latitudes. The two main sources of available nitrogen are atmospheric depositions and biological nitrogen fixation (BNF). Nitrogen depositions are low (< 3 kg $N.ha^{-1}.yr^{-1}$) in most of the Canadian boreal forest (north of the 50th parallel), so the main source of new nitrogen is BNF. Deadwood contributes to biodiversity: more than 25% of forest species are dependent of this deadwood (e.g. bryophytes, lichens, insects, saprophyte fungi). It also plays an important role in both the nitrogen and carbon cycles. It contributes to the carbon sink by storing ~40 Tg $C.yr^{-1}$. Deadwood also harbors N_2 fixers that supply N to the microbial community during decomposition. However, the contribution of deadwood to net N inputs in boreal ecosystems remains unclear. Indeed, BNF estimates for deadwood are highly variable and range from 0.085 kg $N.ha^{-1}.yr^{-1}$ to more than 2 kg $N.ha^{-1}.yr^{-1}$. Several methodological biases could explain this variability. The main proxy used to estimate BNF is Acetylene Reduction Assay (ARA). Acetylene inhibits methanotrophs BNF, which are abundant in deadwood.

Furthermore, the conversion of ARA data into BNF estimates requires the use of a conversion ratio (C_2H_4/N_2 = R-ratio) which varies between 3 and 0.5 depending on which isoform of the nitrogenase, the enzyme responsible for N_2 fixation, is active. Despite several evidence that several isoforms likely coexist in deadwood, the R-ratio of 3 (corresponding to molybdenum isoform) is systematically applied, dismissing the potential contribution of alternative nitrogenases (based on vanadium and iron only). Finally, underground deadwood, that represent up to 20% of above-ground biomass and decompose in condition that are more favorable to BNF (e.g. moisture, nutrient availability), is rarely considered in estimates. Wei et al (1998), estimated that underground deadwood contributes up to 1 kg $N.ha^{-1}.yr^{-1}$, highlighting the importance to consider underground deadwood. In this study we reexamined the contribution of BNF by deadwood in the boreal forest (balsam fir, black spruce and white birch). We carefully evaluated the contribution of methanotrophs and underground deadwood to BNF. Results showed that methanotrophs and underground deadwood significantly contribute to BNF. BNF activity by underground deadwood was similar to the activity measured in late stage of decay of aboveground deadwood (the most active stage of decay). The addition of methanol to alleviate inhibition of methanotroph BNF by acetylene resulted in most case in the doubling of BNF activity. Analysis are underway to evaluate the contribution of alternative nitrogenase to BNF. In conclusion, our data show that BNF by deadwood has likely been largely underestimated in boreal forests. Even if our corrected estimate suggests that N input deadwood is low, 1kg $N.ha^{-1}.yr^{-1}$, it remains a significant contribution in this highly N limited ecosystem.

AEM-PC02

Metagenomic analysis of virus diversity and relative abundance in a freshwater harbour

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Shotgun metagenomics has rapidly expanded DNA sequence databases and led to ground-breaking advancements in many areas of viral ecology. While marine viruses have been extensively studied over the past two decades, less research has been conducted on viruses in freshwater environments. The goal of our research was to characterize virus communities and capture fluctuations in the communities at nearshore and mid-harbour locations in a freshwater harbour over the mid-summer to early autumn. Water samples were filtered through 0.22 µm pore-size filters, and DNA was extracted and sequenced on an Illumina HiSeq 2500 platform. The resulting 250-bp reads were subjected to quality control and assembled into contigs. Contig alignment and annotation was achieved via BLASTx searches against the NCBI-nr database and relative abundances were obtained by mapping reads back to contigs. Assembled contigs were annotated as belonging to the virus groups *Caudovirales, Mimiviridae, Phycodnaviridae*, virophages (*Lavidaviridae*), unclassified bacteriophages, other dsDNA viruses, and ssDNA viruses. *Caudovirales* infect bacteria and typically comprise 10-40% of aquatic virus communities. *Mimiviridae* (including the so-called 'extended *Mimiviridae*') have been isolated primarily from aquatic environments and are a diverse group of dsDNA viruses that infect protists and eukaryotic algae. Until recently, *Phycodnaviridae* were considered the dominant group of algae-infecting viruses since all known dsDNA viruses of eukaryotic algae belonged to this family, but the recent expansion of the *Mimiviridae* has challenged that notion. Virophages are small viruses that co-infect eukaryotic hosts with some *Mimiviridae* and benefit their hosts by diminishing *Mimiviridae* propagation through the production of inactive *Mimiviridae* virions. The mid-harbour site displayed wide fluctuations in virus relative abundance, while the nearshore site was relatively stable. Contigs annotated as virophages were often the most abundant members of the community, especially at the nearshore site. At the mid-harbour site, virophages exhibited a wide range of abundance from >70% of the community to nearly undetectable. Virophages originally detected in Dishui Lake, China were consistently the most abundant virophage annotations. *Mimiviridae* were the second most abundant family, comprising an average of 21% of the virus community across all samples. Interestingly, though they are presumably intimately associated, *Mimiviridae*

abundances did not fluctuate to the same extent as the virophages. Diverse *Mimiviridae* contigs were detected in the samples, and the nearshore and mid-harbour sites appeared to host distinct *Mimiviridae* communities. *Phycodnaviridae* were a minor component of the virus community regardless of site or sampling date, suggesting that *Mimiviridae* were the dominant algae-infecting viruses in this system. In contrast to other studies of eutrophic freshwater lakes, *Caudovirales* were highly abundant in only 2 of 10 samples. Our results provide insights into virus community composition in eutrophic freshwaters, highlighting the complexity of virus ecology. They also expand the diversity of documented communities and highlight the potential ecological importance of virophages and gaps in our understanding of how these presumably intimately associated viruses interact with their hosts.

AEM-PC03

A model cooling tower harboring *L. pneumophila* and *V. vermiformis*: Is there Legionella in the biofilm?

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Legionella pneumophila is a waterborne bacterium known for causing Legionnaires' Disease, a severe pneumonia. Water systems, such as cooling towers, are major sources of infection, given that they provide ideal growth conditions and produce aerosols for its transmission to human. The conditions promoting growth in water systems are not well understood; however, it is known that *Lp* must grow inside host cells, such as amoebas and ciliates. More specifically, the relationship between *L. pneumophila* and the resident microbes needs to be clarified. Cooling towers that show high heterotrophic plate counts do not necessarily harbor *L. pneumophila* suggesting that such cooling towers could host a microbial population resistant to colonization by *L. pneumophila*. In addition, it is not clear where in the towers *Lp* preferentially grows.

Therefore, we built a lab scale cooling tower model to study the dynamic of *L. pneumophila* colonization in relationship to resident microbiota and spatial distribution. The model was inoculated with water from an actual cooling tower harboring low levels of *L. pneumophila*. After a period of 8 weeks the model was seeded with *Vermamoeba vermiformis*, a natural host of *Lp.* After two more weeks the model was seeded with *Lp*. After two more weeks the model was disassembled, the water was collected and biofilm was extracted from the pipes. DNA was extracted and the microbiome was studied using 16S rRNA and 18S rRNA amplicon sequencing.

The bacterial communities of the biofilm were different then the bacterial communities of the water samples. Interestingly, *V. vermiformis* and other ciliates were present in the biofilm but not in the water of the model. In contrast, *L. pneumophila* was detected in the water but not in the biofilm, suggesting that after growth in host cells *Lp* is released in the water phase.

AEM-PC04

How an Antarctica *Rhodotorula* novel strain adapts to cold environments - a combination of Phenotypic MicroArray, mRNA transcriptomic and small RNA analysis.

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The majority of the Earth's biosphere exists under cold temperatures, permanently below 5°C. These include multiple cryoenvironments, many of which are characterized by the most extreme conditions on Earth. However, cryophilic microorganisms are able to thrive in these harsh conditions, as evidenced by active metabolic respiration and in situ growth. Despite the fact that multiple adaptations to cold temperatures have been documented for microorganisms, the study of cryomicrobiology is poorly understood with regards to the cold temperature limits of bacterial viability, and strategies for overcoming these challenges. These organisms are often characterized as extremophiles. The genus *Rhodotorula* includes many species capable of growing under extreme conditions. *Rhodotorula* are pink-pigmented, unicellular yeasts and can be found in diverse extreme environments such as deeps hydrothermal vents, and glaciers. More recently, a novel *Rhodotorula* specie, *Rhodotorula* sp. JG1b, has been isolated from a permafrost sample of the polar desert University Valley, in Antarctica, and suggest to have novel metabolic capabilities and to have the possibility of expressing novel pathways to thrive under extreme conditions. Here, we assess and characterize the metabolic activity pathways of *Rhodotorula* JG1b under a range of different growth temperatures via Phenotypic MicroArray technology (RT, 0°C, and -5°C), transcriptomic analysis, and small RNA analysis (RT and 0°C). Based on the Biolog PM assay, *Rhodotorula* JG1b has a high diversity of metabolic pathways while incubated at optimal growth conditions (RT). The strain is able to uptake the majority of the carbon sources providesdby the plates and almost all the nitrogen sources. On the other hand, when incubated at cold temperature (0°C and -5°C), the metabolic diversity decrease drastically, and only few substrates are used by *Rhodotorula*. Also, the proportion of small RNA is greater in the 0°C cultures than the RT cultures for a similar stage of growth. For the mRNA transcriptomic analysis, RT cultures express more genes related to the basic metabolism and growth (cellular respiration, ATP synthesis, electron transport chains, ribosomal components, etc.) than 0°C cultures, and those 0°C cultures seem to express more genes related to transport activity than the RT cultures. Put together, these results suggest that *Rhodotorula* JG1b have a shift in metabolic pathways from optimal temperature to cold temperature and the metabolic diversity is highly affected. The transcriptomic results can be linked with the Biolog PM assay as there is a decrease in the substrate consumption and that 0°C culture express less genes related to metabolism and growth than room temperature cultures. At term, this study will show for the first time that small RNA plays a role in adaptation to cold environments in yeast and will inform on the metabolic strategies that eurypsychrophilic yeast develop to face such challenges.

MGCM-PC01

Genome-scale metabolic modelling reveals key features of a minimal gene set for *Mesoplasma florum*

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Studying minimal cells could lead to the description of every molecular function required for autonomous cellular life. In turn, this exhaustive knowledge could facilitate the establishment of fundamental constraints to which cellular systems are submitted. Recently, a genome-scale model of metabolism (GEM) was reconstructed for the synthetic minimal organism JCVI-Syn3.0, a robust working approximation of a minimal cell. The parent of JCVI-Syn3.0, *Mycoplasma* mycoides, is phylogenetically close to the fast-growing, non-pathogenic and near minimal bacteria *Mesoplasma florum*. Here we reconstructed a GEM for *M. florum* and constrained it with experimental data. The set of molecular functions in *M. florum* was extracted using three different computational approaches. Firstly, the structure of 386 *M. florum* proteins were reconstructed. Secondly, enzyme commission numbers (EC) were compared between the sequence-based identification from DETECT-v2 and the structure-based identification from COFACTOR. 117 genes were associated to an EC number with a high-level of confidence. Fianlly, the *M. florum* proteome was compared to that of 4 other

species of mollicutes for which a GEM was available, including JCVI-Syn3.0. 411 proteins were conserved between *M. florum* and JCVI-Syn3.0, with ~90% (139/155) of the JCVI-Syn3.0 model proteins conserved in *M. florum*. The metabolic network of *M. florum* was then reconstructed that contains 209 genes. Dividing the network in 6 functional modules revealed that no JCVI-Syn3.0 homologs were found in the “Glycans” module, and the “Lipids” module only shared half of its gene content. The genes in these modules were also associated with fewer EC numbers and homology models. Integrating the experimental characterization of a semi-defined growth media with the use of flux-balance analysis allowed comparing growth phenotypes on 14 different carbon sources. While the model correctly predicted the growth phenotype on 11/14 sources, maltose, glycerol-3-phosphate and ribose were incorrectly predicted. Reviewing the discrepancies between observation and predictions will provide a basis to re-annotate the *M. florum* genome.

MGCM-PC02

Single-stranded DNA annealing and ATP-dependent DNA unwinding are conserved activities of hairpin telomere resolvases

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In contrast to most prokaryotes, the *Borrelia* and *Agrobacterium* genera possess linear replicons including both chromosomes and plasmids. This configuration of DNA presents a problem with priming lagging strand synthesis at the termini of the 3' ends, referred to as the end-replication problem. These species circumvent this problem through the use of hairpin telomeres, covalently closed hairpin loops at their termini that eliminate the free end. These hairpin telomeres are resolved from a replication intermediate consisting of a DNA dimer with inverted repeat symmetry joined by replicated telomere junctions on both ends. A diverse class of enzyme referred to as a telomere resolvase binds as a dimer to these replicated telomere junctions and performs a two-step cleavage and rejoining reaction to produce two linear replicons terminated by hairpin telomeres.

Previously, our lab focused on the biochemical characterization of the *Borrelial* telomere resolvase, ResT. Aside from its expected property of telomere resolution, ResT was shown to promote the annealing of single-stranded DNA and also possessed a 3'-5' unwinding activity, *in vitro*. These properties are unrelated to telomere resolution and thus, provide evidence for multifunctionality in ResT. Furthermore, these unexpected properties of annealing and unwinding have no known function *in vivo*. We sought to analyze a second member of this diverse enzyme family to determine whether these unexpected activities are unique to ResT or represent a property of the enzyme family, and if these activities were identified to create separation-of-function mutants for these unexpected activities to eventually assess their function and importance in an *in vivo* context. We selected the telomere resolvase, TelA from *Agrobacterium tumefaciens* as it is the closest to ResT in both size and sequence homology and has available structural data.

We found TelA also possessed the ability to promote the annealing of single-stranded DNA and a 3'-5' unwinding activity, suggesting these are conserved features of the telomere resolvase family despite its diversity. Furthermore, we identified two promising candidates for separation-of function mutants. Truncation of the N-terminal domain of TelA abolishes its annealing activity while still retaining both its unwinding and telomere resolution capabilities, and mutation of a serine residue (TelA S238A) renders a severely compromised unwinding mutant that still maintains its annealing and telomere resolution activities. These mutants provide a platform for future *in vivo* functional studies.

MGCM-PC03

Antimicrobial resistance dissemination in *Vibrio cholerae*: mechanistic insights into the insidious role of IncC plasmids

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Cholera is an acute diarrheal disease that leads to severe dehydration and often death in the absence of adequate treatment. This infectious disease remains a formidable affliction worldwide, accounting for millions of cases every year. Furthermore, climate change may alter temperature and salinity in the Gulf of Saint Lawrence and the Pacific coast off British Columbia to the point of rendering these two environments highly suitable for *Vibrio cholerae*, prompting concerns about a future comeback of the disease in Canada.

Since the beginning of the ongoing 7th cholera pandemic, antibiotic resistant isolates have arisen and spread to the extent that they are now ubiquitous in Africa. Development of resistance in *V. cholerae* has been ascribed to mutations and acquisition of mobile genetic elements such as integrative conjugative elements and conjugative plasmids of the IncC incompatibility group.

IncC plasmids are encountered across a broad range of Gammaproteobacteria, among which they act as important conveyors for a variable cargo of antibiotic resistance determinants. In recent years, it has become clear that not only do IncC plasmids spread their own cargo of resistance genes, they also mediate the conjugative transfer of mobilizable genomic islands (MGIs). A key actor in this interplay is AcaCD, the master activator of IncC plasmid transfer. AcaCD binding sites are found upstream of various plasmid- and MGI-borne operons, some of which are known to be involved in conjugation. We previously identified MGI*Vch*Hai6, one such MGI integrated into the 3' end of *trmE* in non-O1/non-O139 clinical *Vibrio cholerae* isolates from the 2010 Haiti cholera epidemic. MGI*Vch*Hai6 excises and transfers at a high frequency in the presence of an IncC plasmid, and confers resistance to β-lactams, cotrimoxazole, tetracycline, chloramphenicol, streptomycin and spectinomycin.

Here we present the experimental validation of predicted AcaCD binding sites in MGI*Vch*Hai6, the localization of its minimal origin of transfer (*oriT*) and the identification of additional factors involved in the conjugative transfer of MGI*Vch*Hai6. Putative AcaCD-dependent promoters were cloned upstream of a reporter gene and colorimetric assays were performed to assess their response to AcaCD expression. Cloning into a non-mobilizable vector and progressive reduction of an intergenic region conserved in MGI*Vch*Hai6-like MGIs, followed by mobilization assays by an IncC plasmid, allowed the circumscription of a minimal *oriT* locus within a 50-bp fragment. Finally, several plasmid- and MGI-borne genes thought to take part in MGI*Vch*Hai6 transfer were independently knocked out to assess their role in conjugative transfer.

Altogether, these results enabled us to elaborate a mechanistic model for the mobilization of MGI*Vch*Hai6 by IncC plasmids, thus providing us with valuable insight into the insidious role played by IncC plasmids through the mobilization *in trans* of antimicrobial resistance genes.

MGMC-PC04

Unique synergistic interaction in *Bacteroides* reveals unexpected hemolytic phenotypes

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Bacteroides is a genus of abundant commensals in the gut flora that confer multiple health benefits to the host. They are currently classified as nonhemolytic. Work with *Bacteroides* isolates lead to the observation, by happenstance, of unexpected hemolytic activity in multiple species. After incubation on blood agar plates, we observed that some isolates were clearly hemolytic, but only when plated in close proximity to certain, 'activating' isolates. When we probed a larger collection 94 of isolates, we identified one which was able to activate hemolysis in 30% of the collection! Furthermore, the asymmetrical patterns of hemolysis were unlike any bacterial synergy reported to date. Using a custom timelapse set-up, we show this zone of hemolysis begins between the two colonies and proceeds in a retrograde fashion towards the 'activated' colony. Seeking to investigate the mechanism behind this pattern, we have performed whole genome sequencing of isolates of interest. We have found several hemolysin orthologs and are using a comparative genomics approach to better understand this unique phenotype.

II0

SalmoSeq, an AmpliSeq-based molecular tool for rapid identification and virulence prediction of *Salmonella*

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Identification of food-borne *Salmonella* and their potential in causing disease and epidemics is based upon species identification and serotyping of which require from 6-10 days. Several whole genome sequencing (WGS) and PCR-based methods have been proposed for diagnostics. Standard microbiological as well as molecular methods have several limitations in terms of cost, specificity, time constraints and do not indicate the levels of virulence. We have developed SalmoSeq, a *Salmonella* AmpliSeq™ tool that uses next-generation sequencing panels as a rapid, cost-effective and on-demand for identification and prediction of the levels of virulence in *Salmonella.* One of the major challenges in developing AmpliSeq™ for rapid diagnostics in infectious diseases is the limitation of biological data clearly linking species identification, virulence, and antimicrobial susceptibility genotypes and phenotypes in a single diagnostic tool. Most gene panels used in the building of oligonucleotide pools for AmpliSeq™ rely on *in silico* bioinformatics data and the biological data from various approaches described in the literature. We have developed a completely different strategy that uses biological data in four different models of infection to clearly define high and low levels of virulence combined with phylogenomics and a series of complementary methods for rigorous statistical analysis aiming at a value of >99% for the identification and the prediction of virulence in *Salmonella*. The biological data obtained from 40 *Salmonella* isolates screened in four models of infection for levels of virulence was combined for further analysis with their high-quality WGS genotypes. This information was used in a combined two-pronged method using phylogeny-based gene clusters and k-mer identification from sequences obtained by WGS. Starting with an initial number of 31-mers of $2{,}59 \times 10^7$ from 40 genomes and their frequency as high or low in virulence, statistical analysis was performed to determine significant association with phenotypes (various thresholds in FDR corrected P values considered). Our approach identified a collection of 49,231 k-mers in over 100 genes that were retained as the most promising candidates for *Salmonella* AmpliSeq.The SalmoSeq based upon k-mer association with virulence has been estimated to be the best approach in developing a new diagnostic tool capable of identifying *Salmonella* rapidly. In addition to its simplicity of use, rapid turnover in time and in implementation, SalmoSeq will be able to identify >99% of *Salmonella* isolates as well as predicting their levels of virulence.

II1

A novel plasmid-encoded fimbriae from extra-intestinal pathogenic *Escherichia coli* (ExPEC) contains a distinct type of P fimbrial adhesin

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Extra-intestinal pathogenic *Escherichia coli* (ExPEC) infect both human and animals. Virulence factors including fimbriae, promote adherence of ExPEC to host cells. We identified a new P fimbriae from an avian *E. coli* strain. P fimbriae are associated with *E. coli* from urinary tract infections. We investigated this new fimbriae and compared it with other types of P fimbriae. This new fimbriae is located on a Col-V plasmid on a cluster of 10 genes. The PapG-like adhesin is very distinct from the previously characterized P fimbrial G adhesins suggesting it belongs to a new adhesin class for P fimbriae. The new P fimbrial G adhesin shares 24% identity to PapG class I, 27% with PapG class II and 31% with PapG class III adhesins respectively. *In vitro* analyses have shown that clones of P-like fimbriae in *fim*-negative *E. coli* K-12 strain produce a filamentous structure on their surface. The same strain was also able to hemagglutinate turkey and human blood. However, PapG class I was able to hemagglutinate pig, rabbit, dog and human blood, class II was only able to hemagglutinate pig and human blood and class III was able to hemagglutinate pig, dog and sheep blood. Results also shown that the P-like and the P fimbriae clones were able to adhere to human kidney and bladder cell lines and form biofilm at 37°C. Further, this novel type of P fimbrial adhesin was also identified in some human ExPEC. Overall, results indicate that this new type of P fimbriae, initially identified from a strain pathogenic to poultry demonstrates, a distinct hemagglutination profile recognizing both human and avian erythrocytes. It will be of interest to confirm it's potential role for colonization or virulence.

II2

A siderophore analog of fimsbactin from Acinetobacter hinders growth of the phytopathogen *Pseudomonas syringae* and induces immunity in *Arabidopsis thaliana*

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Background: Beneficial rhizobacteria aid plants in many ways. One of the several means rhizobacteria help plants is by producing siderophores, which can solubilize iron in the rhizosphere for the benefit of the plants. Siderophores might also be perceived by plants as pathogen-associated molecular pattern (PAMP), prompting activation of plant defenses against phytopathogens. We explored the role of a synthetic siderophore (SID, mixed bis-catechol-mono-hydroxamate) that imitates the chemical structure of a natural siderophore, fimsbactin, produced by the genus *Acinetobacter* in the resistance against the phytopathogenic agent *P. syringae pv tomato* DC3000 (*Pst*) in the plant model *Arabidopsis thaliana.* We here report that the synthetic siderophore SID acts as an antibacterial agent as well as an inducer of systemic resistance in *A. thaliana* against *Pst*.

Materials/Methods: To evaluate the antibacterial activity of the SID against *Pst,* we used Luria-Bertani (LB) broth supplemented with 300 µM of 2,2'-bipyridyl and 50 µg/ml of $CaCl_2$, as an iron depleted medium. For the assessment of the protective role of the SID in *A. thaliana,* roots were pretreated with the SID (100 µM) prior to the inoculation of leaves with *Pst* (~10^5 CFU/mL) 24h later. Entire infected leaves were collected for CFU counts at 0- and 3-days post-infection. To identify the molecular mechanisms of resistance induced by the SID, *A. thaliana* roots were pretreated with the SID (100 µM) as mentioned above prior to the inoculation of leaves with *Pst* (~10^6 CFU/mL). Infected leaves were then collected 8h post-infection for RNA extraction to characterize *A.*

thaliana genes induced by the SID and/or the infection and that are involved in the mechanism of resistance. RT-PCR was used to measure markers of two immune pathways in plants, particularly *PR1* for the salicylic acid (SA) pathway and *PDF1* for the jasmonic acid (JA) pathway.

Results: In the iron depleted medium, *Pst* growth was inhibited by the SID at the concentration of 8 µM. The bacterial growth decreased by about 7-fold (0.69 log10, $p < 0.05$) in the SID pretreated group compared to the control group 3 days after infection. *PR1,* a systemic resistance marker, was strongly expressed in the SID pretreated and infected group ($p < 0.05$ *vs* non-pretreated and/or infected groups).

Conclusions: Our synthetic SID, mimicking a natural SID from *Acinetobacter*, inhibits *in vitro* the growth of the pathogenic agent *Pst,* and helps in a significant reduction of the bacterial growth 3 days post-infection in *A. thaliana.* Furthermore, the *PR1* gene, a marker of the SA pathway, which is involved in the systemic immune response, is strongly induced in the SID pretreated and infected group. These results suggest that siderophores (natural or synthetic) like the SID, could assist in the fight against phytopathogenic bacteria, and consequently help decrease the use of pesticides in agriculture.

II3

Analyse structurale de la protéine TraE impliquée dans la conjugaison bactérienne chez *E. coli*

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Antimicrobial resistance (AMR) against antibiotics is a global issue. A recent study estimates that in 2050, the major cause of death will be due to AMR. Several mechanisms are responsible of bacterial resistance acquisition. One of them is the conjugation which is transfer of genetic material between bacteria. The driving force responsible of this genetic exchange is a macromolecular complex known as the type IV secretion system (T4SS), initially discovered in *A. tumefaciens*. In this model, the T4SS is composed of 12 proteins named VirB/VirD4. In *E. coli*, homologous VirB/VirD complex are known as Tra protein complex.

We cannot solve the problem of AMR just by searching new antibiotics because AMR is a natural phenomenon. Some alternative methods need to be put in place. One of them consist to directly target the T4SS and it is one of the objectives of the laboratory. Some studies show that VirB8 is essential in the process of bacterial conjugation. So, the purpose of my PhD project is to determinethe structure at high resolution of TraE protein by using cryo-electron microscopy.

This project will allow us to know more about the structure and the functioning of the T4SS and also to target some interesting sites in order to destabilize the complex and inhibit horizontal transfer.

II4

Bacterial biofilms and their role in Beta-amyloid agregation and Alzheimer's disease

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Alzheimer's disease (AD) is a neurodegenerative illness and the first cause of dementia worldwide. In AD patients, the degradation pathway of the Amyloid Precursor Protein (APP) shifts towards the Beta-secretase to

generate the Amyloid-Beta (Aβ), a small peptide that aggregates into amyloid plaques and elicit a strong inflammatory response. Recent studies have suggested an antimicrobial role for this peptide. Periodontist is currently one of the more important risk factors for late-onset Alzheimer's disease. *Porphyromonas gingivalis* (Pg) and *Treponema denticola* (Td) are the two main anaerobic bacteria involved in periodontitis. Persistence of these bacteria in the body is directly linked with biofilm formation, an extracellular matrix made mostly of polysaccharides, which protects cells from extracellular stresses. Our hypothesis is that bacterial biofilms might enhance accumulation of the Aβ peptides into amyloid plaques, expediting the neurodegeneration. Thus, we want to observe the interplay of Aβ peptides and biofilm formation by various bacterial species including Pg and Td. First, we will examine the anti-microbial effect of Aβ by co-cultivating biofilms in presence of the peptide. Immunofluorescence imaging of Pg and Td biofilms with the Aβ peptide will allow us to determine if there is accumulation of Aβ within the biofilm, and if so to quantify aggregates present in the biofilm matrix. Secondly, we will co-culture human neural cell-lines with bacterial biofilms to quantify Aβ production by neurons in presence of bacteria, and we will observe the structures formed by the produced peptides. This project will lead to a metabolomics analysis of the supernatant produced by the co-culture, to characterize the molecules produced in this type of bacteria-neural cell interactions. Our ultimate goal would be to determine a metabolic profile hinting at bacterial-induced Alzheimer's disease that would open the door for a non-invasive detection tool allowing early detection and management of the disease.

II5

Characterization of a bacterial feeding strategy based on the manipulation of the host sugar export machinery

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Sugars are among the most abundant nutrients on Earth and is often a target to be acquired by plant pathogens from their hosts. However, how pathogens benefit from this resource during an infection in plants remains unresolved. Successful bacterial pathogens encode an artillery of virulence effector proteins capable of being translocated into host cells to sustain their growth at the host-pathogen interface. Plants have evolved a large number of immune receptors capable of recognizing microbe-associated molecular patterns (MAMPs) at their cell surface, resulting in the activation of an immune response to restrict pathogen growth. To cause disease, pathogens not only need to possess effectors able to disrupt this layer of immunity, but also to establish an environment favourable to pathogen growth at the host-pathogen interface. *Pseudomonas syringae pv. tomato* DC3000*(Pst*DC3000) is able to grow in the extracellular environment of its host cells. Here, we show that *Pst* DC3000 has the ability to induce a massive translocation of sugars from the host cell to the extracellular space, depleting starch stores over time, in a type-3 secretion system (T3SS)-dependant manner. By using a library of *Pst*DC3000 type III effector mutants, we have identified potential effector candidates responsible for inducing the expression of sugar efflux transporters, namely Sugar Will Eventually be Exported Transporters (SWEET), *in planta.*Furthermore, we find that the expression of SWEET transporters is reduced after exposure of *Arabidopsis thaliana*leaves to MAMPs. As such, our results reveal a crucial strategy used by *Pst*DC3000 to hijack the host sugar transport machinery to create an optimal extracellular space for pathogen growth.

II6

Characterization of the host-mediated modification of bacterial effector NleA and its role in virulence

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Enterohemorrhagic and enteropathogenic *Escherichia coli* (EHEC and EPEC) are gastrointestinal pathogens transmitted via the fecal-oral route that pose a significant threat to human health. EHEC and EPEC cause diarrheal illness, which in the case of EHEC can progress to haemorrhagic colitis and to the potentially lethal hemolytic uremic syndrome. EHEC, EPEC, and the related mouse pathogen *Citrobacter rodentium* belong to a group of related Gram-negative pathogens characterized by their ability to form “attaching and effacing” (A/E) lesions on the surface of host cells during intestinal colonization. Upon adherence, these pathogens inject effector proteins directly into cells of the host intestine. Effector proteins alter host cell biology to help favour survival and replication of the pathogen. One of these injected proteins, the Non-LEE Encoded effector A (NleA), is a major virulence factor necessary for EHEC and EPEC virulence. NleA undergoes a mobility shift upon translocation into the host cell, suggesting a host-mediated modification of the protein. It was determined that NleA is not phosphorylated or ubiquitinated in the host cell. Recently, using a modified CHO cell line with a reversible defect in glycosylation (CHO-LDLD), we have obtained evidence consistent with the modification of NleA by mucin-type O-linked glycosylation. This is also supported by a bioinformatics prediction program that predicts a serine- and threonine-rich region of NleA to be modifiable by O-linked glycosylation as well as the localization of NleA to the Golgi apparatus, the host cell site for this type of modification. Importantly, whereas the rest of the NleA protein sequence is well conserved between bacterial isolates, we have noted an expansion or contraction of the serine- and threonine-rich region of NleA in some strains. We hypothesize that A/E pathogens modulate host cell biology using the effector NleA to provoke disease, and that its modification upon translocation into the host cell is important for virulence. By determining the precise functional role of NleA in the virulence of A/E pathogens, we anticipate uncovering novel insights into host-microbe interactions in the intestine, and opening new avenues for potential drug targets and treatment of the diseases associated with these bacterial infections.

II7

Characterizing *Staphylococcus aureus* endovascular infection during vancomycin treatment

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Staphylococcus aureus is a significant human pathogen that can infect virtually any tissue and cause severe life-threatening infections such as pneumonia, endocarditis and sepsis. There have been numerous reports that the standard treatment of methicillin-resistant *Staphylococcus aureus* (MRSA) with vancomycin can be ineffective, despite in vitro susceptibility of the organism to this agent. Reduced susceptibility to cationic peptides and increased biofilm formation have been associated with reduced responsiveness to vancomycin. Additionally, it is believed that the ability of *S. aureus* to survive within host cells also play a role in persistence during antibiotic treatment in clinical and experimental settings.

Previous studies have shown that in macrophages containing *S. aureus* bacterial replication begins several hours after infection and precedes phagocyte death. Thus, we hypothesized that *S. aureus* USA300 also exploits the intracellular environment of endothelial cells for survival and evasion of antibiotic treatment during infection. To investigate the intracellular fate of USA300, we performed gentamicin protection assays on human endothelial

cells (HMEC-1s and HUVEC-TERTs). These assays allowed us to measure bacterial adhesion, invasion and growth over time. Our findings demonstrate that *S. aureus* rapidly invades endothelial cells and resides intracellularly for several hours (6-8h) before replication is initiated. Analysis of the bacterial burden within HMEC-1 and HUVEC-TERT cells revealed that *S. aureus* increases ~6 and ~4 fold respectively. Prolonged exposure of the bacteria to sub-MIC levels of vancomycin before infection impairs adhesion to endothelia – we observed a reduction of ~23% when compared to untreated bacterial cells. In contrast, invasion of endothelial cells is not affected by exposure and occurs in both the presence and absence of vancomycin.

To ascertain the intracellular fate of *S. aureus* in endothelia, fluorescence-based proliferation assays paired with immunofluorescence were performed. These analyses demonstrated that *S. aureus* replicated within infected endothelial cells and the bacteria were constrained by lysosome associated membrane protein 1 (LAMP-1) - positive membranes. Together these data show that *S. aureus* USA300 has delayed replication within non-professional phagocytes, similar to what has previously been reported for macrophages. The mechanism by which this phenomenon occurs is the subject of ongoing work.

Collectively, these data define new molecular mechanisms that *S. aureus* uses to circumvent endovascular barrier function and antibiotic treatment during infection.

II8

Characterizing the inflammatory response following interaction between Ebola virus and host cells

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Ebola virus (EBOV) is an RNA virus of the *Filoviridae* family that is responsible for deadly outbreaks of hemorrhagic fevers in primates with a lethality rate as high as 90%. It has been demonstrated that EBOV primarily targets host macrophages, and this interaction leads to activation and disease pathogenesis. However, most of our understanding of the EBOV-induced pro-inflammatory response is derived from studies of epithelial cells, which are not the primary targets during infection. In the present study, we were interested to further elucidate the cellular and molecular events underlying the EBOV-induced cytokine storm. To study EBOV without biosafety level 4 containment, we generated EBOV virus-like particles (VLPs) expressing the viral glycoprotein (GP). *In vitro* and *in vivo* experiments were performed using these particles to stimulate inflammatory cells in culture and in mice. Results from stimulation of murine B10R macrophages and human THP-1 cells with VLP-GP showed an induced inflammatory response, characterized by increased production of pro-inflammatory cytokines and chemokines compared to control VLPs (no GP expression). Cells obtained from mouse intraperitoneal (IP) lavages were analyzed by flow cytometry six hours post-IP injection with PBS, VLP-GP, or control VLP. An increase in the presence of neutrophils was observed in a GP-dependent manner, accompanied by a simultaneous decrease in the recruitment and presence of $CD4^+$and $CD8^+$T cells and B cells. In parallel, serum from two rhesus macaques infected with EBOV was obtained from collaborators at the National Microbiology Laboratory to study the inflammatory events occurring in progressive EBOV infection. Following size exclusion chromatography isolation, nanoparticle tracking analysis of exosomes released in serum during *in vivo* infection indicated an increase in the total number of exosomes in circulation at the peak of infection. Analysis of inflammatory serum biomarkers by multiplex array also corroborated our *in vitro* work in macrophages. This study highlights the importance of viral factors, such as GP, and host-derived extracellular vesicles in the inflammatory cascade and pathogenesis of EBOV, which can collectively be further exploited for antiviral development. This research is supported by a CIHR operating grant to M.O. and M.C.

II9

Comparative therapeutic analysis of treatment A (Sofosbuvir + Daclatasvir) and treatment B (Sofosbuvir + Ribavirin) in chronic hepatitis C patients from Pakistan

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The objective of this study was to compare the effects of Sofosbuvir plus Ribavirin therapy versus Sofosbuvir plus Daclatasvir therapy in chronic Hepatitis C infected 200 patients across Pakistan. The enrolled patients were randomly divided into two groups, each consisting 100 patients. First group patients (group A) were treated with a combination of Sofosbuvir and Ribavirin for 24 weeks, while the second group patients (group B) were treated with a combination of Sofosbuvir and Daclatasvir for 12 weeks. The change in baseline biochemical and hematological parameters was checked after 4 weeks of therapy. The serum HCV-RNA levels through PCR were performed at the end of the treatment. Gender, diabetes mellitus, prior interferon therapy, decrease in Hb >2g/dl, ALT normalization in and rise in serum bilirubin were qualitative variables, while age, weight, baseline hemoglobin (Hb), baseline alanine aminotransferase (ALT) and baseline bilirubin, Hb at 4 weeks of therapy and serum bilirubin at 4 weeks of therapy were quantitative variables. The analysis of the collected data was done using SPSS version 15. For quantitative variables, means and standard deviations (SD) were calculated and for qualitative variables, frequencies and percentages were computed. Chi-square test was applied to find association of factors at 5% level of significance. Odd ratio with 95% confidence interval (CI) was also calculated for each association. The End Treatment Response (ETR) was achieved in 95% and 97 % in group A & B, respectively. The SVR was 93% in group B compared to 73% in group A. The superiority of group B therapy over group A was observed in statistical analysis. No decline in Hb > 2g/dl (p=0.000), ALT normalization (p=0.000), and no rise in serum bilirubin (p=0.000) were significantly seen in group B patients as compared to group A patients. Decrease in Hb > 2g/dl during first month of therapy was found in 50% of group A patients while only 9% of the group B suffered decrease in Hb > 2g/dl during first month of therapy. Similarly ALT normalization during first month of therapy was seen in 51% of group A and 90% of group B patients. The serum bilirubin became elevated than normal value during first month of therapy in 46% of group A patients and only 7 % of group B patients.

It was concluded that Safosbuvir plus Daclatasvir combination therapy for 12 weeks showed superior PCR results and hence is a superior antiviral therapy for hepatitis C than Sofosbuvir plus Ribavirin combination for 24 weeks in the population under study.

II10

Competitive interaction between bovine enterohemorrhagic and commensal *Escherichia coli* and the impact of physiological stress on the host-bacteria interaction in a gnotobiotic murine model

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Enterohemorrhagic *Escherichia coli* (EHEC) serotype *O157:H7* is an important foodborne pathogen, and cattle are considered the primary reservoir of this bacterium. Stress has been suggested as an important factor in gastrointestinal tract (GIT) colonization by EHEC in cattle, but this has not been experimentally investigated. Furthermore, the impact of colonization resistance on EHEC in cattle is poorly understood. As a highly representative cattle model to elucidate key aspects of the host-pathogen-microbiota interaction does not yet exist, a gnotobiotic (GB) C57BL/6 murine model was used to investigate competitive colonization by a bovine EHEC (FRIK 2000) and 20 phylogenetically-distinct commensal *E. coli* (EC) strains isolated from cattle; commensal strains were grown together or separately. Stress hormones, such as norepinephrine, can induce the expression of certain virulence factors in EHEC strains. The murine stress hormone corticosterone (CORT) was continuously administered via drinking water to induce a physiological stress response. This experiment was arranged as a two (i.e. stress ±) by six (i.e. EC) factorial experiment with three replicate mice per treatment. CORT (100 mg/L) was administered to mice in water for 8 days (CORT+); control mice (CORT-) were only provided water. EC were administered to mice *per os* on day 6. EC treatments were: (1) EHEC alone; (2) EHEC & commensal EC grown collectively (i.e. together); (3) EHEC & commensal EC grown separately (i.e. separate); (4) commensal EC alone (together); (5) commensal EC alone (separate); and (6) phosphate buffered saline. On day 9, mice underwent an open field test, and samples were collected for EHEC and corticosterone quantification, histopathologic analysis, cytokine profiling, fluorescence *in situ* hybridization (FISH) microscopy, and metabolomics. A difference between the CORT– and CORT+ treatments was observed in the open field test for mean velocity and total distance travelled by mice. Increases in fecal and serum CORT concentrations were observed in CORT+ mice. Digesta and mucosal densities of EHEC in cecum, proximal colon, and distal colon were decreased by the presence of commensal EC (grown together and separately). Total histopathological scores and expression of pro-inflammatory cytokines *Tnf*-α and *Kc* were similarly reduced in the distal colon of mice inoculated with commensal EC strains. Furthermore, concentrations of IL-1β, IL-2, TGF-β1, TNF-α, IL-10 and IL-6 were elevated in the liver of CORT+ relative to CORT– mice. FISH microscopy to visualize colonization of EHEC relative to commensal EC strains is in progress. In conclusion, EHEC incited disease in GB mice, and the presence of commensal EC strains effectively reduced intestinal colonization and ameliorated disease, particularly evident in the distal colon, the main GIT colonization site of EHEC in cattle. Notably, physiological stress did not potentiate GIT colonization nor intestinal disease incited by EHEC.

II11

Construction of a multivalent peptide vaccine for dairy cows

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Bovine mastitis, an inflammation of the mammary gland mainly caused by a bacterial infection, is the most frequent disease in dairy cows and leads to significant economic losses. This disease can be caused by various pathogens such as *Staphylococcus aureus, Streptococcus dysgalactiae* and *Streptococcus uberis*. *Salmonella* Dublin can also cause a systemic disease in dairy cows and can be transmitted to humans, thus representing an important public health concern. Currently, only minimal benefits are obtained with available vaccines. Our laboratory has developed a sub-unit vaccine composed of full-length *S. aureus* proteins that are highly expressed during intramammary infections. These antigens can be replaced with immunogenic peptides and stimulate a similar immune response against *S. aureus*. The aim of this work was to develop a multivalent peptide vaccine that will protect dairy cows from infections caused by *S. aureus, Strep. spp* and *Salmonella* Dublin.

At least two proteins implicated in virulence were identified for each target species using bio-informatics tools. Seven proteins were selected for the vaccine: two *S. aureus* proteins (A and B), three *Strep.* spp. proteins (C, D and E) and two *Salmonella* proteins (F and G), and from those, eight peptides consisting each of two epitopes from different proteins connected by a linker were chemically synthesized for vaccination. Mice were divided into five groups for vaccination: a group was immunized with *S. aureus* peptides (A1~B1, A2~B2 and A1~B2), one group with *Strep.* spp. peptides (C1~D1 and C1~E1), one group with *Salmonella* peptides (F1~G1, F2~G2 and F3~G1), one group with all peptides (multivalent vaccine) and one control group received PBS. Three subcutaneous immunizations of the peptide mixtures (50 µg/peptide or 20 µg/peptide for the multivalent group) at two-week intervals were performed in each mouse. Analysis of the immune response in serums against peptides used for vaccination or against the full-length proteins were compared to the PBS group.

Groups immunized with the *S. aureus* peptides and *Salmonella* peptides had strong total IgG responses (response vs A1~B2, $P \leq 0.05$; response *vs* F1~G1, $P \leq 0.001$ and *vs* F3~G1, $P < 0.0001$). For the *Strep.* spp. peptides, this response was only observed in mice immunized with the multivalent vaccine (response *vs* C1~D1, $P < 0.0001$; *vs* C1~E1, $P \leq 0.01$). The sensitivity of the ELISA was not sufficient to detect the IgG response against the full-length proteins in some serums. A significant IgG1 response was observed in groups immunized with the *S. aureus* peptides (response *vs* A2~B2 and *vs* A1~B2, $P \leq 0.01$) and with the *Salmonella* peptides (response *vs* F1~G1 and *vs* F3~G1, $P \leq 0.01$; response *vs* F2~G2, $P \leq 0.05$). As for *Strep. spp*. peptides, mice immunized with the multivalent vaccine produced a significant IgG1 response (response *vs* C1~D1, $P \leq 0.01$ and *vs* C1~E1, $P < 0.05$).

A set of immunogenic antigens for vaccination was identified in this project. The results suggest that vaccination with peptides induce a humoral response against individual peptides after the third immunization and more work is needed to determine the best peptide composition that will induce a strong and balanced immune response against all target pathogens

II12

Current status of hepatitis E virus among blood donors in Qatar

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BACKGROUND: Hepatitis E virus (HEV) is an RNA virus transmitted mainly through zoonotic transmission or fecal–oral route. More than 80% of Qatar's population are expatriates, including many coming from hyperendemic countries; thus, it is important to estimate the seroprevalence and to compare between different nationalities. The results can be useful in alerting blood banks to the importance of HEV screening.

STUDY DESIGN AND METHODS: Samples from 5854 blood donations provided by Hamad Medical Corporation were tested in the period between June 2013 and June 2016. Samples were tested for the presence of anti-HEV immunoglobulin (Ig)G and IgM antibodies and viral RNA using real-time polymerase chain reaction (PCR). Descriptive statistics, bivariate analysis, and multivariate logistic regression were used.

RESULTS: Anti-HEV seroprevalence was 20.7%. A total of 1198 and 38 donations tested positive for IgG and IgM antibodies, respectively. Of the IgM-positive donations four tested positive by PCR. A significant association was detected between HEV seroprevalence with age and nationality.

CONCLUSION: The seroprevalence of anti-HEV was high in Qatar. Since HEV IgM and RNA were detected, this suggests the possibility of HEV transmission by transfusion. Blood banks in Qatar and the region should

consider screening for HEV, especially when transfusion is intended to pregnant women or immunocompromised patients.

II13

Deciphering the regulation mechanisms of the Std fimbrial cluster of *Salmonella enterica* serovar Typhi

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Salmonella enterica serovar Typhi is a human-specific bacterium responsible of typhoid fever. It causes annually 20 million cases and 200 000 deaths. Specific virulence factors include a capsule for immune evasion and a toxin that binds to glycan present only on human cells. Among other virulence factors, *S.* Typhi also has a unique combination of 14 fimbriae that may explain its host-specificity and its pathogenicity. Twelve fimbriae belong to the chaperone-usher (CU) group, which is composed of a dedicated chaperone and usher, a major subunit and an adhesin present at the tip of the structure that interacts with its environment. We have previously demonstrated the production of 6 CU fimbriae at the surface of the bacteria and characterized their role in different steps of the pathogenesis of *S.* Typhi. This work focuses on the Std fimbriae, as it shows a high level of expression and a significant phenotype in the invasion of epithelial cells. Interestingly, the expression of *std* in *S*. Typhi was significantly higher than in the closely related serovar *S.* Typhimurium or *E. coli*. We hypothesize that regulation of the Std is unique to *S.* Typhi. Our objectives are to: (i) Evaluate the role of known and potential regulators on *std* expression in *S*. Typhi and (ii) to identify novel regulators of *std* in *S*. Typhi. We will investigate the role of regulators previously identified to modulate the expression of *std* in *S.* Typhimurium, such as Dam, SeqA, HdfR, RosE and StdEF. The role of several global regulators controlling fimbrial expression in other species, such as flagella regulators, metabolic regulators and two-component systems, will be evaluated. Other regulators predicted by the BPROM software to bind to the promoter region, including ArgR, Fur, Lrp, NagC, SoxS and PhoB, were also investigated. Different regulators were deleted by allelic exchange mutagenesis in *S.* Typhi. We transformed each strain with a high-copy plasmid harbouring the *stdA* promoter region in fusion with the *lacZ* reporter gene and performed ß-galactosidase assays. Regulatory mutants showing a significant difference in promoter expression in S. Typhi, were also evaluated in *S.* Typhimurium. Almost all the known regulators of Std in *S*. Typhimurium seem to modulate the promoter similarly in *S.* Typhi. However, the expression was drastically lower in a *hdfR* mutant strain. Otherwise, Fur, and Lrp were activators of *std* expression, and Dam, FlhCD, NagC, RstA, OmpR and SirA repressed its activity. We also identified Crp as a strong activator of *std* in *S.* Typhi and will further investigate its role.

We screened a transposon-based mutant library for Std expression on MacConkey agar to identify novel regulators. Identification of the transposon insertion site was performed by sequencing and expression of *std* by ß-galactosidase assay was confirmed. We have identified SodB, GlnG, PhoP, CitB and Bcf as potential activators of *std* expression.

Better knowledge of *S*. Typhi fimbriae and differences with expression of this fimbriae in *S.* Typhimurium will bring new insights concerning bacterial restriction to a unique host. Fimbriae are also good targets for antimicrobial or anti-virulence treatment as they are extracellular components. If we know when they are expressed during *S.* Typhi infection, we may be able to target them and lower the burden of typhoid fever.

II14

Design of a multivalent vaccine for control and prevention of Mycobacterium avium subsp. paratuberculosis infections in dairy cattle

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Title: Design of a multivalent vaccine for control and prevention of *Mycobacterium avium* subsp. *paratuberculosis* infections in dairy cattle.

Background: *Mycobacterium avium* subsp. *paratuberculosis* (MAP) is the etiological agent of Johne's disease, causing a severe form of enteritis affecting different types of ruminant worldwide. In dairy cattle, MAP benefits from the immature immune system of the calf to establish an infection. Years later, symptoms appear such as weight loss, reduced milk production, and chronic diarrhea, leading to transmission among the herd and therefore causing substantial economic losses for the industry. Currently, no efficient vaccine or treatment is available to prevent or cure the infection. Besides, vaccines leading to high antibody production could be useful for IgG translocation to the colostrum and to protect the newborn calf. This phenomenon results in passive immunity and could represent a promising way of preventing MAP colonization.

Methods: To identify relevant antigens for immunization, programs such as SEED, BLASTn and BLASTx were used. These platforms allow comparative analysis of genomes and protein sequences. Of particular interest were the conserved genes among the pathogenic strains. To target sequences with antigenic potential, CELLO and TMHMM were employed, providing information on subcellular location and transmembrane helices of the proteins. BCPREDS was then used to identify B cell epitopes of 10 to 16 amino acids in the selected protein sequences.

Results: Several MAP proteins were identified with the bioinformatic tools, most being virulence factors. Because of their conserved extracellular domains and high importance in pathogenicity, three specific proteins were selected for further investigation. Those are involved in host cell invasion and dissemination of the bacteria in tissues. On each sequence, extracellular epitopes generating high scores for immunogenicity were identified. When possible, final selection was directed to epitopes corresponding to functional regions of the antigens. To optimize immunogenicity, epitopes will be joined in pairs in the vaccine formulation. Short amino acids sequences composed of small hydrophilic residues will serve as linkers.

Conclusions: We have identified MAP epitopes that will be part of a multivalent vaccine composition designed to also protect against other dairy cattle pathogens. This work is in progress and the next steps will allow evaluation of the protective potential of the selected MAP antigens in cellular and mouse models.

II15

Development of a virology lab on a chip for the isolation and study of giant viruses infecting amoebae

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Despite continuously improving medical advances, it still remains impossible to pinpoint the causative agent responsible for a large proportion of pneumonia cases. Evidence suggests that giant viruses, such as Mimivirus, are respiratory pathogens who could explain a part of pneumonia of unknown origin. These viruses are remarkable, not only because of their size that allows their observation using a simple optical microscope, but also because they possess several unique capabilities. Given the potential impact of these viruses on human health, it is crucial to learn more about them. Unfortunately, giant viruses are difficult to isolate from their natural environment. No giant virus has been isolated in Canada so far, despite Canadian pneumonia patients having tested positive for Mimivirus and the evidence of giant virus DNA in Quebec lakes and soil. The main strategy currently used to isolate giant viruses is to screen samples in a suitable host, like amoebae, the natural hosts of these viruses. However, one limit of this approach is the small volume samples. A system providing a high volume/surface ratio, increasing the chance of virus-amoeba contacts, could enhance the efficiency of the screening. Microfluidics, namely the use of liquid flow in channels of a micrometer range, present this advantage.

The goal of this project is therefore to isolate giant viruses from the environment using their natural hosts, amoebae, trapped in a microfluidic device as a sort of biological filter. In addition to the isolation of giant viruses, the microfluidic trap, that can be observed using microscopy, will also generate real-time data on the interaction of viruses with amoebae over the course of the infection.

To date, some of the various parameters to be considered in the microfluidic device conception, concerning the materials and channel shape to use, and experimental design, regarding the amoeba behaviors in stress conditions, have been explored.

For the first essays, we used the axenic amoeba *Dictyostelium discoideum*. It was possible to reduce the growth rate of the cells without affecting the phagocytic rate. In flow condition, cells demonstrated a good adherence to tissue culture-treated plastic, allowing a flow rate of at least 0,5 mL/h.

The next steps of the project will focus on deciphering the behavior of amoebae, in flow conditions, when samples of Mimivirus are incorporated to the nutrient medium. Ultimately, the developed microfluidic device will allow the amplification of giant viruses from environmental samples, even if they are found in a very small concentration.

II16

Effect of vaccination against *Staphylococcus aureus* on milk quality

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Milk microbiota is very complex since many conditions like the environment, farm management and diet have a huge impact on its composition. A healthy microbiota should contribute to prevent diseases, increase milk quality and improve milk transformation. Bovine mastitis is an inflammation of the mammary gland that can be caused by different pathogens and *Staphylococcus aureus* is one of the most commonly found bacteria causing such intramammary infections (IMIs). IMIs result in substantial costs for dairy producers due to the use of antibiotics, veterinary fees, reduced milk production and, in extreme cases, premature culling of affected animals. In previous research work, our team has developed a vaccine against *S. aureus*, which was composed of important virulence factors expressed during IMIs. We hypothesized that such a vaccine would not only help to control *S. aureus* IMIs but also maintain milk quality. The main goal of the present study is to characterize the milk quality (bacterial load, microbiota and fatty acid composition) of vaccinated cows.

Experimental design: Twenty cows having a least one quarter naturally infected with *S. aureus* will be separated in two groups. The first group will be immunized twice with the *S. aureus* vaccine ten weeks apart while the second group will receive saline. The study is currently in progress and milk and blood will be collected every two weeks for fourteen weeks. Bacteria and somatic cell counts in milk will be determined at each sampling. For analyzing the milk microbiota, DNA will be extracted using the Powerfood extraction kit from Qiagen. Amplification of the V1-V2 and V3-V4 hypervariable regions of the 16S RNA will allow identification of the bacterial community. The milk microbiota composition and diversity will be compared between the vaccinated *vs* saline groups but also between healthy *vs* infected quarters within the same cow to avoid cow-to-cow variations. To characterize the immune response, total IgG titers as well as those of isotypes IgG1 and IgG2 will be quantified in blood and milk by ELISA. Finally, fatty acid and mineral content profiles will be obtained respectively by gas chromatography and inductively coupled plasma spectrometry.

The study is ongoing but we expect that a *S. aureus* vaccine will not only reduce the incidence of clinical and subclinical mastitis on dairy farms but could also ameliorate milk quality. Vaccinated cows could have a lower risk of milk dysbiosis and could therefore maintain a better health of the udder and show optimal milk composition.

II17

EHEC utilizes two-component systems to modulate expression of the major flagellar subunit protein, FliC, in response to host intestinal cues

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Enterohemorrhagic *Escherichia coli* (EHEC) O157:H7 is a food- and water-borne human enteric pathogen that can cause severe hemorrhagic colitis and may lead to hemolytic uremic syndrome (HUS) which can be fatal. In order to effectively colonize the human gastrointestinal tract, EHEC uses many virulence factors including flagella to reach its site of colonization in the large intestine and infect host epithelial cells. Expression of flagella, along with many other virulence factors can be modulated in response to microenvironmental conditions within the gastrointestinal tract sensed by two-component systems (TCS). We have previously demonstrated that short chain fatty acid (SCFAs) mixes representative of small intestinal levels upregulate FliC expression while mixes representative of large intestinal levels downregulate expression. Here we show that flagella expression is also sensitive to oxygen and bicarbonate levels in combination with nutrient levels of media. To evaluate the role of selected TCSs in these differential responses, we constructed three response regulator mutants, previously shown to affect flagella expression in non-pathogenic *E. coli, - arcA, rcsB* and *uvrY*. These isogenic EHEC mutants were assayed for FliC expression under different SCFA mixes alone and in combination with the other intestinal cues including low oxygen and physiologically relevant bicarbonate levels. Distinctive differences in FliC expression profiles were evident with a loss of the SCFA wild type phenotype linked to the *arcA* mutation. The changes in the expression profile of the mutants compared to wild type confirmed their role in flagella regulation and revealed a complex response system to these intestinal cues. Collectively, these results demonstrate that expression of flagella, which play a key role in mediating motility, are acutely sensitive to host environmental conditions in ways that generate synergies with different treatment combinations.

II18

Enhanced methodologies for the isolation of *Mycoplasma bovis* from beef cattle

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Bovine respiratory disease (BRD) is the most significant disease affecting feedlot cattle in North America. *Mycoplasma bovis* (*Mb*) is among the most important pathogens involved in this disease. Culturing *Mb* is laborious and unpredictable with most laboratories relying on molecular-based methods for its detection and identification. However, bacterial culture is still necessary to define phenotypic traits of individual strains for antimicrobial resistance (AMR) surveillance. We therefore developed methods to optimize the culture of *Mb* which included pH and compositional modifications (pH increased from 7.3 to 7.6; higher beef heart infusion and yeast extract concentrations; pyruvate supplementation) of a commercially available PPLO media. In addition, we developed a robust qPCR-based detection method to monitor the presence of *Mb* throughout the culturing process. Using the optimized, in-house prepared PPLO, deep nasopharyngeal swabs (DNPS) from feedlot cattle were enriched in broth and after 4 days of incubation, an aliquot was added to qPCR tubes containing mastermix and primers for the detection of *Mb*. Cells were heat-lysed prior to qPCR by increasing the initial denaturation time (10 minutes in total, 95 °C). Only enriched cultures that were qPCR-positive were then plated for isolation of *Mb*. The modified PPLO supported vigorous and reproducible growth of both the reference and field *Mb* strains as compared to a standard commercial media. The direct detection of *Mb* from broth culture using qPCR proved to be more sensitive (16.1 CFU/mL) than using DNA purified via a commercial DNA extraction kit from broth cultures (1.61E+03 CFU/mL). The qPCR screening (n= 246 samples) of broth-enriched samples showed 100% sensitivity, 81.9% specificity, and a kappa index= 0.81 (strong agreement), whereas for qPCR of DNPS samples (n= 60), showed a 100% sensitivity, 38.1% specificity, and a kappa index= 0.44 (weak agreement) as compared to *Mb* isolation method (gold standard). The qPCR protocol described here makes it possible to reduce the labour and cost of *Mb* isolation by eliminating the need to process *Mb* negative samples. However, the isolation protocol followed has the limitation that mixed strain cultures of *Mb* may be isolated as a result of cell aggregation when the (three times) cloning and filtering technique is not followed. The extent to which this unwanted outcome occurs is presently being investigated using whole genome sequencing of isolated cultures.

II19

EnvY as a possible negative regulator of the omptin protease OmpT in *Escherichia coli* BW25113

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In order to survive microbial infections, the body utilizes various antimicrobial immune responses. One such response is expression and secretion of host defense peptides (HDPs), which are small broad-spectrum antimicrobials secreted by almost every cell of the human body. Predictably, bacteria have evolved a number of adaptations to resist killing via HDPs. This project focuses on omptin proteases, which are *o*uter *m*embrane *p*roteases that cleave antimicrobial peptides at specific motifs and inactivate them. Through a number of experiments, we have shown that omptin proteases are regulated by the PhoPQ two component regulatory system, which is well-studied for regulating a number of other HDP resistance adaptations. To expand upon this observation, a screen was performed looking at omptin protease activity in a number of clinical *Escherichia coli*

isolates; this showed wide variability in the level of omptin protease activity between strains. This suggests the regulation of this activity may not be so simple, and that there may be many players involved that affect the final output of omptin protease activity. We performed a screen for omptin protease activity in a number of Keio collection mutants lacking key signalling proteins via FRET assay. This assay uses a substrate with the cleavage site found on LL-37, an important human HDP, which fluoresces upon cleavage. We identified two genes *envY* and *rpoN*, which showed negative regulation of omptin activity. Closer inspection of these two signalling proteins is required in order to ascertain exactly the role they play in the regulation of omptin proteases in *E. coli*.

II20

Evaluating the role of GraS in promoting *Staphylococcus aureus* adaptation to combined antimicrobial conditions of human skin

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Staphylococcus aureus is a Gram-positive microbe that asymptomatically colonizes 30% of humans, where it is well adapted to survive on the skin in the presence of innate immune defense mechanisms including antimicrobial unsaturated free fatty acids (uFFA), acidic pH, and cationic antimicrobial peptides (CAMPs). While *S. aureus* is a frequent colonizer of humans, community acquired methicillin resistant *S. aureus* (CA-MRSA) pose a serious health risk due to their ability to cause severe soft tissue infections with rapid community transmission. Frequently, infections arise from strains that were previously asymptomatically colonizing individuals, highlighting the dangers of persistent colonization. To better understand how CA-MRSA colonize skin effectively, we assessed the role of the sensor histidine kinase GraS in responding to combined antimicrobial stress conditions that would be encountered on human skin. Specifically, although it is well established that GraS has a primary role in sensing and responding to CAMPs, its role in responding to other antimicrobial conditions, namely acidic pH and uFFA, has not been investigated.

We hypothesized that, in addition to responding to CAMPs through the extracellular sensor loop, GraS can also respond to wide variety of stimuli, such as acidic pH, through sensing alterations to the phospholipid membrane. Furthermore, we hypothesized that a response through GraS can provide resistance to not only CAMPs, but also to other antimicrobial conditions found on human skin including uFFA.

Using a number of different techniques, including construction of a *graS* deletion mutant, assays of cytochrome C binding to assess cell surface charge, and analysis of an *mprF*::lux reporter construct to quantify expression of the GraS dependent gene *mprF* which promotes lysine modification of phospholipid, we demonstrate that GraS is capable of signalling in response to acidic pH. Furthermore, through complementation of USA300Δ*graS* with wild type and amino acid substitution mutants of *graS*, we found that the acidic amino acids in the extracellular loop that promote recognition of CAMPs are not required for signalling at acidic pH. Signalling through GraS in response to either acidic pH or a model CAMP Polymyxin B also promoted increased resistance to uFFA. Interestingly, deletion of genes important to uFFA resistance at neutral pH, *fakA* and *farER,* had no impact on growth under combined conditions of acidic pH and uFFA. In contrast, mutations to the GraS dependent gene *mprF* resulted in a severe growth inhibition under these same conditions. Together, these findings implement GraS signalling in a novel uFFA resistance pathway that we are currently working to further elucidate.

While the antimicrobial conditions on human skin normally function together to have an additive inhibitory effect on bacterial growth, *S. aureus* appears to have evolved to thrive in this environmental niche through the use of GraS. Our findings demonstrate the integral and diverse role of GraS in promoting growth of *S. aureus* exposed to combined conditions of acidic pH, uFFA, and CAMPs. Our results also indicate a novel sensing mechanism

for GraS that may allow GraS to respond to a more diverse range of stimuli than has been previously established. Together, this work sheds insight into the mechanisms used by *S. aureus* to colonize human skin, which is paramount for new strategies for treatment and prevention of infection.

II21

Evidence of slipped-strand mispairing in a putative sialidase gene of *Gardnerella* sp. W11

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Phase variation is a form of gene regulation in bacteria where the expression of a phenotype is altered between "on" state and "off" state. One of the mechanisms involved in phase variation is slipped-strand mispairing (SSM) in which the template or the daughter strand loop-out causing a mis-match during DNA replication. SSM can occur in genomic regions that contain short, homogenous or heterogenous repeats and may change the length of the homopolymeric tract at each replication. Our lab group has discovered a gene (Gene 2) in *Gardnerella piotii* and *Gardnerella* genome sp. 3 could potentially encode a cell wall attached, extra cellular sialidase. Sialidase is recognised as a virulence factor in some strains of *Gardnerella*. The open reading frame of Gene 2 contains a homopolymeric tract of about 12 cytosine residues. The objective of this study is to determine if the homopolymer region of Gene 2 varies in length in a sialidase positive *Gardnerella* isolate. Primers were designed to amplify the homopolymer region of Gene 2. *Gardnerella* sp. W11 was grown on agar and the homopolymer region was amplified from individual colonies by PCR. Amplicons generated from two isolated colonies were ligated into pGEM-T vector and plasmids were extracted and sequenced. The sequencing revealed that the cloned PCR products had identical sequences apart from the variation in the number of C residues in the homopolymeric tract. In the poly-C region, 12 C's was the most common and the only length that makes the coding region in-frame. When there are 11, 13 or 14 C's, a premature stop codon is generated when translated in-silico. We can conclude that the expression of Gene 2 may be regulated by SSM. Phase variation in a cell wall associated sialidase of *Gardnerella* may play a role in adapting to the vaginal microbiome and evading from host immune system.

II22

Examination of biocide cationic antimicrobial resistance in *Escherichia coli* to identify phenotypic and genotypic adaptations

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Background: Cationic antimicrobial (CA) agents are defined as antimicrobials with a cationic charge at neutral pH and commonly include a wide range of disinfectants such as quaternary ammonium compounds (QAC) and bisbiguanides (BG) we rely on to sterile and disinfect skin and surfaces. CAs also include therapeutic antibiotics such as polymyxins (PMX) used as a last resort therapy for drug-resistant infections. In general, CAs kill bacteria by disrupting the cell membrane causing cells to leak to death. The overuse of CAs can place selective pressure on bacteria to adapt to these compounds and as a result, potentially increase antimicrobial cross-resistance to therapeutically relevant extended-spectrum beta-lactamase (ESBL) and carbapenemase producers. CA adaptation is known to alter bacterial cell membrane compositions (protein and lipid alterations), such as increased efflux pump activity, lipid A modifications, and reduced porin expression, however, it is unclear if all CAs impact phenotype and genotype similarly.

Aim: We have adapted an *E. coli* K-12 BW25113 strain over 20-40 subcultures to one of four CAs: QACs benzalkonium and cetrimide, the BG chlorhexidine and PMX (colistin) in an effort characterize the genotypic and phenotypic alterations caused by prolonged increasing bacterial exposure to commonly used CAs.

Hypothesis: We hypothesize that *E. coli* adapted to different CA will result in reduced growth fitness, unstable antimicrobial resistance phenotypes, cross-resistance to biocide and therapeutic and genetic alteration of efflux pump, porin and lipid-modifying membrane proteins as compared to the unadapted wildtype strain.

Results: As compared to the unadapted *E.coli* strain the final minimal inhibitory concentration of each CA adapted strain after 40 broth subcultures revealed a 2-4-fold increase in all CA adapted strains, except for the 200-fold increase by the colistin adapted strain. Unexpectedly, only colistin and chlorhexidine adapted cultures could stably maintain their respective antimicrobial resistance after 10 subcultures without CA selection, indicating that only QAC adaption was unstably inherited. Growth curve experiments of CA adapted strains in rich and minimal media revealed that only growth in minimal media but not rich media significantly reduced cell growth. Antimicrobial susceptibility testing to determine cross-resistance to other antimicrobials revealed that both QAC adapted strains were cross-resistant to different QACs but not to other classes of antimicrobials we tested suggesting CA adaption may antimicrobial class specific. Chlorhexidine and colistin adapted strains did not identify significant cross-resistance to other antimicrobials and were much more susceptible to Gram-negative antibiotics (vancomycin). Illumina Mi-Seq whole genome sequencing analysis identified few commonly identified single nucleotide polymorphisms (SNPs) in genes and non-coding regions shared between different CAs but SNPs were identified in different outer membrane porin, lipid A modifying genes and efflux pumps systems by each CA adapted strain. QAC adapted strains shared the greatest number of genes and non-coding regions with SNPs as compared to BG and PMX adapted strains.

Significance: This study suggests CA adaption is dependent on how each CA specifically disrupts the membrane layer(s), since QACs, BG, and PMX all have different mechanisms of action. It also reveals new insights into mechanisms of CA resistance.

II23

Examination of iron acquisition strategies employed by *Staphylococcus aureus* small colony variants

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Staphylococcus aureus is a notorious Gram-positive opportunistic pathogen that has a propensity to adopt a small colony variant (SCV) phenotype, characterized by reduced metabolic activity and virulence factor expression. Importantly, *S. aureus* SCVs are routinely isolated from patients with chronic recurrent infections. Survival and growth of *S. aureus* SCVs within the host necessitates they acquire iron, an essential nutrient for almost all known living organisms, however little is known about the iron acquisition strategies employed by *S. aureus* SCVs. To facilitate investigation of iron acquisition strategies by SCV bacteria, a stable SCV was engineered by mutagenesis of the *hemB* gene in methicillin-resistant *S. aureus* strain USA300. The *S. aureus hemB* mutant demonstrated hemin auxotrophy and formed characteristic small colonies on solid media in the absence of hemin, a phenotype that was reversed upon provision of *hemB in trans*. To investigate the mechanisms of iron acquisition employed by SCV bacteria, the staphyloferrin A (SA) and staphyloferrin B (SB) siderophore biosynthetic loci, *sfa* and *sbn* respectively, were inactivated in the stable *hemB* mutant background. Growth analysis under iron deplete conditions revealed that *hemB* bacteria carrying mutations in the *sfa* locus maintain their ability to grow while inactivation of the *sbn* locus abolished *hemB S. aureus* growth. These data imply that *hemB S. aureus* express and utilize SB and fail to produce SA. Consistent with this, lyophilized culture supernatants from *hemB S. aureus* only possess activity corresponding to SB when used in siderophore

bioassays. Interestingly, *hemB S. aureus* are also deficient in utilization of exogenously provided SA and the clinically relevant iron chelator deferoxamine (DFO); *S. aureus* can utilize DFO as a siderophore for growth under iron deplete conditions. The mechanisms by which *hemB S. aureus* are defective in production and utilization of SA and DFO are currently being investigated. This study yields important insight into the mechanisms by which *S. aureus* SCV bacteria acquire iron to cause chronic and recurrent infection in susceptible patient populations.

II24

Examining the role of zinc on the proteome, growth and morphology of *Klebsiella pneumoniae*

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Microbial organisms encounter a variety of environmental conditions including changes to pH, temperature, and nutrient levels, which influence their cellular protein regulation and secretion patterns. Recently, a study investigating the transport and regulation of iron, copper, zinc, and manganese in *Enterobacteria* defined correlations between bacterial strains and the relative importance of different metal transport systems in survival and virulence. Here, we focus on *Klebsiella pneumoniae*, a pathogenic bacterium associated with respiratory and urinary tract infections, which shows nutrient-dependent patterns of regulation. Using *K. pneumoniae* as a model system, we aim to determine how a nutrient-limited environment (e.g. zinc) modulates the cellular proteome and secretome of the bacteria. *K. pneumoniae* strain 52145 (WT) was grown under zinc-limited and zinc-replete (+10 µM zinc) conditions to mid-log phase. Cell pellets and supernatant samples were collected, processed, and digested with LysC/trypsin prior to analysis on a Hybrid Orbitrap-Quadrupole Mass Spectrometer. Raw data files were processed using MaxQuant and data analysis, statistical processing, and visualization was performed in Perseus. Proteins with abundance profiles significantly influenced by zinc were subjected to *in silico* characterization. Our results provide an in-depth analysis of the effect of zinc availability on the proteome and secretome of *K. pneumoniae in vitro*. Here, we identified 2,380 proteins from the total cellular proteome (i.e. cell pellet) and 246 secreted proteins, representing the deepest proteome of *K. pneumoniae* to date. A principal component analysis (PCA) of our data showed the largest separating component between the samples to be associated with the zinc treatment. Statistical analysis using a Student's *t*-test (*p-value* < 0.05, FDR = 0.05) identified 19 proteins from the total cellular proteome and 21 secreted proteins that were noted as significantly different. Within the significantly different proteins, a histidine utilization repressor (hutC) was identified. This protein and its targets have been linked to zinc homeostasis and therefore, represent a candidate for validating our study. Given the function of hutC to repress genes on the histidine utilization (hut) operon, we will confirm its increase in protein abundance by quantifying the suppression in gene expression of the *hutC* targets using qPCR. Simultaneously, an uncharacterized protein, chaB was identified, and upon *in silico* characterization, was proposed to be a cation transport regulator (chaB). To evaluate the potential role this protein has in zinc transport, STRING analysis was performed to identify putative interacting partners, from which, a capsule biosynthesis protein was indicated. Upon knocking out chaB, the proteome and secretome of the mutant will be evaluated to assess the role of this potential transporter on cellular homeostasis. Concurrently, the morphology of the mutant will be compared to the WT strain by using phase-contrast microscopy to detect differences in capsule production and to quantify changes in cell size influenced by zinc. Proteomic profiling of *K. pneumoniae* defines the influence of zinc availability on cellular regulation, protein secretion, and virulence factor production.

II25

Functional role of Resistance-Nodulation-Division efflux pump AdeIJK and its TetR transcriptional regulator AdeN in resistance and virulence of *Acinetobacter baumannii*

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Acinetobacter baumannii is a challenging opportunistic pathogen due to the emergence and widespread of antibiotic resistance. Efflux-mediated resistance by proteins belonging to the Resistance-Nodulation-Division (RND) family is the major contributor to intrinsic antibiotic resistance of *A. baumannii.* AdeIJK is a constitutively expressed efflux pump of *A. baumannii* that confers resistance to multiple classes of antibiotics. Expression of AdeIJK is regulated by AdeN, a TetR-family protein, encoded 813kb upstream of *adeIJK* operon.

Our preliminary data suggest that AdeIJK pump is not only involved in the antibiotic resistance of *A. baumannii* but also its virulence. Further, our observations also show that AdeN may regulate the expression of genes other than *adeIJK,* including those involved in virulence and antibiotic resistance. The objective of this study is to understand the interplay between AdeN and AdeIJK in antibiotic resistance and virulence of *A. baumannii*. *adeN* gene deletion created in *A. baumannii* ATCC17978 displayed a differential expression of more than 100 genes. In order to determine if this differential expression was a result of the absence of AdeN or overexpression of AdeIJK, we also created Δ*adeIJK* and Δ*adeN*:Δ*adeIJK* derivatives of *A*. *baumannii* ATCC17978. Gene complementation was achieved using the single copy gene expression system. Using this system, we were able to regulate the expression of *adeN* without the overexpression of the AdeIJK pump. Conversely, we were also able to control the expression of *adeIJK* independent of AdeN. Antibiotic susceptibility and phenotypes were assessed to understand the impact of *adeN* or *adeIJK* independently.

Our findings indicate that the changes in antibiotic susceptibility were dependent on the AdeIJK pump. We also observed that motility and virulence of *A. baumannii* was dependent on the presence of either AdeN or AdeIJK together. Our work suggests that AdeN and AdeIJK both play an important role in the antibiotic resistance and virulence of *A. baumannii*.

II26

Genetic background influences mean and variability of responses to prolonged antifungal drug exposure in *Candida albicans*

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Antimicrobial resistance is inherently an evolutionary phenomenon: in the presence of drug, resistant individuals arise and spread within susceptible populations. The acquisition of resistance is critically understudied in fungi relative to bacteria. Unlike bacteria, fungal microbes primarily adapt via vertical transmission (mother to offspring) through a range of both genomic mutations and larger-scale karyotypic changes in ploidy level (the number of chromosome sets) and aneuploidy (the altered copy number of one or several chromosomes). We conducted a large-scale experimental evolution study to probe how different strain backgrounds of Candida albicans, a prevalent human fungal pathogen, evolves to fluconazole, the most commonly-prescribed antifungal drug. The results indicate that strain background, mediated in part by the ancestral growth ability in drug, significantly influenced both the mean response and the variability of responses: compared to initially fast-growing strain backgrounds, replicates from strains that initially grew most slowly acquired the biggest improvement in growth

in drug on average, but also exhibited the most variability in a range of measured phenotypes. Importantly, very few replicates acquired clinical drug resistance, though we found frequent changes in both genome size (DNA content) and antifungal drug tolerance, a newly defined drug response that involves the slow growth of subpopulations of cells in drug concentrations that inhibit the growth of most cells. These results demonstrate the large range of variability in drug responses within and between populations of microbial populations adapting to drug and highlight that caution should be exercised when generalizing experimental results from a single strain background.

II27

HcpE is a new pro-inflammatory virulence factor of *Helicobacter pylori*

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Helicobacter pylori (HP) causes gastritis, gastric ulcers and cancers but the mechanisms of virulence are not fully understood. The hallmark of HP infection is chronic gastric inflammation that underlies future disease. HP secretes proteins which may play a role in eliciting gastric inflammation, including the Helicobacter cysteine rich protein HcpE (HP0235) whose biological function is unknown. Our goal was to investigate if HcpE is secreted by *H. pylori* in absence of lysis, if it is involved in host / pathogen interactions, and to identify components essential for its production. This was achieved using a combination of anti-HcpE ELISA and Western blots, knockout mutagenesis, phenotypic analyses and biochemical assays.

Regarding secretion, we demonstrate that HcpE is secreted by many HP strains, including clinical isolates. Secreted HcpE exists both as a soluble protein and in association with outer membrane vesicles, suggesting potential differential roles of both forms. We showed that HcpE comprises many disulfide bonds and identified and characterized DsbK (HP0231) as a folding factor necessary for HcpE production and secretion via its disulfide bond forming activity.

In terms of host-pathogen interactions, we show that infected patients produce anti-HcpE antibodies, indicating in situ HcpE production, although these antibodies do not allow elimination of the pathogen. While this could suggest a simple role of secreted HcpE as a decoy for the immune system, we investigated if HcpE was involved in specific interactions with host cells. We show an important role for HcpE and indirectly its gate-keeper DsbK in host-pathogen interactions, including murine gastric colonization and pro-inflammatory cytokine production in human gastric explants, in human gastric cells and in murine splenocytes. The effects were observed using knockout mutants of HcpE, but also of DsbK, their bacterial culture supernatants or recombinant HcpE. Of note, these effects were observed despite the presence of other Hcp proteins in our HcpE knockout mutant, indicating a preponderant activity of HcpE over other Hcps in our assays. Thus HcpE appear as a promising therapeutic target to treat *H. pylori* infections and to prevent gastric ulcers and cancers.

To harness its therapeutic potential, it is essential to understand the mechanism of action of HcpE. HcpE is exclusively comprised of Sel-like Repeats (SLR) known to be involved in protein / protein interactions. We hypothesize that secreted HcpE interacts with a host protein via its SLR motifs to exert its effects. We use immuno electron microscopy to detect intracellular HcpE in gastric cells and use BirA ligase -mediated *in situ* biotinylation and Western blotting to detect intracellular host partners in gastric cells. Identifying these partners will later on allow assessing the functional outcome of the interaction for example by RNA interference.

Overall, our work provides strong evidence that HcpE, its gate-keeper DsbK and its host partners are potential new targets that could be exploited to prevent HP-mediated gastric disease. This would fulfill an important need for new therapeutic options as since 2017, HP was classified by the WHO as a priority pathogen in light of rising antibiotic resistance that jeopardizes even the recently upgraded quadruple therapy against HP.

II29

Identification of 2 novel T3SA substrates in *Shigella flexneri*

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Shigella spp. are enterobacteria that invade human colonic mucosal cells using their Type Three Secretion Apparatus (T3SA). *Shigella spp*. possess a large plasmid that encodes most of its virulence factors and has been the focus of seminal work that defined the T3SA regulon. The first wave of effectors is produced prior to activation of the T3SA and the second wave is expressed only after activation of the T3SA and depends on the transcriptional regulator MxiE and the chaperone IpgC. Also, strains *ipaB4* and *ipaD* that are deficient in the tip complex are constitutively secreting in vitro, while the WT and the T3SA-deficient strain *mxiD* are not. Thus, a global assessment of the transcriptional response regulated by the T3SA has been lacking. Herein we used RNA-Seq to identify genes that are differentially expressed when the T3SA is active (on-state) versus inactive (off-state).

First, all known genes encoded on the virulence plasmid pWR100 that are controlled by the transcriptional factor MxiE and 10 chromosomal genes were upregulated when the T3SA was active. In the latter group, 3 are novel genes dubbed Gem1, Gem2, and Gem3. Next, we used droplet digital PCR (ddPCR) to measure gene expression in three biological replicates. The ddPCR data confirmed that Gem1 and Gem3 are significantly upregulated in the *ipaD* strain. These results suggest that Gem1 and Gem3 are regulated by the activity of the T3SA, while Gem2 is not. Gem1 and Gem3 have in their promoter a sequence similar to a MxiE box as observed in known MxiE-regulated, which are upregulated when the T3SA is active. To address if the expression of Gem1 and Gem3 is controlled by the transcriptional factor MxiE, we inserted Gem1 and Gem3 and their endogenous promoters in a plasmid allowing their expression with a C-terminus 3xFlag tag and transformed these plasmids in 4 strains of *Shigella*(e.g., WT, *ipaB4*,*ipaB4 ipgC*, and *ipaB4 mxiE*). Western Blot analyses showed that Gem1 and Gem3 were expressed only in the *ipaB4* strain, suggesting that MxiE and IpgC regulate Gem1 and Gem3 expression, similarly to genes from the virulence plasmid.

To verify the secretion of Gem1 and Gem3, we expressed Gem1- and Gem3-3xFLAG using a lac promoter. We transformed these plasmids in the WT, *ipaD*, and *mxiD* strains. We used the total fraction and supernatant proteins precipitation obtained by Trichloroacetic acid (TCA) precipitation. The expression of Gem1 and Gem3 by Western Blot indicated that all these strains expressed Gem1 and Gem3, while only the strain *ipaD* was able to secrete Gem1 and Gem3. These data suggest that Gem1 and Gem3 are genuine T3SA substrates.

Gem1 and Gem3 encode proteins that share similarities at the expression and secretion level with second wave effectors, although their respective primary structure is unique. Future experiments will assess the potential of Gem1 and Gem3 as effectors and their role in *Shigella* pathogenesis.

II30

Identification of Canid herpesvirus 1 entry pathways in canine epithelial cells

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Canid herpesvirus 1 (CHV-1) is a veterinary pathogen that causes respiratory, ocular and genital infections in dogs as well as other canines such as foxes. In neonatal puppies (1–2 weeks of age), the systemic infection by CHV-1 is fatal and frequently affects the entire litter. The virus is a member of the *Herpesviridae* family, *Alphaherpesvirinae* subfamily, and *Varicellovirus* genus*. No effective treatment or vaccine that provides long-term protection is available. The mechanism of entry of* CHV-1 in host cells is unknown. For other herpes viruses

such as herpes simplex virus 1 (HSV-1) and Epstein Barr virus, the entry mechanism varies depending on the host cell. For example, HSV-1 enters by direct fusion of the viral envelope with the plasma membrane in African green monkey kidney epithelial cells (Vero cells) but enters by endocytosis in human HeLa cells. The objective of this project is to identify the entry mechanism(s) of CHV-1 in Madin-Darby canine kidney (MDCK) epithelial cells. We tested the effects of different inhibitors of cellular entry and trafficking pathways on CHV-1 infection. Adsorption of the virus to the cells was carried out at 4˚C in the absence of the inhibitors. Virus entry was then triggered by a temperature shift to 37˚C, where the cells and virus were incubated in the presence of the inhibitors or the vehicle alone for two hours. The impact of the inhibitors on CHV-1 infection was determined by plaque reduction assay. In addition, the viral entry pathway was studied using transmission electron microscopy to visualize epithelial cells at 30 seconds and two minutes post entry. Our results suggest that CHV-1 enters MDCK cells through endocytic pathways. Because virus entry is the first step in the virus-host cell interaction, understanding the entry mechanism will help identify potential targets for the development of antiviral drugs against this pathogen.

II31

Identification of IcsB-like acyltransferase family

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Shigella flexneri is a Gram-negative enteropathogen that causes bacterial dysentery. Its infection starts by invading human intestine using cell-to-cell spread technique. *Shigella* uses a type three secretion system (T3SS) to secrete effector proteins through its needle into the host cell causing the infection. IcsB is one of the secreted proteins that is crucial for *Shigella* to escape its vacuole. In addition, it was recently suggested that IcsB possesses an acyltransferase activity that is critical to this process. It is also known that IcsB expression is toxic in yeast. Prior work has demonstrated that when IcsB is co-expressed with the chaperone IpgA, the toxicity is due to the catalytic domain, as mutation of any of the three residues composing the catalytic triad of IcsB rescued toxicity. Interestingly, using bioinformatic approaches, we were able to identify putative proteins in other bacteria that share homology with the catalytic domain of IcsB. Most of those genes seem to have conservations all over the sequence, and they may thus constitute an IcsB-like acyltransferase family. We found that all three catalytic residues (histidine 145, aspartate 195, and cysteine 306) are conserved in all homologs. Nine out of ten homologs have not been studied yet. Hence, we have decided to use the same type of survival assay to test if they are toxic in yeast as a first step toward determining whether they are also endowed with an acyltransferase activity. First, we have established a yeast cytotoxicity assay that reproduced yeast killing upon galactose-induced expression of IcsB. We have also found out that the N-terminus of IcsB is inducing a dominant cytotoxic effect in the absence of IpgA. We are currently testing the capacity of IpgA to rescue this phenotype.

II32

Identification of regulators involved in class IIb bacteriocin expression in *Streptococcus pyogenes*

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Streptococcus pyogenes is a human-specific pathogen that causes a variety of infections including pharyngitis, skin infections and repetitive exposure to this bacteria can lead to life-threatening diseases such as, rheumatic heart disease. *S. pyogenes* has an arsenal of colonization and virulence factors, one of them being bacteriocins,

which are ribosomally-synthesized polypeptides with antimicrobial activity via pore formation. Our laboratory has discovered two novel Class IIb bacteriocin systems within the M18 serotype strain, MGAS8232, known as *Streptococcus pyogenes* bacteriocins (Spb) JK and MN. These systems have been found to be expressed within an *in vivo* mouse model. Currently the regulation of *spb* expression is uncharacterized, however, recent findings suggest that galactose and elevated levels (5%) of CO_2 are required for expression of the *spb* systems. This proposes that galactose and CO_2 are potential signals of a regulatory pathway involved in the expression of these Spb systems. We hypothesize that the disruption of genes via random transposon insertions will identify potential genes or pathways important for the expression of class IIb bacteriocins in *S. pyogenes*. A mariner-based transposon system known as the kanamycin resistant transposons for massive identification of transposants (Krmit) system will be used to produce a mutant library within the *S. pyogenes* MGAS8232 strain. These mutants will undergo screening via the deferred antagonism bioactivity assay. Mutants involved in *spb* expression could provide loss-of-function or gain-of-function in elevated and atmospheric levels of CO_2, respectively. The mutants involved in *spb* expression will be identified through inverse PCR methods. This project will be able to illustrate other factors in colonization closing the knowledge gap regarding the role of bacteriocins, while also discovering how these peptides contribute to the biology of *S. pyogenes*. Classifying these regulatory genes or pathways can potentially aid in providing future targets for preventative strategies against *S. pyogenes*.

II33

Impact de l'infection par le virus de l'herpès simplex 1 sur la voie d'immunité innée médié par oligoadenylate synthetase-like (OASL)

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Le virus herpès simplex 1 (VHS-1) infecte les muqueuses. Suite à l'infection aiguë, le virus établit une infection latente dans les neurones sensoriels qui est caractérisée par une absence de réplication virale malgré la présence du génome du virus. La réponse immunitaire innée contre l'infection par le VHS-1 comprend, entre autres, la détection de l'ADN viral par le senseur cGAS. L'activation de cGAS induit la production des interférons (IFNs) de type I, et l'expression par la suite des *ISGs* (*IFN-stimulated genes*) qui déclenchent l'état antiviral. Le gène *OASL* (*Oligoadenylate synthetase-like*) appartient aux *ISGs* dont la transcription peut être induite par les voies de signalisation STING/IRF3 ou JAK/STAT. L'OASL suscite un intérêt croissant car il a été récemment confirmé que dans le cas de virus à ADN, il peut y avoir un effet proviral d'OASL via sa capacité de moduler à la baisse la fonction de cGAS et, par conséquent, la production des IFNs. Cependant, le mécanisme par lequel le VHS-1 module l'expression ou l'activation d'OASL reste à être déterminé. Pour mieux comprendre ce phénomène, des cellules épithéliales humaines (HeLa) et des fibroblastes humains (HFF1) ont été infectés avec la souche KOS du VHS-1 ou mock-infectés, puis l'expression d'*OASL* a été évaluée par PCR quantitative à des temps précoces et tardifs de l'infection. Nous avons déterminé que l'expression d'*OASL* est induite dès cinq heures post-infection puis est modulée à la baisse dans les temps plus tardifs. En revanche, les niveaux des transcrits pour l'IFN-bêta ont été modulés à la hausse durant la période tardive de l'infection. Nos résultats concordent avec un modèle où le VHS-1 permet l'expression d'*OASL* dans un premier instant pour promouvoir le succès de l'infection, et par la suite module l'expression à la baisse pour bloquer son effet proviral afin de favoriser l'établissement de la latence. Ces résultats suggèrent un nouveau mécanisme de régulation par des protéines virales tardives sur la réponse immunitaire innée d'une manière OASL dépendante.

II34

A genomic epidemiology approach to investigating a persistent hospital-associated outbreak of vancomycin-resistant *Enterococcus faecium*

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Vancomycin-resistant *Enterococci* are a major hospital-associated pathogen. In 2013, a ward within a Saskatchewan hospital continued to identify VRE-positive cases over a nine-month period despite the implementation of extensive control interventions. Here, we combined whole genome sequencing of 51 VRE isolates and single nucleotide variation (SNV) phylogenomics to evaluate the nature of the outbreak. We used Illumina and Oxford Nanopore sequencing technologies to generate a high-quality reference genome, which guided the construction of a whole-genome phylogeny via the SNVPhyl bioinformatics pipeline. Our analysis revealed concurrent outbreaks originating from a single VRE clone within the ward, with new infections most likely arising from a small number of reservoirs contaminated by the founding clone and its direct descendants. In silico MLST analysis identified the clone as Enterococcus faecium Sequence Type 80, which was a predominant sequence type associated with global cases of VRE between 2014 and 2017. Oxford Nanopore sequencing revealed that acquisition of a plasmid correlated with accelerated genetic diversification and expansion of the outbreak. Our genomics-based approach provided novel insights into this hospital-associated outbreak, such as chains of transmission within the ward, evidence of effective control interventions, and contextualization of local isolates in relation to global cases of VRE.

II35

Impact of Canid herpesvirus 1 infection on the cellular miRNA profile of canine epithelial cells

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MicroRNAs are short, noncoding RNAs that negatively affect gene expression, and among other functions, are known to be involved in host-pathogen interactions. This study investigates the impact of *Canid herpesvirus* 1 (CHV-1) infection on the microRNAs of Madin-Darby canine Kidney epithelial (MDCK) cells. In adult dogs, CHV-1 infection causes respiratory, ocular and genital diseases. In puppies, CHV-1 causes a fatal disseminated hemorrhage leading to death. Our goal is to identify cellular microRNAs whose levels are affected by CHV-1 infection. To do this, the cellular miRNAs expression profiles of either uninfected or CHV-1-infected MDCK cells at 3 and 6 hours post-infection were investigated by microarray analysis.. The microarray was based on the latest version of miRbase (Release 22) which includes 453 mature dog microRNAs. Three independent series of infections were used in the study. The results of microarray hybridization identified several cellular microRNAs whose levels in infected cells either increased or decreased compared to uninfected cells. Identification of the potential target(s) of the cellular miRNAs that were modulated during the infection should provide information about how CHV-1 affects the host cell to prompte viral replication.. This information may in turn contribute to the development of antiviral treatments for CHV-1.

II36

Infection with microsporidia induces an immune response in the offspring of *Caenorhabditis elegans*

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In many invertebrates, infected parents have been shown to transfer immunity to their progeny. This phenomenon, known as transgenerational inheritance of immunity, has been observed with several animal-pathogen pairs, but there is little understanding of the mechanisms that are involved. To further understand this process, we have been studying *Caenorhabditis elegans*, a simple genetically tractable invertebrate model organism, during infection with a naturally occurring microsporidian pathogen, *Nematocida parisii*. Microsporidia are fungal-related obligate intracellular parasites that can infect a wide range of different animals including humans. *N. parisii* only invades and grows inside of the intestine of *C. elegans*, making this an ideal system to study how infection in somatic tissues can cause the transfer of immunity through the germline to the progeny. The progeny from parents infected with *N. parisii* are more resistant to the growth of the pathogen and to the effects of infection compared to progeny from parents that are not infected. This immunity is present one generation after infection and is no longer observable by the second generation. The immunity is strongest in the earliest larval stage and is diminished at later developmental stages. Animals from infected parents are initially less infected, suggesting that the immunity functions by inhibiting the ability of microsporidia to infect intestinal cells.

Upon infection with *N. parisii*, *C. elegans* upregulates a set of genes that provides immunity to infection. Host animals carrying loss of function mutant alleles in either of two genes, *pals-22* and *lin-35*, have an overlapping transcriptional response to wild-type animals infected with *N. parisii*. These mutants are also both resistant to infection by *N. parisii*. Thus, these two strains represent a constitutively activated response to infection. Using *pals-22* and *lin-35* mutant strains, as well as transgenic rescue lines, we show that cross-progeny offspring are more resistant to the pathogen and to the effects of infection. Further, we show that this resistance to *N. parisii* is maternally dependent, and can originate cell non-autonomously. Interestingly, our data suggests that multiple somatic tissues can signal for offspring resistance. We are currently using the auxin-inducible degradation system to deplete PALS-22 and LIN-35 in a tissue-specific and time-dependent manner to test the kinetics of this transgenerational phenotype and further uncover the mechanisms behind it. This work suggests that the initial response to infection is not only potentially able to provide defense against the pathogen for the infected parents, but also to provide immunity for their progeny.

II37

Integrated Rapid Infectious Disease Analysis: A comprehensive platform for public health bioinformatics and AMR surveillance using genomic data

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Antimicrobial resistance (AMR) is a major and growing global health crisis. Development of comprehensive AMR surveillance and rapid diagnostics capabilities with genomic and metagenomic data is fundamental to improving understanding of AMR transmission and stewardship of our limited and dwindling antibiotic arsenal. Unfortunately, effectively using genomic data for public health applications such as this is impeded by a scarcity of user-friendly automated and semi-automated pipelines and data sharing. To address these issues, we developed the Integrated Rapid Infectious Disease Analysis (IRIDA) platform (irida.ca), a user-friendly, decentralized, open-source bioinformatics and analytical web platform to support multi-jurisdictional infectious disease outbreak investigations using genomic sequencing data. IRIDA incorporates quality control, genomics assembly and annotation, *in silico* serotyping, multi-locus sequence typing, and outbreak phylogenetics. This platform also incorporates direct visualisation of results to assist with hypothesis generation in epidemiological investigations. IRIDA allows users to perform secure and local analysis, while also enabling controlled data sharing with trusted partners and public sequence repositories.

Additionally, via built-in Galaxy support it integrates high-quality curated AMR data from the Comprehensive Antibiotic Resistance Database (CARD; card.mcmaster.ca) with state-of-the-art AMR detection methods for genomes and metagenomes such as the Resistance Gene Identifier (RGI) and, in the future, AMRtime. CARD is an ontology-centric database and bioinformatics resource on the molecular and genetic basis of antimicrobial resistance (AMR) that has recently been greatly expanded with Resistomes and Variants surveillance data for over 81,000 isolates, plus extensions to its underlying Antibiotic Resistance Ontology. These improvements have been leveraged to extend RGI to enable rapid read-mapping for detection of AMR genes from metagenomic data, with associated k-mer classifiers for pathogen-of-origin prediction. They have also served as the basis for the development of AMRtime, a highly sensitive and accurate machine-learning based metagenomic AMR classification tool.

This extensive set of genomic epidemiology focused analysis methods and robust data management tools are completely open-source and available to all. Currently, IRIDA is being used by the Public Health Agency of Canada as a primary tool for infectious disease outbreak investigations and is used/installed by other government and academic groups internationally across four continents (including the US, UK, South Africa, and Singapore). IRIDA is freely available at https://github.com/phac-nml/irida and www.irida.ca.

II38

Interaction of the *Escherichia coli* heat-stable enterotoxin b (STb) with the NCI-H716 human intestinal cell line

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The interaction of the *Escherichia coli* heat-stable enterotoxin b (STb) with host cells has been investigated in various cell lines over the past 30 years. A most consistent finding has been the observed elevation of intracellular calcium levels upon exposure of cells to STb. Identification of the receptor on those cells have provided conflicting data, however. It appears that depending on the cell line used, a protein or a glycolipid is implicated as a receptor for the toxin. These include a sulfatide in pig jejunal epithelia cells, STR-200A (70 kDa) and STR-200B (53/77 kDa) glycoproteins in Sprague-Dawley rat jejuna/upper ilea cells, and a 25 kDa protein in CD-1 mouse intestinal villus cells. We report here on the isolation of a putative STb receptor in NCI-H716 human intestinal cell line with an apparent molecular weight of 43 kDa. This putative receptor was isolated from the membrane fraction of the cells. Immunofluorescene microscopy shows that the bound STb is internalized via what appears to be active endocytosis.

II39

Investigating microbial engraftment via comprehensive culture-enriched and culture-independent metagenomics in patients with ulcerative colitis

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Surette lab is part of a team investigating the efficacy of FMT for patients suffering from ulcerative colitis, using a comprehensive set of microbiome data from donors and patients in two large randomized control trials. We have previously shown preliminary success in using FMT for the treatment of patients with ulcerative colitis; however, microbial engraftment at the species/strain level has historically been very difficult to demonstrate in FMT studies, especially given the low resolution provided by 16S rRNA gene amplicon sequencing and the challenges in the refinement of high-quality genomes from metagenomic samples. We used culture-enriched metagenomics (CEMG) approach together with other shotgun metagenomic techniques such as assembly and binning to construct high-quality metagenome assembled genomes (MAGs) from a single donor. We compared the presence of MAGs in a single donor at different time points and created a comprehensive DNA sequence library contains functional annotations and taxonomic assignments. The donor reference database was used to track the engraftment of genes and genome in 8 FMT recipients from our previous randomized control trial using metagenomic mapping for each patient with data from before and after FMT. CEMG approach combined with in silico assembly-based methods allowed us not to only recover 209 number of MAGs from a single donor but also, we were able to predict novel metabolites, and more functional and carbohydrate activities that were not investigated earlier. We were able to predict metagenome assembled genomes, genes, and functions that were not present before FMT but they were detected in high abundance after fecal microbiota transplantation. The combination of CEMG and direct shotgun sequencing tackle some of the challenges present in the metagenomics analysis, particularly by increasing the number as well as the accuracy of the MAGs. Our novel culture-dependent approach provides higher genomic resolution that can predict bacterial engraftment in fecal microbiota transplantation for patients with ulcerative colitis.

II40

Investigation of a staphylococcal secreted product with activity against methicillin-resistant *Staphylococcus aureus*

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Abstract not disclosed.

II41

Lighting-up *Pseudomonas aeruginosa*: reporter system for anaerobic tracking

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Cystic fibrosis (CF) is a genetic disease characterized by a thick mucus build-up in the lungs. This mucus is the niche for persistent microbial colonization, sustained inflammation and recurrent infections. The chronic nature of these infections could be attributed to the complex microbiome inhabiting the CF airway. That complexity lies

in the microbial diversity and the heterogeneity of the physicochemical environment. For instance, various levels of O_2 and anoxic pockets are encountered in the thick mucus layer in the CF lung. *Pseudomonas aeruginosa*, one of the major CF pathogens, is particularly detrimental to CF individuals. Indeed, *P. aeruginosa* is a multi-drug resistant organism, and its ability to form a robust biofilm increases its tolerance towards many antibiotics. Fluorescent reporters to study *P. aeruginosa* have been limited to GFP and its variants. However, GFP strictly requires oxygen which is limited within the CF microbiome. Thus, GFP appears unsuitable as a marker in this context. This project aims to develop a reporter system to monitor *P. aeruginosa* anaerobically within an *in vitro* CF microbiome. We started by establishing the optimal anaerobic growth conditions for our model strain PA14. Then, using the plasmid pSMC21/GFP$^+$, we excised the GFP open reading frame (ORF) and replaced it with the ORFs of seven fluorescent proteins (FPs) purposely designed to fluoresce regardless of O_2 level. The newly generated plasmids were transformed into PA14. All these strains grew similarly to PA14 expressing an empty plasmid except the strain expressing the FPs – iRFP670 and LucY; which grew at a slightly slower rate. Fluorescence experiments are being conducted to evaluate the brightness of these FPs in PA14 cells grown as a planktonic and biofilm culture under aerobic and anaerobic conditions. Biofilm cultures are carried out using a microfluidic device, and the fluorescence is assessed using confocal laser-scanning microscopy. The FPs that perform the best under our conditions will be used as reporters to monitor *P. aeruginosa* anaerobically in "cystic fibrosis-like" conditions. This project will yield a better understanding of the persistence of *P. aeruginosa* in the CF lung.

II42

Mechanism of action of the synergistic combination tomatidine-aminoglycoside against *Staphylococcus aureus* virulent and persistent phenotypes

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Background: *Staphylococcus aureus* (SA) can adopt two phenotypes, prototypical (WT) and its small-colony variant (SCV). These two phenotypes are often recovered from the lungs of cystic fibrosis (CF) patients and are frequently co-isolated with *P. aeruginosa*. Aminoglycosides such as tobramycin or gentamicin (GEN) are often used for prophylaxis against *P. aeruginosa* in CF. Tomatidine (TO), a steroidal alkaloid, exhibits a bactericidal effect on *S. aureus* SCV. Moreover, when TO is combined to an aminoglycoside, the combination shows a strong synergistic activity against WT SA. We recently determined that the molecular target of TO was the ATP synthase subunit c (AtpE) and we report here how TO, with or without GEN, exerts its bactericidal activity against the WT and SCV phenotypes of SA.

Methods: Since TO affects the bacterial ATP synthase, we measured the membrane potential. Bacteria in broth were incubated with various concentrations of antibiotics (TO or TO-GEN). Bacteria were then washed in PBS and the fluorophore $DiOC_2$ was added and incubated for 30 min before flow cytometry. To assess the production of reactive oxygen species (ROS), bacteria were suspended in broth and incubated 2h at 35°C before the addition of 10 µM H_2DCFDA for 1h. Bacteria were then washed and transferred to a 96-well plate containing broth and antibiotics (ciprofloxacin as a control). Fluorescence was measured (λ exc 494_{nm}, λ emi 521_{nm}) over a 13-h period. All results are reported as a percentage of that measured for WT without antibiotic.

Results: TO reduced WT membrane potential in a dose-dependent manner and reached a low of 35% at the highest doses (≥8 µg/mL). On the other hand, SCV membrane potential, which was about 10% of that of WT, further dropped to about ≤2% at very low TO concentrations (≥0.0035 µg/mL). This was also accompanied by 2 times more ROS production than that seen in the no antibiotic control. Besides, there was no difference in the membrane potential of WT when comparing the effect of GEN to that of TO-GEN. However, the combination TO-GEN generated 2.5 times more ROS compared to that caused by GEN alone.

Conclusions: TO is able to reduce the membrane potential of both SA WT and SCV phenotypes, but the membrane potential only dropped to a critical level in the SCV. Also, significant ROS are only produced in SCV, which are highly susceptible to TO. Similarly, only the TO-GEN combination generated significant ROS production in the WT, which explains the strong synergy with aminoglycosides. The TO-GEN combination may represent a novel therapeutic paradigm for lung infections in CF patients, targeting both *S. aureus* phenotypes (WT and SCV) and *P. aeruginosa* altogether.

II43

Molecular epidemiology of Rotavirus in children with gastroenteritis in Qatar

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Objective: Acute gastroenteritis remains a major cause of morbidity and mortality of young children worldwide. The vast majority of diarrhea cases in developing and developed countries are attributable to the viruses and to a lesser extent to bacteria, fungi, and toxins. Rotavirus (RV) is recognized as the most important etiological agent leading to acute gastroenteritis globally. In order to determine the burden and characteristics of RV infections in children in Qatar, profiling of circulating genotypes and their correlation with demographics and clinical manifestations were evaluated.

Methods: A total of 205 RV-positive fecal samples were collected from children suffering from acute gastroenteritis (AGE) during two-year seven months period between June 2016 and January 2019. The age of the subjects ranged between 3 months and 12 years (median of 15 months). Genotyping was performed by amplifying and sequencing both VP4 (P-type) and VP7 (G type) regions. Phylogenetic analysis and evolutionary relationships were performed using MEGA7.0. Fisher's exact test was used to run statistical analysis for the clinical and demographical characteristics of circulating strains.

Results: Overall, RV infections were relatively higher in males than females with a ratio of 1.7:1 (P=0.0082). Most of the RV infections were reported in children between 1-3 years old (54.7%), followed by those <1 year- and > 3 years of age (38.4% and 6.9%, respectively). RV infections occurred throughout the year, with a noticeable increase in summer (42.8 %) and drop in winter (20.1%). RV genotypes G3P[8] (38.5%), G1P[8] (30.2%), G9P[8] (17.3%) and G2P[4] (14%) were the dominant genotypes during the study period. Among the RV-positive cases, 118 (54.3%) had been vaccinated using either of the RV vaccines available. Number of children vaccinated with one and two doses were 49 (41.5%) and 69 (58.4%), respectively. The percentage reduction of disease in a vaccinated group of pediatrics compared to an unvaccinated group of pediatrics was 23%. Of these, 91 (77.1%) experienced diarrhea for less than three days and only eight (6.7%) had diarrhea for more than 5 days. Only 18 among those who were vaccinated stayed long in the hospital (more than 4 days) compared to non-vaccinated children. All vaccinated children showed mild to moderate dehydration except for seven children who were then treated with intravenous fluids.

Conclusion: In this study we detected four different rotavirus G genotypes, 2 different P genotypes, 7 different G-P combinations. G3, G9, G2 and P[8] were found to be the predominant genotypes, followed by P[4]. The high infection rate with RV regardless of vaccination requires further assessment.

II45

Optimizing transmission electron microscopy-based visualization of *Pseudomonas aeruginosa* PAO1 within bronchial epithelial cells

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Cystic fibrosis (CF) is a genetic disorder, resulting from various mutations in the cystic fibrosis conductance regulator gene. These mutations result in a slew of downstream effects, which create an optimal environment for the development of bacterial lung infections. *Pseudomonas aeruginosa*, which is an opportunistic, multi-drug resistance pathogen, is the predominant pathogen that colonizes adult CF patients' lungs during late-stage disease. These infections can lead to pulmonary failure and are life-threatening for CF patients. The most commonly studied mode of persistence of *P. aeruginosa* in the context of CF is its growth in biofilms, which are surface associated communities of microorganisms. Importantly, within the last decade, it was discovered that *P. aeruginosa* is also capable of surviving inside epithelial cells. How *P. aeruginosa* survives intracellularly is still not well understood and understanding this mode of persistence could have significant impacts on patient treatment. High resolution transmission electron microscopy (TEM) sectioning is one technique that we can use to deepen our understanding of intracellular *P. aeruginosa*. TEM allows for the visualization of cellular ultrastructures and will enable us to determine the specific localization and organization of intracellular *P. aeruginosa*. However, this imaging technique needs to be optimized for visualization of this host-pathogen interaction *in situ*. To examine the localization of internalized *P. aeruginosa*, I have modeled early (4-hour) and late (24-hour) co-culture intracellular infections using bronchial epithelial cells and a laboratory stain of *P. aeruginosa*, PAO1. Importantly, these intracellular infections were conducted using a multi-layered cell system grown at the air-liquid interface on transwell inserts to maintain polarity of the cell layer and mimic the host-pathogen interface. As a quality control measure, the presence of a multi-layered cell culture was confirmed in a replicate sample using histological staining and light microscopy before an infection was conducted. At the experimental endpoint, the samples were fixed, embedded, and stained, and the resulting interactions were visualized using TEM. Various stages of each of these procedures were optimized during protocol development, including maximizing the resin penetration through the multiple layer model during embedding. The preliminary images obtained from this protocol have shown the presence of intracellular *P. aeruginosa* and suggest a consistent rate of internalization. Further optimization of the sample preparation protocol will improve image resolution and further elucidate the presence or absence of a membrane surrounding the bacterial cell residing within the cell. Using this technique to visualize intracellular infections could lead to insights into alternative modes of *P. aeruginosa* infections and could impact patient treatment regimes.

II46

Producing a porcine epidemic diarrhea virus virus-like particle in plants for oral administration to pregnant sows and protection of neonate piglets

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Porcine epidemic diarrhea virus (PEDv) causes disease and mortality to piglets worldwide. Most vaccines used to combat the disease have been ineffective live attenuated virus vaccines. The goal of this project was to produce a plant-made virus-like particle (VLP) displaying antigenic epitopes of the PEDv membrane protein. To obtain sufficient protein and subsequent VLP assembly, we used an elastin-like polypeptide fusion with the

membrane protein. The resulting protein accumulated up to 0.8 mg/g of fresh leaf weight when transiently expressed in *Nicotiana benthamiana*. Virus-like particles were observed when the membrane protein was overexpressed, but also when the membrane protein was co-expressed with the envelope protein. In the latter case, the VLPs were slightly larger in size. This represents the first time coronavirus-like particles have been made in plants, and is a first step towards a plant-made oral vaccine for PEDv. We are currently gearing up to test this candidate vaccine in mice and pigs.

II47

Purification and characterization of two new antimicrobial molecules produced by an endophytic strain of *Paenibacillus polymyxa*

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Endophytes are promising producers of compounds with diverse biological potential. Among those compounds, antimicrobial peptides (AMPs) are molecules with a broad spectrum of activity that have been studied as prospective antibacterial and antifungal agents. An endophytic bacterium, isolated from Brazilian tropical savannah trees, presented ability to inhibit pathogenic bacteria. The strain was genetically identified by 16S rRNA analysis. The endophyte *Paenibacillus polymyxa* RNC-D was cultivated in YPM broth under aeration. The biochemical characterization of fermentation broth suggested the peptide nature of antimicrobial molecules. The first step to purify the compounds was a liquid-liquid partition that resulted in organic fraction (OF) and aqueous fraction (AF). OF presented a broad spectrum of activity against a panel of pathogenic bacteria and fungus. Reverse phase chromatography of OF resulted in the separation of the compounds into four peaks named FRP1, FRP2, FRP3 and FRP4. The bioassay showed that FRP4 inhibited the indicator strains *E. coli* and *S. aureus*. The MS analysis of FRP4 revealed the presence of an ion with m/z 3,376.459 Da, whose proposed molecular formula was $C_{182}H_{321}N_{29}O_{29}$. The present study reports for the first time a compound that was named polycerradin, whose spectrum of activity includes Gram-positive, Gram-negative bacteria and fungus. Quantitative assays indicated Minimum Inhibitory Concentration (MIC) range of 1.97 – 15.6 µg ml^{-1}, Minimum Microbicidal Concentration (MMC) range of 7.8 – 62.5 µg ml^{-1}, and MMC/MIC ratio of 2 and 4. The AF, which was active only against Gram-negative bacteria, was sequentially submitted to ion-exchange chromatography, desalting chromatography and reverse phase (RP) chromatography. The compound present in peak IE-1/AF/RP-1, with a RT of 2.45 min from RP chromatography, exhibited antimicrobial activity against all Gram-negative pathogenic strains tested with the exception of *P. mirabilis*. The molecule was named AMP PpRNCD and its primary structure was determined as Gly-Glu-Hyp-Gly-Ala which is an unprecedented reported molecule. In addition, the molecular mass and amino acid sequence were confirmed by MS/MS. The mass spectrum indicates that the molecular mass of the AMP PpRNCD is 463 Da. The presence of the amino acid hydroxyproline (Hyp) was detected first in the peptide described herein produced by a species of *P. polymyxa.* The unusual Hyp confers greater stability to the molecule. In terms of molecule size, it can be considered that AMP PpRNCD is one of the smallest active natural peptides reported, yet.

II48

Reduction of biofilm formation by sub-inhibitory concentration of polymyxin B in *Vibrio cholerae*

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Background: The acute diarrheal disease cholera, caused by *V. cholerae*, remains a significant public health problem, causing large numbers of infections and deaths annually in the world. During the infection, *V. cholerae* colonizes the surface of the small intestine. There are increasing number of evidence that *V. cholerae* is forming biofilm-like structures *in vivo* during the infection of the small intestine. The intestinal epithelium is the site of synthesis of many antimicrobial peptides, whose expression can be constitutive or inducible by microorganisms. In addition, bacteria from the microbiota are also secreting antimicrobial peptides. The aim of this study is to determine the effect of sub-inhibitory concentration in antimicrobial peptides on biofilm formation in *V. cholerae*.

Methods: A determination of the minimal inhibitory concentration in polymyxin B, a standard antimicrobial peptide, in condition of biofilm formation has been performed. After verification of the viability of the bacteria using flow cytometry analysis and growth curves coupled with colony forming unit counting, we determined the work concentration of polymyxin B as ¼ of the minimal inhibitory concentration. Then, standard crystal violet quantification of the biofilm, soft agar motiliy test, electronic microscopy and western blotting analysis were performed.

Results: We observed a significant decrease in biofilm formation by *V. cholerae* in presence of polymyxin B after 24h. Experiments on preformed biofilm demonstrated that the inhibition occurs at the initial step of biofilm formation and no effect of the polymyxin B is observed at the maturation stage. Since the flagella is essential at the initial steps of biofilm formation of *V. cholerae*, we performed western-blot analysis to determine the quantity of flagellin associated to the bacteria. Our results demonstrate that the quantity of flagellin decreases in presence of PmB. Electron microscopy observation of the bacterial cells show a reduction of the number of flagellated cells. This reduction is associated with a significant decrease of the bacterial motility on soft agar plates.

Conclusions: The presence of polymyxin B at sub-inhibitory concentration induces a reduction of the biofilm formation by *V. cholerae* most probably due to a deficience in flagella synthesis.

II49

Resolution and co-occurrence patterns of *Gardnerella leopoldii, Gardnerella swidsinskii, Gardnerella piotii* and *Gardnerella vaginalis* within the vaginal microbiome

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Gardnerella vaginalis is a hallmark of vaginal dysbiosis, but is found in the microbiomes of women with and without vaginal symptoms. *G. vaginalis* encompasses diverse taxa differing in attributes that are potentially important for virulence, and there is evidence that 'clades' or 'subgroups' within the species are differentially associated with clinical outcomes. The *G. vaginalis* species description was recently emended, and three new species (*leopoldii, swidsinskii, piotii*) and nine additional "genome species" were defined within the genus. 16S rRNA sequences for the four *Gardnerella* species are all >98.5% identical and no signature sequences differentiate them. Based on previous demonstrations that the chaperonin-60 (cpn60) barcode sequence is an excellent predictor of whole genome sequence similarity, we sought to determine is this phylogenetic marker could be used to resolve the newly designated *Gardnerella* spp.. We demonstrated that *Gardnerella* species can

be resolved based on cpn60 sequences, with pairwise percent identities of 87.1-97.8% among the type strains. After establishing the resolving power of cpn60 barcode sequences for *Gardnerella*spp., we investigated pairwise co-occurrence patterns of *Gardnerella* spp. in the vaginal microbiomes of 413 reproductive aged Canadian women. Among the most frequently detected species (*G. vaginalis, G. swidsinskii, G. leopoldii, G. piotii* and genome sp. 3), the smallest pairwise Jaccard distances (i.e. the most samples in common) were observed for *G. vaginalis* and *G. swidsinskii*, and *G. piotii* and genome sp. 3. None of the species pairs had fewer co-occurrences than expected, suggesting that competitive exclusion may not be important for describing their relative distributions. cpn60 amplicon sequencing can provide a rapid assessment of the relative abundance of *Gardnerella* spp. in microbiome samples, providing a powerful method of elucidating associations between these diverse organisms and clinical outcomes. Application of cpn60 barcode sequencing will provide new insight into vaginal microbial ecology, and whether *Gardnerella* spp. diversity is an explanation for issues such as treatment failure and recurrent vaginal dysbiosis, or for the failure of antimicrobial treatment in the prevention of preterm birth despite a strong association between vaginal dysbiosis and preterm delivery.

II50

Role of the *Streptococcus pyogenes* adhesion locus in nasopharyngeal infection

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Streptococcus pyogenes is a human-specific pathogen that can cause a multitude of diseases with mild to fatal outcomes, such as pharyngitis or the post-infection sequelae acute rheumatic fever, which can lead to fatal rheumatic heart disease. A crucial step in the initiation of pathogenesis by *S. pyogenes* is its successful adherence to mucosal epithelial cells, thereby enabling infection and subsequent colonization of the host. One genomic locus thought to be involved in this process is the fibronectin-binding, collagen-binding, T-antigen (FCT) region. Among other things, the FCT region encodes a pilus structure (*T18.1*), a collagen-binding protein (*cpa*) believed to be attached to the top of the pilus, as well as a fibronectin-binding protein (prtF2), which may be crucial to the initial attachment of *S. pyogenes* to its host during pharyngitis.

To determine the role of this locus, the FCT region was genetically deleted in *S. pyogenes* MGAS8232 using molecular cloning techniques. The adherence properties of this mutant to the extracellular matrix proteins collagen and fibronectin were then tested by performing a cell-substrate adhesion assay using ELISA. Furthermore, the binding capabilities to the human pharyngeal cell line Detroit-562 was also tested by incubating the bacterial strains with a monolayer of the cells, and enumerating the remaining *S. pyogenes* the following day. We discovered that the FCT region is required in order to bind collagen, and also confers partial binding to fibronectin. The ability to bind human pharyngeal cells, however, was not different between the strains, suggesting that the FCT region genes may not interact with the apical surface of human cells. The wildtype and mutant strains were also nasally inoculated in transgenic mice expressing human-leukocyte antigens (to account for the human-specific tropism), and the nasal turbinates were harvested 24 or 48 hours later. RNA was isolated from the turbinates and qRT-PCR was performed to evaluate quantities of *T18.1*, *cpa,* and *prtF2.* At 24 and 48 hours post-inoculation, *T18.1* and *prtF2* mRNA were not detected, while *cpa* was, suggesting that the collagen-binding protein might have a function in nasopharyngeal colonization. Additionally, the nasal turbinates were processed and plated for bacterial enumeration. There was no significant difference between the bacterial recovery indicating that the wildtype and FCT deletion strains infect the nasopharynx at equal magnitudes.

The study of pharyngitis is critical to understanding the development of acute rheumatic fever and rheumatic heart disease. Findings from this study will help illuminate the circumstances surrounding early stages of pharyngitis, and how S. pyogenes strains can establish pharyngeal infection with the aid of its adherence locus.

Future work would then be able to focus on the hindrance of early colonization thereby preventing initial and repeated infection of this globally-relevant pathogen.

II51

Salmonella enterica serovar Typhimurium induces inflammation to overcome the mechanisms of colonization resistance in a swine model

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Salmonella enterica is one of the most important causes of diarrheal disease in humans, and pigs are a common zoonotic reservoir. It has been proposed that *Salmonella* induces inflammation to overcome the microbiota and thereby influence colonization resistance (CR), a process that is often referred to as the "differential killing hypothesis" (DKH). To test the DKH, we established a model of acute salmonellosis in Landrace-Duroc cross piglets. Pigs were orally inoculated with either *Salmonella enterica* Typhimurium DT104 (challenge treatment, 3.0×10^9 cells/ml) or Columbia Broth (CB, control), and a variety of host and microbial responses were examined at 2, 6, and 10 days post-inoculation (dpi; six replicate piglets per treatment and time). Body temperatures were elevated, feed consumption and weight gain were reduced, and evidence of gross pathologic impacts on the intestine were observed within the infected piglets. Densities of *Salmonella* associated with mucosa of infected animals decreased over time, with higher copy numbers of bacteria in the ileum and large intestine. Significant histopathological changes were also observed in the ileum and along the large intestine. The severity of tissue injury differed as a function of time with the greatest change in epithelial injury and neutrophil infiltration occurring at 2 dpi. In contrast, changes in tissue fibrosis and villous fusion were more prominent at 10 dpi. A variety of new quantitative primers were designed and validated. Between *Salmonella* ± treatments, differential expression of *TNFα*, *IFNγ*, *IL1β*, *IL17*, *IL10*, *PR39*, *MUC4*, *iNOS*, *IL8*, *REGIIIg*, among others was detected in the cecum, spiral colon, or ileum. *IL8* was up-regulated (p<0.050) in cecal mucosa of infected animals at 2 and 6 dpi. Significant up-regulation of cathelicidin (i.e. *PR39*) (p<0.01) and *iNOS* (p<0.050) were detected in the cecum of infected pigs at 2 dpi. Characterization of the enteric microbiota was investigated by anaerobic culture and Illumina sequencing targeting 16S rDNA. *Lactobacillus*, *Blautia*, *Megasphaera*, and various *Enterobacteriaceae* genera were highly associated with inflamed tissues, while others such as *Clostridium sensu stricto*, *Actinobacillus*, and *Terrisporobacter* were mainly associated with healthy mucosa. In conclusion, we incited salmonellosis in piglets that was representative of all stages (i.e. acute, subacute, and recovery) of enteric disease, and the interaction among the host, pathogen, and enteric microbiota was elucidated. Importantly, we showed that salmonellosis in the cecum of piglets was associated with high expression of various host defense responses and differential colonization by enteric bacterial taxa. The information obtained from this study is currently being used to mechanistically test the DKH using murine models of colonization resistance.

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Single-strand HIV-1 RNA induces CCR7-dependent monocytes transmigration through the blood-brain barrier via T LR7 and 8 activation

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Introduction: Monocytes play critical roles in human immunodeficiency virus type-1 (HIV-1) pathogenesis. The recruitment of activated monocytes and subsequent systemic migration contribute to the spread of HIV-1 to reservoir organs such as the central nervous system (CNS). In this study, we aim to examine whether Toll-like receptor type 7 and 8 (TLR7 and 8) activation by agonists and HIV-1 single-strand RNA (ssRNA), can modify the CCR7-dependent transmigration of human monocytes through the blood-brain barrier (BBB).

Methodology: MonoMac-1 (MM-1) cells and freshly isolated human monocytes were cultivated in the presence of HIV-1 ssRNA or the exposition to pseudotyped HIV-1 particles, and TLR7 and 8 agonists. CCR7 mRNA transcription was measured using quantitative reverse transcription polymerase chain reaction, and protein expression was examined using flow cytometry. CCR7 function was monitored using migration and transmigration assays in response to CCL19/CCL21, which are natural ligands for CCR7.

Results: Our results show that stimulation of MM-1 and human monocytes using ssRNA and CLO75 (TLR8 agonist) increase CCR7 mRNA expression. However, the simultaneous stimulation of TLR7 and 8 results in a smaller induction of CCR7 mRNA, whereas the activation of TLR7 (Imiquimod) has no impact. We also demonstrated that TLR8 strongly increases monocyte migratory capacity in response to CCL19 in chemotaxis and transmigration assays in a 2D model that mimics the human BBB, while TLR7 does not affect. Besides, monocytes exposition to pseudotyped HIV particles shows an increase in transmigration through the BBB in response to CCL19.

Conclusion: We demonstrate that HIV-1 dsRNA via TLR8 activation leads to an increase in the CCR7-dependent transmigration of monocytes through the BBB. Understanding the molecular mechanisms of monocyte trafficking in the presence of HIV will help the development of new therapeutic strategies to prevent the deleterious consequences of monocyte infiltration at the sites of chronic inflammation.

II53

Species-level molecular typing of clinical *Pantoea* strains with cpn60

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The Enterobacterial genus *Pantoea* contains both free-living and host-associating species, with considerable debate as to whether documented reports of human infections by members of this species group are accurate. Current phenotypic identification methods commonly used in clinical laboratories are often unable to accurately differentiate between *Pantoea* species, and in some cases, even result in the labeling of other members of the *Enterobacteriales* as *Pantoea*. In this study, we evaluated the utility of the heat shock gene, *cpn60* (*groEL*) for species-level identification of *Pantoea* by comparing to a higher-resolution seven-gene multi-locus sequence analysis phylogeny, and found that *cpn60* reliably reconstructs *Pantoea* species groups. We carried out genetic typing of 64 clinical and environmental candidate *Pantoea* strains using *cpn60*, and found that 47 (73%) were confirmed to be *Pantoea*. Of the 55 clinical isolates that had been identified as *Pantoea*, 27% were misidentified, and included members of *Citrobacter*, *Enterobacter*, *Kosakonia*, *Klebsiella*, *Pseudocitrobacter*, members of the newly described *Erwinia gerundensis* and *Mixta calida*, and even several unclassified members of the *Enterobacteriaceae*. The 40 clinical strains that were confirmed to be *Pantoea* were identified as *Pantoea* agglomerans, *Pantoea allii*, *Pantoea brenneri*, *Pantoea conspicua*, *Pantoea dispersa, Pantoea eucalypti*, and *Pantoea septica* as well as the proposed species group, *Pantoea latae*. Our results indicate that *cpn60* provides adequate resolution for species-level identification of *Pantoea* strains, and that some species groups considered largely environmental or plant-associated, such as *P. allii* and *P. eucalypti* may also be capable of opportunistic human infections.

II54

Structural genomics of proteins mediating Gram-negative cell envelope biogenesis

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The Gram-negative cell envelope is an essential structure for maintaining cellular integrity, shape and for mediating interactions with the extracellular environment. The cell envelope forms a formidable barrier to the penetration of antibiotics and presents a major difficulty for antibacterial drug discovery and development. Genome-wide deletion screens of *E. coli* have discovered genes important for blocking penetration to dyes, metals and/or antibiotics, or, genes important for the maintenance of cell shape. Even for this model organism, many of these gene products have no known function and this hinders a fulsome understanding of the molecular mechanisms of cell envelope biogenesis.

One powerful technique for the discovery of protein function is determination of their 3D structures, which enables hypothesis generation and further testing i.e. via rational mutagenesis. In this study, we aimed to elucidate the molecular function of proteins identified by such genome-wide screens to understand their biological roles.

We undertook a structural genomics approach to express, purify, crystallize and determine the 3D structure of more than 200 proteins that were discovered by 7 different published genome-wide screens, along with the orthologs in Gram-negative pathogens. We present the crystal structures of 27 proteins, which belong to various categories including hydrolase, oxidoreductase, transferase, aldolase, transport systems, transcription regulators, as well as many that remain with unknown function. The structural features of the most interesting representatives of this study will be discussed, particularly an acyl-CoA thioesterase (YbgC), a putative nicotinamide mononucleotide deamidase (YdeJ), a putative intermembrane transport lipoprotein (PqiC) and a protein important in peptidoglycan biosynthesis (ElyC/YcbC).

We found that the *E. coli* YbgC thioesterase demonstrates higher activity towards long-length substrate (decanoyl-CoA (C10)) over short-length (butyryl-CoA (C4)), consistent with a role in the generation of membrane fatty acids. Antimicrobial susceptibility testing showed that the *E. coli* knockouts Δ*ybgC* and Δ*elyC*Δ*ycbC* have higher susceptibility to many antibiotics including ciprofloxacin, a DNA gyrase and topopisomerase IV targeting antibiotic. These results are consistent with the notation that these proteins play important roles in cell envelope permeability.

Ongoing studies include continuing *E. coli* knockout/complementation experiments utilizing structure-guided active site mutations, evaluation of cell integrity, antimicrobial susceptibility testing and membrane lipid and peptidoglycan composition.

Altogether, our results to date provide multiple avenues for further studies into the basic biology of Gram-negative cell envelope biogenesis and highlight potential novel antimicrobial targets.

II55

Structural insights on the mechanism of acquired sulfonamide antibiotic resistance

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The unprecedented worldwide misuse and overuse of antimicrobials has fueled an alarming increase in microbial adaptation and in antimicrobial resistance (AMR). Multi-drug resistant bacteria now pose a serious global threat

in combating infectious disease. AMR mechanisms are diverse, ranging from intrinsic resistance such as the formidable Gram-negative bacteria cell envelope, to acquired resistance mechanisms based on horizontal transfer of genes (for e.g., those present in plasmids, transposons or integrons) encoding antimicrobial resistance enzymes. The efficiency and efficacy of all antimicrobial class drugs in human and veterinary medicine are compromised by the acquisition of such mobile gene elements.

The sulfonamides (sulfas) are the one of the earliest discovered antimicrobials possessing broad-spectrum bacteriostatic activity against both Gram-positive and Gram-negative bacteria. Sulfas target the essential, highly conserved dihydropteroate synthase (DHPS) enzyme, involved in folate biosynthesis, which condenses *para*-aminobenzoic acid (*p*ABA) and 7,8-dihydropterin-pyrophosphate (DHPPP) to form 7,8-dihydropteroate; sulfas are *p*ABA analogs. While sulfas are a well-established class of antimicrobial for humans and livestock, their usage has been threatened by resistance through point mutations in the *dhps* gene or by acquired resistance conferred by mobile genes for divergent dihydropteroate synthases, the so-called *sul* genes (*sul1*, *sul2*, *sul3* and *sul4*).Sul enzymes are able to distinguish its own substrate *p*ABA from sulfonamides and thus acquisition of these mobile Suls restores the dihydropteroate synthesis reaction. Even after nearly 40 years since the initial discovery of the plasmid-borne *sul1,* the molecular basis by which Suls confer drug resistance is not clearly understood.

Our study aims to elucidate the structural and molecular basis of acquired sulfonamide resistance. We have solved the three-dimensional crystal structures of Sul3 in its apoenzyme form to 2.3 Å, bound to its pterin substrate analogue 6-hydroxymethyl-7,8-dihydropterin (DHP) to 2.3 Å and Sul3 bound to both its substrates DHPPP and *p*ABA to 2.9 Å. These structures have revealed molecular insights into the active site conformation and key residues as differentiators between Sul enzymes and the chromosomal DHPS. Four loops in the TIM barrel structure undergo dramatic conformational changes to engage the DHPPP and *p*ABA substrates. Detailed analysis of the protein-ligand contacts suggests that a single amino acid insertion in the active site of Sul3 (Phe177) could be the key determinant blocking sulfa binding. These 3D structures will guide *in vitro* and *in cellulo* studies utilizing key deletion mutations, including enzymatic activity assays and antimicrobial susceptibility (MIC) testing in *E. coli*harboring *sul* genes. Combined, this work provides key insights into the mechanism of mobile sulfonamide resistance and sets the stage for further work including screening for inhibitors of Sul enzymes capable of overcoming sulfonamide resistance.

II56

Study of the interaction between the bacterium *Streptococcus suis* and protozoa of the genus *Tetrahymena*

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Streptococcus suis is an important porcine pathogen and an emerging zoonotic agent that can cause sepsis, pneumonia, endocarditis, arthritis and especially meningitis. Strains belonging to serotype 2 are considered the most virulent. It is known that different types of protozoa may be present in swine facilities. Some bacteria, many of which are pathogenic, resist digestion in the phagocytic pathway of protozoa and are then included in lipid structures called multilamellar bodies (MLBs) that are produced and secreted by protozoa, especially ciliates. MLBs serve as protection for bacteria that remain alive in this packaging. In this study we wanted to better understand the possible interactions between different *S. suis* serotype 2 strains having varying levels of virulence with the ciliates *Tetrahymena pyriformis* and *T. thermophila*.

Co-cultures between bacteria and ciliates were performed in 24-well plates with multiplicity of infection (MOI) of 1: 100 and 1: 1000. The plates were incubated without shaking at 25 or 30 °C depending on the protozoan species used, then observed under an inverted microscope after 3 and 24 hours to detect the presence of MLBs and to observe the morphology of the ciliates. Some co-cultures were also stained with DAPI or viability dye for further investigation.

S. suis is packaged by *Tetrahymena* and there is survival of bacteria in MLBs. However, the amount of MLBs observed is lower than for other bacteria packaged by ciliates. There does not seem to be any link between the coating of *S. suis* and its level of virulence. A more frequent phenomenon than the production of *S. suis* packaging has also been observed in co-culture. It is the association of bacteria in the form of a long chain on the surface of the ciliates. It could be a defense mechanism against phagocytosis.

This study demonstrated that the interaction between *S. suis* and ciliates is more complex than expected and that the phenomenon of chain formation requires further analyses to understand its exact role as well as the underlying mechanisms.

II57

Temperate phage-antibiotic synergy

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Over 100 years ago, Félix d'Hérelle discovered bacteriophages (bacteria-specific viruses) and proposed their usage in treating microbial infections; “Phage therapy”. Interest in this approach dwindled in the face of antibiotics, but the spread of antibiotic resistance has pushed phage therapy back into the spotlight. Virulent phages only undergo a lytic life cycle, rapidly killing their host, but temperate phages can also undergo a lysogenic life cycle, integrating in its host’s genome and lying until exposed to a stressor that forces it to awaken and switch to a lytic life cycle. All clinical phage therapy trials use of virulent phages, due largely to concerns about the modification, by the dormant phage, of the host bacterium. Chief among these is that the dormant phage often protects its bacterial host from further phage infections, which would complicate treatment. However, temperate phages are far easier to find, and, as they are far more abundant in (and on) humans, these might also be better adapted to therapeutic applications. Furthermore, many promising phage therapy targets have eluded us due to failure to identify virulent phages for the causal bacterium. Our project proposes the utilization of stressors such as antibiotics at sub-inhibitory concentrations, which is known to awaken dormant phages. Co-administered with temperate phages, we hypothesize that these stressors would *prevent* lysogeny. Comparing the number of bacteria surviving a phage challenge to those exposed to phage in combination with a sub-inhibitory dose of a fluoroquinolone revealed a 2000-fold reduction in bacterial survivors. PCR specific for the integrated phage confirmed that rates of lysogeny had plummeted almost 3000-fold. This scalable and broadly applicable approach to preventing lysogeny may enable the therapeutic use of temperate phages.

II58

The effect of unfolded protein response on *Pseudomonas aeruginosa* infection

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Cystic fibrosis (CF) is the most common fatal genetic disease affecting Canadian children and young adults. One in twenty-five Canadians carry the copy of the defective cystic fibrosis transmembrane regulator (CFTR) gene. Currently, there is no cure for CF and available CFTR modulator drugs can only be used to alleviate the symptoms of a small subset of patients. In addition, CF patients suffer greatly from recurrent infections that if not treated promptly can lead to death. One of the main problems faced by CF patients is chronic lung infection by the bacterium *Pseudomonas aeruginosa*. *P. aeruginosa* is a ubiquitous, opportunistic human pathogen, which is highly virulent and extremely antibiotic resistant. Previous research showed that certain *P. aeruginosa* virulence factors can trigger the unfolded protein response (UPR) in the host. UPR is a cell stress response that occurs when unfolded protein accumulates in the lumen of the endoplasmic reticulum (ER). This response can lead to different outcomes within the cell; either the recovery of cellular homeostasis or cell death depending on the level of damage. However, the precise role that UPR-mediated signalling pathways play in immunity during a *P. aeruginosa* infection is still unclear. Here, we demonstrate that active infection using *P. aeruginosa* strain PA14 activates several sensors of UPR, including Protein Kinase R (PKR)-like ER Kinase (PERK) visualized using western blotting. In an attempt to understand the exact nature of UPR activation by *P. aeruginosa* PA14, we screened quorum sensing mutants of PA14. Quorum sensing is bacterial cell-cell communication that regulates the production and secretion of virulence factors. We have shown that deletion of any of the three main sensors (i.e. *lasR*, *rhlR* and *mvfR*) of the quorum sensing pathway in PA14 led to a decreased activation of the UPR-mediated signalling pathway with a more noticeable decrease in the triple (Δ*lasR/rhlR/mvfR*) and double mutants (Δ*lasR/rhlR,* Δ*lasR/mvfR* and Δ*rhlR/mvfR*) than single mutants (Δ*lasR,* Δ*rhlR* and Δ*mvfR*). This research will contribute to expanding our knowledge about mechanisms involved in UPR activation and restoration of cellular homeostasis under *P. aeruginosa* challenge. It will also improve our understanding of the link between UPR pathways and bacterial pathogenesis. This could help in finding novel targets to fight *P. aeruginosa* infection, in hopes of preventing the debilitating results it causes in CF patients.

II59

The impacts of urban environments on the pathogenic bacteria of Mountain Chickadees (*Poecile gambeli*)

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To meet the demands required of increasing human populations, we expand our cities by creating housing and developing infrastructure. This process of urban expansion, or urbanization, is evidently beneficial to us but not always for wildlife. Some animals found living near or in urban environments display decreased immunity, and certain pathogens like West Nile Virus and *Salmonella* sp. are more abundant in urban areas when compared to surrounding natural habitats. Recent studies have thus questioned if animals living in cities experience greater pathogen loads than those living in natural or rural areas. A handful of researchers have found this relationship between birds and specific pathogens, but few have examined this at broad scales with entire bacterial communities considered. In this study, feathers from an urban and rural songbird, the Mountain Chickadee (*Poecile gambeli*), as well as swabs from its nest, were collected to assess whole bacterial communities along an urban-rural gradient. Tail feathers were collected during the nesting season in Kamloops, British Columbia, Canada. At the end of the nesting season, we additionally collected swabs of the nest bedding. DNA was extracted from feathers and swabs, where we then amplified the 16S rRNA gene for identifying bacterial taxa. High-throughout sequencing of each feather or swab community was then completed using an Ion Torrent S5 XL. Furthermore, satellite imagery for each nest site was converted to estimates of urbanization using overlaid color polygons and vegetation data. Current results show that urban and rural bacterial communities appear dissimilar, and that a potential relationship exists between the number of pathogens present and the degree to

which an area is urban. Further analyses are planned to determine the strength of this relationship across each bacteria, and to consider other factors and life history variables in these analyses.

II60

The involvement of two small-RNA degrading nucleases in the set up of optimal immune responses

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Despite the lack of a circulating immune system and the sessile state, plants are not easy hosts and passive organisms. In fact, during a continuous co-evolution with a wide range of pathogens, plants have developed an effective and a specific immune system. PAMP-triggered immunity (PTI) represents the first lane of defense in plant immunity. It is based on the recognition of the pathogen molecular pattern (PAMPs) by the pattern recognition receptors (PRRs), this basal immune response can reduce pathogen invasion and limits the infection. From the other side pathogen have developed another system that inhibits the PTI and promotes the infection. This phenomenon is based on the production of specific virulent proteins named effectors and which their recognition by the plants can induce the specific immunity response named ETI (EFFECTOR TRIGGRED IMMUNITY).

Small RNAs, including microRNAs and small interfering RNAs, play important roles in numerous aspects of eukaryotes development and host-microbe interactions. MicroRNAs turnover was shown to be properly controlled by Small RNA Degrading Nuclease (SDN) to ensure normal development; the possibility that this turnover plays crucial roles in plant immunity remains unknown. Here, we show that mutation of two SDN protein coding genes leads to a defective PTI and ETI responses. These data highlight the importance of SDN proteins as a regulatory hub for an optimal immune response.

II61

The pathogenicity of commensals: Immunogenic and genetic comparison between clinical isolates of the *Streptococcus milleri/anginosus* group

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Acute respiratory infections are a leading cause of illness and mortality worldwide. Despite this prevalence, the identification of a defined etiological agent is achieved in only half of the cases predominantly due to the failure of standard clinical microbiology protocols. An unappreciated group of microorganisms recovered during respiratory infections is the Streptococcus Milleri/Anginosus group (SMG). The SMG forms a distinct phylogenetic group of Streptococc*i* composed of three closely related species: *S. anginosus, S. intermedius,* and *S. constellatus*. SMG asymptomatically colonizes various mucosal surfaces in 15-30% of healthy individuals. Nevertheless, the SMG is frequently isolated from abscesses, pneumonia, and pleural empyema. However, with SMG usually being dismissed as a commensal, little research has been performed to investigate the heterogeneity and virulence potential within this group of microorganisms.

Using a multidisciplinary approach, we are investigating the mechanisms of commensal and pathogenic interactions with the host and define whether the manifestation of SMG-mediated infection is characterized by a

common host response. We completed a comparative genomic analysis of 152 SMG genomes to investigate the diversity within and between species. Phylogenetic analysis based on the alignment of 440 core genes revealed that while the three streptococcal species clustered separately, the *S. intermedius* clade seems to be more divergent from the two others species. Interestingly, *S. anginosus* isolates exhibited the greatest within-species diversity. To examine the host response to selected SMG clinical isolates, peripheral blood mononuclear cells were isolated from healthy donors and cytokine responses were measured by ELISA. Variable induction of interleukin-8 and interleukin-1β were seen across the donors, suggesting that a combination of bacterial and host factors are involved in the inflammatory response. Future studies will examine the immune response of patients with SMG infections. Taken together, this project will expand our knowledge of SMG strain diversity and host susceptibility to complex respiratory infections.

II62

The role of *Streptococcus pyogenes* surface virulence factors in colonization and autoimmune disease development

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Streptococcus pyogenes is a globally prominent human-specific pathogen placed among the top ten causes of infection-related mortality in humans. Repeated or persistent pharyngeal infections by *S. pyogenes* may lead to serious post-infection autoimmune manifestations including rheumatic heart disease (RHD). Despite in-depth focus on invasive disease, much remains unknown about virulence factors that facilitate the initial stages of colonization and activate post-infection autoimmune pathology. Three key virulence factors expressed on the streptococcal surface, the hyaluronic acid (HA) capsule, N-Acetylglucosamine (GlcNAc), and M protein, share immunologic relationships with endogenous host proteins in cardiac tissues and are highly presumed to induce destructive autoimmune responses involved in RHD. In this study, we aim to elucidate the roles of these virulence factors during nasopharyngeal infection and to evaluate their autoimmune pathogenic roles in a model reflective of RHD.

In-frame genetic deletions for the HA capsule, GlcNAc and M protein were each successfully created in the highly encapsulated MGAS8232 strain isolated from a patient with rheumatic fever. Mutant strains were assessed for immune evasion capabilities through survival in whole human blood and human serum depleted of complement. M protein and HA capsule mutants showed drastically impeded growth and increased susceptibility to killing by whole human blood, however, presented no differences in survival in human serum depleted of complement, suggesting that these virulence factors do not contribute to bacterial survival through complement-modulating strategies. Mutants were also examined for the ability to adhere to and invade an immortalized human pharyngeal epithelial cell line. While GlcNAc and M protein mutants showed no difference in adherence or invasion compared to wildtype *S. pyogenes,* the HA capsule proved to obstruct both adherence and invasion significantly. Mutants were further explored in a murine model of acute nasopharyngeal infection to determine their roles during early colonization events. Notably, deletion of the HA capsule significantly reduced bacterial burden following intranasal infection, and therefore offers critical survival advantages in order for *S. pyogenes* to establish nasopharyngeal infection. Furthermore, preliminary experiments using a novel murine model of RHD show that mice repetitively inoculated intranasally with wildtype *S. pyogenes* develop cardiac failure, illustrated by significantly lower ejection fractions and greater E/A ratios, symptoms reminiscent of mitral valve dysfunction. These results validate that our novel model induces cardiac pathology reflective of RHD development.

In summary, we revealed virulence factors involved in adherence and invasion of pharyngeal cells and during initial stages of acute nasopharyngeal infection. We aim to exploit powerful model systems to develop a fundamental understanding of the autoantigenic role of streptococcal surface structures in the development of RHD.

II63

The role of streptolysin O and streptolysin S in *Streptococcus pyogenes* nasopharyngeal infection

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Streptococcus pyogenes is a Gram-positive, human-specific pathogen that commonly colonizes the upper respiratory tract and skin, causing a wide variety of diseases ranging from pharyngitis to necrotizing fasciitis and toxic shock syndrome. *S. pyogenes* has a repertoire of virulence factors that promote infection and evasion of the host immune system. This research focuses on two *S. pyogenes* virulence factors known as streptolysin O (SLO) and streptolysin S (SLS), which contribute to pathogenesis through their cytolytic activity. Together, SLO and SLS target immune cells and epithelial cells allowing the bacteria to evade the immune system and effectively cause invasive disease. Although these toxins have been previously studied in invasive models of infection, their roles in acute nasopharyngeal infection has yet to be determined. We hypothesized that the genetic deletions of the streptolysins in *S. pyogenes* will cause a significant decrease in bacterial recovery from mice in an acute model of nasopharyngeal infection. Genetic deletions of the *slo* operon encoding SLO, the *sag* operon encoding SLS, or both the *slo* and *sag* operon were generated in *S. pyogenes* strain MGAS8232. Bacterial growth of the streptolysin-deficient mutants in whole human blood was measured to determine if the streptolysins provide a survival advantage in blood. Interestingly, the streptolysin-deficient strains did not show a significant difference in growth in whole human blood compared to the wildtype strain. To study the role of these virulence factors *in vivo*, a previously established murine model of nasopharyngeal infection was used. Transgenic mice expressing human MHC Class II molecules (HLA-DR4/DQ8) were nasally inoculated with the wildtype or streptolysin-deficient mutants, and the murine nasal passages were collected to determine bacterial recovery. We found that an SLS-deficient *S. pyogenes* MGAS8232 exhibited over a 1000-fold reduction in bacterial recovery from the nasal passages of mice. However, an SLO-deficient strain did not exhibit any defects in nasopharyngeal infection. Therefore, we conclude that SLS is the key streptolysin during acute nasopharyngeal infection. By understanding how SLO and SLS contribute to the virulence factors of *S. pyogenes* in an acute infection model, we can target the initial infection to prevent to the development of invasive diseases.

II64

The tail-specific protease of *Legionella pneumophila* is necessary to survive shift in temperature

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Microbes in the environment can be subjected to rapid change in temperature. Most organisms possess sophisticated response mechanism to deal with this threat, which includes proteases and chaperones. *Legionella pneumophila* is a water-borne pathogen that infects amoeba and ciliates. Its ability to grow inside human

macrophages allows it to cause a pneumonia called Legionnaires' disease. *L. pneumophila* is typically found in hot water distribution system and other engineered water system. There, it can face sudden changes in temperature caused by water flowing through the pipe intermittently. This prompted us to identify genes involved in heat shock response in *L. pneumophila*by screening our bank of mutant for defect in heat shock resistance. Deletion of the tail-specific protease(*tsp*) gene resulted in a severe defect, which was resolved by introducing the gene in *trans*. Next, the ability of the mutant to grow inside amoebas was investigated. No defect was observed when the infection was carried at 37°C. Since heat shock could happen during infection of amoebas in water systems, the infection was also carried at 25 °C for 2 days and then shifted to 37 °C for 5 additional days. In this case, deletion of *tsp* resulted in lower intracellular growth compared to the WT or the complemented strain, indicating that Tsp is required to survive a shift in temperature during infection. Finally, a small regulatory RNA (sRNA) is encoded on the complementary strand of *tsp*. Northern blot and western blot analysis showed that the sRNA and Tsp are expressed in a mutually exclusive manner, wherein the sRNA is expressed in E phase and Tsp in PE phase. This suggests that the sRNA represses expression of *tsp*. Further experiments will aim to uncover the sRNA-mediated regulation of *tsp*during heat shock.

II65

Vaccination with a live-attenuated small-colony variant improves humoral and cellular responses against *Staphylococcus aureus*, an important pathogen in Canadian dairy herds

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INTRODUCTION. *Staphylococcus aureus* is known to produce infections that are particularly difficult to treat and many factors, in addition to antibiotic resistance, are thought to contribute to chronic infections such as biofilm formation, intracellular persistence, and immune system evasion. The current public awareness of the rise of antibiotic resistance and frequent failures of drug therapies call for the development of alternative strategies to combat and prevent *S. aureus* infections. Bovine mastitis affects animal health, milk production and quality, and challenges the economic efficiency of dairy farms in Canada. *S. aureus* is the most commonly found pathogen in clinical mastitis, but is also the cause of subclinical, persistent and difficult-to-treat intramammary infections (IMIs). The development of a vaccine for the prevention of *S. aureus* IMIs thus represents an interesting alternative to drug therapy. It is suggested that a vaccine that allows the induction of a balanced immune response, with strong Th1 and Th17 cell-mediated immunity components, may be necessary for protection against pathogens responsible for chronic infections such as *S. aureus*.

METHODS. In this work, we engineered a live-attenuated *S. aureus* vaccine based on a genetically stable small-colony variant (SCV). Various experimental formulations, such as the live-attenuated SCV vaccine, a heat-inactivated SCV vaccine and a combination of the SCV vaccines with the *E. coli* J5 bacterin, were compared and tested. These were administered in mice by subcutaneous injections and the specific humoral and cell-mediated responses to *S. aureus* were characterized by ELISA, lymphoproliferation and cytokine detection assays.

RESULTS. The live-attenuated strain was found to elicit a strong and balanced immune response in vaccinated mice, by inducing higher specific IgG titers and significantly improving IgG2a/IgG1 antibody ratios. This formulation also induced a strong proliferation of *ex-vivo* stimulated splenocytes isolated from these animals. These splenocytes were also found to release significantly higher concentrations of IL-17 and IFN-γ when compared to every other immunization groups. Inversely, the inactivated whole-cell vaccine, alone or in combination with the *E. coli* J5 bacterin, was apparently incapable of inducing high antibodiy titers or a strong Th1 and Th17 cell-mediated response in the splenocyte proliferation assay. These results indicated that the combination with *E. coli* J5 does not improve and can even dilute specific immune responses against *S. aureus*.

Furthermore, we demonstrated that the live-attenuated vaccine shared enough common components with clinically significant strains, so that the antibodies could also strongly recognize IMI isolates from the major *spa* types found in Canadian dairy herds.

CONCLUSION. A strong and balanced humoral and cell-mediated immune response is the key to handle persistent and subclinical *S. aureus* IMIs. Our results suggest that a live-attenuated SCV vaccine formulation that possesses strong immunomodulatory qualities and which mimic the natural infection process could represent a promising new strategy to improve the protection against *S. aureus* infections.

II66

Versatile use of ubiquitin protein ligase activity by attaching and effacing (A/E) pathogens

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Enteropathogenic *Escherichia coli* (EPEC) and enterohemorrhagic *E. coli* (EHEC) are diarrheagenic bacterial pathogens that cause severe disease in humans and animals. These enteric pathotypes, together with the mouse pathogen *Citrobacter rodentium*, belong to the family of attaching and effacing (A/E) pathogens that form a distinctive histological lesion in the intestinal epithelium. As part of host manipulation strategy these Gram-negative pathogens each encodes a unique ensemble of proteins called “effectors” which are translocated inside the host via type III secretion system to alter host cell processes in favor of infection. The largest family of type III secreted (T3S) effectors conserved in all A/E pathogens are NleG effectors, which feature a variable N-terminal target recognition motif and a conserved C-terminal U-box domain typical of eukaryotic ubiquitin ligase (E3) enzymes. NleG U-box domain is able to recruit host ubiquitination machinery to posttranslationally modify specific host targets recognised by the variable N-terminal motif and trigger their degradation by proteasome. To date, several dozens of NleG effector family members have been identified in highly virulent EHEC and EPEC strains as well as in diverse strains of *Salmonella*, *Shigella* and *Citrobacter*. However, the molecular structure as well as host targets are only known for NleG2-3 and NleG5-1, which initiate ubiquitination and degradation of human hexose kinase and a subunit of MEDIATOR transcriptional regulation complex, respectively. The general role of these and other NleG effectors in infection strategy of these intestinal bacterial pathogens remains uncharacterised. To address this, we focused on the *Citrobacter rodentium* DBS100 strain pathogenic to mice and serving as an *in vivo* model to understand the molecular basis of EPEC and EHEC infections. Full genome sequencing of this strain and transcriptome analysis confirmed the expression and translocation of three NleG effectors (called NleG1, NleG7 and NleG8) as part of DBS100 T3S effector arsenal. Using mass spectrometry assisted affinity purification we were able to identify distinct host targets for two *Citrobacter* NleGs, which confirmed that NleGs represent a functionally diverse family of effector E3 ubiquitin ligases evolved to interfere with different host cell processes. The direct interactions between *Citrobacter* NleGs and identified host proteins were confirmed *in cellulo* by yeast 2 hybrid method. Analysis of individual NleG knockout DBS100 strains for infection in mice also demonstrated that these pathogenic factors play a critical role *in vivo*. Together, these results provide new insights into functional diversification and specialisation of the T3S effector arsenal in A/E pathogens and set the stage for characterisation of similar molecular mechanisms underpinning the infection by clinically important EHEC and EPEC pathogens.

II67

Developing a mucosal vaccine delivery system to mitigate the bovine respiratory pathogen *Mannheimia haemolytica*

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Bovine respiratory disease (BRD) continues to be a challenging health issue resulting in significant economic losses due to morbidity and mortality in the North American cattle industry. Treatment and control of BRD in beef sector are mainly through the use of antimicrobials to control bacterial pathogens and vaccination programs. However, there are public and scientific concerns regarding the overuse of antimicrobials in livestock production and currently available vaccines do not provide complete protection against infection. Novel methods to mitigate BRD-causing pathogenic bacteria are therefore greatly needed. *Mannheimia haemolytica* is the predominant bacterial agent associated with the BRD disease. Despite *M. haemolytica* initiating infection at respiratory mucosa, most of the current *M. haemolytica* vaccines are administered by intramuscular injection which act through induction of systemic immunity but are only moderately efficacious. In contrast, mucosal immunization can induce both mucosal and systemic immunity, providing protection at the site of infection. Recently, bacteria are increasingly being evaluated for vaccine delivery due to their natural ability to interact with host immune cells. In this study, the effectiveness of *Bacillus subtilis* spores as a mucosal adjuvant was evaluated for a vaccine against *M. haemolytica* as one of potential alternatives to metaphylactic antimicrobials. A chimeric gene was synthesized containing a tandem repeat of antigenic epitopes from *M. haemolytica* leukotoxin A and outer membrane protein PlpE. The antigen was adsorbed to *B. subtilis* spores and then analyzed for stability when bound to spore coats, and potential to elicit an immune response in mice. Stability of spore-bound antigen was greatest when adsorption occurred at 4°C. Compared to intramuscular injection of the chimeric antigen, intra-nasal administration of spore-bound antigen resulted in greater amounts of specific secretory IgA in lung tissue. This suggests that spore-bound antigens can induce mucosal immunity against *M. haemolytica* and could be further tested in ruminants.

II68

Environmental and nutritional regulation of the toxic shock syndrome toxin-1 (TSST-1) in *Staphylococcus aureus* MN8

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Menstrual-associated toxic shock syndrome (mTSS) is a rare but life-threatening condition associated with tampon usage and other intravaginal devices. mTSS is an intoxication mediated by colonising *Staphylococcus aureus* strains that produces the superantigen toxic shock syndrome toxin-1 (TSST-1). Although many women are colonised vaginally by *S. aureus*, the incidence of mTSS is low which likely indicates that within the vaginal environment, expression of TSST-1 is tightly regulated. Conversely, expression of superantigens such as TSST-1 has been widely reported in the nasal environment, another common site of colonization by *S. aureus*. This aim of this study is to better understand the regulatory networks that control TSST-1 expression, so we can better understand environmental stimuli that lead to abhorrent TSST-1 expression in the vagina. Using the prototypic mTSS strain *S. aureus* MN8, we analysed *tst* transcription and the expression of TSST-1 protein under multiple culture conditions that replicate both the vaginal and nasal environment. Analysis of TSST-1 expression revealed that it is minimal when *S. aureus* MN8 is grown in vaginally defined medium (VDM), despite high growth in this media. However, in synthetic nasal media (SNM), growth of *S. aureus* MN8 was much lower but TSST-1

expression was high. One of the major differences between these two types of media is glucose concentration (60 mM in VDM and 0.7 mM in SNM), previous reports have indicated that this carbon source is a repressor of *tst* expression. We were able to confirm that glucose is a major repressor of TSST-1 expression by replacing this carbon source with pyruvate in the VDM. This resulted in high levels of TSST-1 expression, we also observed a similar increase in *tst* expression in the nasal media when pyruvate was used instead of glucose. Raising glucose concentration in SNM media to 60 mM resulted in a reduction of TSST-1 expression but it did not abolish expression indicating that other factors in the SNM media contribute to activating *tst* expression. These data demonstrate that TSST-1 expression is tightly regulated and multiple environmental and nutritional signals. This work further advances our understanding of the conditions that may contribute to the increased the risk of mTSS.

AEM1

Bacteriocinogenic Enterococcus: a potential probiotic as an alternative to antibiotics in poultry

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Approximately 13.6 million kilograms of medically important antimicrobials approved for food-producing animals were sold and distributed in the US in 2016 (Cvm, 2017). It has been estimated that by 2050, approximately 10 million deaths a year will occur as a result of antimicrobial resistance, and will cost the world economy $100 trillion annually (Eloit, Silva, & Chan, 2016; O'Neill, 2014). Considering majority of the antibiotics are used in farm animals, and with governments around the world taking an important step in banning the non-therapeutic use of antibiotics, including Canada and the US, it is the ideal time for the poultry industry to shift from antibiotic use to using an alternative such as bacteriocin-producing probiotics especially to target *Salmonella*, a common poultry pathogen which is one of the leading causes of foodborne diseases. Bacteriocin-producing probiotics are low cost, allows maximization of host feed utilization, and able to act as a prophylactic while promoting bird growth comparable to antibiotics. Our aim is to determine whether our previously isolated *Enterococci* from gut and food origin, *Enterococcus* spp, *Enterococcus hirae*, and two *Enterococcus faecalis* strains, show antibacterial activity against five *Salmonella* strains; *Salmonella* typhimurium ATCC 14028, *Salmonella* typhimurium ATCC 13311, *Salmonella choleraesuis* ATCC 10708, and *Salmonella enterica* subsp. *enterica* serotype Abony NCTC 6017. Once the activity has been established, we will characterize the *Enterococcus* strains for their probiotic potential by the determination of the production of inhibitory compounds, tolerance to pH and bile salts, sensitivity to antibiotics, ability to adhere to the mucosa of the gastrointestinal tract, and determination of genes encoding virulence factors.

Key words: antibiotic resistance, poultry, probiotic, Salmonella, alternative to antibiotics

AEM2

"Green" ZnO-interlinked chitosan nanoparticles for the efficient inhibition of sulfate-reducing bacteria in inject seawater

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Antimicrobial agents and corrosion inhibitors are widely used as biocides in the oil and gas industry to disinfect water and inhibit excessive biofilm formation caused mainly by sulfate reducing bacteria (SRBs). However, traditional biocides may induce bacterial resistance and/or be detrimental to environment by forming harmful disinfection byproducts. In this first systematic study, we synthesized a"green" and highly stable biocide formulations composed of ZnO-interlinked chitosan (Ch) nanoparticles (CZNCs) and evaluated their antimicrobial activity against mixed SRBs culture isolated from real oil field sludge. SEM, TEM, X-ray diffraction

(XRD) and FTIR suggested the formation of stable nanocomposites with strong interaction between ZnO and Chnanoparticles. Synthesized nanocomposites showed highly stable behaviors in the high salt concentrations of inject seawater. The inhibition of SRBs activity was concentration-dependent and more than 73% and 43% inhibition of sulfate reduction and total organic carbon (TOC) removal, respectively, was observed at 250 µg/mL CZNCs at 10% initial ZnO loading. Biocompatibility and environmental impact of the nanocomposite was evaluated by analyzing their potential toxicity in vivo using the zebrafish embryos. As evidenced by the acute toxicity assays, exposure of zebrafish embryos to CZNCs (25-200 mg/L) failed to elicit any signs of acute toxicity or mortality, suggesting a hypothetical LC50 higher than the maximum dose employed (250 mg/L). CZNCs, at a concentration of 250 mg/L also showed no cardiotoxic or neurotoxic effects. At the same dosage, a minor hepatotoxic effect was observed in zebrafish embryos exposed to CZNCs. However, the observed hepatotoxicity had no effect on embryos survival even after long-term (10-days) exposure to CZNCs. In conclusion, it is expected that the new nanocomposite can contribute to the development of "green" biocides for oil/gas industries that will be eco-friendly and will have no adverse impact on the environment.

AEM3

A comparative genomic analysis of two avirulent *Pseudomonas syringae* strains belonging to phylogroup 2c

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Pseudomonas syringae (*Psy*) is a ubiquitous phytopathogen associated with a large variety of plant diseases in many agricultural settings. Recently, low virulence and putatively non-pathogenic strains have been found in non-agricultural environments, including snowbanks and river water. To elucidate similarities and differences between *P. syringae* strains of variable virulence, an avirulent and putatively non-phytopathogenic strain (*Pss*508), and one putatively non-phytopathogenic (*Psy*TLP2) were compared to 11 other closely related *P. syringae* strains belonging to the *Psy* phylogroup 2. Complete genomes for *Pss*508 and *Psy*TLP2 strains was sequenced using ONT's MinION sequencer, assembled and polished using a custom OLC pipeline, and de-fragmented with Castor, an in-house, error assessment program.

Between the 13 strains, both *Pss*508 and *Psy*TLP2 genomes are among the smallest of the group, at 5.84 Mb and 5.71 Mb respectively, with only environmental strain *Psy*UB0390 having a smaller genome than both. Collectively, the pan genome consists of 12645 genes with 3333 of these genes making up the core genome. The majority of the remaining genes were lineage-specific from phytopathogenic strains and their diverse set of plasmids. Based on a core genome alignment and orthoANI values >98%, *Pss*508 and *Psy*TLP2 were clustered with sole phylogroup 2c strain, *Psy*642, and *Psy*UB0390 as a single putatively non-phytopathogenic group. This group contained the least lineage specific genes between 150-450 consistent with their smaller genome sizes. Although the majority of these genes are hypothetical, the presence of genes involved with chemotaxis, multi-drug resistance, and siderophores may indicate stronger epiphytic fitness and be contributing factors of reduced pathogenicity. Other lineage-specific genes involved in potential plant-microbe interactions include peroxidases, and a type I RTX toxin. These lineage-specific genes and those shared within the non-pathogenic group were further expanded to compare to other phylogroup 2 strains, and environmental *P. syringae* strains.

Both *Pss*508 and *Psy*TLP2 share an atypical variant of type III secretion system common to phylogroup 2c strains. Similar to other phylogroup 2 strains, the two genomes show a reduced type III effectome, including type III effectors involved in apoplast adaptation, *avrE* and *hopM*. Both strains also share biosynthesis genes for toxins such as syringolin A and syringopeptin. Despite the presence of virulence factors, differences in the accessory genome and the reduced type III effectome are key contributing factors to the pathogenicity of a *P. syringae* strain.

AEM4

A new diagnostic assay for food safety: detection and characterization of *Salmonella* contamination of lettuce by means of a whole genome-culture independent diagnostic test (WG-CIDT)

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Whole genome sequencing (WGS) can be exploited for precision and preventive food safety. To that end, we have developed a sensitive, shotgun WGS protocol capable of detecting ≥1 colony forming unit (cfu) of *Salmonella* Typhimurium spiked on 25 g of lettuce using the Ion Torrent's Ion PGM and Ion S5 sequencers. DNA extracted from the surface of lettuce was globally amplified and subjected to WGS followed by rapid bioinformatics analyses. An average of 4.1 million (M) reads (average length per read = 270 bp) was generated using the PGM for each sample compared to 7.6 M for each of two samples simultaneously analyzed on the Ion S5. All samples spiked with very low (1-9 cfu), low (10-99 cfu), moderate (100 – 999 cfu) or high (>10^3 cfu) were readily detected using the Ion S5 sequencer. Spiked samples were also readily detected with the smaller capacity PGM sequencer although only 80% of the very low contamination samples were detected on the PGM. We concluded that a minimum raw read size threshold, approximately 5.5 M reads is required to ensure a reliable detection of food contaminated with very low number of contaminants. The rapid and precise detection of foodborne microbial pathogens contaminating food, especially those consumed raw such as fresh fruits and vegetables, should facilitate the adoption of this method for use between harvest and consumption and thereby pave the way for preventive food safety testing and a reduction in foodborne illnesses.

AEM5

A One Health approach for monitoring antibiotic resistance development and dissemination in a developing country

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Antimicrobial resistance (AMR) is a global problem requiring collective knowledge and technological efforts to reduce high mortalities associated infectious diseases. The antibiotic resistome comprising of potentially pathogenic, non-pathogenic antibiotic resistant bacteria (ARB), antibiotic resistance genes (ARGs) and antibiotic resistance plasmids (ARPs) is widely distributed across various ecological niches. The ecology of the resistome is poorly understood and remains relatively uncharacterized at a global scale, in many developing countries the overall challenge in managing AMR is mainly due to poor infrastructures and lack of research.

Our research aims to use a 'one health' approach in tracking the sources that facilitate development and spread of antibiotic resistance across humans, animals and the environment, taking into consideration also the socio-economic, ecological and climatic attributes of the country. One of our primary aims is also to increase our

understanding of plasmids, ultimately use them as evolutionary and epidemiological markers for tracking the dissemination of clinically relevant ARGs between clinical and non-clinical environments.

Our initial study areas in Botswana are A) Non-clinical; Gaborone (GWWTP), Palapye (PWWTF) wastewater treatment facilities and downstream environments receiving effluent wastewater. B) Clinical; Palapye (PPH), Nyangabwe (NRH), Letsholathebe (LMH) health care facilities. In this research we applied both culture dependent and independent approaches; selective media and PCR for isolation, detection and characterization of potentially pathogenic ARB and ARGs in water and air samples. We also took a Bioinformatics approach to help determine the correlations between ARPs incompatibility groups and the different environments.

The results reveal high occurrence, diversity and abundance of potentially pathogenic ARB; *Staphylococcus* species*, Escherichia coli, Pseudomonas , Brucella* , *Salmonella*, *Listeria* and *Campylobacter* species, and ARGs to clinically relevant antibiotics; *tet*A (tetracycline), *mph*A (macrolides), *str*B (streptomycin), *sul*1 (sulphonamide), *dfr* (trimethoprim) and *int*1 (mobile ARG cassette) detected from the sample sources in clinical and non-clinical environments. The *in silico* characterization of plasmid incompatibility groups details most completely sequenced ARPs from hosts associated environments specifically clinical samples.

Taken together, the results suggest accumulation of antibiotic resistance determinants in various sources and high risk of rapid dissemination across different environments. The results further raises awareness of the threat posed by antibiotic resistance in a developing country, evidently justifying the need to upgrade infrastructures in health care and wastewater treatment facilities. Through various collaborations within the one health framework; our future research will seek to expand and generate more epidemiological data also targeting other environments; wildlife, agricultural farms and local communities. Establishment and application of high-throughput genomics is also very critical to our research in order to better understand the changing dynamics of the antibiotic resistome. A one health framed research data will ultimately help in the development of surveillance programs and national policies/guidelines that will contribute to the control of global spread of AMR.

AEM6

A soft direct DNA extraction method more effectively extracts the γ-proteobacterial community in soil than Mobio lysis

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As most soil bacteria cannot be cultivated, microbiologists often directly sequence soil DNA or amplicons from it to characterize bacterial communities. Both of these approaches need a method that extracts DNA from all bacteria to give an accurate view of the soil bacterial community.

The most common soil DNA extraction method, the Mobio Powersoil kit, was compared with a soft (enzymatic/SDS) lysis method using 16S rRNA next generation sequencing with a range of primers and sequencing platforms on contrasting soils.

Extraction method had little effect on alpha diversity or how samples clustered, but some bacterial groups were much more effectively extracted by soft lysis. Soft lysis extracted 10-100 times more of several γ-Proteobacterial groups from soil: the Enterobacteriales and Pseudomonadales orders and the Stenotrophomonas genus. These groups together made up more than 10 % of the bacterial community in soils. These results were consistent across contrasting soils and different sequencing platforms.

The robustness of these results were confirmed three ways. Increasing bead beating time and intensity did not increase the yield of these groups using the Mobio kit. DNA yield was measured immediately after lysis and was found to be similar for both methods. A reanalysis of data from two studies which compared commonly used soil DNA extraction methods found that these three groups were either absent or less than 0.3% of amplicons.

The incidence of the Enterobacteriales and Pseudomonadales orders and the Stenotrophomonas genus are consistently underestimated in soil because of extraction method bias.

AEM7

Adaptability of the common scab-inducing species to the hydroxycinnamates content of potato periderm

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Potato periderm is composed mainly of suberin. Suberin is composed of two main domains (polyaliphatic and polyaromatic domains). The aliphatic domain of suberin consists of fatty acid polyesters with esterified ferulic acids. Ferulic acid is believed to cross- link to the aromatic moiety of suberin. The aromatic suberin is composed mainly of ferulic and coumaric acids. Previous research reported the importance of ferulate esters in maintaining the integrity of the periderm which acts as a barrier against pathogens entry. Evidence accumulates that *Streptomyces scabies,* a potato common scab-inducing bacterium, can degrade the aliphatic part of suberin but its ability to degrade the aromatic domain has not been studied. A time course study of both *trans*-ferulic and *p*-coumaric acids depletion was carried out by sampling periodically culture supernatant of *S. scabies* strains EF-35 and 87.22 grown in the presence of these aromatic compounds. This study revealed that *S. scabies* has the ability to degrade these compounds. In contrast, other common scab-causing species such as *S. acidiscabies* and *S. turgidiscabies* exhibited a poor utilization capacity. *S. scabies* 87.22 showed also higher growth than the other common scab-inducing species when grown alone or in co-culture with *S. acidiscabies* and *S. turgidiscabies* in suberin-containing medium. This study suggests that *S. scabies* is more adapted to its host plant than the new emerging pathogens.

AEM8

Alder bacterial endophytes and their interactions: a symbiotic symphony

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Revegetating profoundly disturbed environments such as mining sites poses significant problems from a biological standpoint. Mine spoils are generally nutrient-poor substrates with low water content and retention or poor drainage and low oxygen permeability. They are often prone to extensive erosion, extremes of pH and the presence of toxic contaminants (e.g. heavy metals, salt). Alders are hardy native shrubs capable of readily colonizing such environments. Since their ability to form symbioses with fungal and bacterial partners improves their fitness in this regard, the study of these plant-microbe associations is of utmost importance to improve revegetation practices. *Frankia* spp are slow-growing filamentous actinobacteria that fix atomspheric nitrogen in alder root nodules, providing a large portion of the nitrogen required for vigorous plant growth. Other bacterial genera are also found in nodule tissues as we demonstrate in the present study. Non-*Frankia* nodular isolates have been purified from gnotobiotic plants inoculated with environmental samples (soil, tailings or crushed nodules) collected on the Sigma gold mine in Val-d'Or, North-Western Québec. These diverse isolates were

found to express putative plant growth-promoting traits *in vitro* (i.e. metallophore production, auxin secretion, phosphate solubilization) and promoted alder growth in gnotobiotic assays. Quantitative and qualitative coculture assays of these isolates with *Frankia* sp. ACN10a have exposed numerous interactions including both inhibition and stimulation of *Frankia* growth, modification of *Frankia* morphology and modification of auxin concentration in coculture supernatant. Gnotobiotic *in planta* assays using these isolates have revealed interesting effects on various morphometric parameters including total plant biomass, aerial/root biomass ratio and nodule biomass. These results demonstrate the plant growth-promotion capacity of the isolates and suggest interactions between *Frankia* and non-*Frankia* nodular isolates in the initiation and regulation of the actinorhizal symbiosis. This study paves the way for further probing into the interplay of alder-microbe associations in the root nodules and their influence on plant physiology and development. Ultimately, better understanding of this plant-microbe model could lead to the development of coinocula to augment alder performance in societally important industrial site restauration efforts.

AEM9

Anti-*Pseudogymnoascus destructans* (Pd) activities from western Canadian bat wings

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White-nose syndrome, an infectious fungal illness in bats caused by the psychrophilic *Pseudogymnoascus destructans* (*Pd*), has decimated North American hibernating bat populations at an unprecedented rate. The fungus establishes on the surface of bats during their hibernation cycle, resulting in their premature arousal from torpor and depletion of vital fat stores required for hibernation. As some species of bacteria have an antagonistic relationship with fungi, the elimination of *Pd* through the native microbiota of bats may serve as a non-invasive prevention method that limits the disruption of hibernacula ecosystems. In this study, bacteria found on batwings were isolated and screened for anti-*Pd* activity. These bacteria could be used to develop a probiotic powder to be placed in hibernacula and on bat colonies in hope to help prevent bats from WNS. Batwing swabs were obtained from captured bats between the summer of 2016 and the summer of 2018. The swabs were plated on blood agar, nutrient agar (NA), Reasoner's 2A (R2A) agar, potato dextrose agar (PDA), and Wallerstein Laboratory (WL) agar. Culturable bacteria were re-streaked and incubated at 25-30°C for 24-48 hours to obtain pure cultures. Using the agar diffusion method, we screened the anti-*Pd* activity for our isolates on Sabouraud Dextrose agar (SDA) at 14°C for 28 days. Among the 585 bacterial isolates tested, five showed full inhibition and 34 showed partial inhibition. The anti-*Pd* bacterial strains were isolated from 8 different bat species across 11 locations in BC and Alberta. The identities of these anti-*Pd* bacteria are being determined.

AEM10

Antimicrobial activity and antibiotic susceptibility of some probiotic bacteria isolated from Egyptian traditional fermented dairy products

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Probiotic bacteria are organisms that improve the microbial balance in the human gut. They are known to produce various antimicrobial compounds that are considered to be important in the biopreservation of food. In the current study, antimicrobial activity and antibiotic susceptibility were tested for five *Lactobacillus* strains *(1- Lactobacillus plantarum, 2- Lactobacillus acidophilus, 3- Lactobacillus rhamanosus, 4- Lactobacillus salivarious, 5- Lactobacillus paracasei) and* three *Bifidobacterium* strains (*1- Bifidobacterium longum, 2- Bifidobacterium adolescentis and 3- Bifidobacterium breve)* isolated from Egyptian traditional fermented dairy products. Agar-well diffusion method was used to test the antagonistic effect against *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa and Candida albicans*, using complete bacterial culture (CBC). All the tested strains exhibited antibacterial activity against the aforementioned pathogens. Moreover, *L. plantarum, B. breve and B. adolescentis* have the heights inhibition zone against *P. aeruginosa* and E. coli. In addition to, *B. adolescentis, B. breve,* and *L. rhamanosus* were the most powerful against S. *aureus* and *C. albicans.* The antifungal activity of lactobacilli and Bifidobacteria were determined by coculture with *Aspergillus niger, Aspergillus flavous, Aspergillus fumigatus* and *Penicillium chrysogenum* using quantitative method. All the isolates showed a variable level of inhibition against the fungal growth. The antibiotic Susceptibility was tested using the agar disc diffusion method. All the isolates were resistant to Ciprofloxacin. Further, the lactobacilli strains were resistant to Vancomycin. By the same token, *L. plantarum* and *L. acidophilus* were resistant to Clindamycin. However, the other strains were intermediate. Four strains *L. acidophilus, L. rhamanosus, L. salivarious, and B. adolecentis were* resistance to Streptomycin and the other were intermediate. All the strains were sensitive to *Penicillin*, Ampicillin, Tetracycline, Erythromycin, Gentamicin, Florfenicol, Chloramphenicol, and Sulfamethoxazole &Trimethoprim. Probiotics having high antimicrobial activity with no antibiotic resistance transfer could be used as natural bio-preservatives in food and therapeutic formulations.

AEM11

Antimicrobial activity of Thai cave bacteria

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Caves have unique geological characteristics that provide special microenvironment. These distinctive characteristics of caves craft adaptation and evolution in microorganisms that live in such habitats. These particular adaptations may lead cave bacteria to produce essentially useful metabolites either already-discovered and/or yet to be identified. Considerably, some cave bacteria may generate metabolites with antimicrobial activity to fight against some pathogens that are now unbeatable with today's medicine. Ninety-two strains of bacteria were isolated from cave wall swabs and water drops inside Muang On Cave, Chiang Mai Province, Thailand. Three isolation media; Reasoner's 2A Agar, Raffinose-Histidine Agar and Water Proline Agar, were used with no antifungal and antimicrobial agents supplemented and incubated at 25°C for one month. The isolated cave bacteria were then tested for inhibitory activity against non-resistant and multidrug resistant (MDR) strains namely non-resistant *Escherichia coli*, MDR *E. coli* (15-102,15-124, and 15-138), non-resistant *Staphylococcus aureus*, *S. aureus* ATCC 4330 (Methicillin-resistant *Staphylococcus aureus*; MRSA), and *Candida albicans*. Out of 60 screened, eight showed positive inhibition against all of panel microorganisms. Based on microscopic and macroscopic morphologies of positive candidates, all of isolates may be members of the family Actinobacteria. Interestingly, two isolates, PROWM01-8 and RM01-20 showed broad-spectra of inhibition. Isolate PROWM01-8 inhibited non-resistant *S. aureus*, MRSA, *E. coli* 15-124 and *C. albicans*. Isolate RM01-20 inhibited non-resistant *E. coli*, all MDR *E. coli* and MRSA but not on non-resistant *S. aureus*. The positive candidates are now being sequenced for further identification. In conclusion, Muang On Cave bacteria demonstrated a promise to be used as a source of antimicrobial compounds inhibiting the growth of multidrug resistant *E. coli* and *S. aureus*.

AEM12

Antimicrobial susceptibility of intestinal bacterial strains to antidepressant drugs

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Background: Major depressive disorder (MDD) is a common psychiatric illness which accounts for almost 10% of all medical disability in Canada. Treatment options used in the management of MDD are antidepressants drugs which are often associated with metabolic side effects. Studies have shown that gut microbiota could be altered during MDD episodes or in response to antidepressant treatment. The studies conducted on the antidepressant treatment were more focusing on their effect on the central nervous system. However, the impact of antidepressant treatments on intestinal microbiota structure remain unexplored. Antidepressants have been increasingly shown to possess antimicrobial properties with possible implication in microbiota-gut-brain axis. The aim of this study was to investigate the antimicrobial effect of commonly used antidepressants drugs on bacterial strains residents in the human intestine.

Methods: The inhibition and growth kinetics of the intestinal strains were assessed by the critical dilution method and the agar well diffusion method. Resuspended cultures of intestinal strains were incubated with a range of antidepressants (Phenelzine, Escitalopram, Venlafaxine, Desipramine, Bupropion and Aripiprazole). The minimal inhibitory concentration of tested drugs was also determined.

Results: The results from this study showed a differential antimicrobial effects of the tested antidepressants. Phenelzine and Desipramine were the most effective one inhibiting (completely or partially) the growth of the intestinal strains (*Enteroccocus faecium*, *Lactobacillus casei* and *Escherichia coli*) at different tested concentrations (600, 300 and 150 µg/mL).

Conclusions: These data illustrate that the antidepressants medication can influence the composition of gut microbiota which can trigger intestinal dysbiosis.

AEM13

Bacillus velenzensis and *Paenibacillus peoriae* isolates as promising biopesticides and biofertilizers in sustainable agriculture

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The heavy and repeated long-term application of agrochemicals in agriculture is driving plant pathogens to develop resistance and insensitivity towards chemical pesticides, thereby making their control challenging. For example, bacterial pathogens like *Xanthomonas perforans*, the causal agent of bacterial spot disease in economically important vegetable crops, has become insensitive to copper-based chemicals. An ecofriendly alternative to chemical pesticides is using biocontrol agents. Also, some biofertilizers are capable of suppressing deleterious microorganisms in plant rhizosphere and stimulating plant growth. Developing more new biocontrol agents with broad-spectrum antagonistic activity against a range of pathogens and biofertilizers is indispensable for green and sustainable agriculture.

To uncover the potential role of some new strains of *Bacillus velenzensis* and *Paenibacillus peoriae* against *X. perforans,* we performed pathogenicity assays of *X. perforans* on tomato seedlings from a non-hybrid cultivar under conditions highly susceptible to disease infection in growth chambers. Disease severity scoring to determine disease progression over time was performed after first appearance of disease symptoms on tomato leaves, which is the 10th day after inoculation followed by scoring once a week for a period of five consecutive

weeks. An estimation of area under disease progress curve (AUDPC) after the 5th evaluation indicated a significant decrease in disease severity in tomato seedlings that were pre-inoculated with *B. velenzensis* and *P. peoriae* 24 hours prior to pathogen inoculation. The same strains when applied on mung bean seeds for 1 hour prior to sowing showed enhanced plant growth when compared to control water-only treated plants. Currently, we are evaluating the biofertilizer potential of these strains in tomato and wheat plants.

Our study requires further greenhouse and field evaluations to validate the efficacy and consistency of these strains as potential biocontrol candidates against plant pathogens and biofertilizer in target crop plants. The outcome will lay a platform to develop new eco-friendly biological products to control bacterial diseases of crops plants and enhance crop productivity without posing risks to human health and environment.

AEM14

Biomineralization by bacteria isolated from popcorn and soda straw speleothem samples of the Iron Curtain Cave in Chilliwack, BC, Canada

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The enzyme urease has been identified as a virulence factor in many bacteria due to its multiple roles in human disease pathogenesis. For example, urease has been observed to contribute to the precipitation of ions resulting in kidney stones. Urease catalyzes the conversion of urea into ammonia and bicarbonate. The bicarbonate ion produced is transferred to the surface of the bacterial cell, where it will then bind with calcium in the environment to form calcium carbonate. The bacteria are sites of nucleation on which calcium carbonate crystals can form. Bacteria that produce calcium carbonate using this reaction, known as microbially-induced carbonate precipitation (MICP), are currently being studied for their potential applications in biocement techniques. However, the role of this pathway in cave formations is not well understood.

The present study aims to investigate the bacterial community composition of popcorn (PCS) and soda straw (SSS) speleothems found within the Iron Curtain Cave in Chilliwack, BC using primarily culture-dependent techniques. A total of eighty-seven bacterial isolates (forty-four from PCS and 43 from SSS, respectively) were isolated using NA, d10NA, and R2A media and were cultured at 8 °C for 7-21 days. These isolates were then subcultured to obtain single colonies for urease enzyme screening. The positive candidates were then grown on B4 medium at 8 °C until calcium crystals were observed under a dissecting microscope. Positive candidates will undergo DNA extraction and 16S rRNA characterization and further molecular identification of genes responsible for MICP activity. Information gathered will help us understand whether these bacteria can be induced to precipitate calcium carbonate crystals in a laboratory setting. Overall, the results may provide supporting evidence that the bacteria may have some degree of involvement in the formation of the cave speleothems.

AEM15

Bioremediation of seafood waste using *Paenibacillus* sp. AD and concomitant production of chitin oligosaccharides having application as biotherapeutic agent

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Huge amount of waste generation is one of the major problem of sea food industry. Ocean dumping, incineration and land filling are some of the commonly used methods for its disposal, however all these methods cause heavy pollution of the environment. Most suitable way to dispose of huge quantities of seafood waste is to do its bioremediation. Biodegradation of seafood waste also lead to the formation of high value products such as chitin oligosaccharides, N-acetyl D glucosamine, peptides etc. *Panibacillus* sp. AD is known to produce chitinase and degrade the sea food waste in submerged conditions.

In present study *Panibacillus* sp. AD was used and conditions were optimized for the degradation of sea food waste in solid state so that process can be applied at large scale. Under standerdized conditions effective degradation of sea food was achieved. Hydrolysate of sea food waste containing number of value added products most important of which were chitin oligosaccharides (COS). COS were analysed using various techniques. COS were shown to have prebiotic properties they also showed antimicrobial activity against various intestinal pathogens.

AEM16

Bovine mammary gland commensal culture collection

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Until recently the bovine mammary was considered a sterile environment and it was thought that the microorganisms found in raw milk were derived from external contaminations such as from the animal's skin or bedding. Recent advances in DNA-based culture-independent techniques have successfully challenged this paradigm, and it is now accepted that the bovine mammary gland houses a microbial community at a low population density of approximately 3-4.7 $\log_{10}$ CFU/mL. Since most of the work done on this interesting bacterial community has been performed using culture-independent techniques, and most culturing takes place on samples from animals with clinical symptoms there are very few non-pathogenic commensal isolates available that were isolated from the bovine mammary gland. This project was undertaken to address this issue and establish a well characterized culture collection of bacteria isolated the healthy bovine mammary gland. Currently, forty unique isolates have been obtained by culturing fresh, raw milk taken from healthy dairy cows and growth on either plate count agar, Modified Chalmer Agar (MCA), Tryptic Soy Agar (TSA), or MacConkey agar. The collection currently represents 5 geniuses including:*Bacillus, Staphylococcus, Aerococcus, Pantoea,* and *Rothia*. Whole genome sequencing of each isolate is underway, and comparative genomics is being used to determine which bacterial characteristics are required for growth in this environment. In addition, a suite of other characteristics such as growth requirements, metabolic abilities, and antagonistic abilities are being characterized. There has been intense interest in developing probiotics for the bovine mammary gland to prevent or treat environmental and contagious mastitis. However, when traditional lactic acid bacteria such as *Lactococcus lactis,* or *Lactobacillus acidophilus*,which are Generally Regarded as Safe (GRAS) are inoculated into the bovine udder it induces a local inflammatory response and results in mastitis like symptoms. This is unsurprising since most of these strains are derived from the gastrointestinal tract and are not adapted to the mammary gland environment. The ultimate goal of this collection is to provide a resource of strains isolated from this environment for the future of probiotic development.

AEM17

Bugs from slugs: exploring the bacterial diversity of nudibranchs by metagenomics

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Nudibranchs, commonly called sea slugs are marine gastropod mollusks. They are considered as one of the most colourful animals with more than 4700 known species. These soft-bodied mollusks shed their shells during the larval stage. Due to their lack of physical protection, nudibranchs have evolved different defense mechanisms to deter predators. Some nudibranchs use camouflage while others use their bright colours to signal predators away. Quite remarkably, several species sequester chemical defenses from their diet. Nudibranchs feed mostly on sessile organisms such as sponges, tunicates, bryozoans and cnidarians. They store different toxic compounds from their prey to render their bodies distasteful to potential predators. In some species, these compounds are selectively localized in different parts of their body, such as the rim, as their primary line of defense. Certain species of nudibranchs are also known to produce defensive metabolites *de novo* and although most of these compounds are suspected to be of microbial origin, no one has investigated these so far.

This study aims to look at the microbiome of different nudibranch species and compare the bacterial composition of specific body parts such as mantle, rim and viscera in selected species. We used 16S rRNA gene-based metagenomics to characterize the bacterial composition of 24 different species of sea slugs mostly nudibranchs, but also including sacoglossans, sea hares and headshield slugs collected from the Great Barrier Reef, Australia. A total of 18 bacterial phyla were identified across the different samples with the phylum Proteobacteria as the most abundant. Analysis of the core microbiome revealed that *Mycoplasma*, *Ruegeria* and *Alteromonas* are the prevalent genera. In some nudibranch samples, a single bacterial species seemed to dominate the microbiome (>98% abundant). The rim body part in one nudibranch showed an abundant bacterial species (most likely a new taxon under f. *Oceanospirillaceae*) not found in the viscera but a minor component of the mantle. This is the first extensive study looking at the microbiome of diverse nudibranchs. With shotgun metagenomic sequencing underway, we are hoping to correlate the microbial producers of the defensive metabolites found in these remarkable animals.

AEM18

Characterization of a carbon source dependent, phytohormone responsive free methionine-(R)-sulfoxide reductase from *Komagataeibacter hansenii*

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Komagataeibacter hansenii is a gram-negative, non-pathogenic acetic acid bacterium that serves as a model for the study of bacterial cellulose (BC). BC, an extracellular, unbranched polymer of ß-1,4-linked glucopyranose residues, is an important component of the biofilm structure of many bacteria, promoting surface attachment and providing protection against environmental stressors. Despite decades of study, relatively little is known about the regulatory pathways that control BC biosynthesis. The production of BC is affected by environmental signals, such as exogenous phytohormones, likely through two-component signal transduction pathways. A bioinformatics search identified a predicted soluble histidine kinase with putative GAF (cGMP-specific phosphodiesterases, adenylyl cyclases, and FhlA) sensor protein. To gain insight into the function of this protein, its structure was predicted using the Protein Homology/Analogy Recognition Engine (PHYRE2). The template

identified for the structural model was a free methionine-(R)-sulfoxide reductase (fRMsr), a protein involved in oxidative defense that reduces free methionine sulfoxide to methionine. Overlap extension polymerase chain reaction mutagenesis was used to knock-out the gene encoding the fRMsr. Growth kinetics and pellicle (BC production) assays were performed for both the wild-type and DfRMsr strains over a seven day period in the presence and absence of phytohormones (ethylene, abscisic acid, and indole-3-acetic acid) and with and without hydrogen peroxide-induced oxidative stress with either glucose or fructose as sole-carbon source. Notably, the growth of DfRMsr was greatly reduced when grown in glucose compared to fructose-containing medium, as was the pellicle wet-weight. Ethylene increased pellicle formation by DfRMsr grown in glucose. Growth in late exponential phase was affected in the presence of ethylene and ABA in glucose but not fructose medium. Particularly, DfRMsr treated with ABA reached a higher optical density compared to the wild-type. Lag phase was longer for DfRMsr in the presence of all tested phytohormones for both carbon sources. DfRMsr cultures supplemented with phytohormones behaved similarly to wild-type when fructose was the sole carbon source. DfRMsr exhibited a faster growth rate under oxidative stress in fructose medium compared with wild-type. In conclusion, phytohormone supplemented DfRMsr cultures recover their ability to grow on glucose, suggesting that phytohormones stimulate alternative pathways for glucose uptake and/or utilization. The mechanism by which fRMsr is involved in the regulation of BC production remains to be investigated.

AEM19

Characterization of antibiotic resistant bacteria from imported plant-based food products in Canada

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The role of imported vegetables, fruits and spices in the epidemiology of antimicrobial resistance in Canada has been inadequately studied. In this study, antibiotic resistant organisms from imported plant-based foods available at retail across Canada were investigated. A total of 143 products primarily from Asian and African countries were purchased from international markets in Saskatoon, Saskatchewan. Samples were selectively cultured for a panel of bacteria where resistance is known to be emerging. The proportion of samples positive for each organism were as follows: *Escherichia coli* (n=13, 9.1%), *Salmonella* spp. (n=2, 1.4%), ESBL producing Enterobacteriaceae (*Enterobacter* spp.: n=2, 1.4%, *Klebsiella pneumoniae*: n=2, 1.4%), , *S. aureus* (n=7, 4.9%) and *Enterococcus* spp. (n=66, 45.5%). Antimicrobial susceptibility testing of isolates was conducted by broth and agar dilution. Based on the susceptibility of each organism, isolates were then screened for resistance genes (β-lactamases, plasmid mediated quinolone resistance and mobilized colistin resistance determinants) by PCR.

Multi-drug resistant (MDR) Enterobacteriaceae (*E. coli, Enterobacter* spp., *K. pneumoniae*) and methicillin resistant *S. aureus* (MRSA) were identified. Broad spectrum β-lactamases and plasmid mediated quinolone resistance (PMQR) determinants were identified from Enterobacteriaceae isolates resistant to β-lactams and fluoroquinolones. Broad spectrum β-lactamase producers were identified from 6/143 (4.2%) of samples tested. β-lactamases including CTX-M-14 (n=1), CTX-M-15 (n=4), CTX-M-27 (n=1), TEM-1 (n=2), SHV-142 (n=1) and SHV-106 (n=1) were identified. The PMQR determinants, QnrB1 (n=1), QnrB2 (n=1), QnrS1 (n=1) and AAC(6')-Ib-cr (n=3), were identified from isolates grown from 2/143 (1.4%) of the samples. The two *Salmonella* isolates were only resistant to sulfisoxazole. Two *S. aureus* isolates were resistant to oxacillin and contained *mecA* confirming MRSA. Among methicillin susceptible *S. aureus*, no multi-drug resistance was found. A wide variety of susceptibility profiles were observed among *Enterococcus* spp. isolates, but all isolates remained susceptible to ampicillin, penicillin and vancomycin. All of the MDR organisms were originated from countries which are recognized to have a high prevalence of resistance. Further study is required to understand the extent of antimicrobial resistance transmission by imported plant-based foods from these countries to Canada.

AEM20

Characterization of antimicrobial resistance in the respiratory microbiota of beef calves from farm to feedlot

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Bovine respiratory disease (BRD) is a significant animal health issue for the beef industry, and accounts for economic losses greater than other diseases. Recent studies have shown an increase in antimicrobial-resistant BRD bacterial pathogens. Given the importance of antimicrobials in managing BRD, studying the development of resistance throughout the beef continuum will aid in effective prevention and treatment of the disease. To investigate the origin of antimicrobial resistance in the respiratory microbiota of beef cattle, calves sourced from three farms (40 steers/farm; total of 120 calves) were sampled by deep nasopharyngeal swabs (DNS) at: 1) the time of first vaccination (spring processing), 2) feedlot entry processing, and 3) after 40 days in the feedlot (feedlots differed by farm). The DNS were processed for isolation of the BRD pathogens *Pasteurella multocida, Mannheimia haemolytica,* and *Histophilus somni*. A subset of these isolates was tested for antimicrobial susceptibility using microbroth dilution, subtyped by pulsed-field gel electrophoresis, and serotyped (*M. haemolytica* only). Antimicrobial resistance genes [*tet*(H), *tet*(W), and *sul*(2)] were analyzed in DNA extracted from the DNS using quantitative PCR. Isolated pathogens varied for each group of calves at different time points but prevalence was generally greater for *P. multocida* (farm 1: 0.0%-32.5%; farm 2: 2.5%-67.5%; farm 3: 0.0%-70.0%), followed by *M. haemolytica* (farm 1: 7.5%-55.0%; farm 2: 5.0%-15.0%; farm 3: 12.5%-42.5%), and *H. somni* (farm 1: 0.0%-22.5%; farm 2 :0.0%-7.5%; farm 3: 2.5%-45.0%). For *M. haemolytica*, most isolates derived from calves originating from farm 1 and 3 were serotype 6 (65.4% and 65.5%, respectively), whereas serotype 2 was dominant in farm 2 calves (87.5%). None of the bacteria isolated at spring processing were resistant to any of the antimicrobials tested. Most *M. haemolytica* isolated throughout the study were susceptible to all antimicrobials. (92.3%). For *P. multocida*, the most dominant resistant phenotype was against oxytetracycline and neomycin (35.4%). However, the majority of resistant *P. multocida* (18/29 isolates) were detected in calves from farm 3 at time point 3 (40 days after feedlot placement), and appeared to be a clonal strain (100% pulsotype similarity). The abundance of *tet*(W) was similar at time points 1 and 2 for all farms, but increased by time point 3 ($P < 0.05$). The abundances of *tet*(H) and *sul*(2) only increased for farm 3 calves at time point 3, compared to the first two sampling times ($P < 0.05$). This study showed that antimicrobial resistance in the respiratory microbiota of beef calves is lower at the farm of origin, compared to calves that have been placed in feedlots for 40 days. However, the development of resistance can vary by feedlot, and can be caused by the spread of resistant bacterial strains. This indicates that feedlot management practices can affect antimicrobial resistance in calves and may be an important point of preventing dissemination of resistant respiratory pathogens.

AEM21

Characterizing soil microbial diversity through freeze-thaw cycles and the winter transition

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Microbial activity in soil persists under snow and ice throughout the winter transition, before reaching its apex during thaw events. With the onset of climate change, the active layer of soils will experience colder temperatures

as snowpack insulation is lost. Consequently, soils and their microbial communities will undergo a higher frequency of freeze-thaw cycles. Evidence of impacts to activity and bulk changes to microbial community structure under winter and freeze-thaw conditions have been identified, but specific changes to microbial phyla over the course of the entire non-growing season remain unclear. The objective of this study was to characterize changes in microbial diversity and bioenergetics through winter conditions, including planned freeze-thaw cycles, as a function of the changing environmental metrics of soil geochemistry and nutrient availability. We dynamically simulated a condensed climate model of the winter transition in laboratory soil columns, allowing for high resolution of depth and temporal sampling. We used agricultural soil to additionally investigate the efficacy of pre-winter fertilizer amendments, as higher frequencies of thaw events may hasten microbial consumption of fertilizer, decreasing fertilizer availability and efficacy in spring. Our microbial analyses included high-throughput community composition profiling and bulk metabolic assessment for community activity. This longitudinal study will provide insights as to the specific development of the soil microbiome through the winter transition and establish causative links to geochemical conditions, clarifying impacts of winter freeze-thaws on agricultural best-practices and microbial impacts on biogeochemical cycling.

AEM22

Clostridium perfringens and other gram positive spore formers persists for many years in soil following an application of swine manure to an agricultural field

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Clostridia, including the human and animal pathogen *Clostridium perfringens,* are abundant in animal manures that are commonly used as a valued crop fertilizer. Compared to far less persistent vegetative enteric bacteria, clostridia could potentially increase the risk of human exposure to antimicrobial resistance for years if antibiotic resistance genes are carried in the genomes of recalcitrant spores. Previously we observed in field experiments, a very large increase in the abundance of Clostridia for up to a decade after application of swine manure. The purpose of the present study was to determine if *C. perfringens* recovered over a 5-year period from a field experiment having received a single application of swine manure were of enteric origin, or were of soil origin and simply increased in abundance due a 'bloom' effect accompanying the application of the manure. If the former, this would be of potential concern with respect to long-term carryover of antibiotic resistance genes of human health concern, whereas if the latter, there would be far less concern. Swine manure was applied to a field plot at an agronomic rate the spring of 2013, and soil from this plot and an unmanured control plot sampled through to 2017. Presumptive *C. perfringens* from the manure and from the soils were enumerated and purified on M-CP media. Collections of the bacteria were subject to 16S rRNA gene and *cpn*60 sequencing, and resistance to selected antibiotics determined phenotypically. The abundance of presumptive *C. perfringens* declined in the manured plot over the 5 years, but were still much more abundant than in the control plots. Based on 16s rRNA gene sequencing, the proportion of presumptive *C. perfringens* isolates that matched by best blast hit to *C. perfringens* in the manure, control soil, and manured soil were ~85%, ~30% and ~60% of the isolates respectively. The remaining ~15%, ~70%, and ~40% of isolates corresponded to a variety of Clostridales and Bacillales species that closely matched the colony morphologies of *C. perfringens* on M-CP agar. The distribution of the various species in the control and the manured soil species were remarkably stable over the 5 years. The frequency of resistance to the antibiotics erythromycin, tylosin, tetracycline, and clindamycin were determined phenotypically using literature obtained minimum inhibitory concentrations. The frequency of resistance in collections of isolates obtained from the swine manure was much greater than in isolates from the control soil whereas it was intermediate in the manured soil. This suggest that both soil and manure *C. perfringens* contributed to the frequency of resistance in the manured soil. These results suggest that the dramatic increase

in the abundance of *C. perfringens* following swine manure application is likely due to both entrainment of enteric *C. perfringens* present in the manure, and a bloom effect that induces growth of soil, and possibly manure, *C. perfringens*. Overall, we conclude that enteric *C. perfringens* can persist in field soils for years following the application of swine manure under climate conditions characteristic of the Great Lakes Basin.

AEM23

Comparing methods to assess the biodiversity of soil microbes

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Plant productivity and community composition varies according to the microbial biodiversity in the soil. This diversity can be monitored using Next-Generation Sequencing: DNA is extracted from the soil of a plant's root system, and genetic barcodes for the microbes of interest, are amplified by PCR. These amplicons can then be sequenced, and the reads analyzed via a bioinformatics pipeline. To mitigate any errors that may have been introduced to the sequence data by PCR, or the sequencing reaction, retained sequences have typically been grouped together into operational taxonomic units (OTUs) based on 97% to 100% similarity. OTUs are usually then matched, or clustered, together into taxonomic groups, capturing the microbial biodiversity represented in the sequence data. However, OTUs simultaneously have high false positive rates—they overestimate diversity—and high false negative rates, by being unable to accurately discriminate real biological diversity from errors near the OTU-defining cut-off. Moreover, OTUs identified in different studies are not directly comparable. The recent DADA2 pipeline, however, identifies taxa sequences with as little biological variation as one or two nucleotides, and correctly discriminates real diversity from errors. Since sequences can always be compared between analyses, unlike OTUs, DADA2's output may be more valuable for future biodiversity studies. Thus, an objective of my project is to evaluate how previously generated OTU data from plant roots relates to the sequences identified in the same data by DADA2. Here, I will present our preliminary results comparing both pipelines.

AEM24

Competitive interactions are common in mixed communities of *Gardnerella* spp.

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Gardnerella spp. are a hallmark of bacterial vaginosis, a loosely defined dysbiosis of the vaginal microbiome. *Gardnerella* has four subgroups based on cpn60 sequences: A (*G. swidsinkii* and *G. leopoldii*), B (*G. piotii*), C (*G. vaginalis*) and D (encompassing several unnamed genome species), which potentially differ in virulence. Although multiple subgroups often colonize individual women, subgroups A and C are most prevalent and abundant. Several factors including interactions between *Gardnerella* subgroups may contribute to their abundance in vagina. When *Gardnerella* are dominant in vaginal microbiomes, interactions between subgroups would be frequent. Our objective was to characterize the types of interactions between subgroups of *Gardnerella* under *in vitro* conditions. Representative isolates of four subgroups were grown in BHI+0.25% maltose for 48 hours in communities of 1, 2, 3 or 4 subgroups (n = 48, 72, 48, or 12, respectively). DNA was extracted, and subgroup-specific qPCR was performed to determine the abundance of each isolate in the initial inoculum and the final population. To determine the types of interactions in the mixed communities, we compared the observed final population sizes with the expected population sizes under the null hypothesis of no interactions between subgroups. Most (70%, 92/132) of the mixed communities exhibited negative interactions ($p < 0.0001$). Subgroup C had a growth rate that was twice that of the other subgroups. Interestingly, the effect of competition differed

among the four subgroups. Subgroups A, B, and C had lower growth rates in the mixed communities, whereas subgroup D had a higher growth rate ($p < 0.0001$). Moreover, the presence of subgroup D as a competitor negatively affected the growth rates of the other three subgroups. Our study suggests that there is non-interfering, exploitative competition among *Gardnerella* subgroups, which may select for subgroups with different nutrient utilization capacity. Examination of whole genome sequence data and ongoing carbon source utilization profiling of the isolates used in this study will provide insight into mechanisms contributing to the observed competition. The outcomes of this study will reveal how non-interfering, resource-dependent competition between *Gardnerella* subgroups -may contribute to their population dynamics in the vaginal microbiome.

AEM25

Contaminant organisms and antibiotic resistance in holy water

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The purpose of this project was to determine whether the running or standing holy water was more likely to contain contaminant organisms, determine the levels of antibiotic resistance among these organisms and the possibility of transferring the antibiotic resistance genes between the different organisms. The samples of holy water were collected on Sundays, December through March from four Catholic churches following the Mass. Two churches had running water fonts and two had standing water basins. The running water had a pH of 8-9 with 0.016% NaCl concentration; the standing water had a pH of 5 with na detectable salt concentration. The presence of other ions was negligible. 18% of standing water and 11% of running water tested positive for coliforms. 11% of standing water and 3% of running water samples tested positive for *S. aureus*. Of the confirmed *S. aureus* isolates none were resistant to vancomycin and rifampin, one was intermediate for Oxacillin (MIC 8 mg/L), two resistant to Penicillin (MIC 64 mg/L), six to Clindamycin (MIC 32 mg/L), and seven to Erythromycin (MIC >1000 mg/L).

AEM27

Creating synthetic wheat holobionts for increased drought resistance

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Drought is a limiting factor in agricultural production and will become a major problem with the predicted climate changes. The interactions between the plant and its microbiota (collectively known as a holobiont) could be the key to overcome this issue. Our objective was to create a synthetic community from a collection of strains thoroughly tested for their beneficial effect on wheat drought tolerance. An extended culture collection of bacteria and fungi was created from leaves, roots, seeds and rhizosphere soils of field-grown wheat plants under reduced or normal precipitation levels (25 or 100% of the natural precipitation). The isolates were isolated on traditional growth media (TWYE,TSB, R2A and PDA) and specialized, environmentally relevant (soil extract, plant extract and artificial root exudates) growth media. We have isolated more than 500 bacteria and fungi. So far, we have characterized the strains for their growth under low water availability and for their capacity to promote root elongation. Synthetic communities will then be assembled and combined with various drought tolerant or sensitive wheat cultivars to create synthetic holobionts. These holobionts will then be compared in term of their capacity to sustain water-stress. Future experiments will further zoom on the metatranscriptomic responses of these holobionts to water stress. Our results are expected to shed light on the microbial community characteristics necessary for improved water-stress tolerance of wheat holobionts.

AEM28

Defining and improving the dairy cattle microbiota to assure consistent and high vitamin B12 concentrations in milk

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Vitamin B12 is an essential nutrient that only bacteria and archaea can synthesize. Humans must acquire vitamin B12 through diet, and most Canadians obtain their recommended daily intake (RDI) of vitamin B12 through the consumption of milk or other dairy products. However, the vitamin B12 concentrations found in a 250 mL glass of milk are highly variable and can provide between 16-56% of the RDI. This variability can result in vitamin B12 deficiency in vulnerable groups despite the regular consumption of dairy products. The goal of this project was to identify bacterial populations that inhabit the rumen, lower intestine, and mammary gland that are associated with either very high- or very low-vitamin B12 concentrations in milk. Vitamin B12 is produced by bacteria and archaea in the rumen and absorbed into the cow's bloodstream in the small intestine, then it is used by the cow for her own metabolic needs and what remains is secreted in the milk. Microbiota present in the large intestine and the caecum can use the vitamin not absorbed in the small intestine, but this microbiota can also synthesize the vitamin. Moreover, possible use of the vitamin by the udder microbiota, affecting the final concentration of vitamin B12 in milk, cannot be ruled out. In this study, we collected rumen, blood, fecal, and milk samples from 96 dairy cows at a single time point. We measured the vitamin B12 present in each sample, and characterized the microbial population in the rumen, fecal, and milk samples using 16S rRNA targeted amplicon sequencing. Several correlation analyses were performed using LEfSe analysis, and we were able to identify specific bacterial taxa that were strongly correlated to vitamin B12 concentrations. Elevated levels of *Proteobacteria* in the rumen were correlated with high vitamin B12 production in the rumen, but this did not correlate well with vitamin B12 concentrations in the milk. In the lower intestine *Rhodospirillales*, *Alphaproteobacteria*, *Desulfovibrionaceae*, and *Gastranaerophilales* were correlated with lower vitamin B12 content – suggesting these phyla may be consuming the vitamin; while *Lactobacillaceae*, *Tannerallaceae*, *Gammaproteobacteria*, and *Succinivibrionaceae* were associated with high-vitamin B12 content in the lower intestine, suggesting that these bacteria may be producing the vitamin, or at the very least not consuming it. This study has provided potential targets for modifying the bovine microbiome to provide consistent and high-levels of natural vitamin B12 in milk.

AEM29

Detection of individual viable microbial components in a unique microbial consortia-based plant biostimulant using ddPCR

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Concentric Ag's microbial-based plant biostimulant contains multiple microorganisms which cover a broad range of taxonomy across prokaryotes and eukaryotes. Plate-based methods are inadequate to sufficiently resolve and accurately quantify these species due to the following limitations. First, it is difficult to visually discriminate some of the microorganisms in the consortium based on their colony or microscopic morphology. For example, *Lactobacilli* are difficult to differentiate from each other. Second, plate-based methods are not ideal for slow growing microbes, such as the *Rhodopseudomonas palustris*, due to a long turn-over time. Third, the CFU range on a plate is 25 to 250, which indicates the LOQ (Limit of Quantification) is 25 CFU. Since the CFU are counted

from the first diluted series (10 times, minimal, or 100 µL of culture in 900 µL dilution reagent), the LOD (Limit of Detection) is 2500 CFU/mL. Last, plating results can be variable due to the interpretations of different technicians. To overcome these shortcomings, we implemented two technologies: 1) PEMAX fixation for excluding non-viable cells, and 2) target-specific ddPCR quantification, to specifically detect target microorganisms. To prove that PEMAX fixation allows quantification of viable cells, representative samples from Concentric's product were treated with/without PEMAX, and then treated at three different temperatures (room temperature, 55°C and 65°C). Total DNA was extracted, and representative prokaryotic species *L. paracasei* and *L. rhamnosus* were quantified by multiplexed ddPCR. Significantly ($P < 0.05$) less *Lactobacilli* DNA was quantified in the samples treated with PEMAX and treated at higher temperatures, than those without PEMAX and treated at lower temperatures, indicating that the PEMAX treatment successfully excluded non-viable DNA. The greatest effect was observed at room temperature, where the non-PEMAX treated samples had 2 to 3 times more DNA copy counts than the PEMAX treated samples. Similar significant results ($P < 0.005$ for the samples with/without PEMAX with higher temperatures) were observed for a eukaryotic representative, *Candida utilis*. In this study, we also demonstrated that the slow-growing component of our product, *R. palustris*, which was below the LOD with our plate assay, was detected in representative batches with the ddPCR method, albeit at low viable copy numbers (10^2 to 10^3 copies/mL). To our knowledge, this is the first report of implementation of a ddPCR system combining viable cell fixation for the quality control of biostimulant products. Overall, the method presented has the potential for broader use in the characterization and quantification of viable microbes in other consortium-based plant biostimulants, as well as for the food industry (wine and cheese) and probiotic products.

AEM30

Developing a synthetic disinfectant for use on chicken eggs

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Antibiotic resistance is a growing problem for both human populations and commercial livestock industries. To avoid the use of antibiotics, the Canadian poultry industry currently uses disinfectants containing chlorine, ammonia, and iodine, which cause respiratory and skin irritation, making them difficult to work with. Among poultry pathogens such as *E. coli* and *Staphylococcus* is the Gram-positive bacterial genus *Enterococcus,* which is known to infect chicken eggs and cause developmental deformities such as hind-limb paralysis, or Spondylitis, making it an especially problematic pathogen for the poultry industry. Recently, a synthetic disinfectant has been developed called phosphopyricin, which is effective against Gram-positive bacteria, including *Enterococcus*, and appears to exhibit no toxic effects in mice. The synthetic nature of phosphopyricin also offers it a large advantage in that any resistances likely to develop would happen at a much slower rate due to the inherently lower instances of exposure. The primary objectives of my research will be to determine the minimum concentration of phosphopyricin and its related compounds needed to the inhibit growth of target pathogens, develop a solvent which allows for long-term storage, assess whether resistance develops after repeated exposure to the compound, and finally develop the compound as a spray or dip for treating eggs. Commercial use of phosphopyricin as a hatchery disinfectant has the potential to help reduce the development of disinfectant resistance and mortality rates in chicken stocks, which will overall lessen costs associated with bacterial contamination in Canadian hatcheries.

AEM31

Development of a biosensor for *Bacillus* species using recombinant antibodies and aptamers

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Bacillus species are used in high quantities for biotechnology applications. Several of them are on the Domestic Substance List of the Canadian Environmental Protection Act (CEPA), which means that they have been approved for use in the manufacturing of biotechnology products. Out of the many species of *Bacillus*, the vast majority rarely cause disease. However, among the pathogenic *Bacillus* species, *Bacillus cereus* causes food poisoning and *Bacillus anthracis* is the etiological agent of anthrax. Along with *Bacillus thuringiensis*, a bioinsecticide, these bacteria are very closely related and belong to the Bacillus cereus group (BC group). The goal of this project is to develop a selective biosensor for rapid detection of BC group members. Current detection techniques for identifying members of the BC group that are based on cell culture or PCR technology are time consuming. With the aim of developing a rapid detection methodology, we intend on adapting a biosensor based on photoluminescence (PL) of GaAs/AlGaAs heterostructures. This method has been successfully demonstrated with *Legionella pneumophila* and *Escherichia coli*. To achieve this goal, we identified two BC group spore coat moieties as targets for antibody production. One of these, Cot-α, was cloned into a shuttle vector and expressed in *E. coli*. We have isolated and purified this peptide to subsequently serve as an antigen for recombinant monoclonal antibody production. In addition to detection with antibodies, we explored aptamers as detection reagents. These synthetic nucleic acids are reported to exhibit selectivity and increased stability at ambient temperature in comparison to conventional antibodies. In this study, we provide an assessment of sensitivity and selectivity synthetic detection reagents, which will be critical for future optimization of the PL-based biosensor technology.

AEM32

Development of a mobile genome sequencing tool for the detection and characterization of biothreats in food

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On-site testing for intentionally contaminated food using a genomics-based approach could herald a new, exciting and effective paradigm in addressing risks to the food chain by achieving rapid detection, swift regulatory intervention and a coherent and comprehensive management of biothreats by all stakeholders. The approach should lead to a fit-for-use test that could universally detect all ranges of microbial pathogens in contaminated food by relying on a well-curated and comprehensive database such as one provided by Cosmos ID Inc. We have developed a protocol for identifying the presence of a virus (M15 bacteriophage), bacterium (*Salmonella*) or parasites (*Cryptosporidium* and *Giardia*) on lettuce spiked with the respective bioagents. DNA molecules present in extracts from the surface of lettuce were subjected to whole genome amplification (GenomePlex® WGA4 kit; Sigma-Aldrich, Oakville, Ontario, Canada) and sequenced using the MinION sequencer (R9.4 flow

cell, Oxford Nanopore, Oxford, UK). Bioinformatic analysis was done by uploading fastq sequence files on to the Cosmos ID web server. The analysis resulted in the sensitive detection of each of the pathogens used to spike the lettuce sample by identifying the closest sequence match in the Cosmos ID database. We were able to accurately and exclusively match the sequence of a strain of pathogen (e.g., *Salmonella*) used to spike lettuce with a strain in the database if the genome sequence was already deposited in the GenBank and included in the Cosmos ID database. Characteristics of each organism was provided by data on the presence of antimicrobial resistance genes (AMR) genes, virulence genes and bacterial prophages. The developed technology provides an opportunity for accurate forensics and tracking of isolates, such as if a laboratory strain is put to malevolent use. The ability to match informative genome markers present in metagenomic sequences developed from extracts from contaminated food with counterpart markers present in a well curated database is opening up a new era of rapid and effective detection of food contaminants with technologies that can also be applied to monitor clean up procedures and certify facilities for quick return to legitimate use.

AEM33

Development of bacteriophage cocktails targeting the *Burkholderia cepacia* complex

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Bacteriophages (phages) are viruses which selectively infect and kill members of the bacterial species to which they are specific, while leaving eukaryotic cells and commensal organisms relatively unharmed. As a result, these viruses have numerous applications ranging from food safety and agricultural pest control to phage therapy targeting antibiotic-resistant pathogens. Although the medicinal potential of phages was realized almost immediately after their initial discovery, early phage-based medicines were rapidly phased-out in favor of newly-discovered antibiotics in Western countries. As a consequence of the global spread of multi-drug resistant pathogens, however, brought on largely by the rampant overuse and misuse of antimicrobial compounds over the last century, research into phage therapy has experienced a renaissance in recent years. While the medicinal use of phages certainly has great potential, the development of phage-resistance in bacterial populations remains a serious problem, and several studies have demonstrated that single-phage therapies are often ineffective in resolving infections, particularly in immunocompromised individuals. In order to maximize treatment effectiveness and circumvent the development of resistance, phage therapy must be administered using polyphage cocktails consisting of phages utilizing diverse mechanisms of infection.

Burkholderia cenocepacia, one of the most dangerous members of the notorious *Burkholderia cepacia* complex (Bcc), is a highly antibiotic-resistant opportunistic pathogen which can cause severe pulmonary disease in immunocompromised patients and those suffering from cystic fibrosis, making it an ideal candidate for phage therapy. Previous work in the Dennis Lab has focused on the environmental isolation and characterization of novel phages capable of effectively killing *B. cenocepacia* and other members of the Bcc, thus creating a library of virulent phages which can be combined into cocktails for therapeutic purposes.

Using two well-characterized strains of *B. cenocepacia*, C6433 and K56-2, both of which are epidemically-spreading respiratory isolates from Canadian cystic fibrosis patients, we developed a high-throughput *in-vitro* screening assay to determine optimal combinations of virulent Bcc phages DC1, KS14, KL1, AH2, KS12, JG068, KS9 and KS4-M, which can be used in the construction of therapeutic cocktails. Using this approach, we identified several phage combinations capable of severely restricting bacterial growth at biologically-relevant bacterial titres under varying environmental conditions such as incubation temperature, growth medium, and multiplicity of infection (MOI). Validation of these phage combinations using the *Galleria mellonella* infection and phage-mediated rescue model will demonstrate the effectiveness of these combinations *in vivo*, providing proof-of-concept for the construction of polyphage cocktails targeting the Bcc, and contributing a stepping-stone towards investigation of these cocktails in murine studies and ultimately human trials.

AEM34

Differential contributions of *Saccharomyces cerevisiae* and *Brettanomyces claussenii* to a Belgian strong beer

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Parallel 49 Brewing Company opened in East Vancouver in 2012 and has become an award-winning microbrewery central to the growing beer culture in British Columbia. One of their most successful beers is brewed with a co-culture of *Saccharomyces cerevisiae* and *Brettanomyces claussenii* using a fairly complex and laborious process to achieve a unique flavour profile; however, due to the time and energy expenses required to produce this beer, production has been halted. At present, Parallel 49 critically needs information regarding this unique brew in order to understand the genetic makeup of these two strains; characterize the metabolites produced by the individual strains in relation to their gene expression profiles; and understand how yeast strains genetically and metabolically interact during mixed-strain fermentations. To achieve these goals, the genomic, transcriptomic, proteomic, and metabolomic profiles of each yeast species are being characterized independently and in co-culture during 10 litre fermentations of wort into beer. Measures of fermentation progress, specifically decreasing oxygen, pH and specific gravity, as well as increasing ethanol content, show that *Brettanomyces* works at a much slower rate than *Saccharomyces cerevisiae* or co-cultures of the two. The rate of flavour compound production, as measured by headspace gas chromatography coupled to a flame ionization detector and a mass spectrometer, differed in each yeast culture. The transcriptomic profiles of yeast in each brew type are being characterized by mapping mRNA transcripts to whole genome sequences prepared during this project. Proteomic analyses are underway in order to characterize the active metabolic pathways leading to the unique flavour profiles of the three brews. By gaining an understanding of underlying biological processes, Parallel 49 will be able to identify key biochemical pathways that lead to the flavour profile of this popular brew. As advances in brewing sciences are made, the company will hopefully be able to tailor their brewing process to allow for use of a single yeast species to make a similar product more efficiently.

AEM35

Differential gene expression of specific metabolic pathways in a marine, methylotrophic, denitrifying biofilm cultured in different conditions

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The biofilm of a continuous, methanol-fed, fluidized denitrification system that treated a marine effluent is composed of multi-species microorganisms, among which *Hyphomicrobium nitrativorans* NL23 and *Methylophaga nitratireducenticrescens* JAM1 are the principal bacteria involved in the denitrifying activities. To assess its resilience to environmental changes, the biofilm taken from the denitrification system was cultured under batch-mode, anoxic conditions at laboratory scale in artificial seawater (ASW) and exposed to a range of specific physico-chemical parameters. The seawater formulation and the NaCl concentrations had a deep impact on the *H. nitrativorans* NL23 population, with its displacement by a new denitrifier, *M. nitratireducenticrescens* GP59. Here, we report the impact of three conditions of the biofilm cultures on the transcript levels of specific metabolic pathways in *H. nitrativorans* NL23 and *M. nitratireducenticrescens* GP59 and JAM1. The original biofilm taken from the denitrification system was acclimated for five weeks in ASW under denitrifying conditions with i) 300 mg NO_3^--N/L at 23°C, ii) 900 mg NO_3^--N/L at 30°C, and iii) 300 mg NO_3^--N/L at 23°C but with NaCl concentration at 0% instead of 2.75%. The metatranscriptomes of these biofilm cultures were generated, along

with the transcriptomes of the planktonic pure cultures of *H. nitrativorans* NL23 and *M. nitratireducenticrescens* GP59 and JAM1 cultivated under denitrifying conditions. Up regulation of the denitrification genes in strain GP59 and strain NL23 occurred in the three biofilm cultures compared to the planktonic pure cultures, along with some of the carbon assimilation pathways. The biofilm metatranscriptomes also revealed the presence of several other microorganisms representing between 5-10% of the reads. Among them are denitrifying bacteria affiliated to the *Stappia* spp., which were metabolically active in the three biofilm cultures, suggesting that the biofilm has the potential to adapt to heterotrophic, non-methylotrophic environments.

AEM36

Discovery of two aptamers that bind to *Legionella pneumophila*

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Legionella pneumophila (*Lp*) are water-borne pathogens whose widespread prevalence is intensified by mismanagement of engineered water systems. *Lp* is transmitted via aerosols from these water systems, causing a severe pneumonia called Legionnaires' disease. Traditional methods for detecting *Lp* in the environment, such as the standard plate count method and qPCR, are tedious and expensive. Aptamer-based biosensors, however, can provide rapid and economical detection platforms. Aptamers are short, single stranded DNA or RNA oligonucleotides that can bind to a wide variety of targets ranging from small compounds to whole cells with high affinity and specificity. We created specific aptamers for detecting *Lp* in complex environmental matrices by using Cell-SELEX. Cell-SELEX is an iterative process which involves incubating *Lp* cells with a random pool of oligonucleotides, separating the cell bound and unbound oligonucleotides and amplifying the cell bound sequences via PCR for the next round of selection. We identified two aptamers called hereafter R10C5 and R10C1. Their affinity and specificity were characterized via flow cytometry and fluorescence microscopy. Their dissociation constants K_D are116nM for R10C5 and 135nM for R10C1. Interestingly, these aptamers bind preferentially to *Lp* cells but not to other bacteria isolated from environments were *Lp* is found. We conclude that our aptamers are specific to and have high affinity for *Lp*. They can therefore serve as viable biorecognition elements in multiple detection assays.

AEM37

Diversity and physiological capabilities of aerobic anoxygenic phototrophs in Lake Winnipeg

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Lake Winnipeg is the tenth largest freshwater lake in the world, with a watershed reaching nearly one million square kilometers in size. Due to the drainage basin's heavy agricultural and industrial use, eutrophication of Lake Winnipeg has been observed over recent decades. Nutrient increase has been monitored, while the naturally occurring lowest end of the food chain, the bacteria, have been overlooked. It has been presumed that algae and cyanobacteria are the sole microorganisms to proliferate due to excess of nitrates and phosphates. However, bacteriochlorophyll containing aerobic anoxygenic phototrophic bacteria (AAP) are commonly found associated with oxygenic primary producers. These heterotrophs are abundant in hot water springs, soil crusts, and marine environments. A few recent studies have investigated their distribution in fresh water systems, but the current strategies have avoided culture based enumerations and identification. This oversight underestimates the heterogeneity of AAP. To better understand the diversity of AAP in Lake Winnipeg, eDNA sequencing of V4

16S rRNA genes by Illumina MiSeq, and taxonomical identification of the isolates was performed from 2016 through 2018. Sequencing of environmental DNA revealed a seasonal fluctuation of AAP from 0.9% to 1.5%, to 0.7%, in the spring, summer, and fall months, respectively. In comparison, AAP represented 27%, 15%, and 6% of pigmented isolates cultured on rich organic medium in the three relative seasons. Here, 13 strains from various genera were selected based on their unique absorbance spectrum of light harvesting complexes as well as partial 16S rRNA gene sequencing. They included strain AJ72, sharing 98.6% sequence similarity to *Sphingopyxis contaminans*, AM19, 99.7% to *Erythromicrobium ramosum*, BD64, 98.0% to *Blastomonas natatoria*, BE100, 99.4% to *Pophyrobacter colymbi*, BK61, 99.1% to *Blastomonas fulva*, AM91, 98.5% to *Porphyrobacter sanguineus*, CK155, 98.1% to *Roseomonas sediminicola*, CL63, 97.8% to *Methylobacterium extorquens*, BA23, 99.0% to *Methylobacterium indicum*, BL67, 98.9% to *Sandarakinorhabdus cyanobacteriorum*, BC100, 96.6% to *Sphingomonas yantingensis*, CN8, 97.8% to *Rhodoferax saidenbachensis* and GM14, 99.3% to *Erythromonas ursincola*. Numerous physiological traits were investigated, including bacteriochlorophyll *a* production, carbon source utilization, vitamin requirements, enzyme activities, pH and temperature ranges, mobility, antibiotic sensitivity, Gram stain, GC content, fatty acid composition, oxidative growth and cell morphology. The pairwise analysis of AAP and their phylogenetic assignment will aid in the understanding of their ecological role in this freshwater community.

AEM38

Does human exposure to antibiotics through food consumption select for resistance in the digestive tract?

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Veterinary drugs, including antibiotics, are used for the treatment and control of livestock diseases. An issue that needs to be addressed for veterinary antimicrobial drugs is the safety of their residues on the human intestinal flora. Different governmental authorities such as the European Union, The Codex Alimentarius Commission (CODEX) and the Canadian Food Inspection Agency (CFIA) have elaborated principles for evaluating the safety of antimicrobial residues in food, established microbiological acceptable dairy intakes (ADI) and recommended maximum residue limits (MRLs) for antibiotic residues in food products. The evaluation of the microbiological ADI are based in relevant scientific information such as minimum inhibitory concentration (MIC) data of predominant human intestinal bacteria. However, it does not include data derived from minimal selective concentration (MSC) predictions, which accommodate information about the lowest concentration of a particular antimicrobial that provides resistant bacteria an advantage over sensitive. Based on these insights, we sought to determine if human exposure to selected antibiotics, in particular through ingestion of foodstuffs, would result in colon concentrations that could exceed apparent MSCs. Nine antibiotics: tetracycline, oxytetracycline, ciprofloxacin, sarafloxacin, erythromycin, spiramycin, tilmicosin, tylosin and lincomycin were selected according to their pharmacodynamic characteristics for analysis. We took two approaches for estimating dietary exposure, one using published measured concentrations in foodstuffs, and a second modelling exposure considering ADI values or food MRLs. Using the ADI, the estimated antimicrobial residue concentrations in the human colon of all antibiotics assessed may be up to three order of magnitude greater than predicted MSCs. When the dietary exposure assessment combined MRLs or measured concentration in foodstuffs values with food consumption data, the estimated concentrations were considerably lower, but still within the MSC range. Taken together, these modelling results suggest that the ingestion of antibiotic residues through food consumption may expose intestinal microbiota to antibiotic concentrations exceeding the MSC boundaries, thus favoring the growth of potential resistant bacteria. We suggest that MRL and ADI values be revised in light of the recognition that antibiotic concentrations significantly below the MIC values may select for resistance.

AEM39

Dynamics of denitrification in saline environments of species *Methylophaga nitratireducenticrescens* and *Hyphomicrobium nitrativorans* in co-cultures

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Nitrate can be a serious problem in closed-circuit aquatic systems such as aquariums because it accumulates quickly and becomes toxic to some organisms. We study the biofilm of a methanol-fed fluidized denitrification system that treated a marine effluent. The biofilm is composed of multi-species microorganisms, among which the main bacteria responsible to the denitrifying activities belong to the alphaproteobacteria *Hyphomicrobium nitrativorans* (strain representative NL23) and to the gammaproteobacteria *Methylophaga nitratireducenticrescens* (strain representative JAM1), both methylotrophs, account for 60-80% of the biofilm. *M. nitratireducenticrescens* JAM1 is capable of growing in pure culture under anoxic conditions by reducing NO_3^- in NO_2^-, which accumulates in the medium. It can also reduce NO and N_2O to N_2. *H. nitrativorans* NL23 is also is capable of complete denitrification from NO_3^- to N_2. We hypothesized that collaboration between the two species must exist in the biofilm to establish optimal denitrifying activities.

Our objective is to study the influence of the *M. nitratireducenticrescens* JAM1 with *H. nitrativorans* NL23 on the denitrifying activities by performing co-culture experiments. Planktonic pure co-cultures were performed under anoxic conditions at 30°C. The choice of the medium was important for both strains to grow. Although strain NL23 was isolated from a marine biofilm, it cannot grow with a marine medium as does strain JAM1. We had to lower the concentration of NaCl in the medium at 0.5% for both strains to grow. Our results showed that strain JAM1 inhibited NL23 growth by rapidly generating nitrite in the medium. This inhibition did not occur when NL23 inoculum was cultured under denitrifying conditions. No growth benefit and denitrification rates was observed in these co-cultures.

Planktonic monocultures to determine the specific nitrate consumption rates of both strains showed that strain JAM1 has greater than strain NL23 for nitrate. This suggests that strain JAM1 has an advantage over strain NL23 by acquiring nitrate more rapidly, and thus generating nitrite more rapidly in the medium. Antagonist tests on agar plates showed no diffusible metabolites or enzymes generated by either strains that could restrain the growth of each other. We did not detect acyl-homosérine lactones, involved quorum sensing molecules, in cultures of both strains. Finally, both strains can develop biofilm in static monocultures in glass, polystyrene and le polypropylene tubes. Biofilm generated by co-cultures did not improve growth and denitrifying activities. All these results suggest that presence of strains JAM1 and NL23 in co-cultures has synergetic or antagonist effect on the denitrifying activities in the conditions tested.

AEM40

Dynamics of *Pseudomonas aeruginosa* biofilm formation in a microfluidic system

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Pseudomonas aeruginosa is a ubiquitous bacterium and an excellent model for the study of biofilms. We have recently demonstrated that biofilm formation in shaking culture conditions can lead to the formation of biofilm-like structures. Depending on the bacterial strain analyzed and the presence or absence of magnesium or zinc ions, the planktonic growth and the formation of adhered biofilm and biofilm-like structures could vary greatly without a clear link between these three processes. Microfluidics, those systems in which liquids flow in channels of the micrometer range, allows the formation of biofilms to be observed and studied in a much more direct way than other methods.

The purpose of this study was to observe the biofilm formation of *P. aeruginosa* PAO1 and PPF-1 strains in microfluidic channels with or without magnesium ions. The PPF-1 strain was isolated in a dental unit waterline while the PAO1 strain originated from a wound.

The biofilm formation was observed continuously by light microscopy inside a microfluidic system whose channel dimensions were 45 µm high by 600 µm wide. Bacteria were exposed to a concentration of 20 mM $MgCl_2$ or no treatment. Each experiment lasted between 60 and 140 hours.

Video analysis demonstrated that the appearance and dynamics of biofilm formation for PPF-1 and PAO1 are very different with a flatter biofilm for PPF-1 and a seemingly lower capability to resist the shear stress than PAO1, which produce the well-known mushroom-like biofilm. The effect of magnesium exposure has then been tested on PPF-1 and enhanced biofilm formation was observed, while promoting massive detachments.

This study demonstrates the promising side of comparative analysis of biofilm formation and evolution in a microfluidic system. The next step of the project will be to carry out other experiments with potential inhibitors of biofilm formation.

AEM41

Ecological interactions among aquatic microbial communities using metabarcoding in Southern Ontario lakes

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Heterotrophic prokaryotes utilize the dissolved organic carbon derived from phytoplankton exudation or cell lysis and mineralization by heterotrophic prokaryotes provides inorganic nutrients for phytoplankton. For this reason, these communities are expected to be closely linked; however, specific ecological features of individual phylogenetic groups and their interactions are largely unknown. To address this, we collected 324 water samples from sixty Southern Ontario lakes. Water samples were filtered and we extracted environmental DNA for NGS-based 16S and 18S rRNA gene fragment sequencing to characterize the MC (combined bacteria communities (BC) and microeukaryote communities (MEC)). Mantel tests was applied to evaluate the correlations between Bray–Curtis dissimilarity of microbial communities and spatial coordinates. To investigate the interactions among the MC, the most prevalent OTUs (more than 500 reads) were selected and combined base on their taxonomic classification (order). A total of 50,339 (BC) and 14,611 (MEC) OTUs were generated, of which 7 and 5 OTU_S were shared by 71% and 89% of the isolates for BC and MEC, respectively. Mantel tests explained only 7% of community variation. Shannon index ranged from 2.96 to 10.07 for BC and 1 to 8.67 for MEC, respectively. Moreover, a total of 84% of the correlations established in the network were positive (SSCC ≥ 0.6). Moreover, 16 % of the interaction were negative (SSCC < -0.3). Microbial network exhibited that most BC or MEC are clustering separately. Most of the negative interaction were bacterial-bacterial or MEC-MEC. For interactions between MEC- BC, only Gaiellales vs Fragilariophyceae; Bacillales vs Intramacronucleata were negatively correlated. Most MC showed strong correlations (35% had r ≥ 0.9). The abundance of each component within

the microbial loop, (i.e. BC, MEC) is organised by some arrangement of top-down (grazing pressure) and nutrient load (bottom-up) mechanisms**.**

AEM42

Effect of biodiversity manipulation on generalist and specialist function of a forest soil

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Protection of biodiversity aims to support ecosystems functioning for the goods and services they provide.Nevertheless, the relation between biodiversity and functionality is complex, especially at the microorganism level characterized by high functional redundancy. Following that perspective, this work was set up to evaluate the sensitivity of two general functions, namely the community-level physiological profile (CLPP) and respiration, and two specialized functions, namely hydrogene (H2) and carbon monoxide (CO) oxidation to an alteration of microbial biodiversity. Biodiversity manipulation was performed using three different ratios of non-sterile:sterile forest soil mixtures (1: 4, 1:20 and 1:40), with or without two antibiotics. Soil microcosms were incubated under controlled atmosphere, ensuring the availability of H2 and CO during soil colonization. PCR amplicon sequencing of bacterial 16S rRNA gene showed that composition rather than species richness of microbial communities, was influenced by the treatments. Despite the absence of species extinction, the specialized functions reach up to a 94% decrease while the general functions were not reduced. In addition, it was observed that oxidation of CO was more sensitive than oxidation of H2. These results show the complexity of biodiversity and ecosystem functionning while proposing the analysis of the susceptibility of functional microbial guilds to an alteration of biodiversity in order to develop new tools of protection of the environment

AEM43

Effects of crude secondary metabolites extracted from *Solanum mauritianum* and its bacterial endophytes on pathogenic bacteria and resistant human cancer cells

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Endophytes are endosymbionts, often bacteria or fungi, residing within plant tissues and are considered to be non-pathogenic to their host plant. The kind of endophyte present in each plant is mostly determined by the geographical location of the plant. Endophytes have many functions in their host plants, which include plant growth enhancement, nutrient acquisition as well as improve the ability of the host plant to withstand environmental stresses such as drought. They are also believed to produce similar bioactive chemical and have similar bioactive properties as their host plant. This study looks at the effects of crude secondary metabolite extracts of *Solanum mauritianum* and the bacterial endophytes isolated from different parts of the plant on pathogenic bacteria and human cancer cells. Fresh; healthy plant parts (leaves, ripe and unripe fruits) of *S. mauritianum* were collected; washed; surface sterilized; macerated in PBS; inoculated in the nutrient agar plates

and incubated for 5 days at 30 °C. Amplification and sequencing of the *16S* rRNA gene was applied to identify the isolated bacterial endophytes. These endophytes were then grown in nutrient broth for 7-14 days; after which sterilized Amberlite® XAD7HP 20-60 mesh resin was added to each culture to adsorb the secondary metabolites which were later extracted using ethyl acetate. The different plant parts were extracted as well using methanol/chloroform (50:50, v/v). Crude extracts from each bacterial endophyte and plant part were tested for antibacterial activity against 11 pathogenic bacteria and two human cancer cell lines. A total of 4 bacterial endophytes were identified from the stem of *S. mauritianum*. The antibacterial test showed that crude secondary metabolites of the endophytes and parts of *S. mauritianum* possessed antibacterial properties against pathogenic microbes such as *Mycobacterium marinum; Staphylococcus epidermidis; Staphylococcus aureus; Klebsiella pneumonia; Pseudomonas aeruginosa* with concentrations showing inhibition ranging from 0.03125 to 8.0000 mg/mL. The anticancer analysis showed an increase in cell proliferation at the highest concentration of 100 µg/mL when A549 Lung carcinoma and UMG87 glioblastoma cell lines were treated with both the plant and endophytes' crude extracts. This study showed that the isolated bacterial endophytes from *S. mauritianum* had inhibitory effects against similar pathogenic microbes as their host plant and showed no inhibitory effect on the human cancer cells tested in this study as also seen with their host plant. These endophytes and plant will be further analyzed using Mass spectrometry to determine if they indeed produce similar bioactive compounds and possible isolation of the bioactive compounds for further pharmacological studies.

Keywords: antibacterial; anticancer; bacterial endophytes; *Solanum mauritianum*; 16S rRNA

AEM44

Effects of insecticide resistance on *Aedes aegypti* microbiota in French Guiana

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Aedes aegypti is an important vector of arboviruses like dengue, yellow fever, zika, and Chikungunya. The females are anthropophilic and breed in water of human-made containers located in urban area. The mosquito life cycle is composed of 4 stages, distributed between aerial and aquatic habitats (i.e. egg, larvae, pupae, and adults). Due to improper uses of insecticides, *Ae. aegypti* has developed resistance mechanisms, such as an increase in degradation enzymes or mutations of cellular targets. However, it is suggested that the insects' microbiota may also play a role in this insecticide resistance. Accordingly, a study carried out on Riptortus pedestris, colonized with a fenitrothion resistant strain of Burkholderia, showed higher tolerance to dermal exposure and ingestion of this insecticide.

Based on this observation, we hypothesize that insecticide exposition leads to modifications in the structure of mosquitoes microbiota, which may trigger insecticide resistance. First, larvae and water from 70 breeding sites were collected in French Guiana. Physicochemical data, such as pH, turbidity, DCO, and nutrients, were collected at each collection point. Moreover, environmental characteristics such as the type of habitat, vegetation or site's material, were also recorded. Bacterial communities were identified by 16S rRNA sequencing on total genomic DNA extracted from larvae and water. Results indicate that the bacterial communities were mainly composed of three classes: Alphaproteobacteria (41.4%), Actinobacteria (10.4%) and Betaproteobacteria (8.6%). Eleven ASVs were found exclusively in water, which suggests specific recruitment of bacteria from larvae. The bacterial community identified in water suggests the presence of two distinct groups.

Following preliminary analyzes, larvae's microbiota were modulated by rarefaction of water microbiota and then confront to deltamethrin, an insecticide largely applied in French Guiana. To achieve this goal, L1 larvae were grown in a series of dilution of breeding site water until L3-L4 stages before exposure to the insecticide. Larvae were collected based on 30% first mortality and 30% last-survival in order to extract DNA and sequence 16S

rRNA. The effect of insecticide on mosquitoes' microbiota will be monitored and compared between larvae that were severely or less affected by the insecticide.

AEM45

Enumeration of probiotic strain *Lactobacillus gasseri* Lg-36 using viability real-time PCR

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Probiotics were reported to have several benefits to human health, especially in supporting intestinal health. The definition of probiotics are "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host". Probiotichealth benefitsare strain specific, thus, it is necessary to have the correct strains in probiotic products when claiming specific health benefits. Additionally, probiotichealth benefits are dose dependent, hence, it is critical to verify the viable cell count of probiotic strains. Culture based methods such as plate count are currently used for bacterial enumeration, however, this method is tedious, time consuming, requires selective growth media and can't distinguish between different strains. An alternative tool is needed to enable accurate, fast and specific identification and enumeration of viable cells in probiotic products. Polymerase chain reaction (PCR) based methods are commonly used in food testing because they are simple, fast, sensitive and can be validated. Viability PCR is a technique that relies on pre-treatment using intercalating dyes such as PMAxx (modified propidium monoazide) to inactivate DNA from membrane-damaged cells so that only viable cells react in PCR. In this study, a viability PCR-based tool was used for the enumeration of probiotic strain *Lactobacillus gasseri* Lg-36using strain specific primers. The effectiveness of PMAxx in viability PCR was first evaluated by testing viable and heat killed cells. PMAxx treatment was able to differentiate between viable and heat-killed cells. The tool was further validated in terms of linear dynamic range, limit of detection (LOD), reaction efficiency, repeatability and reproducibility. The linear dynamic range was established between $5.6*10^5$ to 56 genomes. The LOD was found to be equivalent to 56 genomes, corresponding to 10.8 CFU. The % relative standard deviation (RSD%) at LOD was 5%. The reaction efficiency was determined to be 97%, with R square values above 0.98. The RSD% for repeatability averaged to 0.6%, while that for reproducibility averaged to 2.8%. To further evaluate the performance of this tool, commercial *Lactobacillus gasseri* Lg-36 products were enumerated using viability PCR and bacterial counts were compared to the standard culture based method (plate count). Bacterial counts calculated from viability PCR highly correlated to bacterial counts determined using plate count method with R square values >0.999. This viability PCR-based protocol provides a faster alternative to the plate count method and facilitates accurate and quick enumeration of strain *Lactobacillus gasseri* Lg-36.

AEM46

Evaluating the potential for transmission of enteric bacteria and antibiotic resistance genes from livestock manure to harvested hay

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Hay is an important feedstock in large animal production systems, and is also used for maintaining soil health and productivity on farms. Typically in a season a hay crop will be harvested (mowed and bailed) two or three times in the climate conditions characteristic of the Southwest Great Lakes Basin. Current farming systems often use manure to fertilize hay fields as part of on-farm manure management, and this practice thus represents

a potential route of transmission of antibiotic resistance to animals that are fed hay. The purpose of the present study was to evaluate the abundance of selected antibiotic resistance genes (ARGs) and enteric bacteria on harvested hay following the application of swine or dairy manure onto a standing crop under field conditions over three successive seasons (2016-18). Select antimicrobial resistance gene (ARG) classes (sulfonamides, streptomycin, aminoglycosides, macrolides, extended β-lactamases, carbapenemases, and plasmid identifying markers) were quantified by qPCR. Plate counts on a variety of media were used to presumptively identify and quantify various classes of bacteria, namely total and fecal coliforms, *E. coli*, enterococci, *Aeromonas* spp, *C. perfringens*, *Yersinia* spp. and *Campylobacter* spp. Chromocult medium containing various antibiotics was used to enumerate presumptive antibiotic resistant coliform bacteria (ARB). Swine and dairy manures contained all analysed ARGs. ARG abundance ranged from 10^8 copies/g of manure to less than 10^2 copies/g of manure. Swine and dairy manures contained coliform bacteria resistant to β-lactams, sulfonamides, streptomycin, tetracycline, and chloramphenicol. Swine and dairy manure was applied to field scale plots onto a standing hay-alfalfa crop. The abundance of ARGs and coliform ARBs was determined at each cutting of the hay during the course of the growing season. Over the three field seasons, the gene targets *str*A, *str*B, *sul*1 and *int*1 were often more abundant on the manured crop than the unmanured crop at the first harvest after manure application. There was no difference in the abundance of any other gene targets. None of the enumerated viable bacteria were more abundant on the manured than the unmanured crop. Manuring had no effect on the abundance of any ARGs or ARBs on second or third hay harvests during the season. Overall, these results indicate that there is a potential for transfer of ARGs from manure to hay at harvest, and the significance of this as a potential transmission route to livestock merits further investigation.

AEM47

Evaluation of microbiological contamination of fresh salads and vegetables from farms, processing plants and retail markets in Korea

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Leafy greens are recognized as potential vehicles of foodborne pathogen such as *Escherichia coli* O157:H7. Fresh-cut salad including romain lettuce is considered as one of the high risk produce in terms of foodborne illness, as it is usually manufactured and consumed without thermal processing. The objective of this study is to evaluate the level of microbiological contamination of vegetables and fresh salads from farm lands, producing plants and retail markets. Total 1,371 samples(50 from farms, 21 from plants and 1,136 from retail markets, 164 from environmental specimen) were collected and tested for *E. coli* including 5 types of pathogenic *E. coli* (EHEC, ETEC, EPEC, EIEC and EAEC), coliforms and aerobic cell. The coliforms of vegetables, farm soils and irigation water were found at the level of 2.38 ± 2.16 log CFU/g, 3.38 ± 1.17 log CFU/g and –1.40 ± 00 log CFU/g, repectively. While the total aerobic count of vegetables, farm soils and irrigation water were 5.46 ± 0.95 log CFU/g, 7.15 ± 0.05 log CFU/g and 0.12 ± 1.54 log CFU/g, respectively. *E. coli* and pathogenic *E. coli* were not detected in any vegetables from farms, while 8 samples of producing plants and 1 sample from retail market were positive for *E. coli*. Nine *E. coli* colonies isolated from plants and retail market were tested for the detection of genetic markers of EHEC, ETEC, EPEC, EIEC and EAEC by real time PCR. Total 3 isolates from plants had pathogenic gene of *E. coli*. Two isolates had VT1 (verotoxin) related gene and the other had *eaeA* gene. The result of this study is expected to contribute to the risk assessment of pathogenic *E. coli* in fresh salads and vegetables from farm to table.

AEM48

Exploration of the hidden side of the bacterium *Leptospira*

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Leptospirosis is a worldwide zoonotic disease. One of the main routes of transmission of leptospirosis is the natural environment contaminated by animal urine. Soils and surface waters also harbor a wide variety of *Leptospira* species for which virulence status is not clearly established. The genus *Leptospira* is historically divided in 35 species classified into three phylogenetic groups, believed to correlate with the virulence of the bacteria. Several genomes of this bacterium are currently available, but these come mainly from pathogenic strains of Latin America and Asia. Species with a lower level of pathogenicity were therefore under-studied, despite their importance to better understand the overall evolution of the bacterium.

In this study, we sequenced and analyzed the genomic sequences of hundreds of environmental *Leptospira* strains to highlight the true diversity of this bacterium. By using cutting-edge computational methods, we have been able to make a major taxonomic update by doubling the number of official species. Thanks to this new dataset representative of the evolution of the bacterium, we also identified a new group, exclusively composed of new species, and propose a new classification scheme. The different new species are distributed among the four major phylogenetic groups, confirming that the environment is an important source of *Leptospira* diversity.

The highlighting of the different new species and the new group allowed us to have a new vision of the evolution of *Leptospira*. We have also been able to discover a dichotomy within species considered pathogenic and determine the genetic characteristics specific to each group. Finally, we found that pathogenic species have a more open pan-genome than those from other groups. This suggests a reorganization of cellular functions in pathogenic species by multiple horizontal gene transfers that could have allowed a change in the ecological niche. Although the reason is not yet entirely clear, it is possible to think that the large number of potential hosts that can be infected by these species requires some specificity and that horizontal transfers can be one of the methods allowing rapid adaptation to these hosts by providing a gene repertoire suitable for them.

AEM49

Exploring aromatic compound metabolism in the foodborne pathogen *Listeria monocytogenes* by elucidating the protocatechuate pathway

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Listeria monocytogenes is an ubiquitous gram positive foodborne pathogen and it is the causative agent of the often fatal disease, *listeriosis*. Although much of the literature concerning *L.* monocytogenes relates to its pathogenicity, its persistence in the environment remains poorly understood. Our lab has recently identified genes within the *qui1* and *qui2* operons in *L. monocytogenes* and its sister species *L. innocua* that direct carbon flow towards the biosynthesis of protocatechuic acid (PCA) from the shikimate pathway. PCA is a common aromatic intermediate in the degradation of other larger aromatic compounds such as lignin, though its biological role in microorganisms has not been fully characterized. In certain species of soil microorganisms, there are two known metabolic routes for PCA. Primarily, PCA is shuttled through the β-ketoadipate pathway and the tricarboxylic pathway for energy. Secondly, PCA is utilized as a moiety for a rare class of iron-chelating siderophores called petrobactin. Intriguingly, *Listeria* encodes for a dehydroshikimate dehydratase (DSD) within

its *qui2* operon called QuiC2. Like other DSDs, QuiC2 converts dehydroshikimate to PCA, though *Listeria* has not been shown to utilize PCA in any aforementioned way. Moreover, we have shown that induction of *qui1* and *qui2* genes results in the accumulation of PCA. We propose that PCA's role may not be apparent in mono-culture and that it requires the presence of other microbial species to study. Our work aims to biochemically characterize the protocatechuate pathway enzymes in *Listeria* and to determine the biological role of this pathway in *Listeria* by combining co-culturing and molecular biology techniques. We work to understand *Listeria*'s saprophytic lifestyle and to expand on current knowledge regarding the metabolism of aromatic compounds in soil microorganisms.

AEM50

Exploring microbiomes in traditionally important wild Arctic salmonids

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The Towards a Sustainable Fishery for Nunavummiut (TSFN) project is an integrated partnership with the community of Gjoa Haven, Nunavut, to increase our understanding of fisheries resources that are becoming more accessible in the lower Northwest Passage due to climate change. As part of this project, we are using microbial community analysis to help inform the health of Arctic char (*Salvelinus alpinus*) and lake whitefish (*Coregonus clupeaformis*). Both of these salmonids can be anadromous in this region, migrating annually from the sea to freshwater lakes and rivers in the autumn to escape sub-zero temperatures. As a result, the fish and their associated microbiota must adapt accordingly to their changing environment. Informed by traditional ecological knowledge, more than a thousand fish have been caught by Inuit fishers and youth, sampled aseptically on-site, and the balance of the fish returned to the community for consumption. After shipment of the samples to the laboratory, DNA extraction and high-throughput sequencing of bacterial and archaeal 16S ribosomal RNA gene amplicons generated microbiota profiles for fish skin and intestines. Fish microbial community composition, for both sample types, differed significantly between freshwater and saline fishing sites. Differences in intestinal microbial community composition were noted even within fish sampled at different sites on the same migration route along a salinity gradient. We also confirmed that the microbiota did not solely reflect the aquatic habitat surrounding the fish because these microbial communities were distinct from those derived from the surrounding waters. Our results also demonstrate that host genotype influences microbial community composition because we observed significant differences between the intestinal and skin microbiota of the two salmonid species, even when caught from the same site. Likely reflecting a paucity of microbiome studies focused on wild Arctic salmonids, a substantial proportion of taxa (>10% at some sites) are "unknown" across skin and intestinal profiles. Overall, the observed salinity-associated turnover of both skin- and intestine-associated microbial communities likely reflects a previously underappreciated stressor for these species that could possibly be exploited for biotechnological applications, along with identified taxa with probiotic potential. If so, it is hoped that these could be applied ultimately for the long-term wellbeing of Inuit communities.

Acknowledgements: We would like to acknowledge the fishers of Gjoa Haven NU, the Hunters and Trappers Association, and TSFN (a Genome Canada project partnered with Ontario Genomics), the Government of Nunavut, NSERC Canada, and the Department of Biology at Queen's University.

AEM51

Exploring the efficiency of microcins J25, B17 and L against *Salmonella* clinical isolates

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The advent of antibiotic resistant microorganisms is becoming an alarming issue, especially when taking into consideration that it is spreading faster than the introduction of new compounds. To deal with this health crisis, new avenues of research are being explored such as bacteriocins. Bacteriocins are defined as bio-active proteins with bactericidal action. Furthermore, they are distinguished from other classical antibiotics for being ribosomally synthesized and possessing a narrow spectrum of activity. Microcins are a group of Gram negative bacteriocins which exercise their inhibitory activity through nutrient uptake channels: essentially, the microcin hijacks necessary uptake machinery, thus ensuring the death of natural competitors vying for the same natural resources. This is believed to be the reason for their (i) narrow spectrum of activity and (ii) high efficiency.

In order to determine the potential of microcins as an alternative to antibiotics against multi drug resistant microorganisms (MDR), we investigate the potency of their antimicrobial activity and compare it with other commercial antibiotics. Microtitration plate assays were used to measure the MIC and MBC of different microcins against clinical well characterized *Salmonella* isolates. At the same concentration of 0.1mg/mL, MccL was active against most of the strains 7 out of 8 followed by MccJ25 and MccB17 6 out of 8.

AEM52

Exposure to sub-lethal 2, 4-dichlorophenoxyacetic acid arrests cell division and alters cell surface properties in *Escherichia coli*

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Herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) is used extensively worldwide and in Canada despite its known toxicity and our limited understanding of its effects non-target organisms. I used *Rhizobium leguminosarum* bv. *viciae* and *Escherichia coli* to determine the effects of 2,4-D at sub-lethal concentrations. I used a novel approach combining advanced microscopy and gas chromatography-mass spectrometry-based metabolomics to examine the herbicide effects at low concentrations.

E. coli showed a filamentous phenotype in the presence of 2,4-D, a phenotype that has been previously observed under a variety of stresses. We also exposed a series of genotypically diverse environmentally isolated *E. coli* to 2,4-D and observed the same filamentous phenotype, demonstrating that it is not an artefact shown by the lab domesticated *E. coli*. 2,4-D caused oxidative stress, DNA damage and dissipation of membrane potential along with changes to several central metabolic pathways, all implicating a negative impact on cell division.I developed correlative atomic force microscopy-quantitative imaging-laser scanning confocal microscopy to simultaneously probe cell surface alterations at the pico-nanoscale with details of molecular changes inside live cells in real-time. Using this novel method, I examined the effects of 2,4-D on live green-fluorescence protein (GFP) tagged *E. coli* in real time. In response to 2,4-D, FtsZ-GFP and GFP-FtsA relocalized within seconds, coinciding with the complete inhibition of cell septation and cell elongation. Exposure to 2,4-D also resulted in increased activation of the SOS response as indicated by the increased SulA-GFP fluorescence, which is a direct result of increased oxidative stress. Changes to cell division were accompanied by concomitant changes to surface roughness, elasticity and adhesion in a time-dependent manner. In summary, this was the first study describing the mechanistic details of 2,4-D impacts at the molecular level in bacteria. Our study suggests that 2,4-D arrests *E.*

coli cell division by disrupting the divisome complex, causing oxidative stress induced DNA damage and activation of the SOS response resulting in a filamentous phenotype.

AEM53

Fecal source tracking: the decay dynamics of mitochondrial DNA markers in water

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In water environment, **fecal contaminations** are characterized as diffuse pollutions, mainly caused by anthropogenic activities, which promote emergence and dissemination of pathogens. In 2017, according to World Health Organisation, fecal related diseases accounted for 842 000 deaths worldwide. As such, it is essential to be able to **control at the source** the spread of fecal contamination by putting in place adapted measures. Nowadays, we are able to assess water quality and detect fecal contamination, but identifying its sources remains a **complex challenge** in which multiplicity of fecal contamination and its spread in soils, surface water and groundwater are synchronous. In this project, we propose to use the unique specificity of **mitochondrial DNA (mtDNA)** to trace the origins of fecal contamination in the environment. Epithelial cells of the intestine, containing numerous mitochondria, are extensively expelled with the stool, and indeed markers specific to mtDNA was detected in it. The variability within the mitochondrial genome makes it specific to each species, allowing the development of **species-specific genetic markers**. These characteristics motivate mtDNA application in the survey of fecal contaminations. For the development of source tracking markers, the decay dynamics of the markers has to be similar of the decay dynamics of the waterborne pathogens. The general objective of this project is to determine the **spatio-temporal decay** of the mtDNA markers according to various biotic and abiotic characteristic factors of an environment subjected to fecal contamination.

Water environment was selected for this first experimental approach. Samples from Rosemère wastewater treatment plant and three sites along Assomption River (North-East of Montréal) were collected and used to monitor the decay of mtDNA markers for several weeks according to the parameters: temperature, pH, turbidity, microbiological respiration (CO_2 production), fecal coliforms and the human Hf183 *Bacteroidales* genetic marker. For wastewater samples, the decay coefficient of human mtDNA is greater over the first 15 days. Limited decay was observed afterwards. The same trend was observed for the fecal coliforms and the human Hf183 *Bacteroidales* genetic marker. Porcine and bovine mtDNA markers were not detected at quantitative level in the wastewater sample. The decay dynamics of fecal contamination markers correlate with production of CO_2 in water samples. The decay of the human Hf183 *Bacteroidales* marker was particularly sensitive to temperature (20°C), which was less pronounced for fecal coliforms and the mtDNA markers.

AEM54

Free living *Amoeba*: a potential reservoir and transport vessel for Human Norovirus GII.4

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Human norovirus (HNoV) is a leading cause of acute viral gastroenteritis, primarily spread through the fecal-oral route. Due to its high infectivity, the occurrence of HNoV poses negative implications for sanitation and public health scenarios, especially if virions can persist within and utilize environmental hosts for shelter and dispersal. Of the five HNoV genotypes, genotype GII.4 causes the highest frequency of human NoV infections and was the focus of this project. Free-living amoeba (FLA) are found ubiquitously within all natural and engineered water systems. They act as known replication and dispersal reservoirs for other water-based pathogens such as

Legionella pneumophila, however, FLA interaction with enteric viruses such as HNoV is poorly understood. Since the waterborne pathogens HNoV and FLA co-occur in faecally contaminated environments, we evaluated the ability of FLA to serve as a natural reservoir and vector for HNoV transmission.

Using various co-culturing techniques, we demonstrate for the first time that human norovirus not only survives but also persists within FLA. Viral counts of the clinical isolate HNoV GII.4 during various time points of the co-culture were determined using reverse transcription (RT)-qPCR. Results show the viral counts persist after 6 days in co-culture with amoebae at a multiplicity of infection (MOI) of 100. This persistence is supported by transmission electron microscopy and image stream flow cytometry which revealed viral reservoirs within the amoeba cytoplasm, potentially undergoing multiplication. Ongoing work includes the exposure of co-cultures to various disinfection methods such as UV, heat, and monochloramine, to demonstrate the protective capacity of the internalized virions. Our results support the hypothesis that FLA contributes to the persistence and transport of human-pathogenic NoV by providing a protected intracellular microenvironment for persistence in natural aquatic environments and engineered water systems.

AEM55

Heat resistance in Shiga-Toxin producing *Escherichia coli* from cattle

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Ruminants, including cattle are a primary reservoir for Shiga-toxin producing *E. coli* (STEC). As steam or hot water pasteurization is frequently used to reduce or eliminate these pathogens from carcasses and beef to minimize risk of gastrointestinal illness in humans, *E. coli* showing extreme heat resistance may be a threat to beef safety. This study aimed to determine the heat resistance of STEC strains originating from cattle or their environment using a traditional water bath or heating block and comparing strains with and without the locus of heat resistance (LHR). A total of 34 strains of STEC isolated from cattle in Alberta between 2002 and 2017 were used. A generic *E. coli* which has been benched- marked as heat-resistant was used as a positive control. *E. coli* isolates were exposed in the water bath and the heating block to a defined come up time to 60ºC (T0) and 6 additional minutes (T6) to calculate the D_{60} value. Strains were classified in two groups with (17) and without LHR (17). Isolates positive for the LHR included serogroups O26, O45, O103, O111, O121, O145 and O157. Strains with a ≥ 5 log reduction (cfu mL^{-1}) after 6 min at 60 ºC were classified as heat-sensitive. Those that exhibited a 1 to 5 log (cfu mL^{-1}) reduction were classified as moderately-resistant, while strains with less than a 1 log (cfu mL^{-1}) reduction were considered heat-resistant. Averages, standard deviation and D_{60} values were generated and analyzed (SAS 9.4) to compare the groups using a significance level of 0.05. Using the water bath, D_{60} for all strains ranged from 0.79 to 3.9 minutes (sensitive to moderate heat-resistant), and was higher ($P < 0.05$) when thermo-tolerance was measured with the heating block. With the water bath, D_{60} for LHR strains was higher ($P < 0.05$) than for strains that lacked LHR, but this difference was not apparent with the heating block. The LHR has been described as conferring exceptional heat resistance (D_{60} > 10 minutes), but none of the isolates analyzed with the water bath had a D_{60} > 3.9 minutes. Ultimately, our data suggest, that the assessment of heat resistance is markedly influenced by the method employed, and showed that the LHR conferring moderate heat resistance has been present in *E. coli* from Alberta cattle for at least 15 years.

AEM56

How is the activated sludge community assembled? Experimental decoupling of wastewater substrates and microbial compositions

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In biological wastewater treatment plants (WWTPs) the microbial community composition of the activated sludge (AS) has the largest impact on the performance and stability of the system, however little is known about how these communities are formed. This study has utilized controlled manipulative experiments to focus on the fundamental mechanisms responsible for the assembly of diverse AS-WWTP communities. The potential for influent immigration has been long debated with two conflicting theories being developed in the form of the niche and neutral models. Seventy-two lab scale AS-WWTP reactors received synthetic wastewater supplemented with sewer bacterial communities to independently test the role of influent immigrants. After feeding with synthetic wastewater only, irrespective of the starting inoculum, highly reproducible but less diverse AS communities were formed due to the simpler composition of wastewater. The introduction of influent solids to the synthetic wastewater increased the diversity of the reactors. We identified numerous genera requiring immigration from the influent to be maintained in the AS. By developing a protocol to reproducibly grow AS-WWTP communities in the lab, we have laid down the foundational principals for the testing of operational factors creating community variations with low noise and appropriate replication. Understanding the processes that drive microbial community diversity and assembly is a key question in microbial ecology. In the future, this knowledge can be used to manipulate the structure of microbial communities and improve system performance in WWTPs. Modification of the microbial community could also be used to control the persistence of antibiotic resistance genes within WWTPs, reducing contamination of the environment and the risk to public health.

AEM57

How to incorporate genetic information into models for pesticide degradation in soils

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Traditional biogeochemical models include state variables such as microbial biomass pools that in practice cannot be measured. Modern molecular biology tools can provide quantitative information on functional microbial groups involved in a range of biogeochemical processes. This data can be used to develop models that accurately capture microbial population dynamics and metabolism of microbial driven matter cycling processes. We developed such a model to simulate pesticide degradation in soils. This mechanistic model is formulated as a set of ordinary differential equations. We assume that the pesticide MCPA is the sole source of carbon and energy for bacterial growth. The model includes an expressed gene pool representing the gene encoding for the enzyme tfdA, which is known to be involved in the rate-limiting step of MCPA biodegradation. We describe the activation of these genes by MCPA using a Hill function. The microbial degrader population is divided into three subpopulations: viable cells that metabolize MCPA; viable cells that do not; and dead cells. The consumption of MCPA drives microbial growth and maintenance; a fraction of the carbon in the consumed pesticide is reduced and released as CO_2. The model was calibrated against data on mineralized 14C-MCPA as well as tfdA mRNA and DNA abundance (Baelum et. al, 2008). We compare this genetically-informed model with simpler biomass-based compartmental biogeochemical models to assess the value of genetic observations in this context.

Baelum, J., Nicolaisen, M.H., Holben, W.E., Strobel, B.W., Sorensen, J., Jacobsen, C.S., 2008. Direct analysis of tfdA gene expression by indigenous bacteria in phenoxy acid amended agricultural soil. ISME Journal 2, 677.

AEM58

Hybrid sequencing and genome analysis of four *Bacillus thuringeinsis* strains

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Bacillus thuringiensis (*Bt*) is a naturally occurring soil bacteria which produces parasporal insecticidal Cry proteins during sporulation. *Bt*-derived bioinsecticides and genetically modified crops capitalize on the insecticidal activity and target specificity of Cry proteins to provide an alternative, environmentally-friendly insecticide. Newly isolated *Bt* strains are potential sources of Cry toxins with novel insect targets, allowing for the development of a broader range of Cry toxin insecticides. Despite the agricultural and economic importance of Cry toxins, their exact mode of action is still to be determined.

Four novel *Bt* strains were isolated from soil in Brazil, including one isolate, temporarily named *Bt*BV5, expressing a novel putative Cry8 protein was identified by PCR amplification. Phylogenetic analysis of this novel Cry8 protein indicated strong similarity to Cry8A, but testing showed no insecticidal activity against a known Cry8 target, *Anthonomus grandis*. To explore the potential insecticidal properties of these isolates, each genome was sequenced using a partial lane of Illumina HiSeq and an Oxford Nanopore sequencing technologies minION flowcell.

Sequencing yielded 2.1 million long reads and 40 million short reads, which were assembled using Unicycler. For the *Bt*BV5 isolate, a complete circular genome of 5.3Mb, along with two 500kb and 260kb plasmids, was assembled and a total of 5890 coding sequences were identified using Prokka. Two complete copies of the previously identified putative Cry8 protein were found on the 500kb plasmid in *Bt*BV5, but no other Cry-like protein sequences were identified. Complete circular genomes and small plasmids were assembled for two of the remaining isolates; initial gene annotation did not identify any *cry* toxin genes in these assemblies.

Following genome assembly and gene annotation for all strains, phylogenetic analysis will be used to group the isolates with previously identified strains of *B. thuringiensis*. Pangenome analysis will be used to compare virulence factors present in *Bt*BV5 to other *B. thuringiensis* strains to identify potential target organisms.

AEM59

Identification and characterization of an extracellular amylase conserved in *Gardnerella* spp

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Glycogen is a major carbon source available to vaginal microbiota, and it is digested into smaller products (maltose and maltodextrins) with the help of human and/or bacterial amylase and debranching enzymes that cleave glycosidic linkages. There is limited information about glycogen utilization mechanisms in *Gardnerella* spp. These bacteria are frequently associated with bacterial vaginosis: a dysbiosis of vaginal microbiome, characterized by a shift from lactic acid bacteria to a mixed population of anaerobic bacteria including *Gardnerella* spp. All isolates of *G. vaginalis* tested to date (n= 26, including representatives of four species: *vaginalis, swidsinskii, leopoldii* and *piotii*) have shown extracellular amylase activity on starch or glycogen in agar plate assays. Culture supernatants from these isolates are also amylase positive, indicating that the activity is associated with an enzyme secreted into the medium. Phylogenetic analysis of predicted extracellular amylase

protein sequences from these isolates revealed one ortholog conserved in all isolates. The objective of this study was to determine if this conserved protein is responsible for the observed amylase activity. The gene predicted to encode an extracellular amylase from *G. leopoldii* isolate NR017 was ligated into expression vector pQE-80L and the recombinant vector was used to transform Top 10 *E. coli*. IPTG induced *E. coli* cells containing recombinant vector produced a protein of predicted mass of 62 kDa, which was purified using nickel affinity chromatography. The purified protein showed amylase activity in the starch agar plate assay over the pH range tested (pH 5-7). The results of this study show that *Gardnerella* spp. have an extracellular amylase enzyme active over a range of pH, which could contribute to the pool of nutrients available to the vaginal microbiota. Characterization of additional steps in glycogen utilization in *Gardnerella* spp. will provide insight into whether competition for this abundant carbon source is important in the population dynamics and competition among vaginal microbiota in general, and *Gardnerella* spp. in particular, that result in shifts between eubiosis and dysbiosis.

AEM60

Identification of novel Type VI Secretion Systems in *Salmonella enterica*

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Globally, *Salmonella enterica* is the etiological agent of a foodborne, enteric, bacterial infection that causes 1.3 billion illness and 3 million deaths annually. To access the nutritional and spatial resources required to successfully colonize the gastrointestinal tract, *S. enterica* must overcome the colonization resistance provided by commensal bacteria. *S. enterica* are known to encode several Type VI Secretion Systems (T6SSs) that, when expressed, may mediate antagonistic interactions with the microbiota, thus freeing up space and nutrients for the *S. enterica* cells. In 2009 a search of the *S. enterica* pan-genome, which at the time contained 44 whole genome sequences, was conducted to identify the range of T6SSs encoded by this species and four T6SSs were identified (SPI-6, SPI-19, SPI-20 and SPI-21). At the moment the SalFoS database contains 2,000 novel *S. enterica* genomes, while NCBI holds more than 7,000. In this study we are using an *in silico* approach to search an up-to-date *S. enterica* pan-genome, to identify the range of T6SSs present. The novel T6SSs identified in *S. enterica* will help us understand the host-specificity of certain *S. enterica* lineages and may give direction to novel antimicrobial strategies in the post-antibiotic era.

AEM62

Identifying the target of LP2006, an antibacterial lasso peptide

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New approaches to antimicrobial discovery are needed to combat the escalating threat of antibiotic resistance. Ribosomally synthesized and post-translationally modified peptides (RiPPs) are a promising and diverse group of natural products, many of which have antimicrobial activity. The RiPP superfamily contains the lasso peptides, a family defined by their threaded macrocycle structure which resembles a lasso. Although many antibacterial lasso peptides have been discovered in recent years, the target and mechanism of action of most lasso peptides remain unknown. In this study, we seek to identify the target and mechanism of action of the antibacterial lasso peptide LP2006. We recently conducted a screen of nearly 50 strains of marine bacteria for antimicrobial activity against *Micrococcus luteus*, *Bacillus subtilis*, *Escherichia coli* and *Saccharomyces cerevisiae*. LC-MS-based metabolite profiling revealed that *Nocardiopsis* sp. strain HB141 produces LP2006, an antibacterial lasso peptide.

LP2006 is the only member of the Class IV lasso peptides and has known antibacterial activity against *Enterococcus faecium*, *Bacillus subtilis*, *Bacillus anthracis*, and *Mycobacterium smegmatis*. Since *Nocardiopsis* sp. strain HB141 (=H153) requires long culturing times, LP2006 was heterologously expressed using the chassis strain *Streptomyces coelicolor* M1146. The six genes required for biosynthesis were heterologously expressed in the pSET152 vector under control of the *ermE*p* constitutive promotor. LP2006 was purified from organic solvent extracts of *Streptomyces coelicolor* M1146 cultures using high-performance liquid chromatography and confirmed using mass spectrometry. Culture conditions are being optimized to increase the yield of LP2006 to identify the target and mechanism of action of the antibacterial peptide.

AEM63

Immune enhancing effects of Queso Blanco cheese supplemented with *Lactobacilli* and *Bifidobacteria* in healthy Beagle dogs

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Cheese is a good source of protein, calcium, and vitamins for dogs. Even if cheese has a bad reputation because of high energy or salts, eating small amounts of cheese shouldn't be a problem in healthy dogs. The majority of cheeses is safe for the majority of dogs. Queso Blanco cheese is one of the easier cheeses to make and does not requires careful handling. In this study, we evaluated the immune enhancing effects of Queso Blanco Cheese supplemented with lactobacilli and bifidobacteria in healthy beagle dogs. Twelve healthy dogs were divided in three experimental groups of 4 individuals; Control group, Queseo blanco cheese containing *Lactobacillus* (*L*.) *reuteri, L. fermentum*, *Bifidobacterium* (*B*.) *longum* and *B*. *animalis* (QCLB) fed group, Queseo blanco cheese without lactobacilli and bifidobacteria (QC) fed group. Dogs were everyday fed a cheese for 8 weeks. Blood was sampled on weeks 0, 4, and 8 to analysis complete blood cell count, peripheral blood mononuclear cell (PBMC) count, PBMC proliferative response to concanavalin A (Con A), and lipopolysaccharide (LPS), activation of NK cell, and serum cytokines production (TNF-α, IL-6, FIN-γ, IL-2 and IL-4). After 8 weeks of cheese intake, QCLB group significantly enhanced PBMC proliferation response to ConA and LPS compare with control group. The serum TNF-α production of QCLB (182.6±18.98 pg/mL) was significantly greater than that of QC group (125.9±23.96 pg/mL) at 8 weeks. QCLB group induced 516.2±73.05 pg/mL of IFN-γ, showing significant difference from QC group (415.2±67.77 pg/mL). In addition, NK cell activity in the QCLB group (1488.6±594.40 pg/mL) showed a significant elevation compared to the QC group (513.1±137.71 pg/mL). We demonstrated that dietary supplementation cheese containing lacrobacilli and bifidobacteria result in enhanced PBMC proliferation and induced serum cytokines in healthy beagle dogs. In conclusion, this study demonstrates that cheese with lactobacilli and bifidobacteria may improve immune response of healthy beagle dogs. Collectively, Queso Blanco cheese with lactobacilli and bifidobacteria is suitable for consideration as a functional feed additive for companion dogs.

AEM64

Impact of water distribution systems on microbiological quality of drinking water in First Nation communities in Manitoba, Canada

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The United Nations recognizes the right to water entitles everyone to have access to sufficient, safe, acceptable, physically accessible and affordable water for personal and domestic use yet there are more than 100 short- and long-term drinking water advisories (DWA) in effect for First Nation communities across Canada. Most DWA's are due to microbial contaminants. Many First Nations homes rely on cisterns (water-holding tanks) for home storage of drinking water. We hypothesized that there would be variations in each rural and northern First Nation community concerning the amount of *E.coli* and total coliforms, as well as in the amount of ARGs, depending on the water distribution system available to the community. In collaboration with the community's Chief and Council of each community, we this study collected water from various points along the water distribution systems from three different First Nation communities. Samples included those from the source water, water treatment plant (WTP), water delivery trucks and taps in homes directly piped, or serviced by plastic or concrete cisterns. Samples were enumerated for the presence of *E. coli* and total coliforms and analyzed with qPCR for the quantification of seven Antimicrobial Resistance Genes (ARGs). The results frequently showed free residual chlorine levels were typically less than 0.2 mg/L which is required to suppress microbial growth, subsequently more drinking water contamination in concrete cisterns than plastic cisterns. Further, increased frequency in the spring than winter was observed overall. Each WTP showed capacity to produce post-treatment water that did not contain *E. coli* or coliforms. However, water samples from cisterns contained high number of *E. coli* and coliforms. Although water from piped homes and the water delivery trucks typically tested negative for coliform bacteria, ARGs were detected in these samples namely, *sul2*, ampC*, tetA and mecA.* Antimicrobial resistance is a significant cause for concern in health care worldwide. Presence of these genes in drinking water supplies is concerning because ARGs can indicate the presence of bacteria that are difficult to treat with antibiotics, hence elevating the risk for infectious diseases of individuals exposed to these bacteria.

AEM65

Impacts of the phi027 prophage on the biology and virulence of the epidemic strain *Clostridioides difficile* ribotype 027

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The vast majority of *Clostridioides difficile* (*C. difficile*) strains are susceptible to bacteriophage (phage) infection. Our recent bioinformatic analyses on thousands of *C. difficile* genomes allowed us to identify prophages (phages integrated into their host chromosome) in virtually every strain analyzed. This observation underlines the extent of the phage-host relationship in *C. difficile* and calls for a better understanding of the potential role played by phages in the biology and virulence of this important pathogen.

We recently focused our efforts on studying the phage-host relationship in members of the 027 ribotype strain (R027), which is a very important group of clinical strains responsible for major outbreaks worldwide, and generally described as being hypervirulent. Targeted bioinformatic analyses of R027 genomes allowed us to establish that a particular prophage, namely phi027, is highly specific and conserved within that lineage, since it was found in almost 100 % of R027 genomes, but rarely encountered in all other known ribotypes. Hence, it is very appealing to hypothesize a potential contribution of phi027 in the hypervirulence associated with the epidemic R027 strains.

To study this question, we first inspected a database including 2,800 R027 genomes and identified very few strains (N=6) devoid of the phi027 prophage. Next, the phi027 prophage was induced with mitomycin C from its original host strain R20291 to produce infectious particles. The phage was then reintroduced into the six sensitive

strains lacking phi027 to create new lysogens in order to study various phenotypes. Intra and extracellular culture fractions of the lysogens were collected at 4 and 8 hours, and toxin production was assessed by ELISA and toxicity assays on VERO cell cultures. Preliminary results showed that new lysogens carrying phi027 produced up to 7-fold more toxin A compared to cells without the prophage (ELISA at 8 hours). In VERO cell culture assays, a 2 to 4-fold increase in toxin activity was also observed with lysogens. In parallel, using an allelic exchange approach, we succeeded in deleting the entire phi027 prophage from the genome of the wild-type R20291 strain. As expected, we observed a lower toxin production in the cured strain lacking phi027 compared to the wild-type parental strain, and the phenotype was restored upon re-lysogenization with phi027. These results suggest a contribution of the phi027 prophage in the hypervirulence often associated with R027 strains through modulation of toxin production. Infection assays in a murine model are planned in order to ascertain if this increased toxin production and activity is also observed *in vivo,* and if it is translated in more complicated symptoms and issues.

AEM66

Inclusion of qualitative data to improve predictive capability of MLR model for recreational beach microbial water quality

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Fecal pollution of recreational water is a significant problem at many beaches across the Great Lakes. Swimming in such water may result in gastrointestinal and respiratory diseases. To protect beach goers, models using statistical modelling framework such as Multiple linear regression (MLR) have been widely used and shown to predict *E. coli* concentration with reasonable accuracy. A commonly recorded qualitative weather condition information (such as clear, cloudy, rainy etc.) is widely reported but has rarely been included in such modelling efforts. The current study examines the inclusion of this qualitative weather information and its impact on the predictive capability of MLR based model for recreational beach microbial water quality. E. coli concentrations and water quality monitoring data collected Monday to Friday over 6 years from 2013 to 2018 on Monday to Friday at Sandpoint beach (Lake St. Clair) in Windsor Essex Region, were obtained from the City of Windsor, Ontario, Canada. Quantitative and qualitative hourly weather condition data were obtained from the nearest Environment Canada station. The present study examined the collected water quality and weather data and their various transformations to develop a multiple linear regression model. We used Genetic Algorithm to correlate the weather condition variable with *E. coli* measurement. Model was set up based on data from 2013-2016 and its prediction capability was tested with 2017 and 2018 data with and without qualitative variables. Best model was selected based on creation of the maximizing the R2 value and minimizing the mean square error.

Results show that including weather condition data enhance the total accuracy of the model from 68% to 81% for 2017 and from 61% to 73% for 2018 *E. coli* prediction. In addition, sensitivity (the proportion of correctly predicted exceedances out of all exceedances) and specificity (proportion of correctly predicted non-exceedances out of all non-exceedances) of the model are improved by 42% and 16% for 2017 and by 86% and 5% for 2018 E. coli prediction respectively.

AEM67

Intra- and inter-species horizontal transfer potential of integrative and conjugative elements carrying antimicrobial resistance genes

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The use of antimicrobials in livestock production may contribute to the emergence of antimicrobial resistant bacteria with relevance to both human and animal health. Bacteria acquire antimicrobial resistance genes (ARGs) by a number of means, but integrative conjugative elements (ICE) are of particular interest as they can confer resistance to multiple antimicrobials in a single genetic transfer event. ICE-mediated antimicrobial resistance (AMR) transfer among bacterial pathogens could therefore have serious consequences for the sustainability of antimicrobial therapies in both humans and animals. Multidrug resistant (MDR) members of the bovine respiratory disease complex (*Pasteurella multocida*, *Mannheimia haemolytica*, and *Histophilus somni*) were isolated from North American feedlots. Isolates containing up to 12 ICE-associated ARGs, conferring resistance to up to 7 classes of antibiotics, were used as conjugal donors to evaluate the potential of ICE-mediated horizontal gene transfer to antimicrobial susceptible intra- and inter-species counterparts, and to human bacterial pathogens including *Escherichia coli*, *Klebsiella pneumonia*, *Haemophilus influenzae* and *Salmonella enterica*. Full-length ICE, containing all of its ARGs could be successfully donated intra-species by all three BRD bacteria. *P. multocida* ICE was transferred to all recipients, whereas *M. haemolytica* donated ICE to all recipients except *E. coli*. The ICE from *H. somni* could be successfully transferred to *H. influenzae*, *E. coli* and *M. haemolytica*. Whole genome sequencing of positive transconjugants across all recipient species confirmed the transfer of ICE in their entirety with integration into the leucine tRNA gene in the recipient genomes. The *M. haemolytica* transconjugants however harboured ICE originating from *H. somni* and *P. multocida* donors as an extrachromosomal molecule. This study highlights the promiscuous nature of ICEs within Pasteurellaceae and their potential to transfer ARGs among both cattle and human pathogens.

AEM68

Investigating marine diesel microbiome using culture dependent and independent techniques

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In a recent report, Environment and Climate Change Canada revealed that the country is suffering from warming temperatures at twice the global level on average, with the northern regions being exposed to an even higher rate. The visible impact of global warming on the Artic regions is the loss of ice, which results in the gradual opening up of the Northwest Passage to maritime traffic and the exploitation of oil reserves. These activities increase the risk of oil and fuel spills in the Arctic Ocean waters, which are detrimental to marine ecosystems. Bioremediation is a useful approach for tackling this form of pollution; microorganisms present in the environment can degrade the oil by using its components as a carbon source. This process can be enhanced by modifying the nutrient content. Since the oil itself may already contain microorganisms that can degrade hydrocarbons, we first aimed to investigate if these microorganisms are present in the oil/fuel. DNA extraction followed by PCR and electrophoresis were carried out on marine diesel to determine their DNA content. In addition, enrichment cultures containing nutrient amended marine broth were inoculated with the marine diesel to investigate cultivable isolates present in the diesel. These findings will prove useful in better characterising the bioremediation potential of Artic ocean waters in the context of oil/fuel spills.

AEM69

Investigating seasonal impact on microbial load and antibiotic resistance genes in drinking water from a First Nation community, Manitoba, Canada

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About 20% of First Nation reserves across Canada are under boil water advisories due to poor microbiological quality. Presence of antibiotic resistance genes (ARGs) in these bacteria poses further risk to the community members consuming the water. In this study, we studied the impact of season on the presence of fecal bacteria and ARGs in drinking and source water in a First Nations community. The average free residual chlorine concentration in four seasons of Water Treatment Plant (WTP), water delivery Trucks (T) and Piped (P) homes was within the range typically observed for treated water in Canada (0.4 to 2mg/L). Whereas water samples from taps in homes served by Plastic cisterns (PL) and Cement cisterns (C) always showed unacceptable level of free residual chlorine concentration (<0.2 mg/L). Throughout the four seasons, the treated water at WTP was free of *E. coli*, whereas trucks and piped homes showed the presence of low number of *E. coli* (0 to 2 CFU/100 mL). However, *E. coli* numbers in Plastic cisterns showed a season-dependent variation. Higher numbers were observed in summer and fall samples (1 to 150 CFU/100ml) compared to those from early or late spring (0 to 9 CFU/100ml). A similar trend was seen for Cement cisterns, albeit the numbers were higher than those observed for Plastic cistern (early and late spring, 0 to 40 CFU/100 mL and summer and fall, 5 to >300 CFU/100 mL). Our results also suggest that in addition to chlorine concentration other factors (such as condition of the cistern, cleaning period etc.) may be responsible for high bacterial counts. Besides fecal coliform count, presence of β-lactamase genes (bla_{SHV}, bla_{TEM}, bla_{CTX-M}, bla_{OXA-1}, bla_{CMY-2}) was observed by multiplex PCR in Plastic and Cement cisterns but not in Piped samples. Among carbapenemase genes (bla_{KPC}, bla_{IMP}, bla_{VIM}, bla_{NDM}, bla_{GES}, bla_{OXA-48}), only summer sample of Cement and Plastic cisterns showed positive result for bla_{OXA-48}. In this study, we show that season can have a significant impact on the microbiological quality of drinking water distribution systems in First Nation communities.

AEM70

Investigation of microbial quality of various health functional food categories for reevaluation of microbial criteria

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In this research, we monitored the microbial population and analyzed the results to determine the sampling plan in various health functional foods. Indicator organisms (i.e. total aerobic count, coliforms and *Escherichia coli*) and food-borne pathogen (i.e. *Salmonella* spp. for mucopolysaccharide-protein products) were monitored in fourteen functional health food categories (total items=379, the number of case=1,895). Total aerobic count of ginseng and Korean red ginseng were found with a mean value of -0.35 log10 CFU/g and -0.76 log10 CFU/g, respectively. Total mean value of coliforms in ginseng and Korean red ginseng were -1.4 log10 CFU/g (not detected) and -1.38 log10 CFU/g, respectively. Coliforms and total aerobic count were not found in any other health functional food products (chlorophyll-containing plant, chlorella, spirurina, green tea extract, propolis extract, milk thistle extract, glucosamine, N-acetylglucosamine, aloe gel, aloe whole leaves, chitosan/chitooligosaccharide and mucopolysaccharide-protein products). Any *E. coli* was not detected in all functional health foods categories. These monitoring results could be applied to propose new version of microbial criteria in Health Functional Food Code.

AEM71

Investigation of prevalence and diversity of *Acinetobacter species* in environmental aquatic sources

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Acinetobacter spp. are strict aerobes and ubiquitously prevalent in the environment. Among several species, *Acinetobacter calcoaceticus-Acinetobacter baumannii* complex (ACB) has been identified as important nosocomial infectious agents. The aim of this study was to investigate the prevalence and diversity of *Acinetobacter* spp. in agricultural surface water and treated wastewater effluent samples. In addition, for enhanced recovery and isolation, two *Acinetobacter* selective (Leeds Acinetobacter Medium (LAM) and CHROM agar Acinetobacter) growth media, different incubation temperatures (ranging from 30 °C to 42 °C) and environmental (aerobic and microaerobic) conditions were also assessed. A total of 280 culture isolates recovered from two selective media under variable culture conditions were further confirmed to species-level, where CHROMagar at 30°C under aerobic, and LAM at 37°C under aerobic and microaerobic conditions showed significant enhanced recovery. Based on *Acinetobacter* genus-specific PCR assay, a total of 118 (42%) isolates were amplified and identified as *Acinetobacter* spp. in all samples. The positive isolates were further sequenced for species-level identification using 16S rRNA and *rpo*B genes. The results showed that *A. calcoaceticus* (42; 36%) was more frequently detected than *A. pittii* (15; 13%), *A. oleivorans* (11; 9%) and *A. baumannii* (7; 6%). Some isolates showed close similarity to *A. calcoaceticus*, *A. johnsonii*, *A. soli* and *A. tandoii* but formed independent lineages. These strains are being further investigated for taxonomic classification, characterization and identification of novel species of genus *Acinetobacter*. The study results indicate that *Acinetobacter* spp. are capable of growing under variable culture conditions and contamination of agricultural water and treated wastewater effluent samples with human-associated ACB complex can pose potential health risks to humans. The study results will be helpful for regulators, policy makers and managers to improve existing or develop new farm management practices and wastewater treatment strategies that can efficiently reduce/remove human pathogens to protect drinking, irrigation and recreation water sources from contamination.

AEM72

Investigation of *Pseudomonas syringae* as an organic biocontrol for Canada thistle

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In the Canadian organic farming industry Canada thistle is a weed of significant concern. Small numbers of this hardy perennial can reduce crop yields significantly as the weed competes for water, nutrients, and sunlight. The chemical herbicides currently available to control Canada thistle are not organic meaning they cannot be used by organic farmers. Current organic weed control methods have limited efficacy since Canada thistle can reproduce from small root fragments, which reduces the effectiveness of methods like weed tillage or pulling. *Pseudomonas syringae*is a plant pathogen that stunts growth and induces chlorosis in the leaves of members of the plant family *Asteraceae*, which includes Canada thistle and sunflowers. *P. syringae* shows promise as a potential bioherbicide due to its virulence and limited host range, which does not include common grain and food crops. My research focuses on assessing the virulence of individual *P. syringae* strains as well

as polymicrobial mixtures, as determined by extent of bleaching and stunting. Preliminary testing on sunflowers has identified several promising strains, some of which work synergistically. Recent results have shown infection of pre-emergent Canada thistle is not an effective control method; however, spray inoculations cause infections with a strong bleaching effect on young Canada thistle plants. These early results indicate the *P. syringae* has potential to be a useful organic method of biocontrol for Canada thistle.

AEM73

Isolation and characterization of lactic acid bacteria from starter cultures with high production capacity of γ-aminobutyric acid

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γ-aminobutyric acid (GABA) is a non-protein amino acid considered a neuro-functional biomolecule that can interact and influence brain function via neural signaling communication of the nervous systems. GABA is a metabolic product of microorganisms, commensal gut bacteria, and probiotics. Particularly, Lactic acid bacteria (LAB) are an important source for glutamate decarboxylase (GAD; enzyme converting L-glutamate to GABA), and thus can be regarded as promising GABA-producing candidates with psychobiotic effects, especially as they are "generally-recognized-as-safe" and commonly found in fermented foods. This study aims to evaluate the *in vitro* GABA production capacity of LAB isolated from various starter cultures and fermented foods. Several LAB were isolated and detected for presence of *Gad* gene using PCR and GAD enzymatic assay. The most active strains with high and fast production kinetics were identified, characterized, and include *Streptococcus thermophilus*, *Lactobacillus plantarum*, *and Lactobacillus delbrukii*. The capacity of selected probiotic candidates to grow and produce their active metabolites was assessed in Macfarlane broth, a complex medium with carbon source similar to those found in the colon. In this study, we have identified several psychobiotic candidates having a good capacity to produce GABA, and high and fast product kinetics, desirable features for a future health promoting probiotics.

AEM74

Log transformation and its Impact on the interpretation of linear regression in the assessment of quantitative microbiological methods

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Methods for the detection and enumeration of pathogenic bacteria and contamination indicators in food and environmental samples have advanced with the use of new materials and technologies, and are faster and more sensitive than methods from just a few years ago. Methods are researched, developed and gold standard validated using reference standards while following principles suggested and/or promulgated by regulatory authorities (ISO, AOAC and Health Canada). Enumeration results from plate counts are usually transformed into logarithms to develop a more normal distribution and importantly to stabilize the variance over large ranges of contamination levels. Unfortunately, the implication of statistical results from log transformed data are often confused with those based on the arithmetic domain, as has been observed in certain current guidelines and peer reviewed publications. Using mathematical formulae and true bench enumeration data, we explore the

meaning of the slope and y-intercept from a linear regression curve based on original counts and compare to those based on log transformed data.

The least square linear regression formula, $y = Ax + B$, y is the recovered bacterial count (CFU/g) and x is the contamination level (CFU/g); slope A is referred as sensitivity of the candidate method and can be used to estimate the percent recovery when intercept $B = 0$.

By taking the log of both sides of $y = Ax$ we develop a log transformed regression,

$$\log(y) = \alpha\log(x) + \log A,$$

and we observe that when log(y) is plotted as a function of log(x), the slope α is no longer related to percent recovery (sensitivity). In this case, the slope α is 1, as long as the percent recovery (no matter high or low) does not change across contamination levels, which is actually now common for most newer methods. Instead, it is the intercept log$A$ that measures the percent recovery.

Extending from $y = Ax + B$ with these considerations leads to the new relationship between log of enumeration results and contamination level, which is shown as

$$\log(y) = \log(x) + \log A + \log(1 + B/Ax).$$

Importantly, a perfect linear relationship of the original data will not remain linear after log transformation of enumeration data when the intercept $B \neq 0$. The term log(1 + B/Ax) complicates the relationship between log(y) and log(x) when plotted in logarithmic scale because it can be any positive number (when $B > 0$) or any negative number (when $-Ax < B < 0$), which would bend log(y) up or down at the lower end. Therefore, bias B would need to be removed with a correction in order to obtain a linear regression of the log transformed data.

Log transformation makes the scale of arithmetic data proportional and the mean becomes geometric. The predicted response is therefore slightly lower in magnitude in the regression of log transformed results compared to those responses from both nonlinear and generalized linear models (from enumeration data without transformation). The parameters in the linear regression model from the original data are therefore different from those based on log transformation, in particular the slope for the latter is in fact not a % recovery. Estimation of mean and measurement uncertainty of log transformed data and the statistical procedures used to study those results, we show in this work, require separate and unique considerations, all of which will have significant relevance for the development and validation of regulatory methods intended for the enumeration of pathogens in food.

AEM75

Microbial communities of wastewaters in the Canadian Arctic

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Wastewater management in Arctic communities is challenging due to the extreme climate and a corresponding lack of conventional treatment system infrastructure. As a result, waste stabilization ponds (WSPs) represent the sole form of wastewater management for many of these Northern communities. For these systems, wastewater is collected in a pond year-round. In warmer months, solids settle in the pond and fluids move into the downstream receiving waters, whereas in winter much of the wastewater freezes. During the summer, warmer temperatures thaw the ice and stimulate aerobic and anaerobic degradation processes, thereby passively treating wastewater contaminants. The efficacy of WSPs in removing microbial contaminants is still unclear. In this work, we evaluated the microbial communities in a WSP and interconnected stream and lake systems near Baker Lake, Nunavut. We investigated whether WSP taxa were detectable within the receiving waters, and present or absent in nearby reference lakes, with the goal of establishing a microbial baseline in

advance of future infrastructure upgrades. The microbial communities of the WSP and receiving water stages were sampled in duplicate at two separate time points ten days apart in Spring 2018, beginning approximately three to four weeks after the start of the spring thaw. A baseline microbial profile was established for the system using high-throughput sequencing of 16S rRNA gene amplicons. Approximately 18 million paired-end reads were generated with a MiSeq (Illumina) and analyzed with the QIIME2 analysis pipeline and error correction with DADA2. The SILVA database was used for assigning taxonomy. The microbial community composition in each lake transitioned from being dominated by relatively few amplicon sequence variants (ASVs) at initial time points to having many taxa above 2% abundance subsequently. The ASVs affiliated with the genera *Acinetobacter* and *Pseudomonas* comprised approximately 75% of the WSP microbial community; several characterized species of these genera are known to be opportunistic human pathogens. These ASVs decreased to below 2% abundance in receiving waters. Other ASVs with abundances between 0.1% and 2% in WSP samples were reduced to below 0.1% abundance in downstream lakes. Although 28 other ASVs were observed in the WSP and its receiving waters, these were not detected in any of the reference lakes at an abundance above 0.1%. Although we cannot rule out a simple dilution effect, it appears as though the WSP system may passively remove microbial taxa, which may also include a reduction in harmful bacteria within the WSP microbial community. This baseline characterization of the microbial community composition gives insight into the efficacy of the WSP wastewater management in an Arctic setting and provides important baseline data to assist with monitoring of future operational and infrastructure upgrades.

AEM76

Microbial community composition and evolution in a CSTR digesting municipal sludge subjected to an acid-crash

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A recurrent problem faced by operators of large-scale anaerobic digestion (AD) facilities, is that their digesters are generally not performing optimally. Most of the solutions to optimize performance involve reactor design and operating conditions. Sparse attention is given to the microbiological aspect of the digester, although it is now well-known that microbial community composition and dynamics are essential elements in the performance of a biogas reactor. This study investigated whether it is possible to anticipate any set back in the performance of a digester by monitoring microbiological population shifts in an anaerobic digester that was forced to failure through increased loading of volatile fatty acids (VFAs).

A 10L-Continuous Stirred-Tank Reactor (CSTR) was operated over 110 days at 37°C. The reactor was inoculated with a digestate from a wastewater treatment plant and was fed daily with 300g of non-treated sludge from the same treatment plant, for an organic loading rate of 1.56 gTVS/Lrx.d. The reactor was monitored daily (pH, temperature and biogas produced), and chemical analyses (TS, TVS, COD, VFAs, alcohols and ions) were performed regularly. When the reactor reached a stable and optimal level of performance, addition of butyric acid was started (from 3g up to 16g per day) until the process crashed. The reactor was terminated when the biogas production stopped, after 110 days of operation. Daily samples were submitted to next generation sequencing of 16S rRNA genes (both DNA and RNA) for microbial population characterization and monitoring.

Four phases were observed during the experiment. The production of biogas, after an initial phase of fluctuation (3 to 9.4L per day), reached a stable level of performance, with an average production of 4.3L per day. As soon as butyric acid was added to the reactor (day 69), there was an increase in the production of biogas, reaching

16.3L per day. After 90 days of operation, the production of biogas dropped abruptly, and the production completely stopped after 102 days. Significant changes were observed in the composition of the microbial population and will be discussed in detail. Also, a significant disconnect was demonstrated between the microbial population present in the digester and the active population. Namely, the archaeal genus *Methanosarcina* and the bacterial taxon *Cloacimonetes* appeared to be linked to good reactor performance.

The next generation sequencing approach evaluated in this study allowed the acquisition of critical information on AD processes in terms of composition and dynamics of microbial communities from a digester subjected to performance loss. This constitutes the basis for the future development of AD diagnostic tools capable of anticipating any microbiological and technical/operational problems in a digester operating in a sub-optimal way.

AEM77

Microbial community dynamics in wastewater treatment bioreactors

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The biological treatment of wastewater is one of the largest industries in the world that utilizes biotechnology. Microbial communities play a key role in wastewater treatment, where their metabolic activity and interactions majorly influence the performance of a wastewater treatment plant (WWTP). This presentation will highlight two studies that were conducted in order to study the dynamics of these microbial communities and how they correlate with the efficiency of wastewater treatment.

In the first study, a full-scale WWTP was studied for one year, where high throughput 16S sequencing was performed on 176 samples collected from the aeration tank and anaerobic digester. The performance of the plant was also tracked over the same period. Results showed the plant exhibited excellent biochemical oxygen demand (BOD), chemical oxygen demand, total suspended solids and nitrogen removal efficiency during the study period. The most dominant bacterial phyla present in aeration tank samples were *Proteobacteria, Bacteroidetes Planctomycetes, Patescibacteria, Chloroflexi*, and *Nitrospirae*. The most abundant phyla in the anaerobic digester were *Bacteroidetes, Proteobacteria, Firmicutes, Thermotogae, Patescibacteria*, and *Spirochaetes*, in addition to an archaeal community belonging to the *Euryarchaeota* phylum, which is largely responsible for methane production. Seasonal variation in plant performance was correlated with differences in the abundance of specific microbial populations. The efficiency of BOD and nitrogen removal were significantly correlated with the abundance of *Bdellovibrio* and *Hydrogenophilaceae*, respectively. Gas production from the anaerobic digester was correlated with the abundance of microbes belonging to the *Bacteroides* genus. The results from this first study presented here are the first to provide this level of temporal resolution on microbial community fluctuations in a full scale WWTP in the Middle East. This work provides interesting information regarding microbes that could potentially be used as markers to monitor WWTP efficiency.

In the second study, fluctuations in microbial communities were studied in a wastewater electrobioreactors, which utilize a low-level electric field. The evolution of these microbial communities was also monitered as activated sludge was passaged through control reactors and electroreactors. Phylogenetic analysis revealed stark differences in the development of sludge microbial communities in the control and electro-reactors. These differences are characterized by both the enrichment of certain populations and the depletion of others. for example, applying current densities of 3, 5 and 7 A/m^2 resulted in an enrichment of taxonomic units belonging to distinct functional bacterial families, such as *Nitrospiraceae* and *Rhodocyclaceae*, and correlated with efficient N-removal (>98%) and P-removal (>98%) higher than the control bioreactor (9.6% and 5.0%, respectively). The results reported here could prove to be valuable for process control, optimization and improving WWTPs design and operation.

AEM78

Microbial community responses to mine site reclamation strategies

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Shifts in soil microbial community structure and function can be important indicators of changes in ecosystem health and can be used to monitor and inform environmental reclamation efforts. Here, we applied high-throughput 16S rRNA and ITS gene sequencing to characterize bacterial and fungal community composition in soils at sites undergoing different reclamation treatment strategies and at various stages of recovery following mining activity, at four mining sites across British Columbia. Microbial community structure differed significantly between mining sites and with reclamation treatment and duration. This data is being coupled to measures of plant and insect community composition and soil physicochemistry to improve understanding of the successional processes that lead to improved soil health following mining disturbance. These findings are being used to inform experiments aimed at determining the functional responses of soil microbial communities through metagenomic and metatranscriptomic sequencing, to examine how particular reclamation strategies may be used to achieve rapid and successful recovery of sites being reclaimed following mining activity.

AEM79

Microbial interactions and metabolisms across high Arctic ice-wedge permafrost cores and their consequences on carbon cycling and global warming potential

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Soil microbial communities play a crucial role in biogeochemical cycles. Of these, carbon cycling is of special interest because of its role in global warming and greenhouse gas (GHG) emissions. Anthropogenic climate change has had a disproportionately higher impact on Arctic habitats resulting in permafrost thaw in these regions. With Arctic warming, currently frozen stores of carbon may become available for microbial metabolism upon thaw. We study the microbial communities in high Arctic soils in order to better understand their metabolisms, their community structures and interactions, and ultimately, their roles in biogeochemical cycling, especially the carbon cycle and how this can impact carbon stores and global warming. To achieve these goals, we collected two ice-wedge permafrost cores (67cm and 78cm), which were drilled from an ice-wedge polygon cryosol site in the Canadian high Arctic. We subsampled at 4 (5cm, 25cm, 45cm, 60cm) and 5 (5cm, 25cm, 45cm, 60cm, and 78cm) depths for the 67cm and 78cm cores, respectively, in order to better understand how the microbial community changes with depth and increasingly harsher conditions. The 78cm depth was past the active layer and consisted of the ice wedge itself. In addition, surface 5cm samples were taken at the site in both late winter (May) and summer (July) months to allow for temporal as well as spatial comparisons of the microbial community. The community was characterized via 16S rRNA gene sequencing at all depths in both cores in quadruple replicates (36 samples) and in triplicate for the 5cm surface samples (total n=45). Our analyses are ongoing, with the immediate goal of establishing community profiles. Following this, we plan on performing in-depth network analyses, using a model previously developed in our lab, with the ultimate goal of identifying the keystone microbial members (bacterial and fungal) of the community that appear to be driving the biological interactions at this site. We are preparing to perform metagenomic (Illumina HiSeq) analyses, where our goal will be not only to identify the dominant members of the community, but also to identify the functions and metabolisms that are driving the community dynamics and interactions. We will follow this up with genome

binning in order to identify potentially novel or interesting organisms in these soils, with a special focus on organisms that appear to play a dominant role in carbon cycling. To our knowledge, this is one of the first studies that will look to reconstruct potential genomes (metagenome-assembled genomes) from an actual ice-wedge community. Overall, we hope that our study can inform on the metabolisms, dynamics, and interactions that underly these communities and their continued impact on nutrient cycling and global warming across Arctic environments.

AEM80

Microbial source tracking of waterborne pathogens by new designed nanofluidic qRT-PCR chip

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Freshwater recreational activities such as swimming, fishing, motor-boating etc. are common in North America. The enjoyment people get from water recreation is tempered by the risk of enteric and non-enteric illnesses from exposure to contaminated water. Fast and reliable pathogen monitoring combined with fecal pollution source tracking would decrease both public health risks and unnecessary beach closures. We applied our novel nanofluidic quantitative real time PCR (qRT-PCR) chip to monitor fecal indicator bacteria (FIBs) and bacterial waterborne pathogens as well as to determine the fecal pollution source at two public beaches; Sand Point and Bell River (Windsor, ON). Water samples were collected weekly in June, July and August of 2016 and 2017. No waterborne bacterial pathogens were detected in 40% of the samples with high *Escherichia coli* levels (19% of samples from the two beaches); however, waterborne pathogens were detected in 21% of the samples with low *E. coli* levels (81% of samples from the two beaches). Canada goose and seagull fecal contamination was detected in 42% of the collected samples, while 64% of those samples were positive for waterborne pathogens. Our human source tracking marker detected human fecal contamination in 10% of the samples, and all of those samples were positive for waterborne pathogens. Rainfall events occurring 10-48 hours prior to sampling (seven with precipitation mean of 26.5 mm) were associated with waterborne pathogens and multiple fecal source tracking markers (human, Canada goose, seagull and dog) five out of seven times.

Our results indicate that there is no strong association between *E. coli* levels and the presence or absence of waterborne pathogens. However, precipitation and source tracking markers (including human, Canada goose and seagull) do show significant correlation with the presence of waterborne pathogens.

AEM81

Nesting dolls: The viruses in the bacteria in us

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The gut microbiota is a complex microbial community that impacts human physiology in multiple ways. While most studies on the subject focus on the bacterial inhabitants of the gut, the community also contains bacteriophages: viruses that infect bacteria. These viruses can influence bacteria through infection leading to lysis of the cell or by integrating in the bacterial genomes (after which they are called prophages), providing their host with various fitness advantages. These prophages are particularly common in the gut and represent the vast majority of viruses found in the microbiota. As such, these prophages are certain to greatly impact the ecology of the microbiota and by extension, its effects on human physiology, the specific pathways and

mechanisms they use are still largely unknown. We study a model community of 18 bacterial strains from the human gut. First, we sequence the genomes of the strains, and identify the prophages integrated within through bioinformatics. These results *in silico* are compared to the results of prophage induction experiment where bacterial cultures are submitted to various stressors (*i.e.* mitomycin c, UV) and then filtered to keep only viral particles which are then sequenced. By identifying the prophages, we can then cure the strains of their phages and compare the behaviour of the normal community to the phage-deficient one over long periods of time in continuous cultures. Preliminary sequencing data has already revealed potential prophages in 13 of the 18 strains, a prevalence that, alongside their capacity to influence bacterial physiology, demonstrate that they are a major player in the community dynamics of the gut microbiota and represent tremendous amount of untapped potential for therapeutic intervention through modification of the microbiota.

AEM82

Novel hybrid-network analysis approach for determining microbial species interactions while controlling for abiotic niche filtering

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In natural environments microbe abundances are governed by both abiotic factors and biotic microbial species interactions. Co-occurrence networks have often been used in microbial ecology to understand interactions of species, since most interactions cannot be empirically observed. The rationale underlying microbial network analyses is that if two species co-occur in space and time together more often that by random chance, then they likely interact. Hence, instances of significant species co-occurrence have been used as evidence for positive interactions (biofilms, syntrophy) and instances of significant co-exclusion as evidence for negative interaction between species (competition, antibiotics, predation). However, caution should be taken when interpreting correlation co-occurrence matrixes, as simple correlation between species can be due to niche overlap. Here we present a novel hybrid network approach combining a correlation approach with a general linear model that aims to isolate the effects of species interactions from abiotic habitat filtering effects on microbial composition. We then apply this approach on microbial communities in permafrost affected soils in the Canadian high Arctic. The microbial network analysis was performed on the processed 16S amplicon data. We first calculated Spearman's rank correlation coefficients between each of the OTU pairs. Alongside the Spearman correlations, we performed two general linear models (GLM), with Poisson distributions on the 16S abundance data for each OTU combination. The first model predicted the abundance of each OTU based on abiotic factors alone, the second model predicted the abundance of each OUT based on the combination of abiotic factors and abundance of every other OUT in the environment. We then tested whether the model that included the other OTUs was significantly better at explaining the abundance of the first OTU compared to the abiotic variables only mode. To limit false positives we filtered out interactions that were significant in both the correlation and the GLP approaches. This approach identified numerous species-species interactions in permafrost affected soils. Overall members of Proteobacteria, Candidatus Rokubacteria, and Actinobacteria phyla tended to have positive effects on abundance of other species, while Verrucomicrobia and Acidobacteria members had negative impacts on other species. The advantage of this method over a simple correlation co-occurrence analysis is that we can determine if species have significant interactions with other species while controlling for environmental niche variables, allowing us to potentially detect true species interactions as well as determine the direction of the interaction.

AEM83

Novel Loop-mediated Isothermal Amplification (LAMP) assay development for rapid and quantitative detection of Arcobacter species

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Arcobacter faecis and *A. lanthieri*, closely related to human-associated *Arcobacter* spp., have recently been identified as potential pathogens. In order to rapidly detect and quantify the rate of prevalence of *A. faecis* and *A. lanthieri* in various environmental and food sources, *Arcobacter* genus- and species-specific Loop-mediated Isothermal Amplification (LAMP) assays were developed and optimized by designing primer sets derived from 16S rRNA and *gyr*B genes. The newly developed genus-specific LAMP assay correctly amplified and detected 20 *Arcobacter* spp. and did not amplify 47 other water- and foodborne pathogenic bacterial reference species and strains. Similarly, species-specific LAMP assays specifically detected *A. faecis* and *A. lanthieri* reference strains. The sensitivity of the developed assays was tested using variable concentration of DNA extracted from spiked cells of each target species in an autoclaved agricultural water where a minimum detection of 1 fg μL^{-1} was achieved. Furthermore, the developed assays were validated by detecting putative *Arcobacter* culture isolates recovered from various environmental sources. A total of 70 putative *Arcobacter* cultures were confirmed to genus-level, whereas 37 (53%) and 19 (27%) isolates were identified as *A. faecis* and *A. lanthieri*, respectively. For direct detection and quantification of two species, the sensitivity was further assessed by applying these assays to the DNA extracted from agricultural surface water samples. The results showed that of the total 173 water samples, 161 (93%) were positive for *Arcobacter* spp. whereas *A. faecis* and *A. lanthieri* were detected in 27 (16%) and 2 (1%) water samples with the concentrations ranging from 10^3 to 10^1 cells 100 mL^{-1}. The newly genus- and species-specific assays are sensitive, simple and economical and can be completed in less than 50 min. These novel genus- and species-specific molecular tools will be useful for rapid and on-site quantitative detection of arcobacters in food, environmental and clinical samples.

AEM84

Occurrence of *Campylobacter* species, *Escherichia coli* and total coliforms in Canadian First Nation drinking water distribution systems

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An investigation on the prevalence of human-associated thermophilic *Campylobacter* spp. (*C. jejuni* and *C. coli*) along with an assessment of *Escherichia coli* and total coliform as an indicator for their prediction of occurrence was first time studied in Canadian First Nation drinking water distribution systems. Water samples were collected between 2016 to 2018 from various sources including water treatment plant (WTP) source water (n= 7), treated water from WTP (n= 5), water delivery trucks (n= 6), cisterns (plastic and cement) (n= 56) and chlorine treated

tap water of piped homes (n= 20). In addition, samples were also collected from septic tanks (n= 16) and nearby lagoons (n= 4). *Campylobacters* were detected in each sampling source, although at relatively low frequency. Both *C. jejuni* and *C. coli* were more commonly detected in the treated WTP samples (20%) with a maximum concentration of 2.4x 10^2 cells 100 mL^{-1} than in the WTP source water (17%) samples at the concentration of 1.8x10^2 cells 100 mL^{-1}. *C. coli* was detected comparatively at high frequency in water delivery trucks (28%) and tap water of piped homes (16%), whereas *C. jejuni* was detected with the same frequency (14%) in both drinking water distribution systems. Also, *C. jejuni* and *C. coli* were detected in septic tank samples at the frequency of 13% and 6% with the maximum concentration of 9.3x$10^2$100 mL^{-1} compared to lagoon samples where only *C. jejuni* (25%) was detected with maximum concentration of 9.8x10^2 cells100 mL^{-1}. Detection of these species indicates that there is a potential risk of outbreak due to the consumption of drinking water. The study results also revealed that the *E. coli* and total coliform did not show a correlation with the occurrence of either target *Campylobacter* spp. since they were detected when concentration of each indicator bacteria (*E. coli* and total coliforms) was below the detection limit. Conversely, when the highest concentrations of *E. coli* (5.8x10^2 cells 100 mL^{-1}) and total coliform (1.0x10^3 cells 100 mL^{-1}) were observed in cistern samples, *Campylobacter* spp. were not detected. More importantly, our data show that while *E. coli* and total coliform numbers were impacted by the chlorine concentration in water, *Campylobacter* spp. were not effected by the chlorination of water. This suggests that monitoring *E. coli* and total coliforms alone may not be sufficient for assessing the microbiological quality of drinking water. There is, therefore, a need to investigate physico-chemical and environmental parameters that might impact the persistence of *Campylobacter* spp. in treated drinking water for controlling human campylobacteriosis in Canada's First Nation communities.

AEM85

Optimization of the denitrifying activity of a continuous methanol-fed biofilm bioreactor

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Denitrification takes place in bacterial cells where N oxides serve as terminal electron acceptor instead of oxygen (O2) for energy production when oxygen depletion occurs, leading to the production of gaseous nitrogen (N2). Our laboratory has been working on a denitrifying biofilm taken from a continuous, methanol-fed, fluidized denitrification system at the Montreal Biodome that treated a marine effluent. This biofilm is composed of multi-species microorganisms, among which *Hyphomicrobium nitrativorans* NL23 and *Methylophaga nitratireducenticrescens* JAM1 and GP59 are the principal bacteria involved in the denitrifying activities. We have shown that this biofilm exhibits great resistance in accordance to change in different conditions suggesting that further optimization of the denitrifying activities of the biofilm is possible. We aim in the development a laboratory-scale bioreactor in a controlled environment to improve the biofilm performance.

As the denitrification system at the Montreal Biodome is no longer in function, the biofilm was preserved in glycerol since 2006. A 500 mL fixed-bed bioreactor with free Bioflow carriers was inoculated with biofilm carriers taken from the frozen stock. The medium was the commercial seawater Instant Ocean from Aquatic System Inc. The medium was recirculated for several days allowing biofilm to build on the new carriers. Denitrification was positive after only few days. After few weeks, all the carriers were colonized by biofilm. Up to now, denitrification rate was measured at 8.5 mg-N L^{-1} h^{-1} in recirculation with a flow rate of 30 mL/min. Changing in the operating mode, the hydraulic retention time and the C/N ratio will be performed to optimize the denitrifying activities. The impact of these changes on the microbial community will be measured by deriving the 16S metagenomes.

AEM86

Pan-genome analysis of *Devosia* reveals role for PQQ biosynthesis locus in deoxynivalenol detoxification

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Devosia is a recently designated genus of *Alpha-proteobacteria*, first established by Nakagawa (1996) through the reassignment of *Pseudomonas riboflavina* to *Devosia riboflavina* based upon 16S rRNA sequence alignment. There are presently ~30 validated *Devosia* species and over 20 strains described in the literature that have been recovered from diverse environmental niches, ranging from plants and animals to hexachlorocyclohexane, diesel and mycotoxin-contaminated soil. The availability of 43 genome assemblies has made it possible to explore the functional potential of this genus and uncover novel enzymes with putative industrial applications. For example, several *Devosia* isolates have been found to be capable of biologically detoxifying deoxynivalenol (DON), and we have recently identified the two key enzymes, DepA and DepB, involved in this pathway in *D. mutans* 17-2-E-8. We previously reported the draft genome sequences of seven *Devosia* strains and in this study, have characterized the pan-genome of 40 *Devosia* strains, including two previously unpublished genomes.

Open-reading frame (ORF) prediction and initial annotation of each genome was performed with Prokka, which yielded an average of 4,233 genes per genome. Predicted protein sequences were further annotated with InterProScan, and assigned to KEGG orthology (KO) groups and Clusters of Orthologous Groups (COGs). Further pan-genome analysis and visualization was performed with Roary, PanGP, and Anvi'o software. Orthologous gene clusters were predicted by aligning protein sequences with DIAMOND and clustering with MCL within Anvi'o. A total of 28,351 gene clusters (GCs), consisting of 169,116 genes, were identified among the 40 genomes. The core genome consisted of just 1073 GCs, and an open pan-genome was observed, with an average of 355 singleton gene clusters found per genome (ranging from 1 to 1139), with *D. enhydra*, *D.* str. 66_22 and *D.* str. 67_54 having the greatest number (1139, 1026 and 980, respectively). Clustering of strains based on average nucleotide identity (ANI) placed them into 7 major groups. A subset of eight strains were assessed for their ability to epimerize DON by LC-MS/MS, and enriched GCs within each group (detoxifier and non-detoxifier) identified with Anvi'o. This revealed four strictly conserved GCs in the detoxifier group, which together form a PQQ biosynthesis operon, implicating the potential importance of PQQ as a co-factor in DON metabolism by these strains.

AEM87

Phylogenetic affiliations and functional properties of alginate lyase extracted from actinomycetes associated with Sargassum seaweed wastes

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The eutrophication of the coastal regions of global oceans owing to the loading of nutrients, climate and land-use changes has been eliciting massive seaweed tides. The washing of enormous quantities of *Sargassum* seaweed biomass to the coastline afflicts the regional economy across the Atlantic coast, including West African and Caribbean countries. Additionally, these biomasses are a threat to the public and environmental health by releasing toxic hydrogen sulfide. Recently, substantial efforts have been directed to develop environmentally sustainable and economical biocatalytic processes to deconstruct alginate, the major polysaccharide of the cell

wall of *Sargassum*, into value-added products. Alginate lyases are the enzymes that cleave alginate into oligosaccharides containing uronic acid residues via β-elimination of 1,4 glycosidic bond. Alginate lyase has the potential to be used in biofuel, pharmaceutical, diagnostic, food, cosmetic and agricultural industries. Due to the high cost of commercially available alginate lyase, there has been a burgeoning industrial interest to search for potent microbial biocatalysts. In the current study, the actinomycetes associated with the decomposing *Sargassum* seaweed wastes were examined for their abilities to produce alginate lyase. Actinomycetes were isolated from the decaying invasive *Sargassum* piled off the coast of Barbados using serial dilutions and spread plate methods on mineral agar supplemented with 0.5% alginate (MMA). The resulting actinomycete isolates were purified by restreaking on actinomycete isolation agar and rapid screening for the presence of alginate lyase in these isolates were assessed via spot inoculation on MMA and staining with Gram iodine. Alginate lyase-producing isolates were identified via sequencing the 16S rRNA gene. Alginate lyase was extracted and characterized from selected isolates. The functional properties, including thermostability, tolerance to high salt concentrations and stability toward broad pH range, of extracted alginate lyase indicate the biocatalytic potential of actinobacterial associates of decaying *Sargassum* seaweed to biotransform alginate into value-added oligosaccharides.

AEM88

Plant genotype and soil type influence fungal and bacterial assemblages in the rhizosphere of balsam poplar

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Abandoned unreclaimed mines represent an important environmental issue since they typically remain unvegetated for decades, letting vast amounts of exposed and contaminated soil, waste rock and tailings being spread via aeolian and water erosion. Several factors limit the revegetation of these sites, including extreme abiotic conditions (e.g. very low pH) and low availability of nutrients.

The selection of tree genotypes with an associated microbiome favoring their growth under harsh conditions, such as those prevailing in mine waste environments, is a strategy that could facilitate the restoration of mining sites. Therefore, the aim of this study was to determine how the tree genotype, the soil type and the interaction of these two factors influence the diversity and composition of the rhizosphere microbiome.

Two mining sites in Abitibi were selected for this study: Westwood, an old gold mine containing acid generating pyrite rich waste rock; and La Corne Mine, a former molybdenum and bismuth mine containing neutral nutrient-poor mine tailings. Ten genotypes of Populus balsamifera from cuttings harvested at the mine sites were grown in these mining substrates in a greenhouse experiment. Tree growth was monitored during two growing seasons, after which the soil strongly attached to tree roots (i.e. rhizosphere) was harvested from fine roots to extract DNA and characterize bacterial and fungal communities by sequencing taxonomic markers. Several physicochemical characteristics of the bulk soil were also measured (e.g. pH, C, N, S, exchangeable cations).

Analyzes are underway to establish links between substrate properties, tree genotype and microbial communities of the rhizosphere.

AEM90

Potato suberin increases iron metabolism in *Streptomyces scabies*, a common-scab inducing pathogen

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Potato common scab is a disease affecting tubers whose major causing agent is the soil filamentous bacterium *Streptomyces scabies.* Its pathogenicity relies on the production of toxins called thaxtomins. The production of thaxtomin A has been shown to be induced by the presence of both cellobiose and suberin. While the cellular mechanism by which cellobiose induces thaxtomin production is known, the role of suberin has to be elucidated. A proteomics study was carried out to identify intracellular proteins produced in a minimal medium supplemented or not with suberin, cellobiose or a combination of both compounds. In a first step, the study focused on proteins that were found only in the presence of suberin (142 proteins). Proteins associated with thaxtomin A production pathway (TxtA and TxtC) were associated only with the medium containing both suberin and cellobiose. The proteins produced in the presence of suberin included several cation transporters and proteins linked to iron acquisition. Iron metabolism appeared to be up-regulated in the presence of suberin since pyochelin (a siderophore) biosynthesis proteins, many iron-containing proteins and iron-dependent enzymes, a ferric reductase, transcriptional regulators of heme proteins and of the iron uptake system component EfeO were only found in the presence of this substrate. Suberin also induced the up-regulation of various stress proteins, a protein with a PAS domain, a signaling module that monitors changes in redox potential, a nitrate reductase, nitrate/nitrite transporter and sensor kinase, and proteins that act as anti-oxidants. This study provides new insight about the role of suberin in *S. scabies* pathogenicity.

AEM91

Prevalence and antimicrobial resistance profiles of extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* recovered from natural and conventional feedlots

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The emergence of antimicrobial resistance (AMR) in bacterial pathogens is a serious global concern. Extended-spectrum β-Lactamase (ESBL)-producing *E. coli* (ESBLs) are of particular concern as they pose an important human health risk. Canada is the 6th largest global exporter of beef in the world and antimicrobials are critical to contemporary high-intensity beef production. On a per tonne basis, beef cattle production is the largest user of antimicrobials in the Canadian livestock sector. Recently, there has been growing consumer demand for "raised without antibiotics beef". This is resulting in the emergence of a "natural" production sector in the industry. To date there is little knowledge about the prevalence of ESBLs within natural vs. conventional feedlot production systems. The objective of this research was to determine if the prevalence of ESBLs and their AMR profiles differ between conventional and natural feedlots. Composite fecal samples were collected in Alberta from two natural (n=139) and two conventional feedlots (n=150) over 18 sampling periods between August 2016 and July 2018. Putative ESBLs were isolated by sample enrichment in cefotaxime prior to selective plating onto ceftriaxone-supplemented MacConkey agar and sub-culturing onto ampicillin supplemented tryptic soy agar. Resistance profiles of putative ESBLs from natural (n=43) and conventional feedlots (n=42) were examined using the Kirby-Bauer disk diffusion susceptibility method according to CLSI guidelines. A comprehensive panel

of antimicrobials (n=12) were selected on the basis of use in beef production systems, clinical importance in humans and representation across antimicrobial families. Of the 85 isolates examined, 16% were confirmed as ESBLs from natural feedlots and 28% from conventional feedlots. As putative ESBLs, all isolates were resistant to β-lactams followed by tetracycline resistance (84.9%), aminoglycoside (66%), sulfa drugs (63%), cephalosporin (62%) and phenicol (61%). All of the isolates, regardless of source, were multidrug resistant (MDR; resistant to two or more antibiotics). Within the natural feedlots 29%, 26%, 19%, 7% of isolates exhibited resistance to 8, 7, 3 and 6 antimicrobials, respectively. The most common MDR phenotype within the natural feedlots was AMP-CTZD-AMCL-FLOR-SULF-CTO-STEP-OXYT. Within the conventional feedlots 26%, 23%, 16%, and 13% of isolates exhibited resistance to 7, 4, 5 and 9 antimicrobials, respectively with the AMP-FLOR-SULF-TMZ-CTO-STEP-OXYT as the most common phenotype. Our results show that ESBLs are recoverable from both natural and conventional feedlots following enrichment and the occurrence of ESBL MDR *E. coli* is observed in both natural and conventional feedlots. It seems that at least in the short term, the complete removal of antimicrobials from beef cattle production is unlikely to eliminate ESBLs from the feedlot environment.

AEM92

Prevalence and characterization of plasmid-mediated quinolone resistance in various aquatic sources

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Bacterial isolates found in aquatic ecosystems often carry antibiotic resistance genes (ARGs). These ARGs are often found on plasmids and transposons, which allows them to proliferate throughout bacterial communities via horizontal gene transfer (HGT) causing dissemination of multi-drug resistance. The increase in antibiotic resistance has raised concerns about the ability to continue to use these drugs to fight infectious diseases. The development of novel synthetic antibiotics is, therefore, one critical approach to continue to treat bacterial diseases. Ciprofloxacin, a semi-synthetic second-generation fluoroquinolone antibiotic, was developed to inhibit bacterial DNA gyrase and topoisomerase IV resulting in cell death. Although these synthetic compounds are not naturally found in the environment, bacterial isolates from hospital effluent, wastewater and receiving waters have been found to be resistant. Ciprofloxacin resistance can occur through chromosomal mutations of type 2 topoisomerases or by the acquisition of plasmid-mediated quinolone resistances (PMQR). A particular PMQR, *Qnr* genes, encoding for pentapeptide repeat proteins confer low levels of quinolone resistance and fortify DNA gyrase and topoisomerase IV. These *Qnr* genes have been identified globally in both clinical and environmental isolates.

The aim of this study was to determine the prevalence of ciprofloxacin-resistant bacteria in aquatic environments around Toronto and the potential dissemination of ciprofloxacin resistance. Isolates were collected from various water sources (Humber wastewater treatment plant, Toronto storm drain, Lake Ontario, Buckhorn Lake, and Lake Devo) and selected for either ciprofloxacin resistance or sensitivity to determine potential *Qnr* determinant reservoirs. With the selective pressure of ciprofloxacin, we hypothesize that the occurrence of ciprofloxacin-resistant bacteria (Cip^R) in the environment may carry PMQR mechanisms while the sensitive population (Cip^S) would not carry PMQR genes. Bacterial isolates that grew in the presence of 10mg/L of ciprofloxacin were considered fully resistant. The isolates were tested for resistance to an additional 12 different antibiotics and identified using Sanger sequencing PCR products of the 16S rRNA gene. The most predominately Cip^R isolates belonged to the genus *Brevundimonas* and *Sphingobacterium* while most Cip^S isolates belonged to the genus *Flavobacterium* and *Aeromonas*. To determine which genes are responsible for ciprofloxacin resistance, multiplex PCR of associated *Qnr* genes, *QnrA*, *QnrB*, and *QnrS*, was carried out on 202 environmental isolates. A similar prevalence of *Qnr* genes was found in Cip^R (19%) and Cip^S (14%) populations suggesting that the presence of these genes were not necessarily correlated with the phenotypic resistance to the antibiotic.

Furthermore, ciprofloxacin-resistant bacteria were found in all locations at similar frequencies suggesting that the resistance genes are widespread and could possibly arise from the activation of sensitive strains or through HGT. Currently, we are sequencing the *Qnr* genes from both populations to determine if sequence differences can explain the resistance phenotypes. Overall, determining the underlying cause of ciprofloxacin resistance could help re-establish the effectiveness of these antimicrobial compounds in clinical settings.

AEM93

Primer mixture design for increased diversity detection and quantification of anaerobic hydrocarbon biodegradation gene biomarkers

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Hydrocarbon contamination is an ever-present and ongoing issue that is the centre of intense research. Generally, hydrocarbon-contaminated sites are anoxic; after the initial flourishing growth of aerobic microorganisms, anoxic conditions prevail. Anaerobic microorganisms can utilize nitrate, sulfate, metals, and even CO_2 as terminal electron acceptors, thus, the diversity of organisms is variable and dependent on geochemistry. While degradation, or activation, of hydrocarbons can be carried out using multiple mechanisms (carboxylation, methylation, etc.) under anoxic conditions, the most well studied mechanism is fumarate addition, carried out by alkylsuccinate synthase (ASS) and benzylsuccinate synthase (BSS). This mechanism is most studied owing to its unique and diagnostic metabolites, alkylsuccinates (ASS) or benzylsuccinates (BSS). PCR (polymerase chain reaction) can be used to assay for the genes associated with ASS (*assA*) or BSS (*bssA*), to elucidate the potential for anaerobic hydrocarbon degradation at contaminated sites. However, PCR-based assays to detect and/or quantify these biomarker genes encoding fumarate addition enzymes (FAE) are limited due to the diverse microorganisms and the environments in which they are found. Most primer designs rely on single sequence primers or degenerate bases to introduce diversity, however these approaches are limited in the sequence diversity they can capture. Here, we employed a mixture of non-degenerate forward primers, wherein each primer in the mixture was designed to bind to specific *assA*/*bssA* sequences within that diversity. All of these forward primers bind to the same locus, and produce the same length amplicon. Using this primer mixture, a more targeted approach to capturing diversity can be utilized, where each primer is designed to target a specific cluster of sequence diversity. In this application, the produced amplicon lengths are compatible with qPCR, adding quantification capabilities. As a proof-of-concept work, primer mixtures were designed for both *assA* (6 forward primers) and *bssA* (4 forward primers). Two hydrocarbon contaminated sites in Alberta, Canada, were interrogated with these newly designed primer mixtures and we found quantifiable FAE genes, showing some correlation with hydrocarbon concentration and gene abundance. Furthermore, the captured diversity is on-par with previous literature primer sets that are not qPCR compatible, capturing taxa across the Proteobacteria. Using this directed primer mix design strategy, PCR- and qPCR-based assays into diverse environments can be done reliably and can be tailored to specific environments and conditions.

AEM94

Protective efficacy of recombinant heavy chain fragment of *Clostridium botulinum* D/C

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1. Introduction

Botulism is a paralytic disease of human beings and animals caused by neurotoxins produced by *Clostridium (C.) botulinum*. Botulinum neurotoxins (BoNTs) B, C/D, and D/C, were the most common cause of cattle botulism in Korea. Vaccination has been reported to be the most effective way to control cattle botulism. The aim of this study was the expression of the recombinant heavy chain fragment with D/C strain (rBoNT-D/C-Hc). And, the purified antigen was also evaluated with the mouse protection test against a native BoNT D/C challenge.

2. Materials and Methods

A 1,230 bp DNA fragment encoding the BoNT-D/C-Hc gene was amplified from total DNA of *C. botulinum* D/C using PCR. The PCR product was sub-cloned into TA- and pMAL-vector, and transformed in *E. coli* BL21 expression host. Recombinant protein was over-expressed and purified by maltose binding protein (MBP) affinity chromatography. The purity of the recombinant protein was confirmed with SDS-PAGE and western blot, respectively. Mice (n = 10) were vaccinated subcutaneously with 5 μg of recombinant protein. After 2 weeks, booster injection was also performed. One week after booster injection, mice were challenged intraperitoneally with 25 LD_{50} of D/C neurotoxin.

3. Results

The results showing in this study demonstrated that recombinant protein was successfully cloned and expressed in *E. coli* BL21, and the MBP-tagged recombinant protein was purely obtained by MBP affinity columns. Mouse survival rate with rBoNT-D/C-Hc was confirmed as 100% whereas the survival rate in the negative control group was 0%.

4. Conclusions

In this study, we established *E. coli* clone which produced rBoNT-D/C-Hc. Recombinant protein was successfully over-expressed and purified. The purified protein was able to protect against 25 LD_{50} with a dose as low as 5 μg of BoNT D/C.

AEM95

Recovery of nutrients from *Fusarium*-damaged grain using solid-state fermentation and insect culture combinations

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Fusarium Head Blight (FHB) is one of the most significant causes of economic loss in cereal crops. Every year Fusarium-infected crops result in a loss of $50 - 300 million to Canadian agriculture. The infected grain (containing Fusarium damaged kernels - FDK) is lower in quality and nutrients than uninfected grain. More importantly the FDK contain a high level of mycotoxins, such as DON, making the grain unsuitable for human and animal consumption. Despite the presence of FDK, the grain still contains a considerable amount of nutrients. A method to recover the nutrients without the toxins should be beneficial for the agricultural economy. In our previous study, we showed combinations of solid-state fermentation and insect culture can recover >90% of the nutrients from inedible agricultural by-products. We hypothesized that the insect does not accumulate mycotoxins in its body, but uptakes the proteins, lipids and other nutrients from the FDK and uninfected grain. In this study our objective was to determine the recovery rate of the nutrients in relation to mycotoxin accumulation in the insect. The FDK were soaked in water to 50% (w/w) moisture and inoculated with *Aspergillus oryzae* and or *Lactobacillus plantarum* (solid-state fermentation; SSF). The inoculated kernels were incubated at 30°C for 4 days. The SSF-kernels were then provided to 50 young, black soldier fly larvae (BSFL; ~5 mm; 2nd instar), which were allowed to feed on the fermented grain for 12 days. A sample of the larvae and the spent media sample were taken at every 4 days, and protein, lipid, and ash contents were determined, as well as the weight gain of

the BSFL. Each BSFL sample was analyzed for its DON content. The results indicated that the BSFL used the nutrients in the grain efficiently. After 12 days of insect culture, the SSF-grain supported a weight gain of the BSFL of approximately 250 mg (dry weight basis)/larvae, which is about 20% higher than larvae fed with unfermented FDK. During the active growth period (Day 0 to Day 8), the larval biomass contained DON at considerable levels, however, by Day 12 when the larvae were in the pre-pupal stage and were no longer consuming feeds, the amount of DON in the insect biomass became the baseline amount, i.e., undetectable. Thus we concluded the combination of BSFL and SSF can be developed as a method to recover DON-free nutrients from FHB-damaged grain to add value to unmarketable grain.

AEM96

Souches microbiennes bio-protectrices comme alternatives aux antibiotiques dans la filière porcine

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En santé animale, l'utilisation des bactéries lactiques est une alternative possible à l'antibiothérapie. Certaines souches issues du microbiote intestinal peuvent inhiber des microorganismes pathogènes grâce à la production de métabolites d'intérêt (bactériocines, acides organiques…).

L'objectif de cette étude est d'isoler des souches lactiques d'intérêt utilisables en santé porcine dont les pathogènes principaux posent des problèmes d'antibio-résistance.

Des échantillons de fèces de 9 espèces animales (Zèbre, Rhinoceros, Muntjac, Capybara, Binturong, Tapir, Tatou, Alpaga et Cacatoès) ont été collectés par le zoo de Lille. Après un criblage à haut débit sur ces échantillons contre une sélection de bactéries cibles (*E. coli*, *Clostridium perfringens*et *Salmonella enterica* serotypeNewport) afin de rechercher des activités antagonistes. 11 isolats ont montré une inhibition, 2 contre *E. coli*, 9 contre *Clostridium perfringens*. Les souches ont par la suite été identifiées par séquençage de l'ADN codant pour l'ARN 16S. Enfin une analyse phylogénétique par RAPD-PCR a été réalisée afin de ne pas garder de clones d'une même souche. Parmi les 11 isolats, 5 souches ont été identifiées. Les antibiogrammes de ces 5 souches ont été effectués et aucune résistance non naturelle n'a été trouvée. La cytotoxicité et la virulence des souches ont également été vérifiées, les 5 souches ne sont pas cytotoxiques mais 3 sont porteuses d'activité hémolytique bien qu'isolées d'animaux sains. Les tests de biocompatibilité entre les souches ont permis de choisir des consortiums possibles pour la suite des travaux. Ces travaux ont permis de sélectionner des souches au potentiel probiotique intéressant et après la vérification de leur survie en milieu intestinal, les tests *in vivo*nous permettront de connaitre leurs effets sur le microbiote intestinal du porc et sur sa croissance.

AEM97

Stability of microbial community profiles associated with compacted bentonite from the Grimsel Underground Research Laboratory

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In order to assess the microbiology and corrosion potential of engineered components of a deep geological repository for longterm storage of high level nuclear waste, the Materials Corrosion Test is being conducted at the Underground Research Laboratory in Grimsel, Switzerland. Test modules containing metal coupons surrounded by highly compacted MX-80 bentonite, at two dry densities (1.25 and 1.50 g/cm^3), were emplaced within 9 m deep boreholes and the first modules were retrieved after 13 months of storage. Bentonite and environmental materials were sampled, and microbial communities and their distributions assessed using high-throughput 16S rRNA gene sequencing and phospholipid fatty acid (PLFA) analysis. The borehole fluid was dominated by OTUs affiliated with *Desulfosporosinus* and *Desulfovibrio*, which are putatively involved in sulfate reduction. The relative abundance of these OTUs was lower for samples taken from the inside of the borehole module and were almost undetectable in samples of the inner bentonite layer. The dominant OTU in case and filter sample sequence data affiliated with *Pseudomonas stutzeri*, yet its relative abundance decreased towards the inside of the borehole module. *Streptomyces* spp. OTUs were relatively abundant in all bentonite core sample data, prior to emplacement and after 13 months of storage, presumably as metabolically inactive spores or extracellular “relic” DNA. The PLFA concentrations in outer and inner layer bentonite samples suggested cellular abundances of 1 to 3 × 10^6 cells/g with similar PLFA distributions among all bentonite samples. Together, our results demonstrate consistent microbial communities inside the saturated borehole module, providing evidence for microbial stability under conditions that mimic engineered components of a deep geological repository.

AM98

Stability of nitrogen and phosphorus cycling genes in activated sludge revealed by quantitative genomics

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Wastewater treatment plants (WWTPs) are essential in limiting negative anthropogenic effects on aquatic ecosystems by minimizing the release of biologically available nitrogen and phosphorus as well as microbial pathogens. Most large-scale WWTPs rely on the activated sludge (AS) process, a form of biological nutrient removal that relies on the metabolic activities of its microbial community. Small disruptions in these communities can lead to operational problems like wastewater bulking and foaming, which are often accompanied by large reductions in treatment efficiency. Despite the microbiological nature of the AS environment, it has been relatively uncharacterized from a microbial ecology perspective. This study explored the microbial community structure within AS, especially its stability in an outdoor Canadian prairie environment that experiences large temperature fluctuations. Activated sludge samples were collected from the WWTP in Regina, SK at 4 points throughout the year. DNA was extracted from each sample and sequenced using 3 lanes of an Illumina HiSeq, generating 20 million paired end reads per sample. Abundance of microbial taxa and genes of interest were assessed using Metaphlan2 and Humann2. Regardless of sampling date, the microbial community was consistently comprised of phyla Actinobacteria and Proteobacteria (>90%). Changes in the community composition between sampling

dates were most apparent on a species level. The species present in the highest abundances were *Afipia sp, Thiomonas sp., and Tetrasphaera sp*. The abundance of genes involved in denitrification, nitrification, and phosphorus accumulation remained consistent despite fluctuations in environmental conditions and in the microbial community composition. These findings indicate that although the microbial community composition may vary in response to environmental conditions, the abundance of genes that are essential to wastewater treatment functionality remain relatively stable, in spite of the large temperature fluctuations that are common on the Canadian prairies.

AEM99

Structural and functional study of the bacterial acetylcholinesterase ChoE from *Pseudomonas aeruginosa*

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Mammalian acetylcholinesterase (AChE), one of the most efficient enzymes of nervous system, hydrolyses the neurotransmitter acetylcholine into choline and acetate and plays a pivotal role in cholinergic neurotransmission.

Acetylcholine hydrolyzing activity has also been recognized in prokaryotes although they lack a nervous system. This was especially the case in the bacterial *Pseudomonas* genus, which are able to inhabit a large range of environments with many species like *Pseudomonas aeruginosa* being pathogens for many different hosts. It was reported that *P. aeruginosa* is able to use acetylcholine as a carbon and nitrogen source. In this work, we have structurally characterized the bacterial acetylcholinesterase ChoE at 1.85Å. Site-directed mutagenesis of ChoE followed by activity assays has confirmed the role of Ser38, Asp285, and His288 in the catalytic triad and Asn147 of oxyanion hole. Furthermore, a PAO1 mutant lacking this ChoE gene was investigated in various media, confirming that the mutant strain is unable to use acetylcholine as a carbon and nitrogen source as the wild-type strain. This study reveals the first structural insight into the bacterial acetylcholinesterase, indicating the important role of ChoE in the metabolic pathways of *P. aeruginosa*. Furthermore, our analysis also provides an overview of biological evolution of acetylcholinesterases of both prokaryotic and eukaryotic origins.

AEM100

Structural and mechanistic insights into phenolic acid decarboxylases

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Phenolic acid decarboxylase (PAD) is an enzyme present in bacteria, notably *Bacillus pumilus*. This protein is characterized by a ten strand beta-barrel adopting a lipocalin-like fold flanked with three alpha helices. This fold is mainly known for binding small hydrophobic molecules. PAD shares a high similarity with the Ferulic Acid Decarboxylase (FAD), where both are involved in detoxification processes through the decarboxylation of small aromatic molecules of the hydroxycinnamic acids family such as ferulic, p-coumaric and caffeic acids.

In organic chemistry, decarboxylation is an important process for the elimination of carboxylic acids, mostly achieved through the use of progressive heating up to 200 degrees C. While this process is simple, high temperature may be detrimental for more complex molecules. Achieving decarboxylation through biocatalysis is thus an interesting goal. The bioconversion of ferulic acid, the most abundant hydroxycinnamic acid found in

plants, leads to the formation of many value-added products such as flavoring agents vinyl guaiacol and vanillin, and biopolymers.

An engineered PAD protein, designated SAD for sinapic acid decarboxylase, was previously developed in order to use sinapic acid as a substrate to produce the antioxidant canolol [Green Chem., 2013, 15, 3312]. Further development of a larger variety of substrates relies on an ultimate understanding of the enzymatic mechanism.

Here, we report the crystal structures of wild-type and mutants SAD with substrate analogues present in the catalytic site. Interestingly, its binding mode in the pocket is different from those previously reported in the literature. Based on these structural results as well as mutagenesis assays data, we hereby suggest an alternative mechanism for the decarboxylation reaction by phenolic acid decarboxylases.

AEM101

Study of conservation and diversity of mobile genomic islands in *Vibrionaceae*: detection, identification and classification

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One of the major threats weighing on nowadays food and health safety systems worldwide is the rapid emergence and spreading of antibiotic resistance among pathogenic microorganisms. This phenomenon is mainly carried out by diverse and still largely unknown mobile DNA structures called mobile genomic islands (MGI). To allow an easier detection, recognition and possibly categorization of these elements, it is necessary to understand if they share common grounds regarding their general composition, organization and origins. This project aims to provide some answers by studying MGIs composition, phylogeny, evolution, their rearrangement dynamic and history by robust computational methods in *Vibrionaceae*. We propose to use specific characteristics (insertion sites, repetitive motifs, mobility genes and origin of transfer) to widen the detection and prediction scope, currently limited to variations in GC content, dinucleotide bias, insertion in *tRNA* genes only and genomic comparison to already known genomic islands. Our methodology proposes first to gather as many relevant and updated curated genomic islands data as possible into a simple custom database, annotate their sequence from scratch, explore their diversity and identify possible trends. Then, build a pipeline to detect and locate the MGIs using the characteristics mentioned above and the database data as test sets. Finally, benchmark the results with other programs and pipelines, to further propose its incorporation in existing open tools if relevant. This study has the potential to offer new avenues to prevent the dramatic increase in bacterial infections linked to multidrug-resistant strains, first cause of death predicted in 2050.

AEM102

Systems and evolutionary biology of the *Pseudomonas aeruginosa* population structure

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Pseudomonas aeruginosa is a ubiquitous opportunistic pathogen resistant to most classes of antibiotics. It can be found in the environment (soil and water) and causes infections in animals and in humans with a high

prevalence in Cystic Fibrosis individuals. Phylogeography and wide distribution in the environment indicates remarkable adaptive biology. We used a data set of 1,311 high-quality genomes from the human pathogen *Pseudomonas aeruginosa*, 619 of which were newly sequenced, to show that a pan-genomic approach can greatly refine the population structure of bacterial species, provide new insights to define species boundaries, and generate hypotheses on the evolution of pathogenicity. The 665-gene *P. aeruginosa* core genome presented here, which constitutes only 1% of the entire pan-genome, is the first to be in the same order of magnitude as the minimal bacterial genome and represents a conservative estimate of the actual core genome. Moreover, the phylogeny based on this core genome provides strong evidence for a five-group population structure that includes two previously undescribed groups of isolates. Comparative genomics focusing on antimicrobial resistance and virulence genes showed that variation among isolates was partly linked to this population structure. Finally, we hypothesized that horizontal gene transfer had an important role in this respect, and found a total of 3,010 putative complete and fragmented plasmids, 5% and 12% of which contained resistance or virulence genes, respectively. This work provides data and strategies to study the evolutionary trajectories of resistance and virulence in *P. aeruginosa*.

AEM103

The effects of moisture and nitrogen content on nitrous oxide emissions and nitrifying and denitrifying gene abundances in orchard soil from the Okanagan Valley

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Nitrous oxide is a major greenhouse gas that contributes to climate change and stratospheric ozone depletion. Agricultural soils are the main source of anthropogenic N_2O emissions. The two main N_2O generating processes from soil are microbial nitrification and denitrification. The goal of this study was to evaluate differences in N_2O emissions from two different soil depths (5-15 and 15-30 cm) as affected by soil moisture and nitrogen content. Agricultural soil was collected from an apple orchard with drip irrigation and a soil texture of 59.7 % sand, 29.7 % silt, and 10.6 % clay. Microcosms containing 50 grams of dried soil were subjected to varying moisture content (0, 30, 50 and 80 %) and nitrogen content (0, 25, 50 and 75 mg of urea/ 50 g dry soil). N_2O emissions were measured on days 0, 1, 3, 5, 7 and 9, and soil DNA was extracted and evaluated on days 0 and 9. Bacterial and archaeal *amoA* (codes for the first enzyme in the nitrification pathway), and bacterial *nosZI* (codes for the last enzyme in the denitrification pathway) genes were quantified and correlated to soil properties and treatments to understand the role of microbial processes in N_2O emission. The overarching aim of this research is to develop soil texture-specific agricultural practices that aid in reducing N_2O emissions. Future studies will include more soil, irrigation and crop types

AEM104

The milk storage effect on milk microbiome and an optimized bacterial DNA extraction protocol for *Staphylococcus aureus* mastitic milk

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Bovine mastitis is a disease of inflammation of cow udder, that results in an annual loss of $600-million from the dairy industry in Canada. The changes to the milk microbiome due to mastitis have been explored in previous studies, however, there is a massive knowledge gap surrounding what happens to the microbial population

resident in the bovine udder prior to the first case of mastitis. Our group is conducting a longitudinal study to determine what changes occur in the bovine milk microbiome prior to the first mastitis infection. However, to accomplish this, milk must be archived in such a way that does not change the microbiota over time. In order to understand the potential alteration in milk microbiome, due to long-term storage, we are investigating how the microbial population changes in the same milk sample that was stored three different ways: milk immediately used for DNA extraction, frozen milk at -20℃, and milk stored with 2-Bromo-2-nitropropane-1,3-diol. Also, to extract bacterial DNA consistently from both healthy and mastitic milk for longitudinal study purpose, we suggest optimized bacteria DNA extraction procedure for *S. aureus* mastitic milk by comparing enzymatic lysis to mechanical lysis method.

AEM105

The production of pyrrolnitrin and phenazine by *Pseudomonas chlororaphis* PA23 is involved in the amoebicidal activity against *Acanthamoeba castellanii*

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Pseudomonas chlororaphis PA23 is a biocontrol agent that is able to protect canola against the pathogenic fungus *Sclerotinia sclerotiorum*. A number of metabolites contribute to fungal antagonism including pyrrolnitrin (PRN), phenazine (PHZ), hydrogen cyanide (HCN) and degradative enzymes. Beyond pathogen suppression, the success of a biocontrol agent is dependent upon its ability to persist in the environment and to avoid the threat of grazing predators, including protozoa. The focus of the current study was to investigate whether PA23 is able to resist predation by *Acanthamoeba castellanii* (Ac) and to define the role of antifungal compounds in the bacterial-protozoan interaction. Our findings revealed that the antibiotics PRN and PHZ contribute to PA23 toxicity towards Ac trophozoites, either by killing or inducing cyst formation. Chemotactic analysis showed that Ac preferentially migrates towards regulatory mutants devoid of extracellular metabolite production, namely the *gacS* mutant, and two quorum-sensing deficient strain (PA23-*phzR* and PA23-AI). Moreover, a PRN biosynthesis mutant (PRN^-) showed increased Ac attraction compared to the wild type, indicating that PRN acts as a repellent. Next, we examined the effect of purified PRN and PHZ on amoeba viability. Both exoproducts exhibited toxic effects on amoeba in a dose-dependent manner. Collectively, our findings indicate that both PRN and PHZ are involved in amoebicidal activity and through production of these molecules, PA23 is able to avoid the threat of predation. Such findings bode well for the persistence of this biocontrol bacterium in the environment.

AEM106

The spore formation of the PGPR *Bacillus subtilis* is directly influenced by the plant

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Several bacteria such as *Bacillus subtilis* and *Pseudomonas fluorescens,* known as PGPR (Plant Growth-Promoting Rhizobacteria), live in the rhizosphere and provide beneficial activities for the plants. *B. subtilis* and *Bacillus velezensis* are PGPR that are used as fertilizer in organic agriculture, but their efficiency is still inconsistent. Those bacteria are able to persist in the environment by forming endospores, characterized by an inactive metabolism which do not produce plant-beneficial molecules or activities. Endospores can withstand many environmental stresses. Thus, we hypothesize that part of the inconsistency in *Bacillus* beneficial effects on plants could be explained by the predominance of inactive state on plants. Indeed, the dynamic of germination and sporulation of *Bacillus* on plants is mostly unexplored. We used a hydroponic system and a soil system to

study the dynamic of sporulation of *B. subtilis* on seedlings over time. We observed that spores germinate rapidly in the first three days following their inoculation on the plant. However, the vegetative cells are predominant only for a few days before reverting back to spores around the ninth day. Also, we identified the germinant receptor GerK for being responsible for the germination of spores on seedlings and the histidine kinases KinA and KinB for the spore formation on plants. Since sporulation is influenced by nutrients starvation, we hypothesized that root exudates released by seedlings could impact the sporulation level of *B. subtilis*. Surprisingly, when plants are inoculated with *B. subtilis*, free-living cells around the roots sporulate more rapidly than those associated with plants. However, direct contact between plant and bacteria is required for the induction of sporulation of the free-living cells. This study is relevant for our understanding of interactions between *Bacillus spp*. and plants, and particularly for a more efficient usage of *B. subtilis* as a biofertilizer.

AEM107

Understanding the probiotic potential of GABA producing bacteria using an ex-vivo continuous fermentation model

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Gut microbiota contributes to colon health through variable metabolites including neuromediators such as gamma-aminobutyric acid (GABA). GABA is the major inhibitory neuromediator of the enteric nervous system with potential role in modulating the immune system in many health disorders. Recently, many GABA-modulating bacteria have been identified among the colon microbiota. GABA was also found to be an essential growth factor that solely can induce the growth of some unculturable organisms. Furthermore, some gut microbes can grow only in the presence of *Bacteroides fragilis* members due to its GABA production capability. Here, we postulate that enhancing the microbiota GABA production through supplementing the gut microbiota with GABA-producing probiotics will modulate gut microbiota functionality and promote gut health. This study aims to investigate the growth, GABA production, and competitiveness of probiotic candidates in the presence of human gut microbiota ex-vivo in a model of a colon mimicking fermentation simulating physiological and microbiological conditions of a human large intestine. Currently, the impact of GABA-producing *B. dentium* on the growth, and metabolite production of fecal microbiota from 2 adults with different enterotypes are being evaluated ex-vivo. Characterization of gut microbiota composition and metabolic functions is in progress using Illumina MiSeq sequencing of 16S rRNA libraries and gas chromatography. Results from this study will unravel the probiotic potential of GABA producing bacteria and their impact on gut microbiota structure and functionality.

AEM108

Using the microbiome to detect geographic food fraud in seafood

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Rapid improvements in the high-throughput sequencing technologies, curated and annotated databases, and novel informatics techniques are leading to innovative ways to verify food authenticity. While genomic DNA from various economically important “seafood” species can be used to confirm the identity of the organism that produced the meat, it is more difficult to verify that the food was produced in the advertised geographic location. It has been proposed that the microbiome of certain food products, if properly analyzed, would able to confirm

where the food was produced. In this study we have obtained several clam samples harvested from different locations on the East coast of Canada and attempted to determine if we could differentiate between clams sampled from each location on the basis of the microbiota. While correlations could be identified for freshly harvested mollusks, the ability to differentiate clams based on geographic source was not possible using mollusks purchased from retail environments. The microbiota of retail mollusks was dominated by Proteobacteria, and the proliferation of Proteobacteria during retail storage masks any signal of the native microbiota that could be used to identify the geographic origin. Although, the potential for using the microbiota to determine the origin of retail clams is low, there is a potential to use this technique to determine the origin of fresh seafood – and we have demonstrated potential to use this technique to detect this unique type of food fraud.

AEM109

Vancomycin polymeric nanoparticles for the destruction of methicillin-resistant *Staphylococcus aureus* biofilms

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The bacterial biofilm is an association of microorganisms whose extracellular products adhere to biotic or abiotic surfaces. This microbial structure is related to chronic infections and multiresistance to antibiotics. Conventional antibiotic treatment has no effect on the biofilm structure, which increases the costs of an inefficient treatment that can lead to the death of the patient. Strategies for the disruption and elimination of biofilm formation have been studied, among them, the use of polymer nanoparticles. The objective of this study was to evaluate in vitro the nanoparticles of PLGA polymers containing vancomycin for the disorganization of biofilms produced by strains resistant to methicillin of Staphylococcus aureus. In the present study were used PLGA nanoparticles containing vancomycin, produced by the double emulsification-evaporation method and properly characterized. The microorganisms used in the study were strains resistant to methicillin from Staphylococcus aureus. Initially the capacity of biofilm formation was verified. After analysis, the nanoparticles were tested in biofilm and observed by microdilution in broth, optical microscopy and AFM. Regarding the results of the multiresistant evaluation of microorganisms in studies, these studies showed that the bacterial strains studied presented resistance to free antibiotics and presented high adhesion and high capacity of biofilm formation. The formed biofilms presented predominantly protein composition. When the action of NPs on biofilms was evaluated, a disturbance was verified, besides the elimination of planktonic cells. The results found in the present study show the potential of the PLGA nanoparticles developed with the antibiotics facilitating the delivery and release of the drugs in an efficient way for the de-structuring of biofilms of multiresistant microorganisms.

AEM110

Wild blueberry rhizophere microbiome

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Canada is the largest producer of wild blueberries in the world. The crops are grown in boreal regions using two species belonging to the Ericaceae family: *Vaccinium angustifolium*, known to be very productive but sensitive, and *Vaccinium myrtilloides,* hardy and more resistant.

The main edaphic properties of ericaceous culture are high carbon to nitrogen ratio and low pH. The contribution of ericoid mycorrhizal fungi is essential as they increase the nitrogen and phosphate supply, by accessing non-assimilable sources by the plants. Field studies on microbial diversity associated with wild blueberry roots, especially ericoid mycorrhizal fungi, remain fragmentary.

This part of the project aimed to characterize the fungal and bacterial communities of the wild blueberry rhizospheric environment. It consists of the first step in order to identify bacterial and fungal species correlated to productivity in blueberries.

Nine plots were selected, with the advice of the blueberry producer, based on their estimated yields within three commercial blueberry fields in the Lac St-Jean region of Quebec. The sampling consisted of extracting blueberry shrubs including their shoots, roots and adhering soil, using a shovel. Five rhizospheric soil samples of *V. angustifolium* were collected per plots, for a total of 45 samples. We proceeded to the DNA extraction and amplification of 16S and ITS regions. Sequencing was done using Illumina MiSeq and DADA2 was used for bio-informatics analyses. 2964 Amplicon Sequence Variants (≈zOTU) were obtained for ITS, and 9013 for 16S. PLFA analyses on the soil were also undertaken to have an estimation of the microbial biomass. Finally, the nitrogen content in the shoots was measured as an indicator of plant health.

The results obtained show a greater variability in terms of structure in the fungal community than in the bacterial community. The bacterial community is mainly composed of *Rhizobiales, Acidobacteriales and Acidobacteria.* The fungal community is mainly composed of *Helotiales*, *Valsariales* and *Chaetothyriales*. Finally, the bacterial PLFA markers indicated a larger presence of Gram negative than Gram-positive bacteria. The stability of these communities will be evaluated in future studies.

AEM111

Worldwide limitation of biological nitrogen fixation by molybdenum availability: implications for trace metal and nitrogen biogeochemistry in natural and anthropogenic ecosystems

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Biological nitrogen fixation (BNF) is one of the most important biological processes on earth. BNF is catalyzed by the enzyme nitrogenase. This metalloenzyme requires molybdenum (Mo) and iron (Fe) at its active center to reduce atmospheric dinitrogen (N_2) into bioavailable ammonium. In addition to the canonical Mo nitrogenase, two alternative nitrogenases, using vanadium (V) and Fe-only instead of Mo are known to fix N_2. Despite the discovery of these alternative isoforms more than 40 years ago, BNF is still believed to primarily rely on Mo. This reflects our poor understanding of the importance of trace metal dynamics on BNF activity and the concomitant question of the contribution of alternative means of N_2 fixation, using V as metal cofactor, in natural and anthropogenic habitats.

Here, I will first summarise the pilling evidences of a worldwide limitation of BNF by Mo availability. Over the last 3 decades Mo limitation of BNF has been reported in ecosystems ranging from tropical to subarctic latitudes, in both natural (e.g.., forest, tundra) and anthropogenic (e.g., agricultural soils) environments. The widespread limitation of BNF by Mo challenges current views on the importance of alternative nitrogenases to the global N budget. I will thus also summarise recent studies highlighting the ubiquitous distribution of alternative nitrogenase genes and the contribution of alternative nitrogenases to BNF in a wide range of ecosystems.

I will then discuss how the contribution of alternative nitrogenases to BNF challenges our understanding of the role and importance of trace metal biogeochemistry on BNF. V has long been considered a toxic metal with

limited biological purpose. Our understanding of V acquisition strategies deployed by N_2 fixers to manage V limitation and toxicity remains elusive strongly limiting our ability to fully comprehend the biogeochemical cycling of this important, but mostly ignored, biometal. The widespread contribution of alternative nitrogenases to BNF also invites to re-evaluate N input estimates in many ecosystems. Indeed, the most common methods used to estimate BNF over the last half century (acetylene reduction assay) significantly underestimate N_2 fixation by the Mo-independent nitrogenases and thus likely net N input estimates.

Finally, I will discuss future research avenues and methodological developments required to fill current knowledge gaps in nitrogenase metal cofactors biogeochemistry, including V acquisition at the soil-N_2 fixers interface, trace metal dynamics in complex biological communities (i.e., biofilms) and environmental factors controlling the expression and use of alternative nitrogenases.

AEM112

Antimicrobial activity of bactofencin, nisin, pediocin and reuterin against multi-drug resistant mastitis associated Staphylococcus and Streptococcus isolates

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Bovine mastitis also known as intramammary infections (IMI) is the most common and costly disease in milk production. In the dairy industry, IMI is the leading cause of antimicrobial use. With the rapid increase of antibiotic resistance, it has become of great importance to develop new alternatives to be used alone or in combination with existing antibiotics. Bacteriocins are antimicrobial peptides synthesized by a wide variety of bacteria and inhibiting other microorganisms in their competitive environments. This study aimed to evaluate the inhibitory activity of bacteriocins and reuterin against clinical IMI isolates. A collection of IMI clinical isolates of *Staphylococcus aureus* (n=20), *Streptococcus dysgalactiae* (n=18) and *Streptococcus uberis* (n=19) were selected from the Mastitis Pathogen Culture Collection of the Canadian Bovine Mastitis and milk quality Research network. The antimicrobial susceptibility test was conducted by disk diffusion method against fourteen antibiotics currently used to treat IMI . The susceptibility of these isolates to bactofencin, nisin, pediocin and reuterin was then evaluated by determining the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) using the CLSI microplate dilution essay. Among tested isolates, 47% were resistant to penicillin, 30% to cefoxitin and 47% were multi-drug resistant (MDR). Bactofencin showed inhibitory activity against *S. aureus* isolates exclusively with MIC values varying between 2 and >125 µg/ml. The MIC values of nisin against all tested strains varied between 0.8 and >50 µg/ml. Those of reuterin varied between 70 and 560 µg/ml. MCB values for bactofencin were four to thirty-two times higher than MIC values indicating a mostly bacteriostatic effect, whereas MCB values for nisin were one to ten times higher than MIC values indicating a bactericidal effect. MCB values of reuterin were one to twenty-four times higher than MIC values, suggesting that this compound exhibits a bactericidal or bacteriostatic effect depending on the isolate. This study demonstrates that bactofencin, nisin and reuterin exhibit significant inhibitory activity against MDR clinical IMI isolates suggesting high potential of these natural molecules to be used as an alternative to antibiotics in the control of IMI associated MDR microorganism. *In vivo* studies will be performed to further investigate the potency of these natural antimicrobial compounds as an antibiotic alternative for the treatment of IMI.

AEM113

Association of individual prey specialization with the gut microbiome of an Arctic seabird

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The role of the gut microbiome is increasingly being recognized by ecologists due to the importance of microorganisms in the life cycle of many animals. At the same time, there is growing awareness of the presence of individual prey specialization within populations of the same species in which individuals from the same population use prey resources differentially. Variations in the gut microbiome could be the result of individual prey specialization. We evaluated the hypothesis that diet alters gut microbiome by testing the predictions that gut microbiome varies with sex and diet types. We present the first description of the fecal microbiome of the thick-billed murre (*Uria lomvia*), an Arctic seabird, through a metabarcoding survey of the V4-V5 hypervariable regions of the 16S ribosomal RNA gene from samples collected in Coats Island, NU in 2017. We show the presence of large inter-individual variation in the composition of the gut microbiome occurring in murres. This supports the idea that a generalist population can be comprised on different types of individualists. The murre microbiome is dominated by bacteria belonging to the genus *Catellicoccus*, ubiquitous in the guts of many seabirds and presumed to be an intestinal symbiont. We also observed differences in Shannon diversity and Pielou's evenness among diet types that can be explained with the possible role of bacteria in uric acid recycling which aids their avian host in compensating for nutritional deficiencies that may be encountered during starvation periods. Differences between the feeding behaviour of males and females during the reproductive season were also associated with changes in the fecal microbiome in terms of phylogenetic diversity.

AEM114

Characterizing hydrocarbon biodegradation along the shorelines of Canada's Northwest Passage

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As global warming drives the opening of Canada's Northwest Passage, the Arctic is at an increased risk of an oil spill. Proactively characterizing how microorganisms respond to oil exposure and determining the extent to which oil is naturally degraded will inform relevant bioremediation approaches. This research will characterize both the microbial communities present on Arctic beaches, as well as the hydrocarbon biodegradation potential within Arctic beach sediments. It is hypothesized that shifts in the existing Arctic shoreline microbial communities will be observed following exposure to hydrocarbons, reflecting increased community hydrocarbon metabolism. Intertidal beach sediments were collected from eight beaches in the Arctic in the summer of 2018. Enumeration of culturable bacteria within these sediments was determined by counting total colony forming units (CFU)/g sediment after four weeks of incubation at 10˚C on various marine salt and low nutrient agar medias: R2A and seawater, 10% R2A and seawater, marine broth. These results will be complimented by the quantification of the *rpob* gene abundance using a digital PCR platform to determine total microbial counts within the beach sediments. Community composition was assessed through 16S rRNA gene sequencing on an Illumina MiSeq platform. The potential for hydrocarbon biodegradation within the beach sediments will be measured through microcosm experiments. Radio respiration assays of ^{14}C-labelled naphthalene, toluene and acetate will be conducted to monitor microbial degradation of these compounds through $^{14}CO_2$ production. Moreover, the biodegradation of marine diesel oil with and without the addition of fertilizers will be assessed by determining remaining fractions of oil through gas chromatography mass spectroscopy (GC-MS) and correlating to the quantification of biodegradative genes of interest using digital PCR and to shifts in the microbial community composition through 16s rRNA gene sequencing. Ultimately, the results of these microcosm experiments will serve as guidance for best bioremediation strategies that will then be tested *in situ* in a small controlled fuel spill on a tidal beach in the Northwest Passage to evaluate natural attenuation under actual environmental conditions. The significance of this research will be to provide an accurate understanding of both the advantages and limitations of natural attenuation processes in the Arctic. This characterization will serve to inform optimal

bioremediation approaches and provide a policy framework for implementing bioremediation strategies in the future as the Arctic undergoes environmental and anthropogenic changes.

AEM115

Culture-dependent bioprospecting of antibiotic producing bacteria isolated from the Canadian high Arctic

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Antibiotic resistance is increasing at an alarming rate and novel antibiotics are urgently needed to combat this threat. Underexplored microbial communities, such as those in extreme environments, are potential sources of novel antibiotics. The goal of this study was to isolate, screen, and characterize Arctic microbial isolates capable of inhibiting the growth of foodborne and clinically relevant pathogens. Arctic bacteria were isolated using (1) the cryo-iPlate, an innovative *in situ* cultivation device and (2) bulk soil plating by undergraduate students that applied a standard cultivation approach to isolate bacteria from twelve different Arctic habitat samples. To decrease the possibility of identifying isolates with already-known antibacterial activities, a cell-based dereplication platform was used. Ten out of the twelve Arctic habitats tested were found to yield antibiotic producing cold-adapted isolates. Twelve cold-adapted Arctic isolates were identified with the ability to inhibit the entire dereplication platform, suggesting the possibility of novel mechanisms of action. Two promising isolates displayed antibacterial activity either against foodborne pathogens at refrigeration temperatures and clinically relevant pathogens, suggesting that cold-adapted Arctic microbes may be a promising source of novel antibiotics for use in both industrial and medical settings.

AEM116

Developing enzyme biotechnology to remove filter cakes during the hydraulic fracturing process

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Hydraulic fracturing (fracking) is a widely used technique to extract hydrocarbons from unconventional reservoirs. In this technique, gelling agents are added to fracturing fluid to help proppant reach the fractures, keeping them open. These highly viscous gelling agents (polymers) tend to accumulate at the reservoir fracture faces and block them, forming what is known as a filter cake, preventing gas (or oil) flow thus impeding recovery.

Chemical oxidizers are commonly used to remove filter cakes, but their toxicity remains a serious concern. They are also used in combination with strong acids, potentially leading to corrosion of equipment. These problems highlight a need for a more sustainable and safer technology to degrade filter cakes.

In this study, we investigated the use of a biotechnological tool as an alternative to chemical filter-cake degradation. Specifically, we sought to identify enzymes capable of degrading carboxymethyl cellulose (CMC),

a cellulose-based polymer used as a gelling agent. To date, no known work has been done on enzymes degrading CMC as a gelling agent under fracking conditions.

We detected enzymatic CMC biodegradation in the supernatant of a mixed bacterial culture enriched from cattle manure. Viscosity, a parameter directly affecting filter cake formation, was quantified as an indication of activity. Within 5 days, viscosity is reduced by 90.5% compared to controls. Characterization of these CMC-degrading enzymes indicate that they are extracellular, stable between 50˚C and 80˚C, at pH values 5 - 8, at high pressure (1000 psi) and can tolerate salinity up to 18% (w/v). Significant viscosity decrease was also measured when enzymes (in cell-free extracts) were tested on oilfield produced water samples, showing that this approach holds promise for use in the field. Our results present an encouraging non-toxic, environmentally-friendly alternative for effective fracking treatment protocols.

AEM117

Évolution de la qualité microbienne de l'air circulant dans les centrales de traitement de l'air (CTA) d'un centre hospitalier nouvellement construit

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Les bioaérosols dans les hôpitaux peuvent contenir des microorganismes provenant des patients, des visiteurs, du personnel et de l'environnement. Les centrales de traitement de l'air (CTA) sont des unités qui permettent de filtrer et conditionner l'air avant qu'il ne soit acheminé aux différents secteurs du bâtiment. L'échantillonnage d'air dans les CTA permet de suivre la qualité du traitement de l'air sans perturber les activités qui se déroulent dans l'hôpital. Le présent projet a pour but d'identifier des marqueurs d'air intérieur et extérieur qui permettront de suivre les performances du système de ventilation et l'évolution des bioaérosols avec le temps. Les échantillonnages seront effectués sur une période d'un an dans le but de vérifier les variations saisonnières. Six CTA couvrant des secteurs différents seront échantillonnés (cafétéria, blocs opératoires et salles d'urgence). L'échantillonnage aura lieu à six points dans chaque CTA afin de détecter les microorganismes, notamment à l'entrée d'air extérieur, au retour d'air intérieur et lors de son traitement. Les bactéries et les moisissures seront quantifiées et identifiées par culture et par biologie moléculaire. Un compteur de particule sera utilisé et des paramètres physico-chimiques seront mesurés. L'utilisation des méthodes de séquençage à haut débit permettra d'évaluer la diversité des espèces bactériennes et fongiques. Quatre campagnes d'échantillonnage se sont déroulées jusqu'à présent, couvrant l'automne et l'hiver. La variation saisonnière a influencé la concentration de microorganismes à l'entrée d'air extérieur. Une diminution des bactéries et des moisissures cultivables et totales est observable entre l'entrée d'air extérieur et l'air filtré soufflé dans l'hôpital. Ce projet a permis d'élaborer des stratégies d'échantillonnage en air dynamique et à haut débit. Il permettra de proposer une stratégie assurant le suivi de la performance du traitement de l'air dans les CTA à long terme, sans perturber les activités qui se déroulent à l'intérieur de l'hôpital.

AEM118

Growth phase-dependent physiology of bacterial isolates from the Human gut microbiota

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The human gut microbiota is a complex community of microorganisms heavily implicated in human health. Much of the current understanding of this community relies on metagenomic techniques, which have identified many of the bacteria commonly found in the gut, but do not provide any information about the physiology and metabolism of these bacteria. Here, we use 10 bacterial isolates from the human gut microbiota and link bacterial physiology to growth by employing single-cell techniques used in other microbial ecosystems. We use the relative bacterial nucleic acid content, determined by staining with SybrGreen, as an indicator of bacterial activity, and we assess cell membrane damage as another component of bacterial physiology. We hypothesized that bacterial physiology, as characterized by nucleic acid content and cell damage, was dependent on growth phase for these bacterial isolates. Our results indicate that some bacteria remain invariably low nucleic acid bacteria (LNA) throughout their growth, while others are more dynamic and switch between high nucleic aid bacteria (HNA) and LNA. Bacterial growth was not limited to HNA bacteria, although HNA have been identified as the more active bacteria in other systems. This suggests that replication is physiologically distinct from relative activity, and that LNA bacteria remain part of the metabolically active community. Cell damage, when present, was highest in stationary phase, when growth is absent or at a balance with cell death. Overall, these findings show that cell damage, when present, is growth phase-dependent, and that nucleic acid content is growth phase-dependent for most bacterial isolates. We identified 2 more static isolates that were invariable LNA, and may be an invariable part of the less active subset of the human gut.

AEM119

Investigating the occurrence of *Micromonospora* in nodules and rhizosphere of actinorhizal plants growing on mine sites in Québec

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Our research laboratory is currently studying the symbiosis between the nitrogen fixing organism *Frankia* and actinorhizal plants, leading to nodule formation. Despite the fact that this ecological niche is narrow, plants recruit bacteria, mainly known as endophytes or PGPB (i.e. Plant Growth-Promoting Bacteria), that can provide beneficial effects. Filamentous actinobacteria that belong to genus *Micromonospora* are often found in root nodules. Although this genus appears to be a frequent occupant of nodules, both in leguminous and actinorhizal plants, its precise role is not clearly defined. Moreover, in regards to the presence of *Micromonospora* in the nodules of plants that establish symbiosis with *Frankia* sp., most studies have been conducted on plants on the European continent. The present study focussing on *Micromonospora* is the first performed on actinorhizal plants from North America.

To study the prevalence of *Micromonospora* in the nodules of plants growing on and around mine sites, we carried out the isolation of this actinobacterium from the following plant species: *Alnus viridis* ssp. crispa, *Alnus incana* ssp. rugosa, *Hippophae rhamnoides*, *Elaeagnus angustifolia* and *Shepherdia canadensis* from a gold mine site and also *Alnus incana* ssp. rugosa and *Myrica gale* from a lithium mine site, using the osmium tetroxide surface sterilisation. We have attempted the same isolation process with the rhizosphere of those plants, but by using a soil dilution method. Here, we describe the isolation of fourteen strains of *Micromonospora* whose were found to belong mainly to two species: *Micromonospora tulbaghiae* and *Micromonospora siamensis*. Those isolates have been described with several physiological characteristics, such as colony colour, growth temperature range and NaCl tolerance. This study aims to describe the biodiversity and prevalence of *Micromonospora* in actinorhizal plant nodules, and begin to decipher their role, notably through the characterization of plant growth-promoting traits.

AEM120

Is hybridization an adaptive force in response to UV conditions?

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The environment is heterogeneous and includes extreme conditions such as high ultraviolet (UV) radiation. High UV radiation represents a particular challenge in terms of adaptability because it affects the viability of organisms by directly damaging the genome while also challenging future generations by increasing mutation rate. Extremophiles thriving in high UV radiation conditions have been reported, suggesting that adaptation to these conditions is possible. It has recently been proposed that hybridization may facilitate the colonization of new ecological niches and increase the probability of evolutionary rescue. This could be caused by increased genome plasticity in hybrids, as reflected by alterations at the nucleotide and chromosomal levels, which could boost adaptability in stressful environments. We directly test if hybridization promotes adaptive evolution in response to DNA damage, here represented by conditions that mimic UV radiation using *Saccharomyces* yeast hybrids as models. We exposed hybrid and parental strains to UV-mimetic conditions for 200 generations and measured growth continuously. Although we found that adaptation occurs in most strains, contrary to our expectations, yeast hybrids achieved a similar adaptation potential than the two parental lines. We hypothesize that this result is caused by increased genome instability in hybrids that is further enhanced by the UV conditions, preventing adaptation. We are currently examining the instability of the hybrids by measuring ploidy changes and changes in copy-number variation along the genome. Altogether, our results show that hybridization may reduce adaptive potential in extreme conditions if these conditions accelerate genome evolution.

AEM121

Isolating and characterizing novel Arctic microorganisms

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Polar cryoenvironments harbor complex, active microbial communities living at the cold temperature limits of life. Understanding how microbes survive in such conditions requires their cultivation, yet 99% of bacteria are uncultivable using standard technique. The Cryo-iPlate is a new technique being developed for in situ cultivation. This technique is based on the ichip, which has been shown to increase the cultivable component of microbial communities to 20-30%. Additionally, the genomes from unculturable microorganisms can now be assembled from metagenomes through binning, although this has only rarely been attempted in polar microbiology. The objective of this project is to employ the Cryo-iPlate as a tool to elucidate polar microbial diversity and adaptations through the isolation and characterization of highly novel cryophilic microorganisms and to obtain and characterize genomes of uncultured cryophiles by combining the Cryo-iPlate with genome binning. During the July 2018 field season, six Cryo-iPlates were incubated in diverse sediment and soil samples, as well as a seal carcass found in Resolute Bay in the Canadian high Arctic. Following incubation, microbial growth was observed on the surface of the iPlate wells using a dissecting microscope. This microbial growth was collected and used for subcultivation on standard media and for identification of non subcultivable strains. Cultivable isolates are currently being identified through 16s rRNA gene sequencing and will be screened for their cryophilic growth capabilities and novelty (<97% similarity). The best candidate strains will be selected for genomic and transcriptomic analyses in parallel with metabolic assays at sub-zero temperatures. The gellan plugs from the wells that did not produce subcultivable isolates are being targeted for metagenomic sequencing for binning. The genomic and metabolic traits from microorganisms that we can culture and those we cannot will be compared.

AEM122

Isolation and characterization of marine Myxobacteria clade for the identification of novel antibiotics

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The overuse and misuse of antibiotics has resulted in the widespread development of antibiotic resistance, increasing the risk of morbidity and mortality. The ever-increasing threat of antibiotic resistance has driven researchers to find solutions to address this problem. One of which is to identify novel antibiotics, such as bactericidal or bacteriostatic compounds produced by bacteria.

Myxobacteria are Gram-negative bacteria that use secondary metabolites to kill their prey and other bacterial rivals. Myxobacteria inhabit both terrestrial and marine environments, and it has been demonstrated that unique environments can result in the production of novel secondary metabolites. Thus, we chose an estuary environment with unique conditions to study secondary metabolite production of marine myxobacteria present in the sediment.

The objective of this research is to isolate strains from the marine myxobacteria clade, then extract the compounds that they produce. Sediment samples were collected from the Saint Lawrence estuary, which were then used as raw material for metagenomic and metatranscriptomics studies. Experiments conducted on the DNA content of the sediment samples determined that previously designed primers could prove the presence of marine myxobacteria. Additionally, we further determined that these primers amplified an unrelated clade of marine bacteria. By studying the RNA content, we were able to conclude that marine myxobacteria and these newly identified unculturable strains were metabolically active in the sediment. These findings can help us with the cultivation of marine myxobacteria clade and further isolation of the secondary metabolites present in the medium. Structural findings on these compounds and their mechanism of action can lead us to the identification of novel antibiotics.

AEM123

Legionella pneumophila and the microbiome of cooling towers

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Legionnaires' Disease is a severe pneumonia caused by the bacterium *Legionella pneumophila*. The disease is acquired through the inhalation of aerosols contaminated with the bacterium. Consequently, urban water systems are the main reservoir and vector for the spread of the disease. Cooling towers are by far the major source of large outbreaks, as they contain high amounts of water and produce vast quantities of aerosols. The mechanisms by which outbreaks occur are not well understood. However, it is believed that the resident microbes of the cooling tower play an important role in the colonization, survival, and proliferation of *Legionella pneumophila*. Consequently, we characterized the bacterial and eukaryotic communities of several cooling tower across Quebec in order to analyse their relation to *Legionella pneumophila*. To do this, we used a *16S*and *18S rRNA*targeted amplicon-sequencing strategy. Furthermore, we measured several chemical, physical, and biological parameters in order to compare cooling tower conditions and their respective microbiomes. The results indicate that continuous treatment of chlorine promotes the growth of a *Pseudomonas*population inhibiting the *Legionella* population. This inhibition would occur either by direct negative interactions between *Pseudomonas* and *Legionella*, or, indirect interactions between Pseudomonas and positively correlated species with *Legionella*. Finally, the presence of several positively correlated species with *Legionella* seems to

indicate a potential pool of microbial interactions that have yet to be characterized, and potentially the discovery of biomarkers. These biomarkers could help increase the robustness of current surveillance programs.

AEM124

Leveraging macroecological concepts for microbiological processes: modelling global co-occurrence of methane cyclers

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Microbial ecology has undergone a revolution in the quantity of data available for environments of interest, with sequence dataset growth due in large part to the accessibility and lowered costs of deep metagenomic sequencing. This data explosion presents new challenges for empirical hypothesis testing, where a focus on developing modern analysis techniques is needed. Our work here focused on applying statistical frameworks from macroecology to microbiological systems, with a focus on the global distribution of key genetic functional markers for the methane cycle. Our work seeks to advance the statistics available for metagenomic data and to answer important questions regarding the environmental fate of methane, a potent greenhouse gas.

Our main goal was to assess the co-occurrence of methanogens and methanotrophs across globally distributed environments. To answer this question, we borrowed occupancy modelling from macroecology, a technique designed to address an important question in ecology: how can detection failures be accounted for? In macroecology, this is accomplished by repeatedly sampling sites to inform detection probabilities. In metagenomics, we instead use multiple marker genes in order to emulate the repeated sampling required for occupancy modelling. This provides a way to take repeated statistical samples of a site without the need to sequence multiple metagenomes per site.

Marker genes for methanogenesis and methanotrophy were retrieved from 9,629 metagenomes based on KEGG annotations. These detection profiles were converted to presence/absence data. These data were then analyzed using occupancy models to better understand where methanogens and methanotrophs occur as well as possible co-occurrence patterns for these important groups. This research not only summarizes and connects the distribution of methane cycling microorganisms across Earth's myriad environments, but also pioneers an established statistical approach for the field of metagenomics, allowing us to provide sound statistical support of our results.

AEM125

Microbiological investigation of lava tube ice for determining its habitability on Mars

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To date most of the research characterizing the habitability of Mars has been largely confined to the surface. However recently it has become widely accepted that if extant/extinct life does exist on Mars it is much more likely to be preserved in the subsurface. Lava tubes identified on Mars provide access to the subsurface and may provide many of the conditions necessary for the persistence of life or the preservation of biosignatures. Specifically, the notion that water in the form of ice may be stable within the lava tubes hints at their habitability. Hypothetical Martian microbes could remain viable/active within the thin microscopic channels of liquid water formed in the ice due to local melting by solutes. It has been our goal as part of the "ATiLT:

Astrobiology Training in Lava Tubes" funded CSA FAST grant to characterize the microbial community of lava tube ice to determine if this environment could support a microbial ecosystem on Mars.

The microbial community of various lava tube ice samples taken from Lava Beds National Monument in California have been analyzed. The community appears to be cold adapted with 24-74% of cultured microorganisms capable of growth at 5°C (classified as psychrophiles) and abundant with cell counts ranging from 10^6-10^9 cells/ml. Analysis of the community composition using 16S rRNA gene sequencing has revealed the community to be diverse and dominated by members of the phyla Actinobacteria, Bacteroidetes, and Proteobacteria. Preliminary work to elicit the functional profile of the microbial community by linking individual OTUs to their closest related whole genomes has suggested that the community contains many facultative or obligate anaerobes as well as pathways related to autotrophic energy metabolism, specifically the use of CO_2 as a carbon source. These findings were reinforced by *in situ* gas flux measurements showing a net decrease in CO_2 over time from the ice surface within one of our study caves.

To quantify the level of activity of the microbial community within our cave ice samples we've set up biomineralization assays measuring the conversion of acetate to CO_2 using radiolabeled C^{14} in microcosms incubated at 5°C and -5°C. To date we've measured activity in all our 5°C incubations but not in our -5°C incubations. Alternative methods to measure activity in ice will be explored to confirm these results and ensuring negative results are not due to the difficulty in CO_2 diffusing out of the ice.

Taken together these results shed light on the diversity of the microbial community within lava tube ice and hint at the microbial survival strategies required for growth within the ice. The finding that the community contains many psychrophiles which appear capable of chemoautotrophic growth is relevant to the search for life in Martian lava tubes where heterotrophic metabolism using oxygen is unlikely to occur.

AEM126

Potential of pre-harvest UV treatment as an ecological approach for the control of *Xanthomonas campestris* pv. *vitians* in lettuce

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Xanthomonas campestris pv. vitians (*Xcv*), the causal agent of bacterial leaf spot, is responsible for significant economic loss in lettuce worldwide. The control of this disease remains difficult because consumers, concerned about their health and environmental sustainability, denounce the presence of residues on horticultural products and the abusive use of chemical pesticides in agriculture. The horticultural industry is therefore constantly on the alert to develop and propose innovative alternatives to the use of pesticides. Extensive work carried out on crops at the post-harvest stage indicates that short-wavelength ultraviolet (UV-C) treatments could represent an environmentally friendly approach and could also improve the quality components of fresh horticultural products. However, only a few studies have been conducted suggesting that this approach could also control the development of diseases on growing plants while affecting quality. In this study, two independent trials were conducted to evaluate the effect of UV-C on *Xcv* control in lettuce and the impact of such treatment on yield. The first trial consisted of four UV-C treatments with a dose of 0.4 kJ/m^2 on 3-week-old lettuce plants followed by leaf inoculation with a virulent strain of *Xcv* 48 hours after the last UV-C treatment. The second trial consisted of three weekly UV-C treatments at a dose of 0.4 kJ/m^2 at 48-hour intervals from the 3rd week after planting to the harvest of lettuce at the commercial stage. UV-C treatments on growing lettuces resulted in a decrease in their sensitivity to *Xcv* compared to controls. The severity of the disease was reduced by 90%, 30% and 10% to 4, 8 and 14 days respectively after inoculation with *Xcv*. The resistance induced by UV-C treatments apparently declines in time, suggesting that repeated treatment with UV-C during plant growth may be necessary to maintain effective protection. To verify the potential effect of continuous treatment throughout growth , the second trial was

conducted. A non-significant decrease in yield of fresh UV-C treated lettuce compared to controls was observed, however, the dry matter content of UV-C treated lettuce was 11% and significantly higher than that of controls. These preliminary results therefore suggest that the UV-C dose of 0.4 kJ/m^2 has positive effects on growing lettuce.

AEM127

Predicting the bacterial hosts of the gut viral dark matter with CRISPR spacers

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Phages are important members of the human gut ecosystem and together with other viruses, they form the virome. Unlike the gut microbiota, the virome is still poorly characterized and phage-bacteria interactions in the gut along with their influences on our health remain unclear. This is mostly caused by the difficulty in identifying new phages, which is hampered by their sequences exhibiting few to no matches in public databases. One of the approaches recently developed to overcome this problem is the use of CRISPR spacers to predict the bacterial host of unknown phage genomes, via homology between spacers and short regions in the phage genomes. Here, our goal was to characterize the gut virome of 1-year-old children by identifying novel phage sequences and predicting their hosts with CRISPR spacers. We sequenced by Illumina the gut viromes of 700 1-year-old children enrolled in the COPSAC 2010 cohort, which generated 266 824 phage contigs. A CRISPR spacers database of more than 2 million spacers was constructed by extracting the CRISPR loci from more than 170 000 bacteria on NCBI. Using a simple homology search between CRISPR spacers in the database and the phage contigs, we were able to predict the host of 9 399 phage contigs. These include phages infecting dominant members of the microbiota, such as ~ 1000 *Clostridium* phages, 700 *Bacteroides* phages and 500 *Ruminococcus* phages of previously unknown sequences. Interestingly, some phage contigs were predicted to have multiple hosts belonging to different genera and families, suggesting that these phages exhibit a broad host range. Additional work, including the identification of CRISPR spacers from the gut metagenomes of the same children and other host prediction approaches will contribute to further characterize the virome.

AEM128

Quantification of moss-associated cyanobacteria using phycocyanin pigment extraction

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Biological Nitrogen Fixation (BNF) by bacteria constitutes the main entry of nitrogen in the boreal forest. In this ecosystem, feather moss can cover 70-100% of the ground and participate up to a third of the total boreal forest primary productivity. Cyanobacteria establish symbioses with feather moss and have an essential role in C and N cycles in boreal ecosystems because they can support forest primary productivity by contributing up to 50% of nitrogen input through BNF. The study of cyanobacteria communities living on moss is essential to our understanding of BNF drivers and their response to climate change. However, quantifying cyanobacteria

biomass associated with moss is challenging. Techniques usually performed (e.g. microscope counting) are time consuming and biased due to the difficulty to separate bacteria from the host plant. Here, we tested a new approach using the extraction and quantification of phycocyanin (i.e. a photosynthesis pigment produced by cyanobacteria) as a proxy for moss-associated cyanobacteria biomass assessment. This approach is inspired from a technique commonly used to monitor aquatic cyanobacteria bloom. Phycocyanin was extracted from moss samples in a sodium phosphate buffer solution using freeze-thaw cycles followed by sonication and was quantified by fluorescence emission measurement. We demonstrate that phycocyanin mass is strongly correlated with cyanobacteria cell quantity in pure cultures (R^2> 0.93 and P< 0.002 for all strains used). In our study, no significant matrix effect is detected and the relation between moss-associated cyanobacteria density and phycocyanin concentration is linear over a wide range (R^2> 0.82 and P< 0.01). We also show that there is a significant relation between phycocyanin concentration and BNF rates for two dominant boreal feather moss species (*Pleurozium schreberi* and *Ptilium crista-castrensis*). We conclude that phycocyanin extraction is an easy, fast and efficient tool to estimate moss-associated cyanobacteria quantity.

AEM129

Social motility selects for quorum sensing mutants in the opportunistic pathogen *Pseudomonas aeruginosa*

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Bacteria can move in different environments using various types of locomotion. For instance, swarming motility is a social and coordinated movement of a group of bacteria on a semi-solid surface, which requires the expression of a functional flagellum and production of a surface-active (wetting) agent. In *Pseudomonas aeruginosa*, rhamnolipids act as the wetting agent and the production is regulated through quorum sensing (QS), a mechanism used by bacteria to communicate between each other and coordinate their behaviour. To better understand the regulation of swarming motility, we are investigating the *hptB* gene of *P. aeruginosa*, which is involved in regulating the switch between planktonic and surface lifestyles. This mutant possesses a functional flagellum and produces sufficient rhamnolipids for swarming motility, but still has a defect in its swarming capacity, suggesting that additional unknown elements are necessary for this a phenotype. To identify that missing element, we experimentally evolved *P. aeruginosa* for swarming motility. After three transfers, the Δ*hptB* mutant recovered a swarming motility phenotype. Genome sequencing of several evolved clones revealed mutations, including in the key QS regulator *lasR*. This was unexpected since QS is considered important for such a social behaviour. To verify whether evolution of QS mutations was specific to the Δ*hptB* background, an experimental evolution was performed with the wildtype strain. Unexpectedly, spontaneous mutations in *lasR* also appeared in this background supporting the hypothesis that swarming motility is a selective pressure for the emergence of mutants with a QS defect. To explain the adaptive benefits of such mutants under swarming conditions, several hypotheses are investigated. One favorite explanation is the loss of cooperativity by a subpopulation of «cheaters» exploiting shared resources («public goods») useful for swarming.

AEM130

Use of an adsorptive matrix to assess microbial biodegradation of aromatic hydrocarbons

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Tenax-TA is a well-known adsorptive material used to trap volatile organic compounds (VOCs) in airborne applications and aqueous solutions. Benzene and toluene are important VOCs associated with petroleum contamination and are model aromatic compounds whose microbial biodegradation processes are of interest to researchers. Tenax-TA is also an effective matrix for microorganisms to colonize and grow upon. Using Tenax-TA resin beads, we sought to assess the potential for bioremediation in a hydrocarbon-contaminated aquifer in Alberta, Canada. Microcosms prepared under different electron-accepting conditions were established using Tenax-TA pouches, water from a contaminated site, and with benzene and toluene as the carbon sources. After 80 days of incubation, aerobic microcosms were shown to readily degrade both toluene and benzene. Anoxic and no electron acceptor-added microcosms degraded toluene but not benzene. Molecular analysis of microcosm fractions resulted in considerably more genomic DNA recovered from planktonic than sessile (Tenax-TA) communities. This was particularly evident in microcosms that degraded toluene slowly, while microcosms that degraded hydrocarbons in 14 days had far more biomass. This study revealed that trapping benzene and toluene on the Tenax-TA matrix resulted in microorganisms growing in direct contact with normally volatile and hydrophobic aromatic hydrocarbons. It also allowed a unique opportunity to characterize these microbial communities when growing under varying redox conditions, providing promising insight into the optimal conditions necessary to achieve *in situ* bioremediation of hydrocarbons in this contaminated aquifer.

AEM132

Response of temperate forest soil microbial communities to drought and precipitation events

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Despite constant logging, forests cover ca. 30% of Earth's landmass; are among the most important carbon sinks and host a wide variety of ecosystem services ranging from biogeochemical cycling to climate regulation. While plants are the main drivers of carbon dioxide fixation, soil microbes play an essential role in the turnover of carbon (C), nitrogen (N) and other elements within forest ecosystems. Altered environmental conditions associated with global change, including increasing drought events, global warming and the recurrence of forest fires, are threatening the integrity of the entire biosphere, namely terrestrial ecosystems such as boreal and temperate forests. Understanding how such ecological stresses and perturbations will affect the ecology and biogeochemistry of forest ecosystems is of utmost importance in order to minimize their drawbacks in the short and long term. A part of this project aims to determine how water availability shapes temperate forest ecosystems by alleviating drought stress via the induction of artificial precipitation events in a primary beech forest naturally subjected to drought. In this regard, our goal is to determine if hot moments induced by these precipitation events (perturbation) are sufficient to maintain traits associated with an absence of drought. Similarly, another beech-dominated forest will be subjected to drought in order to understand the long-term effects of water deprivation (stress). Continuous carbon dioxide fluxes will be measured throughout the dry season in order to follow changes in net respiration associated with precipitation events. Soil microbial community structure, namely the distribution of the most abundant archaeal, bacterial and fungal taxa, will also be recorded several times during the dry season. Preliminary results have shown that soil moisture and some of its associated impacts last for a few days following each precipitation event. Alleviation of drought is thus expected to increase microbial biomass and enzymes activity in the short term, leading to the promotion of organic matter turnover in the long term when compared to soils facing drought stress.

AEM133

Effect of bio-protective culture on the surface microbial flora of pork loins

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Pork production and transformation is an economically important activity in Quebec, representing one-fifth of the Canadian export market valued at $ 1,638 million. A large proportion (39%) of products is exported to the Asian continent. This long distance transport involves a robust control of the meat shelf life. However, during long trips, temperature fluctuations influence the microbial flora of fresh meat, providing opportunities for pathogenic and spoilage microorganisms growth. Obtaining a safe product that complies with the customer's organoleptic demands is a major challenge. To solve this problem, the meat industries have been resorting for several years to the addition of chemical preservatives. To adapt to the increasing consumer demand for “Green Label” products, companies are interested in new alternatives such as bio-protective cultures. Thereby, this project, in collaboration with Olymel, aims to evaluate the impact of a bio-protective ingredient (*Carnobacterium divergens* M35 culture + and its antimicrobial peptide Divergicin M35) on the surface flora of vacuum packed pork loins. The ingredient and has been approved by Health Canada as an additive since 2016 in salmon and smoked trout. *Carnobacterium divergens* M35 is able to grow and produce its bacteriocin at low temperatures with antimicrobial activity against the main pathogenic and meat spoilage bacteria. The modification to the microbial ecosystem of pork loins was evaluated by a metabarcoding approach over a 10-week period along with physicochemical parameters. On one hand, results were processed by a QIIME 2.0 pipeline to determine the bacterial species present in our samples at different storage points. On the other hand, pH and exudation were monitored to evaluate the quality of the product. This work highlights the complete dominance of *Carnobacterium divergens* M35 on the endogenous microbiota during storage. The use of such bio-protective culture could serve to extend the shelf life of fresh pork while preserving its organoleptic properties.

AEM134

Transcriptional response of *Rhizobium leguminosarum* bv. *viciae* 3841 to exposure of 2,4-dichlorophenoxyacetic acid (2-4-D)

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2, 4-Dichlorophenoxy acetic acid (2,4-D), a structural analogue of plant hormone indole 3-acetic acid (IAA), is a widely used herbicide targeting broadleaf weeds. In this study, we have used transcriptomics and fluorescence microscopy to determine the the response of *Rhizobium leguminosarum bv. viciae 3841* (*Rlv*) to 2-4-D exposure. A comparison of gene expression profiles of *Rlv* cultures treated with formula control and 2,4-D using RNA-Seq revealed an enrichment of differentially expressed (at ≥ 1.5-fold) genes in response to 2,4-D treatment within the plasmid portion of the *Rlv* genome. Genes involved in glycerol-3-phosphate metabolism (pRL90074-81), and EPS production and export were significantly up regulated (12-44 fold) on addition of 2,4-D. The effect of 2, 4-D on *Rlv* phenotype was monitored by studying the response of *Rlv*-FtsZ-GFP tagged cells to treatments with formula control, 2,4-D, IAA and no treatment. Our work advances the understanding of the biological impacts of 2, 4-D on *Rlv* at genetic and phenotypic level.

MGCM1

A bifunctional ATPase drives tad pilus extension and retraction

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Molecular motors convert chemical energy directly into mechanical work and are found in all domains of life. These proteins are critical to intracellular transport, motility, macromolecular protein assembly, and many other essential processes. A subset of related ATP-hydrolyzing molecular motor proteins (ATPases) in bacteria drive the polymerization of protein subunits into extracellular fibers including type II and type IV secretion systems (T2SS or T4SS), competence pili of Gram-positive organisms, archaeal flagella, and type IV pili (T4P). Retraction of the fibers of T4SS and T4P is important for their function, as is also hypothesized for the T2SS and Gram-positive competence pili. Despite its importance, the retraction mechanism has remained elusive for most of these systems as they possess a single ATPase, which is required for extension. The type IVa pili (T4aP) represent an exception to this rule, since they possess an antagonistic ATPase, derived through an early evolutionary duplication event of the extension motor, that drives fiber retraction. Here we use the tight adherence (tad) T4cP as a model to elucidate the retraction mechanism for the systems that lack a retraction ATPase. We show that a bifunctional pilus motor, CpaF, drives both activities through ATP hydrolysis. We find that mutations within the ATP hydrolysis active site of CpaF in *Caulobacter crescentus* result in a correlated reduction in extension and retraction rates. Furthermore, mutations that cause a decrease in rates result in a correlated decrease in ATP hydrolysis by *in vitro* assays, and the force of retraction in those mutants is reduced. We show that the mechanism of motor bifunctionality extends to another genus of tad-yielding bacteria, but that extension and retraction are uncoupled in the dual-motor containing T4aP competence pili in *Vibrio cholerae*. Together, these results demonstrate a novel mechanism for processive T4P retraction and increase our understanding on the utility of widespread molecular motor proteins.

MGCM2

A distinctive new class of conjugation system widely distributed in the rhizobia

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Plasmids and chromids make up a substantial portion of the genomes of many rhizobia, and their horizontal transfer by conjugation has contributed extensively to genetic diversity and evolution. Several distinct types of conjugation system have been identified on plasmids and genomic islands of members of the Rhizobiaceae, and each has different required components and regulatory elements. The best- studied systems (Type I) show similarity to the *Agrobacterium tumefaciens* Ti plasmid interbacterial conjugation system. Type II systems are regulated by RctA repressors. We have characterized Type IV rhizobial conjugations systems, which are found on various Sym and non-Sym plasmids in *Rhizobium* spp., and were first characterized on plasmid pRleVF39b, from *R. leguminosarum* strain VF39. We have identified the genes required for self-transfer of pRleVF39b by mutational analysis and expression studies using gene fusions and qRT-PCR. In addition to the relaxase gene,

traA, and genes involved in production of the type IV secretion system (*mpf* genes), we have demonstrated that at least six additional genes in the vicinity of *traA* are required for optimal transfer. These accessory genes are co-regulated with *traA* and the *trbN* operon as part of the TrbR (repressor) regulon. The presence of pRleVF39a, which encodes an AHL-independent Type I conjugation system, enhances the rate of transfer of pRleVF39b, and affects expression levels of TrbR regulated genes. Among the available complete and draft genome sequences of members of the Rhizobiaceae (484 total), we have found over 80 genomes that contain evidence of the presence of a Type IV rhizobial conjugation system. Genera in which this type of system was detected include *Rhizobium*, *Neorhizobium*, *Agrobacterium*, *Sinorhizobium/Ensifer*, *Aminobacter*, *Mesorhizobium* and *Phyllobacterium*. Multiple species of the genus *Rhizobium* contain strains with Type IV conjugation systems, including *R. leguminosarum*, *R. phaseoli*, *R. etli*, *R. pusense*, *R. laguerreae*, *R. alamii*, and *R. sullae*. For complete genomes, the conjugation systems were always located on plasmids, but were associated with a variety of different types of *repABC* genes, indicating that the plasmids belong to different incompatibility groups. Symbiosis genes were found on the same replicon as the Type IV conjugation system in a minority of cases. Overall, the synteny of the genes conserved in this type of system was maintained even in relatively unrelated species, but there were instances of incomplete sets of genes, and of insertions of other genes, along with deletions of individual genes, in the cluster of genes encoding the Mpf system and Dtr system (DNA transfer and replication). There were some instances of plasmids carrying additional conjugation systems as well as the Type IV one on the same replicon. Phylogenetic analysis shows that Type IV systems are only distantly related to the other conjugation systems found in the Rhizobiaceae

MGCM3

A DNA junction superstructure regulates Type III Secretion System gene expression and virulence in *Vibrio parahaemolyticus*

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Bacterial pathogens temporally upregulate virulence gene expression to promote infection, subverting host defenses, leading to disease. Efficient repression of virulence gene expression is also a requirement for bacterial fitness in the environment, outside of a host. Consequently, bacteria have evolved genetic mechanisms that rapidly switch between virulence gene repression and activation.

In the case of pandemic *Vibrio parahaemolyticus (Vp)* strains, two **T**ype **III** **S**ecretion **S**ystems (T3SS), along with a variety of toxins contribute to acute enteric disease in humans. Previously, we reported that *Vp* derepresses the expression of its T3SS-1 master transcriptional regulator, ExsA, by encoding a HlyU regulator during infection. Specifically, HlyU dimers act as DNA binding transcriptional regulators to relieve *exsA* virulence gene silencing mediated by the histone-like protein H-NS. Critically, we demonstrated that *Vp hlyU* null mutants are abrogated for T3SS-1 gene expression and are significantly deficient for host cell cytotoxicity, implicating HlyU as a key virulence regulator.

Here we demonstrate that a HlyU dimer binds the *exsA* promoter within a 56-bp DNA region that contains an inverted repeat separated by a 14-bp A/T rich palindromic sequence. *In silico* modelling lead us to hypothesize that a 4-way stem loop DNA cruciform structure forms at the inverted repeat, and that this DNA superstructure plays a key role in T3SS-1 gene regulation. T7 endonuclease I digestion, which specifically cleaves at 4-way DNA cruciforms, revealed that the *exsA* promoter DNA does indeed form a real 4-way DNA superstructure. Moreover, the DNA cruciform superstructure was precisely restriction mapped to the inverted repeat sequences and stable cruciform formation was strongly dependent on supercoiled DNA. Importantly, genetic deletion of each inverted DNA repeat or palindrome sequence completely prevented cruciform formation.

Next, to investigate the biological role of the DNA cruciform superstructure in regulating *Vp exsA* gene expression, we performed quantitative *in situ* real-time transcriptional assays using a bioluminescence reporter system. As expected, *Vp* with the native *exsA* promoter supported high bioluminescence. In sharp contrast, bacteria with mutated *exsA* promoter DNA in either the inverted repeats or palindrome sequence were all significantly impaired for bioluminescence. Lastly, electrophoretic mobility shift assays (EMSA) demonstrated that purified HlyU protein efficiently bound *exsA* promoter DNA, whereas inverted repeat mutants were deficient for HlyU binding.

Overall, the data highlight a biologically relevant DNA cruciform superstructure within *Vp* that serves to regulate virulence gene expression with the cooperation of HlyU DNA binding. Similar conserved DNA sequence elements are found in other *Vibrio* species suggesting that DNA superstructures may be widely used to regulate gene expression. This study demonstrates that bacterial virulence gene regulation goes beyond traditional protein activators and repressors by implicating DNA junction superstructures in gene expression.

MGCM4

A locus on the *Sinorhizobium meliloti* pSymA megaplasmid that restricts phage growth

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Sinorhizobium meliloti is a nitrogen-fixing bacterium that forms root nodules on alfalfa and other leguminous plants. Its genome consists of a 3.7 Mb chromosome, a 1.7 Mb chromid (pSymB), and a 1.4 Mb megaplasmid (pSymA). We isolated soil phages that could form plaques on *S. meliloti* strains lacking pSymA, but whose growth was restricted on wildtype strains carrying both the pSymA and pSymB replicons. By examining the phage sensitivity phenotypes of strains containing various deletions covering regions of pSymA, we have identified a locus on the pSymA megaplasmid that restricts the growth of some *S. meliloti* phage (e.g. ϕ2S and 5A) but not others (ϕM12). This locus is sufficient to confer bacteriophage resistance when introduced into an otherwise phage-sensitive strain lacking pSymA. Although phages can adsorb to both resistant and sensitive cells, they do not form plaques on strains containing the pSymA defense locus. We are currently characterizing this locus with the goal of defining how it provides protection against phages. To this end, we are also examining and will report on the genome sequence of several of the phages.

MGCM5

A novel computational approach for the investigation of human associated microbiota

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With growing recognition of the importance of the human microbiome in health and disease, there is an increasing need for strategies that allow for investigation of all members of the human microbiome, including unclassified bacteria. Traditional methods of identifying members of the human microbiome are often focused on the more abundant, previously classified members of the community, which does not provide a complete picture of the human microbiome. Two of the challenges of identifying and comparing these unclassified members across thousands of datasets are the computational requirements and time needed for *de novo* OTU clustering of 16S RNA sequences, which is amplified as research moves towards more comprehensive and larger size sample sets. To address this issue, we developed a novel computational approach that allows for the quick recognition of unclassified members of the human microbiome across a large number of samples. Our technique involves sequential filtering based on taxonomic assignment and clustering of unclassified sequences using the metagenomic data processing tool VSEARCH, reducing computational time. This produces a set of

OTU-like clusters, called “super-OTUs” that are collections of OTU representative sequences obtained from individual microbiome samples. This technique allows for insight into the relative abundance of super-OTUs across different microbiome samples, and will help reveal more information about an often overlooked but influential part of the human microbiome.

MGCM6

A pH-regulated integral membrane protein inhibits an unusual transcription module

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σ factors mediate cognate promoter recognition by RNA polymerase (RNAP) holoenzyme and play other integral roles in transcription initiation. A subset of σ factors known as extracytoplasmic function (ECF) σ factors regulate gene expression in response to environmental physical and chemical stimuli. The proteins SigO and RsoA constitute an atypical two-subunit ECF σ factor in *B. subtilis* that is activated under acidic growth conditions and after exposure to certain cell-wall acting antibiotics. To isolate the factors that regulate SigO-RsoA activity, we reconstituted the system in a heterologous host (*Escherichia coli*). SigO and RsoA can productively associate with *E. coli*RNAP and activate transcription from a cognate promoter *in vivo*. We also functionally reconstituted the integral membrane protein (RsiO) that negatively regulates SigO-RsoA activity. RsiO specifically interacts with the SigO subunit and the pH-dependent loss of this interaction results in the activation of transcription from cognate promoters. This unusual transcription module (SigO-RsoA-RsiO) responds to acid stress in *E. coli* as it does in the native host (*B. subtilis*), suggesting that the anti-σ factor RsiO responds to acid stress and raises the possibility that it is also a direct sensor of acidic environmental conditions. Current investigations are focused on identifying the mechanism by which RsiO is inactivated at low pH. Anti-σ factors like RsiO play a pivotal role during environmental sensing by bacteria. The results of this investigation lend insight into non-canonical pathways for transcriptional responses to environmental stressors.

MGCM7

Acetone and isopropanol production by the hyperthermophilic archaeon *Hyperthermus butylicus*

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Hyperthermus butylicus is a strictly anaerobic archaeon producing 1-butanol as a fermentation product. This sulfur reducing hyperthermophile has been successfully grown and studied to determine other solvents produced throughout its growth. Samples were taken at different growth time points for analyses using both high performance liquid chromatography (HPLC equipped with a Refractive Index Detector) and gas chromatography (GC equipped with an Flame Ionization Detector), respectively. The results showed that *H. butylicus* also produced acetone, ethanol, and isopropanol in addition to 1-butanol. Growth of *H. butylicus* was further optimized by using different pHs, resulting in much higher production of acetone, ethanol and isopropanol. Solvent detection was determined from ethyl acetate extracted samples using GC. The metabolic pathways for the solvent production is currently unknown, however, the presence of several solvents brings us closer to understanding the capability of solvent production and mechanism of *H. butylicus*, which ultimately aids in the understanding of archaeal alcohol metabolism at high temperatures.

MGCM8

Adaptation of a reduced-genome *Escherichia coli* strain to different growth conditions

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Many reduced strains of *Escherichia coli* have been created, bearing deletions up to 39% of the parental genome. Considering the smaller amount of DNA to replicate and transcribe, it has been hypothesized that a reduced strain should at least have the same growth rate as their parent, if not superior. In reality, most of those strains have an impaired growth capacity even in the richest medium. The mechanisms underlying these growth defects are still unclear, and constitute a challenge to further reduce bacterial genomes towards minimal organisms.

In order to understand the causes of growth defects and to develop more robust versions of reduced strains, we initiated accelerated evolution studies on min*E coli* [AC1] (36% of reduction) and its parent strain *E. coli* W3110. Using an inducible mutagenesis plasmid (MP6), we introduced mutations that improved growth in LB medium as well as two others defined media (MOPS minimal and EZ-rich). We next sequenced the complete genomes of evolved strains with the objective of finding medium-specific mutations increasing growth rates in this reduced strain compared to mutations observed in W3110 under the same conditions. The impact of these mutations will be investigated using RNAseq and their metabolism will be analyzed using an *in silico* genome-scale model.

MGCM9

An alternative toxic model of *Streptomyces*-Insect interactions in nature

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Insects and bacteria have played a key role in each other's evolutionary history for >3.5 billion years. Many examples of insect-microbe interactions exist. In particular, bacterial of genus *Streptomyces* has a ubiquitous association with various insect species that is beneficial to the insect by providing antibiotic-driven defences against insect pathogenic species. Using the model fruit fly (*Drosophila melanogaster*), we demonstrate that *Streptomyces* can harbour toxic properties to larval development. These toxic properties are linked to their ability to produce insecticidal compounds. In particular, the anthracycline metabolite Cosmomycin D is shown to have potent inhibitory activity against larval development and is linked to the inherent toxicity of an environment species of *Streptomyces*, WAC-288. At the cellular level, spores of toxic *Streptomyces* strains cause deterioration of microvilli along the gut lining of larvae and causes apoptosis of cells within various tissues. This work provides an alternative model to the *Streptomyces*-insect relationship whereby harmful strains are capable of being lethal to insect larvae and thus drive insect evolution.

MGCM10

Anti-*Neisseria* drugs screening start revealing the Achilles' heel of *N. meningitdis* and *N. gonorrhoeae*

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Neisseria meningitidis and *Neisseria gonorrhoeae,* constitute major human treats. There are both highly related species that emerged from a common commensal symbiont ancestor. Vaccine and antibiotics are currently minimally limiting a devastating global epidemic. Unfortunately, some strains are rapidly evolving to escape both types of human interventions. *N. gonorrhoeae* is evolving rapidly and is generating resistance with a high risk of untreatable infection emergence. As a result, it is now urgent to develop new avenues of treatments to fight these bacteria (WHO).

We undertook an anti-*Neisseria* drugs screening with a library of molecules from different origins. Interestingly enough, a group of molecules harbouring a common feature were all highly effective to inhibit the growth of *Neisseria meningitidis*. By deciphering this effect, we realized that it was not only bacteriostatic but also bactericidal at concentration near 10µM or less. In fact, we could not recover any live bacteria after 3h of exposure with these molecules and this for *N. meningitidis* but also *N. gonorrhoeae*. Surprisingly, this effect seems to be highly specific for these two species. In fact using increased doses (5 times or more), we were not able to kill as a similar rate, the closely related species *N. lactamica*, on more distant *Neisseriaceae* and even on completely unrelated species. We are now deciphering why these two species are so sensible to this series of compound using different technics such as isolation of naturally evolved resistants, ICP-MS and microarrays to understand the mechanism of action. Preliminary results may suggest that envelope properties of these two pathogens may be responsible of this hypersensitivity. Zebrafish-based model of toxicity did not reveal any strong harmful effects.

We discovered a chemical motif with very strict activity against pathogenic *Neisseria,* a narrowed selectivity that has rarely been achieved for any antibiotics.

MGCM11

Armeniaspiroles are pan-proteosome inhibitors targeting ClpYQ and ClpXP in Gram-positive bacteria

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Antibiotics are an indispensable aspect of modern medicine enabling a wide array of medical interventions including treating serious infections, surgery, and cancer chemotherapy. Due to the rapid rise in antimicrobial resistance, many antibiotics are being rendered ineffective, leaving few clinical options to treat serious infections. Armeniaspirole, a novel polyketide with a spiro-[4.4]non-8-ene core, was isolated from *Streptomyces armeniacus* and shown to be active against drug resistant Gram-positive bacteria. Here, a combination of chemical biology, *in vitro* biochemistry, and proteomics are used to decipher the mechanism of action for this exciting and structurally unique natural product. Three potential targets were acquired by affinity-based protein profiling through synthesis of an armeniaspirole-derived probe in *Bacillus subtilis*: YfkN, MtnU, and ClpY. Subsequent *in vitro* biochemical assays showed potent inhibition of the ClpYQ proteasome. Furthermore, lesser extent inhibition of the ClpXP proteasome indicates that armeniaspirole functions as a pan-proteasome inhibitor with a complex, polypharmacological mechanism of action. To assign a physiological mechanism for armeniaspirole antibiotic activity, dimethyl labeling proteomics was used to quantitatively study changes in protein levels in *Bacillus subtilis*. While armeniaspirole effects deviated from the proteomic signatures of known classes of antibiotics, armeniaspirole inhibition appears to be heavily implicated in cell division, showing significant increases in related proteins. As a result, inhibition of the ClpYQ and ClpXP proteasomes represent new bacterial targets for antibiotic development.

MGCM12

Arrested Development: investigating DNA damage in the filamentous microorganism *Streptomyces*

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The soil microorganism *Streptomyces* is a filamentous, hyphal bacteria. *Streptomyces* is initially a substrate bound organism but will eventually rise and sporulate. Their morphology and growth processes pose a striking contrast to bacteria such as *E. coli* and *B. subtilis.* However, at the molecular level, their cell division processes are largely conserved. Thus, while *Streptomyces* utilize much of the same cell division machinery that a rod-shaped bacterium does, their differences in growth implicate the presence of novel bacterial cell biology.

Cell division regulation is of utmost importance to an organism. If left unchecked, detrimental effects like DNA damage can be lethal, triggering the SOS response. This response is regulated by the RecA-LexA system. While Streptomyces rely on this response, they also have an uncharacterized DNA damage response independent of RecA-LexA control.

Completion of Streptomyces sporulation relies on several genes. One of these genes, *ssgB*, is putatively involved with the recruitment of the divisome to sites of incipient cell division. A null mutant for *ssgB* leads to a block in sporulation.

Previous work in the Nodwell Lab has generated preliminary RNA-Seq results that indicates *ssgB* may have a significant role in the *Streptomyces* DNA damage response. The addition of the nucleotide cross-linking molecule mitomycin-C to *Streptomyces* showed a precipitous drop in the expression of *ssgB*. I hypothesize that *ssgB* plays an additional role in the streptomycete DNA damage response. Using the model *Streptomyces venezuelae,* my work looks to assess the transcriptional response of streptomycetes to DNA damage as well as delineate a potential role for *ssgB* during DNA damage in Streptomyces cell division.

MGCM13

Assembly and export of the serotype O4 polysaccharide antigen from *Klebsiella pneumoniae* O4

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Klebsiella pneumoniae infections are a serious health concern due to the emergence of multi-drug resistant strains. Immunotherapeutic strategies offer a potential solution to combat such infections and the ability to create protein-conjugate polysaccharide antigens by *in vivo* protein glycosylation is an important enabling technology. Lipopolysaccharide O-antigens present an attractive target for immunotherapeutic efforts due to the relatively low number of O serotypes among clinical isolates of *K. pneumoniae*. In order to develop the appropriate neoglycoprotein antigens, a full understanding of the structure and biosynthetic pathways of the clinically relevant O antigens is required. Currently 9 O-antigen serotypes are proposed for *K. pneumoniae*, but genome sequences from large collections suggest this number could increase. The biosynthesis of some known O serotypes has not been examined and this study focuses on one of the last remaining examples, serotype O4. Analysis of the gene cluster directing O4-antigen production indicates it is exported by an ABC transporter. Furthermore, a putative carbohydrate-binding module within the transporter is consistent with biosynthetic processes involving a chain-terminating residue. The O4 O-antigen repeat unit consists of a disaccharide of α1,2-linked galactose

(Gal) and β1,4-linked ribofuranose (Rib*f*) terminated by a α2,2-linked 3-deoxy-D-*manno*-oct-2-ulosonic acid (Kdo) residue. The predicted biosynthesis gene cluster encodes two glycosyltransferases, a protein of unknown function, a hydrolase and an epimerase. Here we propose a biosynthesis model for the O4 antigen based on bioinformatics, glycan structure, and mutagenesis experiments to establish the essential gene set. The recombinant O4 antigen was expressed in *Escherichia coli*, providing an essential resource for ongoing studies to identify the biochemical functions of all of the individual proteins involved in biosynthesis. Preliminary analysis indicates that the protein of unknown function incorporates the terminating α-Kdo transferase, which shares no sequence similarity to known Kdo transferases, and a coiled-coil molecular rule to establish O-antigen chain length. Further understanding of these mechanisms will provide a platform required for immunotherapeutic strategies targeting *K. pneumoniae*.

MGCM14

Assembly of the *Klebsiella pneumoniae* O1 and O2ac polysaccharide antigens

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Klebsiella pneumoniae is a Gram-negative, opportunistic pathogen gaining attention for its widespread resistance to last-resort carbapenem antibiotics. Treatment of nosocomial *K. pneumoniae* infections requires rapidly acting therapeutics, and passive immunization by administration of therapeutic antibodies has been proposed. Developing precise immunotherapies requires a fundamental understanding of the structures of vaccine candidate antigens, and their expression in 'safe' recombinant host bacteria depends on a clear understanding of the relevant assembly components. Cell surface polysaccharides have been shown to be effective in generating protective antibodies against several bacteria, and the O-polysaccharide (O-PS) component of lipopolysaccharides is considered a target in *K. pneumoniae*. O-PS is composed of diverse repeating sugar units and divides the *K. pneumoniae* species into distinct O serotypes. Among the most prevalent of serotypes is the O2 serotype, which is actually a collection of serotypes that all contain a modified version of the O2a repeat unit backbone of: [→3-β-Gal*f*-1→3-α-Gal*p*-1→]. Two subtypes of O2 (O1 and O2ac) employ unique strategies for antigen diversification, by adding a new polysaccharide structure to the non-reducing end of the O2a polymer. The O1 system adds a [→3)-α-D-Gal*p*-(1→3)-β-D-Gal*p*-(1→] disaccharide, which defines the O1 antigen, and the O2ac system adds a [→3)-β-D-Glc*p*NAc-(1→5)-β-D-Gal*f*-(1→] disaccharide, defining the O2c antigen. Interestingly, the formats of these modifications differ biochemically. WbbY is the sole glycosyltransferase (GT) required for polymerization of the O1 antigen; it , contains two active sites, and interacts with the O2a polymerase WbbM. In contrast, O2c polymerization requires two enzymes, WbmV and WbmW, which interact with one another, but do not form any apparent interaction with WbbM. Using site directed mutants coupled with *in vitro* assays using fluorescently labelled glycans, the N-terminal domain of WbbY was found to be a UDP-Gal*p*-dependent GT-B galactosyltransferase, while the C-terminus is a UDP-Gal*p*-dependent GT-A enzyme. Using the same approach, the functionalities of WbmV and W were elucidated with WbmV identified as the UDP-GlcNAc transferase and WbmW representing a UDP-Gal*f*-dependent enzyme; both GT-A members. These studies provide detailed insight into a novel mechanism used to achieve serological diversity.

MGCM15

Biosynthesis of a conserved glycolipid anchor for Gram-negative bacterial capsules

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Bacterial cell-surface glycoconjugates play critical roles in host-pathogen interactions. Lipopolysaccharide is an essential component of the outer membrane of Gram-negative bacteria. It is assembled on a conserved glycolipid terminus, making in it an attractive therapeutic target. The glycolipid terminus is linked through one or more α-Kdo (3-deoxy-D-*manno*-oct-2-ulosonic acid) residues to hypervariable glycan structures which are surface exposed. Recently, another major class of cell-surface glycoconjugates, capsular polysaccharide (CPS) layers forming "group 2" capsules, has also been discovered to contain a conserved glycolipid terminus. This CPS assembly strategy has been identified in extraintestinal pathogenic *Escherichia coli*, (ExPEC) and several other important Gram-negative pathogens. The hypervariable regions of these CPS molecules are assembled on a β-Kdo oligosaccharide which is attached to a phosphatidylglycerol (PG) moiety. Here, we present the definitive structure of this glycolipid anchor from *E. coli* through the *in vitro* functional characterization of three CMP-Kdo-dependent glycosyltransferase (GT) enzymes responsible for its synthesis. In this assembly pathway KpsS adds a single Kdo residue to PG, initiating synthesis. An alternating β-(2→4)- and β-(2→7)-Kdo oligosaccharide is then synthesized by KpsC, a single enzyme containing two β-Kdo GT modules with different linkage specificities. Interestingly, homologs of these GTs exist as separate polypeptides in a thermophilic organism, *Thermosulfurimonas dismutans*. Crystal structures were determined for the β-(2→4)-Kdo GT, revealing two α/β Rossmann-like domains with a helical bundle inserted between them. Both Rossmann-like domains are reduced compared to canonical GT-B folds in GT enzymes and the N-terminal domain, responsible for acceptor binding, only shows a small remnant of a Rossmann-fold. This structure reveals interesting mechanistic insights for β-Kdo GTs and provides the first example of the new GT107 family. The β-(2→7)-Kdo GT was also structurally characterized and shows an overall architecture very similar to the β-(2→4)-Kdo GT. However, the N-terminal Rossmann domain shows significant remodelling, likely contributing to the difference in linkage specificity between these two GTs. Chimeric constructs were generated, swapping acceptor binding domains between enzymes, to confirm that the acceptor binding domain alone is responsible for linkage specificity. The KpsSC enzymes involved in synthesis of this conserved glycolipid anchor represent viable therapeutic targets as interference to synthesis would uncloak these high-profile pathogens from their capsular layers and render them susceptible to host defences. This opens the door for a potential "antivirulence" therapeutic strategy to treat infections. Establishing the assembly pathway and components involved in glycolipid synthesis provides an essential foundation for these efforts.

MGCM16

Burkholderia cepacia complex's clinical strains are able to produce 4-hydroxy-3-methyl-2-alkylquinolines and their Hmq system could be negatively and positively regulated

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The *Burkholderia cepacia* complex (Bcc) comprises twenty-one similar species of bacteria, several pathogenic. Multiple infections have been reported in immunosuppressed individuals (e.g. chronic granulomatous disease) and cystic fibrosis patients. Among Bcc species, some strains of *Burkholderia ambifaria,* and *Burkholderia cepacia* produce 4-hydroxy-3-methyl-2-alkylquinolines (HMAQ) - structurally analogous to 3,4-dihydroxy-2-heptylquinoline (the *Pseudomonas* Quinolone Signal) from *Pseudomonas aeruginosa*, a quorum sensing signal

important for the virulence of this opportunistic pathogen. The *hmqABCDEFG* operon encodes functions required to synthesize HMAQ. Based on the homologies between the PQS and Hmq systems, we hypothesize that HMAQ also play a role in the virulence of opportunistic *Burkholderia* pathogens as signaling molecules.

The prevalence of the Hmq system in Bcc, its regulation – solely studied in *B. ambifaria* strain HSJ1 - and its role are still unknown. We investigated the prevalence of the *hmqABCDEFG* operon by bioinformatic analysis and screened 223 clinical strains for their ability to produce HMAQ and express the *hmqABCDEFG* operon.

From the genome sequences available on *Burkholderia* genome database (www.Burkholderia.com), the *hmqABCDEFG*operon distribution was determined within the Bcc group. Homology and orthology analyses were complementarily used. Results of the *in silico* analyses show that (1) not all species and (2) not of all strains within these species of Bcc, carry the *hmqABCDEFG* operon. To confirm bioinformatic analyses, the presence of the *hmqABCDEFG* operon in 223 clinical strains was verified by PCR targeting *hmqA*and *hmqG*genes using degenerated primers. Ten out of sixteen tested Bcc species were found to have the *hmqABCDEFG* operon, but not all strains within a species. Then, their ability to produce HMAQ, in tryptic soy broth from overnight cultures, was assessed. HMAQ were quantified by liquid chromatography coupled to mass spectrometry. More than half of the strains, having the operon, produce HMAQ. For the strains where HMAQ production was not detected, the *hmqABCDEFG*operon expression was analyzed by RT-qPCR. None of the tested strains expressed the *hmqA*gene, supporting the hypotheses that (1) these strains should not produce unknown HMAQ and (2) the Hmq system is under complex strain-dependant regulation.

Current studies will elucidate the regulation of the Hmq system and if HMAQ are involved in Bcc virulence like PQS in *Pseudomonas*.

MGCM17

Characterization and quantification of *Pseudomonas aeruginosa* biofilms

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Pseudomonas aeruginosa is a Gram-negative opportunistic pathogen that can severely affect immunocompromised individuals, such as individuals suffering from cystic fibrosis (CF). Prolonged and chronic infection is due to the ability of *P. aeruginosa* to form biofilms within the CF lung. Biofilms consist of a community bacterium that synthesize extracellular polymeric substances (EPS) such as proteins, lipids, polysaccharides and extracellular DNA. Since there is evidence that this bacterium can form biofilms within a CF lung, there is an effort to try to understand P. aeruginosa biofilms in the context of this disease. Although there are many studies evaluating biofilms, these studies do not include a correlative approach between the quantitation of two methods of visualization of biofilm structure and proteomic analysis in parallel. Confocal microscopy will allow for visualization of these microbial communities. Using confocal microscopy will also demonstrate the localization and quantity of these EPS components. On the other hand, mass spectrometry (MS) based proteomic analysis can provide information on protein abundance and protein-protein interactions within the biofilm matrix. The primary goal of this study is to use the methods of confocal microscopy and shotgun proteomics to be able to characterize and quantify biofilms of common lab strain P. aeruginosa PAO1 and Liverpool Epidemic Strain isolates. This project will allow the visualization of differences in biofilm structure among these isolates, quantification of the different components in biofilms, and the identification of unique microbial proteomes.

MGCM18

Characterization of exclusion mechanisms in the conjugative plasmid TP114

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Various mechanisms promote genetic variability in bacteria. Bacterial conjugation is a type of horizontal gene transfer that can rapidly disseminate antibiotic resistance genes in microbial populations. Most conjugative plasmids have evolved strategies to prevent the acquisition of plasmids from the same incompatibility group. Surface and entry exclusion are mechanisms used by conjugative plasmids to avoid redundant transfers, which would result in a waste of resources and energy. Here, we present a new approach based on high-efficiency mutagenesis to identify the genes involved in exclusion using the $IncI_2$ plasmid TP114 as a model. We first validated that the transfer of TP114 to a TP114-containing recipient bacterium is decreased by over three orders of magnitude compared to an otherwise isogenic strain. Transposon mutagenesis next identified gene product TP114-05, hereafter renamed ExcI, as the main exclusion determinant in this plasmid. We next performed iterative rounds of random mutagenesis to identify mutants that can bypass exclusion by ExcI. Transfer rates gradually increased for 5 consecutives mutagenesis rounds, reaching levels equivalent between recipient cells expressing or not ExcI. The populations of mutants along with isolated clones from each mutagenesis round were sequenced to identify the specific proteins and residues mediating exclusion. This strategy could be applied to other conjugative plasmids, and can be used to rapidly identify genes that are important for various aspects of bacterial conjugation.

MCGM19

Characterization of the replicating gut bacterial community in a mouse model of colitis

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The naturally occurring microorganisms in the intestines, called the gut microbiota, are essential for host health. Mainly consisting of bacteria, the composition of the gut microbiota is altered in disease. However, little is known about how these changes arise, as changes in overall bacterial diversity only reflect total changes in bacterial replication and death. As such, it is imperative to conduct studies assessing both bacterial replication and community changes during disease development. Furthermore, bacterial replication itself is associated with the production of various microbial metabolites which are released into the gut, such as central carbon metabolism intermediates. Changes in composition or metabolites are known to affect host health; as such, gut bacterial replication is an important aspect to consider when studying the gut microbiota. However, there are no established techniques for identifying individual replicating bacterial members in a complex community such as the gut.

In this study, we aimed to address this gap by optimizing an experimental pipeline to identify the replicating gut bacteria using 5-ethynyl-2'-deoxyuridine (EdU) click chemistry coupled with flow cytometry. EdU is a thymidine analog that incorporates into replicating DNA, and fluoresces upon a copper-catalyzed reaction with a fluorescently labeled azide. Fluorescent cells (EdU^+ cells) can then be quantified using flow cytometry. As a proof of concept, we first conducted EdU labeling of three known gut bacterial isolates. We show that during exponential phase, up to 77% of bacterial cells are EdU^+ cells. We then applied our approach to whole fecal bacterial communities from healthy C57BL/6 mice, and show that up to 47% of bacterial cells are EdU^+ cells. Next, we separated EdU^+ and EdU^- cells using fluorescence-activated cell sorting (FACS) and identified the number of cells required to accurately reflect gut bacterial diversity. These findings confirm the validity of EdU labeling for identifying replicating gut bacteria.

We next aimed to determine the changes in replicating bacteria over time during the development of disease, by following EdU^+ cells in stool samples from C57BL/6 mice during DSS-induced colitis. Before, during, and after DSS administration, we isolated fecal bacteria from these mice and sorted the EdU^+ and EdU^- cells using FACS. To characterize these populations, as well as the composition of the whole unsorted community, we isolated the genomic DNA from these samples and conducted 16S-rDNA sequencing. Our results indicate changes in the replicating subset of bacteria in the gut during DSS exposure, reflecting the development of colitis.

Conducting temporal analyses of the gut bacteria as it changes from health to disease will further elucidate the microbial dynamics underlying this condition. This will deepen our understanding of bacterial activity in intestinal disease, which can aid in the development of more informed treatments.

MGCM20

Characterization of the role of the Cpx stress response pathway in the regulation of proteolysis at the inner membrane of *Escherichia coli*

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The Gram-negative bacterial cell envelope is a complex multicomponent structure that functions as a protective barrier, communicates with the external environment, and maintains the shape, stability and rigidity of the cell. Under conditions of impaired cell envelope biogenesis, bacterial cells react by activating several stress response systems to detect damage and restore homeostasis. The Cpx envelope stress response mediates adaptation to stresses caused by periplasmic or inner membrane protein misfolding. It consists of the membrane-localized histidine kinase CpxA and a cytoplasmic response regulator CpxR, as well as the auxiliary regulator CpxP. It was recently demonstrated in enteropathogenic *E. coli* that Cpx-mediated downregulation of large inner membrane protein complexes is important for adaptation to protein misfolding, specifically for the protein complexes of the electron transport chain. The Cpx response directly represses the transcription of the *nuo* (NADH dehydrogenase I) and *cyo* (cytochrome bo3) operons as part of the adaptation to stresses that compromise envelope integrity. In this research project, we sought to test whether proteolysis of these protein complexes also plays an important role in Cpx-conferred stress adaptation and identify which protein folding and degrading factors play a role. To monitor the effect of Cpx-regulated proteolytic enzymes and chaperones on levels of the NADH dehydrogenase I (NDH-I), we constructed a tagged version of the NuoA protein and showed that it complemented a Δ*nuoA* strain. In this strain background, we generated strains carrying wild type, disabled (Δ*cpxR)* or constitutively activated (*cpxA24*) Cpx stress response systems and examined growth under conditions where the cells must respire. Our preliminary data demonstrate that constitutive activation of the Cpx response diminishes growth on minimal media containing a non-fermentable carbon source. Additionally, we showed that activation of the Cpx response increased degradation of exogenously expressed NuoA and CyoA subunits and that overexpression of several Cpx-regulated proteins, including proteases DegP and HtpX together with chaperones PpiD and YccA, altered the levels of these same exogenously expressed NuoA and CyoA proteins. We propose that Cpx-mediated upregulation of proteases and chaperones that control the stability of inner membrane proteins is an important component of adaptation to envelope stress.

MGCM21

Characterization of the small regulatory RNA lpr0050 in *Legionella pneumophila*

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Legionella pneumophila (Lp) is a facultative intracellular pathogen and the causative agent of Legionnaires disease, an acute form of pneumonia, and Pontiac fever, milder flu-like illness. *Lp* is commonly found in most water systems including freshwater bodies, rivers, and lakes as well as in engineered water systems and man-made water distribution systems such as cooling towers. Free-living amoeba in aquatic environments are the primary reservoir of *Lp*. It can be transmitted to human's lung by inhalation of contaminated aerosols. Upon entry into the human's lung, *Lp. pneumophila* infect and replicate inside alveolar macrophages and potentially cause LD in susceptible individuals. The bacterium establishes its intracellular niche by forming the *Legionella*-containing vacuoles (LCVs). *L.p* governs the formation of the LCV and intracellular growth through the Icm/Dot type IVB secretion system. Icm/Dot is able to translocate around 300 protein effectors in the host cell allowing *L. pneumophila* to modulate many signalling and metabolic pathways of the host to its benefit. It is believed that Small Regulatory RNAs (sRNAs) are major players of regulation of virulence-related genes in *Lp.*

We investigated the role of the sRNA *lpr0050* which is encoded on the complementary strand of the Icm/Dot effector SdeA. Northern blot analysis revealed that this sRNA is expressed in both exponential and post exponential phases. To study the role of lpr0050, a fragment of *sdeA* complementary to the sRNA was cloned under the *Ptac* promoter. This produce an RNA complementary to the sRNA, called the trap, that can block the sRNA function by titration. The expression of the sRNA and SdeA was then measured by Northern Blot and qPCR, respectively.

We showed that the expression of the trap reduce expression of SdeA. In contrast, homologs of SdeA were induced upon expression of the trap, suggesting co-regulation of SdeA and it is homologs through *lpr0050*.

MGCM22

Characterizing the mouse gut microbiota and developing an in vitro model of the mouse colon

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It has become increasingly evident that human health is largely influenced by the gut microbiota. To study the magnitude of this influence, *in vitro* and *in vivo* models are commonly used. One of the most common *in vivo* models used in gastrointestinal and gut microbiome studies is the murine model. Mice are inexpensive, have high reproductive rates and a short life cycle. However, the murine and human gut microbiomes are known to be dissimilar, for the most part because of differences in diet, genetics and environmental factors between the mouse and human hosts. Compounding these differences, it is also known that the murine gut microbiota varies according to husbandry; even genetically identical mouse lines raised in different animal facilities tend to display different gut microbiomes. Collectively, these variations can cause studies to be irreproducible and inconclusive, and efforts to standardize the microbiome through the use of e.g. Altered Schaedler Flora have not really considered the true complexity and diversity found within the murine gastrointestinal tract, further confusing the issue. If we are to continue using murine models as a proxy for human disease, we must improve murine models by introducing a standardized, murine-derived gut microbiome of sufficient diversity to drive physiologically-relevant metabolism as well as compositional stability. This project takes the first steps towards these goals by characterizing the mouse gut microbiota, and developing an *in vitro* model of the mouse colon.

To date, we have characterized and, through microbial isolation strategies, derived defined experimental ecosystems from the gut microbiome (feces and cecal contents) of representative animals from six mouse lines housed either at The University of Guelph's Central Animal Facility or The Division of Comparative Medicine. Mouse lines investigated include: four lines of C57BL/6 origin (IL10$^{-/-}$, APC $^{min-/-}$ Msh2$^{-/-}$, C57BL/6 N/N WT, and fat-1), one line of CD-1 origin (Nephrin-Y3F), and a wild-caught mouse (*Mus musculans)* from Algonquin park.

Unsurprisingly, each animal tested displayed a unique gut microbial composition, with only three core microorganisms identified.

In order to test the metabolic outputs of these various microbial communities, we have also successfully developed *in vitro* bioreactor conditions representative of the mouse colonic environment which we show to be capable of supporting both fecal and defined microbial communities for extended periods of time. Metabolic output and microbial composition of these in vitro models were used as metrics of success.

The ultimate objective of this work is to provide a uniform reference point for mouse model work in the future, that takes into account the need for experimental reproducibility as well as translatability to human health.

MGCM24

Chemically perturbing the regulatory network for secondary metabolism in *Streptomyces coelicolor*

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Streptomyces are known for the production of antibiotics and other biologically active secondary metabolites. These secondary metabolites are produced by biosynthetic pathways encoded in discrete gene clusters. The expression of these clusters is controlled by factors that include growth rate, signaling molecules, metabolism, and physiological and environmental stresses. These diverse signals along with signal transduction proteins and transcription factors constitute a complex regulatory network that mediate various types of responses including the induction of secondary metabolism. Understanding this regulatory network is important for the discovery and exploitation of these biologically active compounds. One method of disturbing the cell's regulatory network is through the use of chemical elicitors. I am exploring the effects of the ARC2 elicitor, which we identified in a previous small molecule screen. We have shown that ARC2 induces the production of the blue-pigmented secondary metabolite actinorhodin in *S. coelicolor*. Our most recent work demonstrates the global effects of ARC2 on both primary and secondary metabolism. We also show that the ARC2 signal is relayed through two pleiotropic regulators to induce actinorhodin production.

MGCM25

Come out, come out, wherever you are! Characterizing colonic microbiome metabolites as triggers of prophage induction

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The recent surge of research into the bacterial residents of the human gastrointestinal tract has brought to light two critical findings. First, that a diverse and copious population of bacteriophages reside in the human gastrointestinal tract. Second, that a distinct lack of antagonism exists between gastric phages and their bacterial hosts. ~90% of colonic bacteriophages are temperate and demonstrate remarkable genetic stability over time. The prevalence and stability of colon prophages has evolutionary consequences. Mutualism may evolve between the two foes, as the fitness of a prophage becomes tied to its replicating host upon integration. There is now little doubt that phage populations are intimately linked to the state of the colonic bacterial ecosystem, though the degree to which gut bacteriophages may influence the healthy functioning of both bacterial and human hosts remains to be fully defined.

Classical virology states that integrated prophage are triggered to excise and replicate when host fitness is threatened. It is therefore common practice to use stressors such as mitomycin C (among other antibiotics), UV radiation, sunlight, temperature, and pressure to induce prophage excision *in vitro*. However, the mechanisms controlling prophage induction *in vivo* may be more nuanced. Dedicated colonic prophages that have evolved mutualistic relationships with their bacterial hosts may induce in response to specific biochemical triggers.

In the gut, microbe-microbe biochemical interactions are largely facilitated by the substrates and products of carbohydrate and protein metabolism. The ubiquity of these compounds and their demonstrated role in both bacterial and human host fitness make them prime candidates for speculative prophage induction triggers. The current research aims to explore how varying concentrations of several common microbiome metabolites influence the breadth and magnitude of prophage induction.

MGCM26

Coupling between bacterial physiology and mutation rate

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Bacteria are mechanistically very complicated, but when they are observed as a population and grown in conditions that allow for exponential growth, simple empirical relations emerge. The most studied of these relations is how physiological attributes, such as DNA, RNA, volume, and protein per cell, change with the growth rate. One very important physiological attribute that has not been systematically studied in this context is the mutation rate. Using Luria-Delbruck fluctuation tests one can infer the average mutation rate of bacteria based upon the distribution of spontaneous mutants that are resistant to a selective screen, such as antibiotic challenge. I perform Luria-Delbruck tests with the model organism *Escherichia coli* in different growth conditions. Special care is taken to ensure that the cells are in exponential growth for the duration of the experiment and that they are grown to the same final population numbers in order to facilitate comparison among growth conditions. Experimental data is then fitted to a stochastic model of bacterial growth with mutations in order to estimate the mutation rate. Results could give insight into how to better combat antibiotic resistance, as well as how evolutionary dynamics couple to environmental conditions.

MGCM27

Covariance analysis reveals important residue-residue contacts in the *Escherichia coli* division protein FtsK

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Cell division is a vital cellular process. In rod-shaped bacterial cells, cytokinesis is carried out by the divisome; a highly intricate, multi-protein complex. In *Escherichia coli* the divisome consists of at least 12 essential proteins, with multiple accessory proteins. It is responsible for septum formation, constriction of membranes, and synthesis of septal peptidoglycan. The divisome protein FtsK (filamentous temperature sensitive protein K), is an integral-membrane protein that coordinates cell division and chromosome segregation in *E. coli.* The N-terminal portion of FtsK ($FtsK_N$) is implicated in cell division and is of unknown structure. This project will use covariance-guided *ab initio* modeling to derive constraints on the structure of $FtsK_N$. Recently, *ab initio* folding in Rosetta using residue-residue contact prediction constraints derived from sequence co-evolution has been shown to allow predictions of protein structures where a large set of diverse homolog sequences is known. Here we will

test the hypothesis that intraprotein residue contacts, predicted by covariance analysis, can be experimentally tested by showing that deleterious mutations in one site can be rescued by a complementary mutation in the interacting residue. Recent work has identified residue contacts which disrupt cell division and could consequently play a crucial role in the structural integrity of FtsK, or mediate contacts with other divisome members. Additionally, a select number of variants display a novel *in vivo* phenotype which has never been observed previously. The approach taken in this project will give insight into the structure of *E. coli* $FtsK_N$ and the overall assembly of the divisome complex. Improving the understanding of the structural roles of each of the divisome members and how they interact together as an integrated unit, will not only further our understanding of a very fundamental process, but may also elucidate novel antibiotic targets in this essential bacterial cellular machinery.

MGCM28

Development and application of tunable CRISPRi gene silencing in *Burkholderia*

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Genetic tools are critical to dissecting the mechanisms governing cellular processes, from fundamental physiology to pathogenesis. Species of *Burkholderia* have emerged over recent decades as both important opportunistic pathogens in immunocompromised patients and as a rich source of secondary metabolites for biotechnology. With large, high GC-content genomes, there are limited tools available for genetic manipulation of *Burkholderia* species. To address this, we have developed CRISPR interference (CRISPRi) technology for gene silencing in the cystic fibrosis pathogen *B. cenocepacia* K56-2. Tunable expression was provided by placing a codon-optimized *dcas9* from *Streptococcus pyogenes* under control of a rhamnose-inducible promoter, which was validated by immunoblot. Expression from a multicopy plasmid caused a growth defect; therefore, the mini-CTX single-copy insertion system was used to deliver *dcas9* to the chromosome, resulting in wild-type growth. As a proof of concept, the *paaABCDE* operon controlling genes necessary for phenylacetic acid degradation was targeted by introduction of plasmid-borne sgRNA, resulting in near complete inhibition of growth on phenylacetic acid as a sole carbon source. This was supported by reductions in *paaA* mRNA expression. The utility to probe endogenous, poorly understood functions was expanded upon as we show for the first time in *B. cenocepacia* that knocking down *phbC* and *fliF* dramatically reduces polyhydroxybutyrate granule accumulation and motility, respectively. As a hallmark of the mini-CTX system is the broad host-range of insertion, current work includes introducing our CRISPRi system into other species of *Burkholderia*, *Acinetobacter baumannii* and *Achromobacter xylosoxidans*. In addition, we are optimizing the rhamnose-inducible promoter to increase dynamic ranges of *dcas9* expression. Future research will explore the use of CRISPRi systems to rapidly annotate genes of unknown function in under-studied organisms.

MGCM29

Development of a high-efficiency genomic integration platform for near-minimal bacteria

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The near-minimal bacterium *Mesoplasma florum* constitutes an attractive model for systems biology and synthetic biology. While sophisticated methods such as whole-genome cloning and transplantation have been developed for *M. florum*, additional genetic tools would help exploit the full potential of this microorganism. We

have designed a high-efficiency genomic integration system based on serine recombinases for site-specific addition of DNA cargo in *M. florum*. The approach requires the introduction of cognate *attB* and *attP* sequences, respectively in the genome and on the DNA molecule to integrate. We have introduced *attB* sites in *M. florum* by transposon mutagenesis, and used the fluorescent protein NeonGreen as a reporter gene using this system. We have also observed that the number of transformants obtained using this site-specific integration can reach thousand per assay. We are now engineering a specialized strain to facilitate and standardized genomic integration at specific neutral loci using this strategy. We are also builiding large libraries of synthetic promoters and will soon test their activity in *M. florum*. We will leverage this information to characterize standardized DNA parts that will be used to create and fine-tune the expression of synthetic gene circuits. High-efficiency site-specific integration will constitute an important molecular tool towards the characterization of genetics elements and the creation of a programmable chassis based on *M. florum*.

MGCM30

Development of CRISPR interference system for investigation of the role of the natural antisense transcript in *Toxoplasma gondii*

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Toxoplasma gondii is an intracellular eukaryotic parasite of the Apicomplexan phylum and is responsible for the infection that affects more than 50% of the human population. Our research focuses on the molecular mechanism underlying the pathogenesis of this unicellular parasite. In particular, we are investigating a post-translational regulation pathway known as SUMOylation which involves the addition of a small ubiquitin-like modifier (SUMO) to a protein substrate. This pathway is critical for parasite invasion and the formation of protective cyst structures in its host cells. A key enzyme of the pathway, ubiquitin-like protease 1 (Ulp1), is required for both SUMOylation and deSUMOylation. We previously showed that the gene encoding Ulp1 in *T. gondii* (*Tg*Ulp1*)* is regulated by its natural antisense transcript (*TgUlp1*-NAT). Therefore, *TgUlp1*-NAT is hypothesized to exert its regulatory effect on the SUMOylation pathway. To characterize and investigate the function of *TgUlp1*-NAT as a potential regulator of SUMOylation, we have adopted CRISPR Interference (CRISPRi) technology, which is a modified version of CRISPR (Clustered Regularly Interspaced Short Palindromic Repeat). The CRISPRi system consists of two components: the guide RNA and the dCas9 protein. The former has been designed to specifically bind target genomic sequences of *T. gondii*. The latter, as an inactive enzyme, has an affinity for the designed guide RNA but lacks ability to edit the genome. The expression of dCas9 has been constructed to be inducible upon addition of anhydrotetracycline. As a proof of concept, we have generated a transgenic line of *T. gondii* expressing green fluorescent protein (GFP) which is used with the engineered CRISPRi system specific to the promoter controlling GFP expression. It was observed that GFP expression was reduced upon induction of CRISPRi system expression. Various experimental parameters, including transformation conditions, plasmid and anhydrotetracycline concentrations, induction and drug exposure periods, are being optimized to obtain suitable conditions that ensure parasite viability at a maximal knockdown effect. Assessment of knockdown effect has been carried out by fluorescence microscopy, immunoblotting, and qRT-PCR analyses. The system will subsequently be used in the study of *TgUlp1*-NAT as a regulator of SUMOylation pathway. If *TgUlp1*-NAT is crucial for the regulation of SUMOylation, the knockdown of *TgUlp1*-NAT expression should reduce the invasion efficiency and/or the survival of the parasite within the host cells. We will discuss the results and the application of our engineered CRISPRi system.

MGCM31

Effects of alignment on contact based cell-cell interactions

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Colonies of *E. coli*, when grown in a monolayer, are known to spontaneously self-organize, transitioning from a disordered colony to one where the cells are aligned in one direction. The subsequent increase in packing efficiency has the potential to affect contact based cell-cell interactions. We used agent-based and partial differential equation (PDE) simulations to investigate the role this alignment plays in two contact dependent processes: plasmid transfer and contact-based killing via the type VI secretion system. Our agent-based simulations were carried out using the CellModeller software package, which realistically represents cell morphology and physical interactions (in contrast to simpler cellular automaton models). Alternatively, we spatially coarse-grain the system to arrive at a system of PDEs. Standard coarse-graining approaches account for local cell density, while local orientation is described using the tensor order parameter, borrowed from the theory of liquid crystals. These simulations accurately capture colony dynamics, and reveal the role of cell morphology (and consequent ordering) on colony-level cellular growth and interactions.

MGCM32

Efficient inter-species conjugative transfer of a CRISPR nuclease for targeted bacterial elimination

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The specific control of pathogenic bacteria in complex microbial communities requires new and innovative tools. Clustered Regularly Interspaced Short Palindromic Repeat (CRISPR) systems have been effective in specifically targeting bacteria, but a key problem is efficiently delivering the system to a target species of interest. We developed plasmids based on the RK2 conjugative system that can efficiently conjugate a TevSpCas9 dual-endonuclease in a two-species system from *Escherichia coli* to *Salmonella enterica*. We have shown that a *cis*-conjugating plasmid (pNuc-*cis*) encoding its own conjugative machinery conjugates more efficiently than a *trans*-conjugating plasmid (pNuc-*trans*), which requires conjugative machinery provided in*trans*. We observed increased conjugation frequencies approaching 100% for pNuc-*cis*in conditions that enhance cell-cell contact and mimic biofilms. We found that induction of the TevSpCas9 nuclease can kill *S. enterica* with varying efficiencies depending on the single guide RNA (sgRNA), and that multiplexed sgRNAs perform better as a group than their constituent sgRNAs. After evaluating several aspects of sgRNA targeting, we have found that killing efficiency was generally higher when sgRNA identity to the donor *E. coli* genome was minimized. Overall, our data provides a proof-of-principle for the use of *cis*-conjugating plasmids as a CRISPR delivery vector for the targeted elimination of pathogenic bacteria in microbial biofilms. Our data also supports the potential use of conjugation as a delivery mechanism of other microbial-altering agents to microbial populations.

MGCM33

Elucidating the molecular basis of AmpC-mediated ceftazidime/avibactam and ceftolozane/tazobactam resistance in *Pseudomonas aeruginosa*

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Pseudomonas aeruginosa is an opportunistic nosocomial pathogen notorious for causing chronic and often fatal infections in people afflicted with cancer, cystic fibrosis, severe burns, or immunodeficiencies. In fact, the morbidity and mortality rates associated with this bacterium are among the highest of all clinically-relevant Gram-negative pathogens. This is attributed, in part, to its remarkable multidrug resistance profile.

β-lactam antibiotics are among the suite of antibiotic classes against which *P. aeruginosa* evolves resistance. These drugs possess a β-lactam ring, a reactive four-membered cyclic amide that impedes bacterial cell wall synthesis by interrupting peptidoglycan cross-linking. β-lactam resistance is a consequence of chromosomal mutations that lead to rapid hyperproduction of the β-lactamase AmpC. AmpC is a particularly robust group 1, class C cephalosporinase that deactivates many β-lactam antibiotics through hydrolysis of the β-lactam ring. Since β-lactam antibiotics constitute over 50% of all clinically-used drugs, resistance to them is a major threat warranting immediate attention.

β-lactamase discovery necessitated the development of β-lactamase inhibitors such as tazobactam and clavulanic acid. Unfortunately, most β-lactamase inhibitors are ineffective against AmpC. One exception is the recently approved inhibitor, avibactam, which is co-administered with the cephalosporin ceftazidime. Ceftolozane (administered with tazobactam) is also a new front-line β-lactam effective against *P. aeruginosa*.

Recently, however, clinical isolates of *P. aeruginosa* expressing AmpC variants that are resistant to both ceftazidime/avibactam and ceftolozane/tazobactam have emerged, indicating that the evolution of reistance to these novel β-lactam/β-lactamase inhibitor combinations is already being selected for in the clinic. Specifically, our clinical collaborators (Oliver et al. 2017) recently identified three AmpC variants (T96I, E247K, and a 19-amino acid deletion, G229–E247). Not long after that, Chen et al. (2017) discovered one as well (G183D). Currently, it is unclear as to how these mutations confer resistance. I hypothesize that these mutations either prevent the inhibitor (avibactam) from binding and inhibiting AmpC or they augment the ability of AmpC to hydrolyze the antibiotic (ceftazidime or ceftolozane).

To understand the molecular basis behind this AmpC-mediated resistance, I have expressed and purified each AmpC variant (as well as the wild-type) and am currently crystallizing them in order to visualize their 3D structures by protein X-ray crystallography. Molecular models of these variants bound to the antibiotics and inhibitors will be subsequently generated to gain structural insight into how their mutations confer resistance. In parallel, the Michaelis–Menten parameters of each variant are being determined to elucidate the effect of each mutation on the ability of AmpC to hydrolyze ceftazidime or ceftolozane, and on the inhibition of AmpC by avibactam as described. To contextualize the effect of each mutation on the propensity of AmpC to evade the drug/inhibitor combinations, the structural and kinetic data of each variant will be compared to that of the wild-type AmpC enzyme. The knowledge gained from this study could inform new derivatives of these compounds that are less susceptible to mutation-based resistance.

MGCM34

Entry exclusion of conjugative plasmids of the IncA, IncC and related untyped incompatibility groups

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Over the past 50 years, the emergence and spread of multidrug resistant bacteria have considerably increased globally in both environmental and clinical settings. This phenomenon is greatly facilitated by conjugation, a mechanism of horizontal gene transfer that allows the directional translocation of DNA between two bacterial cells in direct contact through a type IV secretion system (T4SS). Conjugative plasmids of the IncC incompatibility group (formerly known as A/C_2) spread antibiotic resistance genes in several species of *Enterobacteriaceae* and *Vibrionaceae*. They can also mobilize different families of mobile genomic islands, such as the multidrug resistance-conferring *Salmonella* genomic island 1 (SGI1). SGI1 was shown to reshape the T4SS encoded by these plasmids and consequently evade IncC entry exclusion, a mechanism that blocks DNA translocation between cells bearing identical or highly similar plasmids. Entry exclusion is an essential feature of conjugative plasmids, as it protects them from loss due to incompatibility, and prevents futile energy consumption during transfer between two donor cells. Here, we investigated the entry exclusion system of IncC plasmids and its interactions with SGI1. Using a collection of deletion mutants in mating assays, we identified EexC as the entry exclusion factor in the recipient cell. Its interacting partner in the donor cell is $TraG_C$, an IncC-encoded T4SS component. SGI1-mediated substitution of $TraG_C$ by $TraG_S$ abolishes IncC entry exclusion mediated by EexC, thereby enabling transfer of SGI1 between two cells containing an IncC plasmid. Phylogenetic analyses and experimental validation showed that IncC, IncA and related untyped conjugative plasmids encode EexC and $TraG_C$ homologues that are divided in three entry exclusion groups. IncC and IncA plasmids belong to the C exclusion group, while untyped conjugative plasmids isolated from *Aeromonas* sp. form two other exclusion groups (D and E). Substitution of fragments of EexC with corresponding fragments of the homologous protein EexE encoded by the untyped conjugative plasmid pAsa4c of *Aeromonas salmonicida*, showed that the C-terminus of these proteins determines the selectivity of the entry exclusion group.

MGCM35

Evading antimicrobial resistance: Using synthetic organophosphorus compounds to slow evolution

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Antimicrobial resistance has become a global health crisis and is expected to become much worse in the coming years. New antibiotics are not being developed at a rate fast enough to combat antimicrobial resistance. To make matters worse, almost all antibiotics are derived from the natural environment in one form or another. Therefore, bacteria have evolved alongside these molecules for millions of years, which has selected for resistance mechanisms. My research focuses on synthetic organophosphorus molecules as a means to combat antimicrobial resistance. These synthetics are of particular significance because they do not occur naturally in the environment. This means they will likely resist evolutionary processes because they are foreign to bacteria. Initial studies indicate some of these molecules are highly potent in vitro (2 µg/ml) in inhibiting a variety of gram positive pathogens and are not acutely toxic to mice. In my work, I have tested and characterized many new organphosphorus molecules. Photosensitivity, minimum inihibitory concentrations, and mutagenic properties have been assessed in all molecules showing antimicrobial activity against a human pathogen.

MGCM36

Examining the intraspecific interactions in mixed-strain biofilms containing the prairie epidemic strain and unique clinical isolates of *Pseudomonas aeruginosa*

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Pseudomonas aeruginosa is the predominant species causing chronic lung infections in adult patients with the genetic disease cystic fibrosis (CF). *P. aeruginosa* can form biofilms that provide protection from host immune- and antibiotic-challenges, allowing it to persist in the CF lung environment. Previously, it was believed that CF patients acquire unique *P. aeruginosa* strains from their environment, with no concerns of transmissibility. However, strains that are highly transmissible among CF patients have recently been discovered. One example, the prairie epidemic strain (PES), was identified in CF patients throughout the prairie provinces of Canada. Importantly, the PES was found to be capable of replacing less virulent unique *P. aeruginosa* isolates pre-existing in the lungs of CF patients.

A high-throughput biofilm formation assay was used to evaluate multi-isolate biofilms produced by concurrently inoculating a 96-well microtiter plate with equal amounts of PES isolates and unique clinical *P. aeruginosa* isolates that were either stable or replaced by the PES. The resulting biofilms after 24h were stained with crystal violet, which was then solubilized and the absorbance at 590nm was determined. The amount of absorbance is positively correlated to the amount of biofilm produced. This screen identified a unique replaced isolate that promotes biofilm formation, and a unique stable isolate that inhibits biofilm formation, when cultured with the PES. To deduce the type of intraspecific interaction(s) occurring, digital droplet PCR (ddPCR) was used to quantify the isolate concentrations within the biofilms using a subtractive-ddPCR approach where one primer set determines the total number of *P. aeruginosa* cells in the sample, while a second primer set amplifies a region unique to the PES. The number of unique clinical *P. aeruginosa* cells are then determined by subtracting the concentration of PES cells from the total number of cells ([All *P. aeruginosa*] – [PES] = [Unique Clinical Isolate]). Fluorescence microscopy was also used to visualize the co-culture biofilms in a glass-bottom 96-well microtiter plate after a 24h incubation period. This allows us to verify the ddPCR results and determine the spatial arrangements of the isolates within the biofilm. This study will provide insight into CF-relevant intraspecific interactions and strain-replacements, which may have implications in the treatment of bacterial lung infections.

MGCM37

Generation of a large set of small plasmids for synthetic biology

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Plasmids are circular, self-replicating and stable extrachromosomal units of double-stranded DNA; they can be found in bacterial cells and occur in some eukaryotes. They often carry genes that provide bacteria with genetic advantages such as resistance to antibiotics and can be transferred between cells. Synthetic plasmids constitute an important tool in molecular biology and are used as a vector to insert genes into bacteria through recombinant technology. Plasmids are usually between 2000 to 10,000 base pairs, and their size is often increased by the presence of "junk" DNA sequences that are not essential for their function. Transformation efficiency being proportional to plasmid size, this junk DNA is detrimental to the generation of large DNA libraries necessary for protein engineering, synthetic biology and biotechnology efforts. Therefore, we propose the creation of a set of small plasmids which only contain the sequences necessary for their maintenance in the bacteria. The most common method for plasmid construction is ligation of DNA fragments of interest via DNA ligase following

digestion with restriction enzymes. Due to its flexible design and efficiency, we instead used Gibson assembly to construct our plasmids from 4 pre-designed DNA fragments (modules) that can be easily mixed and matched to expand the variety of possible plasmids. A set of approximately 30 pUdO plasmids will be assembled using 4 origins of replications with different incompatibility groups, and 7 selection markers. The design also includes transcriptional terminators, universal primers, and a versatile multicloning site that include type IIs restriction site for Golden Gate cloning. Plasmids were screened by colony PCR. Positive clones were confirmed by Sanger sequencing and stored. These plasmids will be made publicly available for widespread use in downstream applications.

MGCM38

Generation of a *Streptomyces venezuelae*-derived chassis strain and investigation of global repressor Lsr2-deficient *Streptomyces* species

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The antimicrobial resistance crisis is implicated in upwards of 700,000 deaths a year worldwide, and this number is estimated to rise to 10 million by the year 2050. Historically, new antibiotics were discovered by isolating compounds directly from cultures of microorganisms. Indeed, *Streptomyces* bacteria produce approximately two-thirds of all naturally-derived antibiotics discovered to date. A major challenge associated with this discovery strategy is the majority of the gene clusters directing the production of novel antibiotics are not expressed under laboratory conditions. We are working to develop complementary solutions to this production barrier. One approach involves employing a surrogate host, or chassis strain, for the heterologous over-expression of predicted, novel biosynthetic gene clusters. The second involves genetically manipulating *Streptomyces* strains of interest, to relieve the repression on antibiotic biosynthetic clusters.

The development of a *Streptomyces venezuelae*-derived chassis strain is a major goal of this work, and this requires removing the endogenous biosynthetic gene clusters. Minimizing the native biosynthetic activity of our chassis strain is expected to both reduce competition for precursor molecules and limit endogenous antibiotic activity. As our parent strain, we employed a strain of *S. venezuelae* in which a global repressor of secondary metabolism, Lsr2, was removed. This was expected to alleviate repression of any heterologously introduced cluster. We have gone on to delete multiple biosynthetic clusters from this strain, and in moving forward, are prioritizing those with the highest level of expression for subsequent deletion from the genome.

In tandem, we have been screening through a library of wild *Streptomyces* species, in which Lsr2 activity has been knocked-down, for new antibiotic activity. These genetically engineered strains were tested for antibiotic activity against a range of indicator microorganisms, including but not limited to *Saccharomyces cerevisiae* (fungus), *Micrococcus luteus* (Gram-positive bacterium), and *Escherichia coli* (Gram-negative bacterium). It follows that any of these wild *Streptomyces* species demonstrating new antibiotic activity upon Lsr2 knock-down would be interesting candidates for further investigation.

MGCM39

Genetic regulation of the locus of heat resistance in *Escherichia coli*

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Escherichia coli are exposed to diverse stressors including oxidative stress, osmotic stress, and high temperature, and have acquired resistance mechanisms. Stress resistance of *E. coli* may particularly compromise control of this organism in food processing. Heat resistance of *E. coli* is mediated by a genomic island called the locus of heat resistance (LHR), which consists of 16 predicted open reading frames. The LHR occurs in high frequency in *E. coli* isolated from beef, raw milk cheese, and chlorine treated wastewater; the genomic island contributes to heat, high pressure, and chlorine resistance. Recent studies elucidated the role of the LHR on the survival under different types of stress but the regulation of LHR-encoded genes in response to stress remains unclear. This research aimed to investigate the regulatory mechanisms of the LHR. Previous studies predicted that EvgA, which is part of a two-component system with EvgS, is a regulator of the LHR because it induces *yfdX* expression and the LHR contains two hypothetical proteins from the YfdX family. To confirm the role of EvgA, EGFP was expressed under the control of an LHR derived promoter fusion in *E. coli* MG1655 and an EvgA deficient derivative. Deletion of *evgA* decreased fluorescence compared to the wildtype. Overexpression of EvgA/EvgS in LHR positive strains decreased heat resistance of the strains, which further support that EvgA/EvgS affects the expression of LHR and consequently heat resistance. CNNPromoter was used to predict six additional promoter sequences regulated by Crp, OmpR, PurR, FadR, RpoD or OxyR within the LHR. Overexpression of these regulatory proteins using plasmids from the ASKA library and measuring gene expression using quantitative RT-PCR of downstream genes from the predicted promoters were used to determine promoter activity. Crp, OmpR, PurR, FadR, and RpoD increased the expression of LHR genes. The role of RpoD in LHR expression is consistent with the heat resistance of exponentially growing LHR-positive strains of *E. coli.* A role of OxyR in the regulation of the predicted promoter confirmed by quantifying expression of the downstream gene, *trx,* in response to $H_2O_2$and chlorine, two known inducers of the OxyR regulon. Confirmation of the role of Crp, OmpR, PurR, and FadR in regulation of LHR-encoded genes remains subject to future investigations. In conclusion, the LHR is regulated by multiple regulatory proteins, which accounts for its high expression at diverse growth and stress conditions and matches its protective role against multiple stressors.

MGCM40

HcpE and DsbK effects on the pro-inflammatory immune response

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Introduction: *Helicobacter pylori* (*HP*) chronically colonizes half the world population, causing gastric inflammation that can lead to gastric ulcers and cancers. Efficient control of *HP* infections is key to treat gastric ulcers and prevent gastric cancers. HcpE is a Helicobacter Cysteine-rich Protein secreted outside of *HP* which contains Sel-Like Repeats (SLR) signatures for protein / protein interactions that are involved in signal transduction across kingdoms. HcpE may affect *HP*'s virulence via interaction of its SLRs with host proteins. Moreover, DsbK (DiSulfide Bond forming protein K) is vital for HcpE's secretion outside *HP* and is crucial for disulfide bond formation in HcpE.

Hypothesis and Objective: HcpE, and its gate keeper DsbK, are important for host/bacterium interactions and hindering their activity or production might alleviate gastric colonization and inflammation. Thus, our aim is to identify the pro-inflammatory effects of HcpE and DsbK and identify a host interacting protein partner with HcpE, using human adenocarcinoma gastric cells (AGS).

Methods: To assess the pro-inflammatory effects of HcpE and DsbK, AGS cells will be exposed to wild-type *HP*, and *hcpE*- and *dsbK*- knock out mutants, and their complemented syrains. IL8 pro-inflammatory cytokine released by AGS cells will be assessed by ELISA. Also, bacterial culture supernatants and recombinant HcpE will be used to avoid signaling from other bacterial components. Immuno-Electron Microscopy (IEM) will be used to demonstrate the intracellular location of HcpE; as such, AGS cells will be exposed to each strain, fixed, thin

sectioned, incubated with anti-HcpE serum, followed by protein-A bound to gold. Finally, to identify interacting partners for HcpE in gastric cells, we will implement *in situ* biotinylation of interacting partners by BirA ligase using a HcpE-BirA fusion transfected in AGS cells. The biotinylated partner will be pulled down using streptavidin beads and identified by Mass spectrometry.

Results: There was significant HcpE- and DsbK-dependent stimulation of IL8 secretion when AGS cells were exposed to bacterial supernatants. In addition, our preliminary IEM data showed significant intracellular HcpE labeling for AGS cells incubated with wild-type *HP* or transfected with HcpE (positive control) but not for AGS cells alone. Furthermore, we have detected by Western blot (WB) biotinylated protein/s as potential partner/s for HcpE, which are yet to be analyzed by mass spectrometry.

Discussion and Conclusion: The HcpE- and DsbK-dependent stimulation of IL8 secretion from AGS cells elucidate their roles as stimulants of the immune response. In the absence of DsbK, HcpE is not be secreted outside the bacteria and can not thus be able to interact with a host partner protein. Moreover, detecting intracellular HcpE in AGS cells supports that HcpE reaches the host cell's cytoplasm; thus, the search for an intracellular partner is warranted. Finally, a biotinylated protein detected by WB could potentially be a host interacting partner of HcpE. These results suggest that there might be some interaction between *HP* and host proteins. Discovering HcpE's interacting partner may yield a better insight of HcpE's mechanism of action and will highlight host cellular pathways that could be used to prevent HcpE-mediated effects of *HP* infection.

MGCM41

Hide and seek with bifidobacteria: Improving microbial profiling techniques in the infant gut microbiome

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Bifidobacterium species such as *B. longum*and *B. breve* are dominant members of the infant gut microbiome, comprising up to 90% of bacterial profiles in some young infants. *Bifidobacterium* species are the first colonizers of the gut and are important for the development of the immune system, host metabolism, and protection from pathogens. Shotgun metagenomics can help us understand the role of bifidobacteria within microbial communities over time through inference of community diversity and overall function, however, the infant gut microbiome poses unique challenges for these methods. We hypothesize that the high amount of diversity in closely related bacterial species, such as the bifidobacteria, is an obstacle to good metagenomic assembly. In this project we investigate potential errors in metagenomic data assembly that would lead to the drastic underestimation of bifidobacteria in the infant gut and propose to optimize these methods to allow us to make an effective analysis pipeline, specific to the infant gut microbiome. In addition to N50 scores, we relied on Illumina 16S rRNA gene profiles and culture-based techniques to validate the metagenomic assembly results.

Three stool samples from breastfed infants were used to obtain shotgun metagenomic sequencing data, 16S rRNA gene profiles, and cultured bacterial isolates. Taxonomic profiles generated for each sample were the same between culture-based, 16S rRNA gene profiles and unassembled metagenomic data but inconsistent with assembled metagenomic profiles. While bacteria of the genus *Bifidobacterium* were observed as dominantly present in culture, 16S profiles and raw metagenomic reads, no *Bifidobacterium* species were found in assembled metagenomic profiles generated with multiple *de novo* assemblers. Twenty additional public infant gut metagenomic datasets were obtained to understand the impact of bacterial strain diversity on analysis techniques. Multiple genome assembly and annotation techniques were compared to interpret and improve the considerable discrepancies in the presence of the bifidobacterial strains, and development of a final analysis

pipeline for infant gut microbiome data is underway. The results from this study will have implications for the methods currently utilized to analyze metagenomic data from the microbiome, in addition to contributing to the body of knowledge pertaining to the diversity of bacteria in the infant gut.

MGCM42

Host-plasmid interactions in antibiotic resistant *Escherichia coli*

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Antimicrobial resistance (AMR) is a major public health concern with the potential for both massive economic and human costs. While traditionally thought of in terms of chromosomal mutations, AMR can be conferred by the gaining of a plasmid encoding AMR genes, and the ability for plasmids to transfer between bacteria horizontally is problematic for the spread of resistance. However, these elements may be costly in the absence of antibiotic selection, although much work done to suggest this uses common laboratory strains and vectors, rather than those more commonly responsible for clinical infections. Further, this cost can be mitigated by the interactions between host and plasmid, such that one plasmid on one genetic background may produce a cost with a certain plasmid but no cost in another background. Here, we constructed various host-plasmid combinations using clinically-relevant strains of *E. coli* and measured their fitness and final antibiotic resistance. We find that the plasmids produced a slight average relative fitness cost of 0.97±0.05. Further, we determined that there was a statistically significant interaction among the host-plasmid combinations using a two-way ANOVA ($F(19,65)=6.295$, $p<0.001$). We were then interested in seeing if this interaction would apply to the fold change in antibiotic susceptibility, measured using a minimum inhibitory concentration (MIC) assay using ampicillin, a drug which each of the clinical plasmids conferred resistance to. Here, an interaction was observed between the host-plasmid combinations, but to a lesser extent than that found for fitness ($F(14,24)=2.769$, $p=0.0137$). However, we note that host genetic background appears to most strongly influence this relationship, and that the change in resistance may be a result of diminishing-returns epistasis. We conclude here that host-plasmid genetic interactions play a role in determining the fitness and resistance outcomes in these strains of *E. coli*. This agrees with other studies which suggest that this interaction occurs, but this study suggests this relationship also applies to more clinically-relevant bacteria.

MGCM43

How Streptomyces explorers move and what they need to do it

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Streptomyces are soil bacteria with a complex and well-defined life cycle. Recently, however, we discovered that in response to glucose depletion and amino acid abundance, Streptomyces can deviate from theiris classical life cycle and exhibit a novel mode of growth termed ‘exploration’. Our work is focussing on understanding the conditions that influence exploration in Streptomyces venezuelae, specifically focussing amino acid effects.

Exploration requires abundant amino acids. We have discovered that exploring colonies behave in drastically different ways depending on which amino acids are supplied. Our data further suggest that exploring colonies have different amino acid preferences depending on how long they have been growing, and that explorers can sense and respond to different amino acids, and direct their growth towards preferred compounds. We are working to understand how amino acids are being differentially utilized and how this leads to unique behavioural effects.

We have also been investigating exploration in a broad developmental context, to determine whether exploration is a terminal phenotype. We have probed whether it is possible to switch between exploration and more traditional developmental trajectories. We have determined that S. venezuelae can switch back and forth between growth strategies when nutritional conditions change.

The ultimate goal of this work is to elucidate the role of exploration in the environment and to further understand how exploration differs from conventional Streptomyces development at a mechanistic level.

MGCM44

Identification of a novel mechanism of surface-induced expression of RsmZ in *Pseudomonas aeruginosa*

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Pseudomonas aeruginosa is a versatile bacterium mainly studied as an important opportunistic human pathogen. It is characterized by its vast number of virulence factors, antibiotic resistance mechanisms and regulatory pathways encoded in its genome. One such system is the Gac/Rsm pathway, directed by the GacS/GacA two-component system. Following activation from the binding of a yet-unknown signal, the sensor kinase GacS activates the response regulator GacA, promoting the transcription of the small non-coding RNAs (sRNA) *RsmY* and *RsmZ*. Both sRNAs will bind to and sequester the global post-transcriptional regulator RsmA, which acts mainly as a translational repressor of mRNA involved in the adoption of a sessile lifestyle, such as biofilm formation. The Gac/Rsm pathway stands as the main player in the motile-to-sessile transition and plays a great role in the pathogenesis of *P. aeruginosa*. Previous results from our lab showed that a double *hptB-gacA*- mutant of *P. aeruginosa* PA14 overexpresses *RsmZ* specifically when grown on a surface, indicating the existence of a GacA-independent pathway that can induce surface-only expression of *RsmZ*. With the aim to identify unknown pathways capable of modulating *RsmZ* transcription, a screening using Tn*5* transposon mutagenesis was conducted. Transposants were identified in multiple loci, but only an insertion in the glycosyl transferase FgtA affected *RsmZ* expression in a surface-dependent manner. Results suggest a complex multifactorial control of *RsmZ* and brings us closer to a better understanding of the surface adaptability of this important human pathogen.

MGCM45

Identification of genes involved in autoagglutination and biofilm formation in *Escherichia coli* strains O157: H7

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Enterohemorrhagic *E. coli* (EHEC) O157:H7 strains are important foodborne human public health pathogens. These bacteria have a higher capacity to form biofilm, allowing their persistence in different environments. Among O157:H7 strains of seropatothype A, Sakai reference strain displays a distinctive capacity of autoagglutination and strong biofilm formation. The aim of this work is to identify genes involved in this phenotype, in Sakai strain as a model.

A transposon mutant library was generated in Sakai and the insertion sites were identified by high-throughput sequencing of a pool of 22 non-agglutinating and weak-biofilm-forming mutants. *csgB* and *csgG* genes involved in curli formation, were highly represented. Complementation was performed using the pTrc99a expression plasmid. Individual mutants and complements were evaluated for biofilm formation and autoagglutination, as well as colony morphotype and yeast mannose-independent agglutination. *csgB* and *csgG* mutants displayed less biofilm and non-autoagglutination phenotypes, as well as the loss of mannose-independent yeast agglutination. While Sakai colonies exhibited an RDAR (red, dry and rough) morphotype, the mutant colonies showed a SAW (smooth and white) morphotype.

The complementation of *csgG* mutant restored the wild phenotype, with a strong autoagglutination and biofilm formation, RDAR morphotype, and mannose-independent yeast agglutination.

Also, the relative expression of curli genes was evaluated by qRT-PCR in Sakai 24h biofilm compared to that of weaker biofilm producer strain EDL93. *csgA, csgD* and *csgG* genes were significantly over-expressed in Sakai biofilm. In addition, eleven isolates O157:H7 were tested for the curli formation, and the Sakai-like strains were high curli producers, while EDL933-like strains were low curli producers. These results suggest that the production of curli amyloid extracellular fibers is involved in the autoagglutination and strong biofilm formation phenotype in a subset of O157:H7 strains and it could be an interesting target to counteract the persistence of these bacteria in natural and industrial environments.

MGCM46

Identifying prophage-mediated responses to bioactive compounds

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Bacteriophages (bacteria-specific viruses) almost certainly play a role in regulating the bacterial microbiota. Many are temperate phages, which can lie dormant in their hosts, a ticking time bomb waiting to be induced by an external signal. This external signal is conventionally associated with the activation of the bacterial host's SOS response. Recent studies show phages sensing other signals as they can respond to nutrient availability, can eavesdrop on their host's quorum sensing signals and even directly communicate with other phages. This ability of phages raises the question: what else can they sense? Screening the bioactive compound library at the McMaster HTS facility to induce two very different dormant phages, HK97 and Mu, uncovered new triggers of induction. The non-overlapping list of compounds inducing each of the two model phages indicates a clear phage-mediated response to the compounds, as the bacterial genomes are essentially identical except the temperate phage. The 185 of HK97-inducing compounds range from the expected fluoroquinolones and β-lactams, but also surprising anti-inflammatory and neurological drugs. By contrast, 6 of the bioactive compounds tested induced Mu, a phage previously considered to be uninducible. These results may lead to us to the discovery of currently undetectable phages, as well as offer clues as to what systems and signals are modulating the human microbiome in a phage-mediated manner.

MGCM47

In the pits with pap: Translational coupling of a bacterial phosphate uptake system

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Phosphate (PO_4^{-3} or Pi) is an essential molecule necessary for sustaining life and it plays important roles in nucleic acid and cell membrane integrity. However, phosphate is found in growth-limiting concentrations in most environments. Bacteria have developed a diverse set of transport systems to uptake and scavenge phosphate from their environment for use in cellular processes. In the soil bacterium, *Sinorhizobium meliloti,* one such Pi transport system is the Pap-Pit system. Pit is a membrane transporter for Pi, and is associated with a cytosolic protein of unknown function known as Pap. Interestingly, the stop codon of *pap* overlaps with the start codon of *pit* by a single nucleotide. The *pap* gene appears to be required immediately upstream of *pit* in an operon for functional Pi transport. Thus in *pap* mutant backgrounds, when *pap* is present *in trans*, there is no Pi transport. This suggests a possible translational coupling mechanism between Pap and Pit, in which the translation of Pap is required for the translation of Pit. Here, a *gusA* translational reporter encoding the enzyme β-glucuronidase was fused to Pit as a measure of its translation and to understand the role of translational coupling in the Pap-Pit system. Various mutations in both *pap* and *pit* have also been constructed in an attempt to determine the function of Pap in Pi uptake.

MGCM48

Integrative and systems biology of the *Borrelia* species and Lyme disease complex in a One Health perspective

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Lyme disease caused by the spirochete *Borrelia burgdorferi* has become a global public health problem by rapid expansion and a medical challenge in long term human and animal therapy. This emerging infection is expanding rapidly in Canada. Climate change and rapid perturbation of forest ecosystems is facilitating the migration of rodents and ticks, the main arthropod vector infecting humans and other animals. We are using a global integrative and systems biology approach to study the spirochetes *Borrelia burgdorferi*, the causative agent of Lyme borreliosis and other *Borrelia* species. A collection of 30 genomes from *B. burgdorferi*, *sensu stricto, garnii, afzelii, americana, andersonii, bissetii, carolinensis and kurtenbachii* were analyzed by whole genome sequencing (WGS) using Illumina MiSeq. A subset of representative species done with long reads WGS using Oxford Nanopore and hybrid assemblies gave unique circular chromosomes of 0.9 Mb and linear and circular plasmids (6-21, depending on species). An automated integrated pipeline was developed for the study of *Borrelia* phylogeny, genome evolution, antibiotic resistance, the pan-genome and the gene-encoding proteins of the secretome. Metadata, including phenotypic as well as genomic data for isolates of the collection are provided through the *Borrelia* systems biology database (SystBorr) available online. Our data will be used to draw potential links between strains found in the human host, in different tick species and in other animals. The ultimate goals are to 1) understand how *B. burgdorferi* and other *Borrelia* species evolves over time, 2) improve the accuracy of diagnostics, 3) develop control methods in the field, 4) identify prognostic markers for vaccines and evidence-based decisions in epidemiology and surveillance in a one-health perspective.

MGCM49

Investigating changes to the *Pseudomonas aeruginosa* proteome upon exposure to antibiotics

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Pseudomonas aeruginosa is a Gram-negative bacterium known for causing antibiotic resistant infections in immunocompromised patients, including chronic lung infections in individuals with cystic fibrosis (CF). *P. aeruginosa* possesses a number of intrinsic mechanisms of resistance that allow it to survive treatment with antibiotics. In recent decades, epidemic strains of *P. aeruginosa* have been discovered and have been suggested to have higher levels of antibiotic resistance than non-epidemic *P. aeruginosa* strains. We aim to use proteomics to identify the different responses to antibiotic treatments between clinical epidemic isolates and laboratory strains. Our work has focused on isolates of the Liverpool Epidemic Strain (LES) of *P. aeruginosa*. This epidemic strain was first discovered in the UK, and has since been found in individuals with CF in Ontario. Uncovering the molecular mechanisms responsible for higher levels of resistance in these clinical isolates could lead to improved treatments for CF lung infections.

To determine conditions for our proteomics experiments, we first conducted minimal inhibitory concentration (MIC) assays to characterize the levels of resistance in eight LES isolates and the laboratory strain PAO1. Our results confirmed that many of the LES isolates show higher levels of resistance to various classes of antibiotics compared with the laboratory strain PAO1. A subset of the LES isolates showed increased levels of resistance particularly when treated with β-lactam antibiotics, which target the cell wall. Our proteomics experiment will compare PAO1, two LES isolates that showed increased resistance to β-lactam antibiotics, and one LES isolate susceptible to β-lactam antibiotics when treated with three β-lactam antibiotics, the non β-lactam antibiotic tobramycin, and H_2O_2 as a stress response control. To determine the time points and antibiotic concentrations for proteomics samples, time kill assays were performed for the four strains and five treatments. Bacterial cultures were exposed to various concentrations of each treatment and CFU/mL determinations were made at 0, 1, 2, 3, 4, 6, and 20h. Preliminary samples of PAO1 untreated or treated with the β-lactam aztreonam for 4h have been analyzed by HPLC-MS/MS, which successfully identified 1695 proteins in these samples. Upon exposure to aztreonam, 342 proteins showed differential levels of expression, with 177 proteins significantly increased in the aztreonam samples and 165 significantly decreased compared with the untreated PAO1. Analysis of all proteomics samples will allow us to determine how the clinical isolates differ in their responses to antibiotic treatments. We will then further characterize proteins of interest identified using proteomics to determine their role in the antibiotic resistance of clinical epidemic isolates.

MGCM50

Investigating several putative mitochondrial pumps in *Saccharomyces cerevisiae*

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Resistance to antifungal drugs is an increasingly significant clinical problem. The most common antifungal resistance encountered is efflux pump-mediated resistance. There are two main families of efflux pump proteins in fungi, the ATP-binding cassette (ABC) and major facilitator superfamily (MFS) transporters. Members of theMFS are more prevalent than the ABC multidrug transporters among *S. cerevisiae* and clinically important fungi. Moreover, MFS of the non-clinical *S. cerevisiae* have homologues in the clinically important fungi. On the other hand, bacterial MFS efflux pumps have associations with resistance to antibiotics. Surprisingly enough,

even though fungi can withstand various environmental selective pressures due to their ecological, morphological and biological plasticity, there is no established association of antifungal resistance with fungal mitochondrial MFS. The xenobiotics, antibiotics, fungicides, pesticides and toxic chemicals in their natural habitat might have selectively favored the fungi to develop efflux pumps in mitochondria. More importantly, clinical fungal mitochondria might have drug efflux pumps, as current classes of drugs against animal and plant fungal infections also include drugs targeting mitochondria. Here,we have hypothesized that fungal mitochondria will contain efflux pumps of the major facilitator superfamily (MFS) that could be involved in antimicrobial resistance. We are interested in investigating this phenomenon in fungal, plant and animal mitochondria. In one approach, we are interested to express a set of *S. cerevisiae* Polyamine transporters (TPOs) and Hexose transporters (HXTs) of the MFS, found to be localized in mitochondria, in a bacterial expression system. Among the polyamine transporters TPO1 is the most potential candidate for mitochondrial drug efflux, since, a number of studies have already showed TPO1 to be a multidrug efflux pump localized in the plasma membrane. Moreover, its expression conferred resistance to herbicide (2, 4 D), Cycloheximide, Caspofungin, Quinidine and Non-steroidal anti-inflammatory drugs (NSAIDS) such as Indomethacin. A strategy for cloning and expressing these genes in *E. coli* has been established and a yeast-*E. coli* shuttle vector has been developed and is being used for recombination-based cloning of potential pump genes. Following optimization of expression in *E. coli*, we expect to carry out the functional analysis of these putative pumps to determine the specific substrates, including antibiotics, antifungal and herbicides, and to study the structure of these pumps as potential antifungal therapeutic targets. If successful, we anticipate our study to be a starting point for more sophisticated in-vivo models to look into mitochondrial drug accumulation and efflux mediated resistance in fungi. Understanding mitochondrial efflux of drugs and the regulation of proteins involved may be important factors towards the development of new antifungals and anti-cancer drugs.

MGCM51

Investigating the genetic determinants for *Escherichia coli* group 2 capsule assembly

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Many Gram-negative bacteria are coated in a protective layer of high molecular weight polysaccharides called a capsule. Capsular polysaccharides (CPSs) are firmly attached to the cell surface and play a role in cell survival and virulence within the host, by allowing evasion of immune defenses. *Escherichia coli* group 2 CPSs are synthesized by an ATP-binding cassette (ABC) transporter-dependent pathway, which is a strategy shared by many important human pathogens. The biosynthetic and export machinery for this system is encoded by the *kps* locus and is generally well-defined, but neither the essential cellular (“housekeeping”) components that support CPS biosynthesis, nor the global genetic connectivity of this pathway, have been established. High throughput genetic screens offer an unbiased approach and allow the discovery of gene interactions that can not be inferred from existing knowledge. By transferring the *kps* locus into the Keio collection of mutations in all non-essential *E. coli* genes, a diverse set of genes required for proper CPS biosynthesis was identified. This gene set includes uncharacterized genes, and genes involved in other cell envelope processes like enterobacterial common antigen (ECA) biosynthesis and the multidrug efflux pump component, TolC. The influences of these CPS-essential genes on the biosynthesis pathway are being investigated to elucidate the complex interactions of CPS assembly with other cell processes. Given the attenuated virulence of unencapsulated mutants in a murine model, the CPS biosynthesis, export, and regulation machinery in an attractive target for therapeutics. This study seeks to provide an unprecedented level of understanding of capsule assembly as a foundation for these "antivirulence" strategies.

MGCM52

Investigating the potential use of diketopiperazines as anti-virulent agents in combination with antibiotics to treat *Burkholderia cepacia* complex infections

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Opportunistic pathogens, particularly species within the *Burkholderia cepacia* complex (Bcc), pose a serious threat to patients with cystic fibrosis (CF) as infection can lead to rapid lung tissue deterioration and the development of fatal bacteremia. Their ability to form highly tolerant biofilms is a major contributing factor to antibiotic resistance and chronic persistence within the lungs. Treatment options to combat *Burkholderia* infections are therefore limited; and with the increasing emergence of multidrug resistant strains, alternative therapeutic methods are needed. The use of anti-virulent agents, such as diketopiperazines (DKPs), offers a promising new approach for controlling bacterial infection by decreasing the virulence phenotype, including biofilm production. DKPs are a highly diverse class of cyclic dipeptides shown to have many important biological functions, including the ability to interfere with a major virulence regulatory system known as quorum-sensing. This study focuses on investigating the effect of DKPs on the formation and treatment of *B. multivorans* biofilms by measuring viable cell counts and total biofilm biomass. DKPs cyclo(ala-val), cyclo(pro-val), cyclo(leu-pro) and cyclo(phe-pro) were shown to have no effect on biofilm formation of various *B. multivorans* clinical isolates gown in the Calgary Biofilm Device. However, the addition of DKPs was shown to enhance antibiotic treatments of *B. multivorans* biofilms, suggesting a possible synergistic effect. Our results indicate that this effect may be a result of changes in the structural integrity of the biofilm matrix. Whether these combination treatments hold clinical significance *in-vivo* is still yet to be determined. Together these findings offer insights into the potential use of DKPs as anti-virulent agents in alternative treatment methods of *Burkholderia* infections.

MGCM53

Investigating the predator-prey relationship between *Bdellovibrio* and virulent *Fusobacterium nucleatum*

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Bdellovibrio and like organisms (BALOs) are relatively small bacteria that prey upon other Gram-negative bacterial cells. BALOs are highly motile, enabling them to hunt down their prey with persistence. Once they perforate the prey cell's outer membrane, BALOs grow within the periplasm and feed on their unwilling host's cellular components. BALOs present an attractive potential application in human infection and disease as [1] BALOs do not damage human cells and [2] BALOs are proficient at invading and disintegrating bacterial biofilms, penetrating sessile bacterial cells far more efficiently than antibiotics or bacteriophage.

Fusobacterium is a genus of Gram-negative bacteria that is well-versed in biofilm formation. Long, spindle-shaped *Fusobacterium* cells can serve as bridging units between early- and late-colonizing bacteria during biofilm formation. *Fusobacterium nucleatum*, for example, typically lives as a commensal anaerobic species within the human mouth, contributing to oral biofilms. In healthy individuals, *F. nucleatum* is rarely isolated outside the oral cavity—yet it has been identified in multiple body sites in cases of infection or disease. As BALOs are predatory bacteria that feed upon other Gram-negative bacteria, BALOs pose an attractive potential application in eradicating virulent strains of *F. nucleatum*.

To date, a limited number of studies have investigated the potential predator-prey relationship between BALOs and *F. nucleatum*. The current research serves to shed more light on the effect of different environmental conditions during BALO-*F. nucleatum* co-incubation, and the range of *F. nucleatum* which BALOs may predate. *F. nucleatum* exhibits a high degree of intraspecies variation; it is subdivided into five subspecies—*animalis*, *fusiforme*, *vincentii*, *polymorphum*, *nucleatum*—that have been recently proposed for reclassification as separate species based on genomic distances. Furthermore, these five subspecies are differentially linked to human infection; not all strains of *F. nucleatum* are virulent. As such, investigating the predatory-prey relationship between BALOs and *F. nucleatum* must include the range of potential *Fusobacterium* prey strains. Additionally, the anaerobic environment of *F. nucleatum* is not propitious for aerobic BALOs; as such, predation co-bacterial incubation assays must also be optimized. This exploratory investigation will further elucidate the potential for BALO-based targeting of virulent human-associated *F. nucleatum* strains.

MGCM54

Keep your enemies close: Secretion of a *Pseudomonas* anti-bacterial effector protein

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Pseudomonas aeruginosa is a Gram-negative opportunistic pathogen with intrinsic resistance to many antibiotics and the ability to adapt to a variety of environments and form biofilms. *P. aeruginosa* mediates both anti-bacterial and anti-eukaryotic activity through delivery of effector proteins (such as nucleases, peptidoglycan hydrolases, and lipases) by a specialized secretion system call the type VI secretion system (T6SS). The genome of *P. aeurginosa* encodes three T6SSs, called H1-H3. The H1-T6SS primarily targets bacterial cells, whereas the H2- and H3-T6SSs may deliver effectors to both bacterial and eukaryotic cells. Targeting of effector proteins to the T6SS apparatus during secretion system assembly is not well understood. We aim to delineate the mechanism of delivery of an effector protein by the T6SS, as well as characterize its activity and identify its biological target. Here we show that the proteins encoded upstream of the effector act as secretion chaperones for the effector and are required for its delivery. We also identified components of the T6SS tip complex specifically required for delivery of this effector. The effector has anti-bacterial activity, and we are currently pin-pointing the cellular target and mechanism of the effector and testing whether the effector is also active against eukaryotic targets.

MGCM55

Lack of a functional Csa quorum-sensing (QS) system in *Pseudomonas chlororaphis* strain PA23 does not directly affect biocontrol activity

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Pseudomonas chlororaphis strain PA23 is able to inhibit fungal phytopathogens, primarily through production of excreted secondary metabolites. Antibiotics, including phenazine (PHZ) and pyrrolnitrin (PRN), degradative enzymes and volatile organic compounds make up the arsenal that combat pathogenic fungi. Production of these exometabolites is tightly controlled via a regulatory hierarchy, in which lie various signalling systems, working directly and indirectly with one another. We have previously demonstrated that the Phz quorum sensing (QS) system plays a direct role in regulating expression of genes encoding secondary metabolites during the late-exponential and early-stationary phases of growth. To discover the global influence that QS has on PA23 gene expression, RNA-seq analysis was conducted on two QS-deficient strains, namely PA23AHL and

PA23*phzR*. Transcriptomic profiling revealed that between 5.5% (PA23*phzR*) and 7% (PA23AHL) of the PA23 genome is under QS control. Expression of genes encoding PHZ, PRN, HCN and protease were downregulated in the absence of the Phz QS system, consistent with our phenotypic analysis. *P. chlororaphis* PA23 has multiple QS systems, including the Csa (cell surface alterations) QS system, which has been characterized in the closely related *P. chlororaphis* 30-84. In strain 30-84, the Csa system was found to regulate cell surface components and rhizosphere competence. At present the role of the Csa QS system and its contribution to PA23 biocontrol remains to be discovered. To address this knowledge gap, mutations were generated in genes encoding the transcriptional regulator (*csaR*) as well as the AHL synthase (*csaI*). A number of phenotypic assays have been executed, showing evidence that the Csa QS system plays a more significant role in cell and colony morphology and motility, in comparison to the production of secondary metabolites, which coincides with what has been reported for *P. chlororaphis* 30-84. Pairing results from the phenotypic assays with analysis of biosynthetic and regulatory gene expression in the *csaI* and *csaR* mutant backgrounds will provide a better understanding of the role of the Csa QS system in PA23 physiology.

MGCM56

Light inducible secondary metabolite from the fungus *Hyphodiscus hymeniophilus*

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Hyphodiscus hymeniophilus is a fungus characterized by the production of a striking red pigment, although little is known about this red molecule and the other secondary metabolites produced by this microbe. In this study, the genome of *H. hymeniophilus* was sequenced and assembled to reveal that this microbe has a genome size of 35.1 Mba and is capable of producing over 20 distinct secondary metabolites. Furthermore, we discovered that the production of the characteristic red secondary metabolite is light inducible. By combining both genome analysis and small molecule purification techniques, we have progressed towards identifying the structure of this novel red metabolite along with the gene cluster responsible for its biosynthesis. This work gives insight on the molecular diversity of fungal secondary metabolites and the genomic features responsible for the production of these molecules.

MGCM57

Metabolism of D-arabinose and L-fucose in *Sinorhizobium meliloti* 1021

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The ability to utilize different carbon sources is important for diazotrophic bacteria to form an effective symbiosis with their host plants. During symbiosis, soil bacteria invade host tissues forming organs on the plant roots called nodules where they fix nitrogen into a form that can be used by the plant for growth. The bacteria, termed rhizobia, benefit from this relationship by receiving carbon from the plant; in this way rhizobia avoid having to grow saprophytically in the highly competitive soil environment and are nourished by the plant. Bacterial strains that are unable to catabolize certain carbon sources can be deficient in their ability to compete for nodule occupancy or establish symbiosis. However, the catabolic pathways for many substrates as well as their relationship to symbiosis have yet to be elucidated. In this study, we use genetic techniques to identify the genes involved in D-arabinose and L-fucose utilization and define the enzymatic reactions that breakdown these substrates in the model organism *Sinorhizobium meliloti*. D-arabinose is metabolized through a forked pathway involving genes found on a chromosomal locus previously associated with polyol metabolism as well as a set of

genes found on a distinct replicon called pSymB. The pSymB genes encode proteins that can metabolize L-fucose in addition to D-arabinose using a diketo-hydrolase type pathway. Our results enrich the knowledgebase of the metabolic capacity of the model organism *S. meliloti* and further efforts to engineer symbiosis in other organisms.

MGCM58

Modulating the microbiome through CRISPR interference

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Microbiome dysbiosis is correlated with numerous disease states, such as gastrointestinal cancers, metabolic diseases, respiratory diseases, psychological disorders, and autoimmune diseases. The ability to control and regulate specific populations within the microbiome would alleviate the negative outcomes associated with non-specific antimicrobials, such as the rise of antibiotic-resistant bacteria. Conjugative machinery allows the transfer of foreign DNA into microbial populations and utilizing the CRISPR/Cas9 system allows for sequence-specific targeting of bacterial chromosomes through the use of a single guide RNA (sgRNA). We have demonstrated that a TevSpCas9 dual nuclease delivered from *E. coli* by an IncP RK2-derived conjugative system can kill *S. enterica.* We are now exploring the capability of CRISPR interference (CRISPRi) to transiently repress specific genes in the microbiome. CRISPRi relies on a catalytically inactive Cas9 (dCas9), which binds sequences complementary to the sgRNA to repress transcription. Past studies have shown repression levels approaching 99% when targeting the promoter or an exonic coding strand of genes. Our first target of interest is the beta-glucuronidase gene, which encodes a protein that cleaves glucuronic acid from aglycones. Many compounds such as bilirubin, dopamine, estradiol, testosterone, and nicotine are targeted for excretion via glucuronidation; microbial beta-glucuronidases can reactivate these compounds in the gut, resulting in localized and occasionally detrimental outcomes. One such aglycone is the chemotherapeutic drug, irinotecan, which is often prescribed for late-stage colorectal cancer. Localized reactivation of irinotecan by microbial beta-glucuronidases causes dose-limiting diarrhea in a subset of patients, preventing sufficient treatment and worsening prognosis. Using a modified Miller assay, we have begun testing transconjugants with plasmids encoding dCas9 and sgRNAs targeting *E. coli* beta-glucuronidase *in vitro* to demonstrate the efficacy of repression. We propose that CRISPRi will be an effective method of repressing specific genes, possibly from specific bacterial species, in the microbiome to alleviate adverse conditions resulting from specific bacterial products.

MGCM59

New S-adenosylmethionine riboswitch variants reveal a surprising versatility of their structures and mechanisms

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Riboswitches are structured RNA elements typically located in the 5′ UTRs of bacterial mRNAs and are characterized by their ability to switch ON or OFF a nearby gene by directly binding a specific metabolite and causing a conformational rearrangement responsible for their activity. Riboswitches are composed of an aptamer ligand-binding domain and expression platforms that use different mechanisms to control gene expression. Through bioinformatics searches that take pseudoknots in consideration, we have found numerous new instances of S-adenosylmethionine (SAM) riboswitches with a many new variations in structures of the aptamer domains. We hypothesized that these various structures represent fine-tuning adaptation to the genetic context

where these riboswitches are found, with several sub-families of structures each potentially representing a variation in their gene regulation mechanism and folding pathways. Indeed, we have results supporting an alternative structure that appears to compete with the aptamer conformation, but may also favor the correct folding of the aptamer. Also, comparison of three different SAM riboswitches found upstream of three genes involved in SAM and methionine metabolism in *Burkholderia* provide evidence of widely different modulatory responses.

MGCM60

One Health genomic investigation of the rise in gentamicin resistance in *Salmonella* from human and chicken sources in Canada, 2014-2017

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Objective: Following a poultry industry ban on preventative use of ceftiofur in 2014, increased use of gentamicin and lincomycin-spectinomycin was reported. Simultaneous increases in gentamicin resistance (gen-R) rates in *Salmonella* from human and chicken sources were also observed. Our objective was to carry out a One Health genomic investigation of gen-R in human and chicken *Salmonella* isolates to identify potential transmission. This study focused on the five human *Salmonella* serovars from 2014-2017 that displayed the highest rates of gen-R. **Methods:** The Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) collected *Salmonella* isolates from provincial public health laboratories, broiler chicken farms, abattoirs, retail stores and other sources across Canada. Antimicrobial susceptibility testing was carried out by broth microdilution using the Sensititre™ Complete Automated AST System (ThermoFisher Scientific) and whole genome sequencing was carried out by Nextseq™ (Illumina). For human isolates, susceptibility testing was carried out on the ten most prevalent serovars. Phylogenetic analyses were carried out with SNVPhyl pipeline v1.1, genomes were assembled with SPAdes v0.5, and resistance genes were identified with staramr v0.3. **Results:** In human isolates of *Salmonella* (n=10621) collected between 2014-2017, 196 isolates displayed gen-R. Five serovars accounted for 87.2% (n=171) of occurrences, including *S*. Heidelberg [34.2%, n=67], *S*. 4,[5]12:i:- [21.4%, n=42], *S*. Kentucky [12.8%, n=25], *S*. Infantis [10.7%, n=21], and *S*. Typhimurium [8.2%, n=16]. From 2003-2017, susceptibility data was collected for 46951 human isolates and 15643 chicken (animal and food) isolates. For these five serovars, the rate of gen-R doubled from 2.3% (n=278) in 2003-2013 to 4.6% (n=171) in 2014-2017. Increases in gen-R rates in these five serovars were also seen in broiler chickens and retail chicken meat compared to previous years. In chickens (n=4962), gen-R doubled from 1.1% in 2003-2013 (n=46) to 2.4% in 2014-2017 (n=26). Similarly, among retail meat (n=4093) gen-R rates more than doubled from 1.4% in 2003-2013 (n=47) to 3.9% in 2014-2017 (n=33). Eight known gentamicin resistance genes were found amongst gen-R *S*. Heidelberg, 4,[5]12:i:-, Kentucky, Infantis, and Typhimurium isolates from humans and chickens between

2014-2017. Four of these genes (*aac(3)-VIa*, *aac(3)-IId*, *aac(3)-IVa*, *ant(2")-Ia*) were common to both humans and chickens, accounting for 84.3% of human gen-R and 96.7% of chicken gen-R. The *aac(3)-VIa* gene was the most frequently identified gene in both human (49.4%) and chicken (85.2%) isolates. Phylogenomic analyses showed multiple related clusters of human and chicken isolates for each serovar except *S*. Kentucky, with some differing by as few as 1-50 single nucleotide variations. *S*. 4,[5]12:i:- and *S*. Infantis showed particularly tight clustering, with some clusters differing by only 0-5 single nucleotide variations. **Conclusion:** Recent increases in gen-R in humans and chicken isolates were most commonly attributable to *aac(3)-VIa* and for four serovars, isolates from both humans and chickens were closely related suggesting potential transmission. The poultry industry in Canada committed to stopping preventive use of antimicrobials of high importance to human medicine (Category II), including gentamicin and lincomycin-spectinomycin, by the end of 2018.

MGCM61

Optimal characterization of physiologically-aware gene regulatory models

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Synthetic gene regulatory networks are a promising tool in many application areas, including metabolic engineering and biomanufacturing [1]. Construction of these networks often requires many experimental iterations to implement a functioning design [2]. Mathematical models can be used to guide the design process and reduce the experimental burden [3]. Existing models usually neglect the effects of the cell's physiological state on gene expression [4], and thus cannot be used to predict expression across varying contextual factors like nutrient sources, antibiotic selection and expression burden [5]. These external factors alter the cells internal state affecting growth rate, cell volume and the availability of resource like ribosomes and RNAP [4,6]. In past work, we developed a model that could account for the effects of nutrient-limitation on gene expression and designed optimal dynamic induction experiments for model fitting [7]. This approach allowed us to mathematically decouple physiological effects from the underlying gene expression parameters governing the regulatory behaviour. However, the dynamic experiments are difficult to implement and the model considered only nutrient perturbations. In this work we develop an extended model to accomodate a wider variety of physiological perturbations. We have also designed new steady-state and step-decay experiments for parameter estimation which are easier to perform experimentally.

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MGCM62

Oxygen-dependent role of copper metabolism in the modulation of drug resistance of the opportunistic yeast *Candida albicans*

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Candida albicans is a natural component of the human microbiota, but also an opportunistic pathogen responsible of several superficial infections of digestive and gynaecologic mucosa and can also cause systemic, life-threatening infection in immunosuppressed patients. There are presently a limited number of antifungal molecules due to the eukaryotic nature of *C. albicans* and also to the increased incidence of clinical resistance. Recently, we have shown that the chromatin remodeling complex, SWI/SNF, modulates the sensitivity of *C. albicans* toward azoles and polyenes antifungals specifically under low oxygen environments (hypoxia). This finding showed, for the first time, that tolerance/resistance to antimicrobials is affected by oxygen status. Furthermore, our data uncovered that transcriptional control through chromatin remodeling activity of SWI/SNF link hypoxic adaptation to drug resistance.

To understand the role of SWI/SNF complex in controlling antifungal sensitivity under hypoxia, we performed RNA-seq to characterise the transcriptome of the mutant subunit *snf5* exposed to the antifungal fluconazole and amphotericin-B under hypoxia. For both class of antifungals, the transcriptional profiling data showed that transcripts level of genes related to copper metabolism were altered in *snf5* mutant. Genes of the copper transporter *CTR1* and the master regulator of copper metabolism *MAC1* were activated in *snf5* while the copper-efflux pump *CRP1* was repressed. This transcriptional signature is reminiscent of cells experiencing copper deprivation. This suggest that a defect in copper utilization of *snf5* might contribute to the sensitivity of this mutant to antifungals under hypoxia. In accordance with this hypothesis, growth defect of *snf5* under hypoxia and in the presence of antifungals was reversed to a level similar to that of the wild-type strain by supplementing the culture medium with free copper.

We also found that the mutant of the transcription factor Mac1 that control copper regulon exhibited a differential sensitivity to fluconazole and amphotericin B only under normoxic conditions. This reinforce our previous finding that copper metabolism plays a significant role in regulating drug sensitivity in *C. albicans*.

Genetic connection map of Snf5 and Mac1 with all known regulator of drug resistance in *C. albicans* will be presented. We will also present data related to genome-wide occupancy of SWI/SNF in hypoxic cells treaded with different antifungal or growing in media depleted or repleted with copper. Our data is expected to reveal how chromatin remodeling activity link copper metabolism to hypoxia adaptation to influence drug sensitivity in *C. albicans*.

MGCM63

Prevalence and characterization of antimicrobial resistance among *E. coli* causing canine urinary tract infections: Passive surveillance of laboratory isolates in Saskatoon, Canada 2013-2018

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Rationale: Urinary tract infections are common in veterinary medicine. The urinary tract is the most common source of multidrug resistant *E. coli* in canine patients and a potential treatment challenge. Surveillance to detect the emergence of antimicrobial resistance in companion animal pathogens is lacking in Western Canada.

Methods: From October 2013 to 2018, 624 non duplicate canine urinary *E. coli* isolates were collected from a Western Canadian diagnostic laboratory in Saskatoon, Saskatchewan. Susceptibility testing was performed against a panel of 14 antimicrobials belonging to 8 different drug classes and isolates with appropriate phenotypes were screened for production of extended spectrum b-lactamases (ESBLs), AmpC b-lactamases and plasmid mediated quinolone resistance (PMQR) genes. Epidemiological relationships were assessed by multilocus sequence typing (MLST).

Results: 80% of isolates were pan susceptibility to all antimicrobials tested. There was no significant change in the proportion of isolates resistance to any of the tested antimicrobial during the study period. Resistance to ampicillin was most common (13.8%). Overall, thirteen isolates harbored CMY-2 type AmpC b-lactamases, and seven produced CTX-M type ESBLs. A single isolate was found to harbour the *aac(6')- Ib-cr* PMQR gene. The *qnr* and *qep*A determinants were not detected. A single isolate belonging to the pandemic lineage ST131 was identified.

Conclusion: Canine urinary *E. coli* in Western Canada remain largely susceptible to first line therapies, though resistance, particularly to the aminopenicillins, warrants continued monitoring. This is the first report of *E. coli* ST131 from a companion animal in Canada.

MGCM64

Probing *E. coli* growth in different carbon environments

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There is still a lack of clarity in how each gene in the model organism, *Escherichia coli,* is affected by the environment the bacteria are grown in, despite extensive research efforts on bacterial central metabolism in past decades. Previous studies in bacterial metabolism typically used nutrient-rich growth conditions, but recent studies suggest minimal media may be more representative of the nutrients available during a host infection. It is imperative to understand how gene essentiality patterns change during the growth in different media since bacterial growth *in vivo* rarely reflect typical lab conditions. Here, we have grown the *E. coli* single non-essential gene deletion collection (Keio collection) in minimal media containing 30 different sole carbon sources that enter central metabolism at different points. We have generated growth phenotypes for each gene knockout in every carbon condition. Using this set of data, we can generate hypotheses for genes of unknown function related to carbon utilization, experimentally validate predictions from metabolic models, and further our understanding on known metabolic genes. This high-quality data set is a valuable resource for studying bacterial metabolism allowing for the identification of genes connected to the cellular response to different carbon environments.

MGCM65

Production and identification of the unknown *Burkholderia cenocepacia* biosurfactant

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The opportunistic pathogen Burkholderia cenocepacia is a member of the Burkholderia cepacia complex (BCC). BCC is a group of very similar bacterial species, sometimes infecting the lungs of cystic fibrosis individuals. We typically find these species in the environment and they possess an intrinsic tolerance to several antibiotics. Several bacteria produce surface-active agents that lower the tension between cells and the surface, promoting the flagellum-driven social motility called swarming. The surfactant produced by Pseudomonas aeruginosaand

various Burkholderia species (such as B. pseudomallei, B. thailandensis, B. glumae) is composed of rhamnolipids. Intriguingly, B. cenocepacia possesses a gene cluster containing homologues of rhlA, rhlB and rhlC (around 80% homology with those of B. pseudomallei), that would normally permits to also produce rhamnolipids. However, even by precise and sensitive methods to characterize and identify glycolipids by HPLC coupled to mass spectrometry, we have not been able to detect any rhamnolipid or other surfactant produced by B. cenocepacia. Still, a rhlA- mutant in strain K56-2 loses its ability to produce swarming motility, strongly suggesting that a surfactant dependant on the rhl gene cluster is indeed produced by this BCC species. In attempts to identify this biosurfactant, we confirmed its production on blood agar and on Nutrient Broth containing dextrose, but levels are too low for further characterization. The first objective of this project is to determine the optimal culture conditions for the production of the biosurfactant by constructing a luxCDABE reporter under the control of the rhlA promoter region, in order to correlate the production of the biosurfactant with the reporter activity. Our hypothesis is that by optimizing the culture media, it should be possible to maximize the production of the unknown biosurfactant with a high enough concentration to permit its identification and its physico/biochemical and structural characterization. We believe this new biosurfactant would permit to better understand the pathology of the BCC group.

MGCM66

Random mutagenesis of self-conjugating plasmid to identify super-conjugative variants

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Conjugation is a bacterial horizontal gene transfer process that allows for the transfer of DNA, such as plasmids, between bacteria. For this reason, it is an attractive method to introduce genes of interest throughout bacterial populations such as the human gut microbiome. More specifically, we use a self-conjugating plasmid to introduce a CRISPR-Cas9 system that is capable of selectively killing strains within diverse populations. Previously, we observed that cis plasmids encoding the conjugative machinery and origin of transfer have higher conjugation frequency compared to the trans set up where these components are separated over two plasmids. For this system to function effectively in a therapeutic application, the initial rate of conjugation needs to be improved, especially at low donor concentrations. To increase the rate of conjugation for our self transmissible plasmid, we performed random chemical mutagenesis of the plasmid with EMS. From here, rounds of conjugation were done between *E. coli* donors and *E. coli* recipients to enrich for plasmids with mutations that increase the conjugation rate. We expect to identify plasmid variants that have a higher rate of conjugation and will allow for more efficient introduction of a strain specific antibiotic CRISPR-Cas9 cassette.

MGCM67

Regulation of fatty acid efflux pump FarE by TetR family transcriptional regulator FarR in *Staphylococcus aureus*

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Staphylococcus aureus is a Gram-positive bacterium that colonizes approximately 30% of the population asymptomatically. Community-acquired methicillin-resistant S. aureus (CA-MRSA) emerged in the 1990s and are hyper-transmissible, hyper-virulent and pose a threat to both immunocompromised and healthy individuals. One strain of CA-MRSA, USA300, is now the leading cause of all staph infections. The success of USA300 can

be attributed in part to their enhanced ability to overcome innate defenses of the skin including sebum which is composed of unsaturated free fatty acids (uFFA).

We have identified the divergently transcribed *farE* and *farR* genes that confer resistance to uFFA in S. aureus, respectively encoding an RND-family efflux pump and a TetR family regulator (TFR) required for expression of farE. Previous work has identified three operator sites within the intergenic segment of *farER*. O_{farE} lies downstream of the farE promoter, contains the pseudopalindrome PAL2, and is situated in a position that would be consistent with a role in activation of *farE* expression. OfarR overlaps with the promoter of *farR*, is downstream of the +1 transcription start site of farE, while O_{PAL1} lies in-between the two, contains the pseudopalindrome PAL1, and overlaps with O_{farR}. The position of both O_{farR} and O_{PAL1} would be consistent with a role in auto-repression.

As reported for other TFR's, FarR is subject to auto-repression, such that nucleotide substitutions in O_{farR} and O_{PAL1} both caused de-repression of *farR* expression. Western blots revealed that stronger de-repression was caused by substitutions in PAL1, and this was supported by EMSA experiments, where nucleotide substitutions in PAL1 also abrogated binding to the adjacent O_{farR} site. Nevertheless, although *farE* cannot be expressed in the absence of FarR, de-repression of *farR* did not promote increased resistance to antimicrobial fatty acids. Additional EMSA were done with either minimal PAL1 or PAL2 motifs. FarR was able to bind to a minimal PAL1 probe but not to a minimal PAL2 probe. Only when the minimal PAL2 motif was extended with additional nucleotides, was FarR able to bind. This result is consistent with the expectation that the addition of a ligand will facilitate the binding to a minimal probe.

Since induction of *farE* expression in response to unsaturated fatty acids is dependent on a fatty acid kinase FakA, we expect that an acyl phosphate is the physiologic ligand that modulates FarR activity. Work is in progress to co-express *fakA* and *farER* in *Escherichia coli* so as to determine the minimal requirements for *farE* expression in a heterologous host. As infection is usually caused by the colonizing strain, a better understanding of the mechanisms by which *S. aureus* persists on the human skin may lead to insights on new therapeutic targets that can prevent colonization and subsequent infection.

MGCM68

Regulation of *rpoS* expression by the small regulatory RNA Lpr10 impacts survival of *Legionella pneumophila* in water

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Introduction: *Legionella pneumophila* (*Lp*) is the causative agent of a fatal pulmonary infection known as Legionnaires' disease. This pathogen infects human hosts through the inhalation of contaminated aerosols that are mainly generated from water distribution systems. Once inside the lungs, *Lp* infects and grows within alveolar macrophages. The Type IVb secretion system is the most important virulence factor of *Lp*. In recent years, small regulatory RNAs (sRNAs) have been identified as important regulators of virulence and other cellular functions. While more than 600 small regulatory RNAs have been identified in *L. pneumophila*, only a few of them have been studied and characterized.

Objective: The objective of this study is to understand how the known sRNAs are regulated and to identify their function. Lpr10 was selected because it is expressed during the post exponential phase, which mimics the transmissive phase during infection.

Results: Expression profiles of this sRNA were analyzed in mutants of known virulence regulators. Lpr0010 is regulated by RpoS, RelA, SpoT, and CpxR. Since Lpr10 is differentially expressed during *Lp*'s growth phase, a mutant was constructed for further characterization. The mutant showed similar intracellular growth in THP-1 human macrophages than the wild-type. The Δ*lpr0010* mutant was able to survive longer in water at 25°C which

suggests that it represses genes involved in this condition. The mutant strain's transcriptome was then analyzed by microarray and the results were confirmed by qPCR. RpoS, the sigma factor required to survive various stresses, including survival in water, and necessary for the switch from replicative phase to the transmissive phase is upregulated in the Δ*lpr10* mutant. Finally, a putative binding site for Lpr10 was identified upstream of *rpoS*.

Conclusion: Lpr10 mediated control of RpoS expression likely facilitate the switch between transmissive phase back to replicative phase, which is necessary for maximum growth inside cells. The mechanism will be further studied by western blot and by analyzing the stability of the *rpoS* mRNA in the wild-type and Δ*lpr10* mutant.

MGCM69

Ribonuclease interacting partners and behaviour in *Streptomyces venezuelae*

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Ribonucleases (RNases) are enzymes that degrade or process RNA. They are involved in a multitude of critical cellular functions including RNA maturation, RNA metabolism, and ribosome quality control. The majority of research on the behaviour of RNases in bacteria has been done using model organisms like *Escherichia coli* and *Bacillus subtilis*. This project moves beyond these model organisms to investigate RNase behaviour in the Gram-positive actinobacteria *Streptomyces venezuelae*. *Streptomyces* produces several specialized metabolites, including many antibiotics, and a better understanding of RNases in these bacteria will facilitate the development of methods for manipulating *Streptomyces* biosynthetic clusters to stimulate high level expression. Here, a bacterial adenylate cyclase two hybrid system was used to screen an *S. venezuelae* genomic library for proteins that interact with *S. venezuelae* RNases of interest. In particular, screens were conducted against RNase J (SVEN_5394), YbeY (SVEN_2322), and the C-terminal scaffold domain of RNase E (SVEN_2380). A screen against YbeY showed interactions with fragments of six different *S. venezuelae* proteins. These include proteins involved in siderophore biosynthesis and proteins with as-of-yet unknown functions. Ongoing work includes RNA stability assays investigating the impact of RNase deletions on RNA profiles in *S. venezuelae*. In addition, pull down assays with overexpressed and purified RNase proteins, as well as investigations using catalytically inactive RNases, will offer a chance to uncover more binding partners of these RNases and identify their specific targets.

MGCM70

Role of a DEAD-box RNA helicase, named TgHoDI, in translational repression and the life cycle of *Toxoplasma gondii*

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Toxoplasma gondii is an obligate intracellular parasite infecting approximately 1/3 of the human population worldwide. To initiate an infection, *Toxoplasma* enters a host cell and creates a parasitophorous vacuole (PV). Inside the PV, the parasite can either exist as slow growing bradyzoites and form a tissue cyst, or as rapidly growing tachyzoites. Tachyzoites continually replicate and eventually cause lysis of the host cell. The released parasites then invade neighboring cells, initiating a new round of infection. During the search for a new host cell, translation is suppressed. Like other eukaryotic organisms, during translational repression non-translating mRNAs and various interacting proteins form ribonucleoprotein complexes and granules (mRNP granules).

While the formation of mRNP granules has been shown to improve parasite survival, invasion, and growth, the responsible mechanisms are poorly understood. Therefore, the translational arrest initiated in extracellular *Toxoplasma* offers an excellent model for the study of translational suppression and its cellular implications. Previously we identified an important member of the DEAD-box RNA helicase family named TgHoDI, and showed that TgHoDI is an interacting protein of mRNP granules. Specifically, the formation of TgHoDI-containing granules can be induced by extracellular exposure and/or translational repression. Moreover, as the single *Toxoplasma* homolog that harbors all significant features of a DEAD-box RNA helicase similar to other significant homologs, including yeast DHH1, human Rck54, and *Plasmodium* DOZI, it has thus been hypothesized that TgHoDI plays an important role in mRNA homeostasis. To investigate the function of TgHoDI in mRNP granules, a loss-of-function analysis was performed. The CRISPR gene editing technique was adopted to create a knockout strain, referred to as HoDI-KO. While HoDI-KO parasites showed no significant change in growth, their ability to survive in extracellular media was greatly reduced in comparison to the parental strain. Interestingly, extracellular HoDI-KO parasites can still form as many mRNP granules as the parental strain, suggesting that mRNP granule formation alone is not sufficient for survival. Our working hypothesis is that TgHoDI is involved in mRNA processing or mRNP remodeling during translational repression. It was also noted that despite being the only homolog, the survival deficiency of HoDI-KO parasites was diminished during the multiple rounds of culturing, suggesting that other proteins can complement the essential role of TgHoDI. Complementary functional analysis is ongoing in the HoDI-KO line that exhibits a survival deficit using various constructs whose amino acid residues are predicted to be critical to its function in mRNA processing or mRNP remodeling. The results and their implication will be discussed.

MGCM71

Streptococcus milleri group in cystic fibrosis airways: geographic variability and contribution to disease progression

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Cystic fibrosis (CF) is the most common, fatal genetic disorder with pulmonary exacerbations (PE) accounting for >90% of mortality. Increased frequency of PEs results in permanent loss of lung function, decreased quality of life, and ultimately, mortality. The CF microbiome is often underestimated and largely oversimplified leaving many clinically relevant organisms unstudied. The *Streptococcus Anginosus/Milleri* Group (SMG) has been highlighted as key player in PEs due to its correlation with clinical status, as previously proven by the Surette lab. Since that time, SMG has been closely monitored and treated in adult CF patients in the Calgary clinic. SMG had a 40% prevalence in exacerbating patients, and when therapeutically targeted, patients stabilized. However, since SMG is not routinely cultured in clinic, their role is underestimated in pulmonary infections. A comparison of SMG prevalence in CF patients at distinct geographical locations has not yet been explored. A retrospective study was conducted to identify SMG within clinical labs in Ontario using MALDI-TOF. To further characterize SMG, we conducted an assay to observe variability in activity in clinical SMG isolates from a single CF patient throughout their disease. This will help us understand its role in PEs, and will also allude to possible bacterial interactions promoting increased virulence and pathogenesis. We also examined microbe-microbe interactions among isolates for potential bacterial interactions influencing pathogenesis. Isolates were assayed for antibiotic resistance and potential transmission events were identified by comparative analysis using pulse field gel electrophoresis. Overall, we found that SMG is prevalent across chronic airway diseases (ex. asthma and COPD) with a high prevalence in CF. The bacterial group presents high phenotypic variability and heterogeneously interacts with common CF pathogens. This study further characterizes the role that SMG plays in CF exacerbations and disease progression enabling better management of disease.

MGCM72

Structural and biochemical studies of ribosomal frameshifting in Porcine reproductive and respiratory syndrome virus (PRRSV)

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To maximize the coding capacity of their polycistronic genome, arteriviruses like porcine reproductive and respiratory syndrome virus (PRRSV) employ multiple forms of “translational gymnastics”, one of which being programmed ribosomal frameshifting (PRF). The 5’-proximal region of the PRRSV genome is occupied by the large open reading ORF1a that encodes for polyprotein pp1a, which is subjected to an autoproteolytic cleavage cascade that yields 10 non-structural proteins (nsp). Nsp1b, acts to facilitate a -1 and -2 PRF event in the nsp2-coding region of ORF1a. In conjunction with the ribosome and the host cell protein poly(C)-binding protein 2 (PCBP2), this mechanism exposes two novel open reading frames, producing proteins that show relevance for viral replication and virulence. The trimeric complex involving the ribosome, PCBP2 and nsp1b interacts with a conserved “slippery site” and a downstream highly-conserved 11-nt C-rich motif in the nsp2-coding region of ORF1a to exploit the -1 and -2 ORFs.

The exact nature of how nsp1b, PCBP2, the ribosome and the viral genome interact remains unclear, however the complex between nsp1b and PCBP2 has a higher affinity for nucleic acid than either protein alone. Former studies defined a 58-nt stretch of the RNA genome that was essential for inducing PRF and nsp1b/PCBP2 complex formation. With the aim of structural and functional characterization of this protein-protein-nucleic acid complex, electrophoretic mobility shift assays aided in truncating the nucleic acid molecule to 34-nt. Using a combination of affinity purification and size exclusion chromatography we have now purified a stable complex containing nsp1b, PCBP2 and 34-nt DNA or RNA molecules. Several nsp1b mutants have been created to further explore the importance of certain amino acid residues in complex formation. These mutations have been created within the theorized RNA binding domain within a highly-conserved alpha helix as well as within the dimer interface of nsp1b. Dynamic light scattering, analytical ultacentrifugation and small-angle X-ray scattering have been used to characterize the complexes, which are guiding full structural and biochemical characterization of the novel complex by X-ray crystallography.

MGCM73

Study of *thiC* riboswitch transcriptional pauses

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Riboswitches are non-coding RNA located in the leader regions of messenger RNA. These sensors alternate between two conformations in response to ligand binding to modulate gene expression. Riboswitches have been found to rely on transcriptional pausing to perform ligand binding and the co-transcriptional formation of their structure. The pausing mechanism corresponds to RNA polymerase arrests at pause sites during transcription elongation. However, little is known about the molecular mechanisms involved in the riboswitch transcriptional pausing process.

Recently a NET-seq approach has highlighted the presence of a consensus sequence at translation start sites important for a pause signal. The role of this pause signal has been studied in the context of the *Escherichia coli thiC* riboswitch where it was found to modulate Rho transcription termination and translation initiation. Here, we

performed a study to decipher the role of the sequence and structure of the riboswitch in the formation of the pause signal. We used mutants of the *thiC* riboswitch that lock the structure in the ligand-bound state to characterize the influence of the structure upstream of the pause signal. These assays allowed us to determine the half-life and efficiency of the pause signal at the vicinity of the translation start site. Our results suggest that the structure and consensus sequence are important for the pause signalling as we have observed the decrease of mutants pause efficiency.

MGCM74

The minimal pSymA symbiotic nitrogen fixation gene region of *Sinorhizobium meliloti*

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Sinorhizobium meliloti is a model bacterium for the study of symbiotic nitrogen fixation (SNF). It infects the roots of alfalfa as well as some other legumes and differentiates into N_2-fixing bacteroids within the plant cells of specialized nodule organs. To understand the genes essential for SNF and, in the longer term, to facilitate the manipulation of this SNF process for agricultural purposes, it is highly desirable to construct the minimal genome for SNF in this organism. *S. meliloti* harbors two replicons essential for SNF: a 1.7-Mb chromid (pSymB) and a 1.4-Mb megaplasmid (pSymA). A previous deletion analysis identified that only four loci, accounting for <12% of the total sequences of pSymA and pSymB, were essential for SNF, and each replicon contained two SNF-essential regions. The two SNF-essential regions in pSymA are A117-118 ~103 kb (402136 ~ 505335 nt) and A121 ~52 kb (624863 ~ 677157 nt), respectively. Here we report the cloning of these two regions as a 155 kb fragment in a plasmid, pTH3255, in *Escherichia coli*. The symbiotic phenotype of a ΔpSymA *S. meliloti* strain carrying pTH3255 (RmP4291) suggests that this 155 kb pSymA region is sufficient for near-full N_2-fixation with alfalfa. This 155 kb region provides a new starting point towards construction of the minimal genome essential for SNF in *S. meliloti*. Towards that end, we have demonstrated that a 12.5 kb sub-region from the A121 ~52 kb region can fully complement a ΔA121 mutant for SNF.

MGCM75

The role of scaffold protein Cst5 in the *Candida albicans* pheromone response pathway

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Saccharomyces cerevisiae cells and *C. albicans* opaque cells release pheromone to stimulate cells of opposite mating type. The pheromone response pathway in *C. albicans* shares some similarities with its cousin's pathway but exhibits some important aspects that are not yet fully understood. First, both alpha and beta subunits of the heterotrimeric G protein are required for the transmission of the mating signal, while in S. *cerevisiae* the alpha subunit down-regulates the signalling pathway. In *S. cerevisiae*, the scaffold protein (Ste5) interacts with the G protein and directs the signal and acts enzymatically to ensure that the MAPKK Ste7 phosphorylates and activates the MAP kinase Fus3. The situation in *C. albicans* is much less clear: the catalytic part of Ste5 involved in Fus3 activation is not found in the *C. albicans* scaffold protein (Cst5), implying the regulation is different. Because the architectural structure of Cst5 is different from its ortholog Ste5, we decided to investigate whether the localization of Cst5 and its relationship with the pheromone receptor, heterotrimeric G protein subunits and MAPK elements are also altered. We constructed a set of mutant strains lacking the α-pheromone receptor Ste2, the G protein subunits Cag1 and Ste4, and the MAP kinases Ste11 and Hst7 in which the scaffold protein was tagged or not with GFP or with a CAAX box in order to direct Cst5 to the membrane. Artificially directing Cst5 to

bind to the membrane did not suppress the absence of any element of the mating pathway. In the absence of pheromone stimulation, opaque cells of *cag1Δ/Δ* and *hst7Δ/Δ* mutants expressed Cst5-Gfp at a lower level than the wild-type did, and differed from *ste2Δ/Δ*, *ste4Δ/Δ* and *ste11Δ/Δ* mutants which presented significant Cst5-Gfp background in the absence of stimulation. Although *ste2Δ/Δ*, *ste4Δ/Δ* and *ste11Δ/Δ* still expressed some Cst5-Gfp background in response to pheromone, the intensity levels were far below the response presented by the WT that localized Cst5 signal in punctate structures at the tips of the sexual projections. Intriguingly, *ste11Δ/Δ* not only expressed Cst5-Gfp at high levels under no stimulation but also exhibited comparable signal concentrated in puncta despite the absence of shmoos. These findings suggest that the pheromone response pathway in *C. albicans* may be regulated by the effector players. In order to expand on these observations, we are planning to construct double mutants to test the boundaries of Ste11 in relation to regulation of Cst5 functions and verify the interaction of Ste11 with the MAP kinase Cek1.

MGCM76

The Type IVb Pilus of conjugative plasmid TP114 is required for conjugation in the mouse gut microbiota

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Type IV pili (T4P) represent one of the most common surface appendages in bacteria. These thin flexible pili are involved in different processes such as adherence, motility, competence and pathogenesis. Several conjugative plasmids also encode T4P. For example, $IncI_2$ plasmid TP114 encodes eleven *pil* genes involved in the biogenesis and filament structure of a pilus from the type IVb family. The *pilS* and *pilV* products form the major and minor prepilin, respectively. The C-terminus region of the *pilV* gene is subject to DNA rearrangements by a shufflon, which is composed of various cassettes that can be exchanged by a tyrosine recombinase also known as a shufflase (*rci*). The shufflon acts as a biological switch to modify the specificity of the donor bacterium towards recipient cells. We have identified eight *pilV* variants in TP114, of which three are specific for *E. coli* Nissle 1917 in conjugation assays. We have also observed that the T4P of TP114 is required for conjugation in liquid media but not on solid support. Moreover, we performed *in vivo* conjugation assays with TP114 and a *pilS* deletion mutant. These experiments revealed a strong dependency of the T4P for conjugation in the mouse gut microbiota. We hypothesize that T4P encoded by conjugative plasmids stabilize mating pair during DNA transfer in liquid or unstable environments.

MGCM77

Transient replication of a mobilizable resistance island in the presence of its helper plasmid

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The mobilizable resistance island *Salmonella* genomic island 1 (SGI1) is specifically mobilized by IncC plasmids. Both SGI1 and IncC plasmids drive the dissemination of multidrug resistance in pathogenic enterobacteria. SGI1 uses and reshapes the conjugation pore of the IncC plasmid to ensure and enhance its dissemination in cell populations (Carraro *et al.* 2017). Moreover, the IncC conjugation master activator AcaCD, stimulates the expression of several SGI1-encoded proteins including the recombination directionality factor (RDF) Xis (Carraro *et al.* 2014). Remarkably, although SGI1 needs a coresident IncC plasmid to excise and transfer, these elements seem to be incompatible (Huguet *et. al* 2016). We monitored the stability of population of cells bearing both elements using fluorescence-activated cell sorting (FACS) by inserting the red and green fluorescent reporter

genes *mCherry* and *neonGreen* on SGI1 and the IncC plasmid pVCR94, respectively. While antibiotic selection for both SGI1 and pVCR94 allowed co-retention of both elements in 95% of the cells after overnight growth, without selection 95% of the cells segregated in two populations containing either SGI1 or pVCR94. Independent deletion of most of the 44 genes of SGI1 had no effect on incompatibility. However, deletion of *int*, *xis* or *S003* abolished incompatibility, and allowed stable coexistence of SGI1 and pVCR94. *int* and *xis* encode the integrase and RDF of SGI1, respectively, thereby indicating that excision of SGI1 from the chromosome is essential for incompatibility. *S003*, which is under the control of AcaCD, encodes a protein of unknown function with a predicted RepA_C domain (Pfam 04796 involved in plasmid replication). Furthermore, cells containing both pVCR94 and SGI1 (or most of its mutants) exhibit high red fluorescence (SGI1). In contrast, cells containing pVCR94 and one of the three SGI1 mutants (*int*, *xis* or *S003*) exhibit low red fluorescence. A high level of red fluorescence suggests a high copy number of SGI1 per cells resulting in increased mCherry production, whereas low red fluorescence corresponds to a single copy of SGI1. These data suggest that SGI1 excises from the chromosome and undergoes transient conditional replication that likely undermines IncC plasmid stability. Our on-going efforts aim at identifying the IncC plasmid-encoded factors required for SGI1 replication and the mechanism by which SGI1 replication impairs the stability of IncC plasmids.

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MGCM78

TxtH is a key component of the thaxtomin biosynthetic pathway in the plant pathogen *Streptomyces scabies*

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Bacteria from the genus *Streptomyces* are best known for their morphological complexity and for their ability to synthesize a wealth of bioactive specialized metabolites with useful applications in medicine and in agriculture. In addition, some *Streptomyces* spp. are able to function as aggressive plant pathogens and to cause economically important diseases of root and tuber crops. The principle pathogenicity determinant produced by such species is a phytotoxic cyclic dipeptide metabolite called thaxtomin A, which functions as a cellulose synthesis inhibitor. The biosynthesis of thaxtomin A involves two nonribosomal peptide synthetases (NRPSs), TxtA and TxtB, which together produce the thaxtomin backbone using L-phenylalanine and L-4-nitrotryptophan as substrates, respectively. TxtA and TxtB are large proteins consisting of multiple enzymatic domains, of which the adenylation (A-) domain is responsible for selecting and activating the specific amino acid substrate for incorporation into the peptide product. Another protein that may be involved in thaxtomin A biosynthesis is TxtH, which is a small (7.4 kDa) protein belonging to the MbtH-like protein (MLP) family. MLPs typically function as chaperones required for the proper folding of the A-domain of NRPS enzymes, and/or they function as activators to stimulate the adenylation reaction catalyzed by the A-domain. *txtH* is conserved in all thaxtomin-producing *Streptomyces* spp. and is located downstream of *txtB* in the thaxtomin biosynthetic gene cluster. In addition, the genome of the best-characterized phytopathogenic species, *Streptomyces scabies,* contains two other MLP-encoding genes that are localized in NRPS gene clusters producing a siderophore and a predicted lipopetide metabolite.

The objective of this study was to investigate the importance of the TxtH MLP in thaxtomin A biosynthesis in *S. scabies*. Biochemical studies in *Escherichia coli* showed that TxtH is essential for promoting the soluble

expression of both the TxtA and TxtB A-domains, suggesting that TxtH functions as a chaperone in *S. scabies*. Site-directed mutagenesis of TxtH revealed several amino acid residues that are required for the solubility-promoting activity of the protein. Deletion of the *txtH* gene in *S. scabies* significantly reduced thaxtomin A production, though some production could still occur. Notably, deletion of the two additional MLP–encoding genes in the *S. scabies* genome completely abolished phytotoxin production, and heterologous expression of all three MLPs could restore thaxtomin A production in the triple MLP-deficient strain, whereas expression of MLPs from other *Streptomyces* spp. could not. The constructed *S. scabies txtH* mutant displayed a reduced virulence phenotype compared to the wild-type strain in a potato tuber bioassay, and the virulence of the MLP triple mutant was even more severely affected. The results of our study demonstrate that TxtH plays a key role in thaxtomin A biosynthesis and plant pathogenicity in *S. scabies*, and that other MLPs encoded in the *S. scabies* genome can functionally replace TxtH in the thaxtomin biosynthetic pathway.

MGCM79

Understanding the role of glycerol in the exploration of *Streptomyces venezuelae*

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Streptomyces are ubiquitous marine and soil dwelling bacteria that produce over 60% of the world's clinically useful antibiotics. Streptomyces are also notable among bacteria for their complex multicellular life cycle. Similar to fungi, dispersed spores germinate under favourable conditions, growing to form a branching vegetative mycelium. In unfavourable conditions, aerial hyphae are produced that mature into chains of stress-resistant spores, which may then be dispersed to new environments. Recently, it has been shown that in nutrient depleted conditions or upon physical interaction with fungi, some Streptomyces species can escape this classical life cycle and enter a new mode of development called 'exploration'. Exploring cultures spread rapidly outwards from a central point by extending long unbranching hyphae, distinct from those formed during vegetative growth or aerial development. Exploring cultures also produce metabolites that are not detected in their classically growing counterparts, a possible tool for stimulating production of novel compounds.

While many environmental cues that initiate and influence exploration have been determined, regulation and changes at the genetic level for entering exploration are less clear. To uncover changes in gene expression during exploration, RNA was harvested from exploring cultures at different timepoints throughout exploration and sequenced. We found that over one third of all genes in S. venezuelae were differentially expressed between two and nine days of growth. Four of the most significantly differentially expressed genes encoded an operon predicted to function in glycerol uptake and metabolism. Adding glycerol to exploration-promoting media enhanced the rate of exploration, altered colony growth, and led to overproduction of a novel pigmented metabolite. To probe how glycerol is being used by exploring cultures, mutational and metabolomic analyses are being undertaken. Understanding how glycerol can manipulate growth and metabolism in these cultures may provide insight into how production of novel compounds can be stimulated in Streptomyces.

MGCM80

Using the fluoride riboswitch as a molecular tool to offset reporter gene toxicity while screening for potent bacterial promoters

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Bioreporter systems based on detectable enzyme activity, such as that of green fluorescent protein or luciferase, are key in novel bacterial promoter discovery. While these systems permit quantification of gene expression, their use is limited by the toxicity of the expressed reporter enzymes in a given host. For example, when screening for transcriptional activity of potential promoter sequences inserted upstream of the autonomous bioluminescence cassette *luxCDABE*, a potent promoter may be overlooked if its activity causes a lethal overproduction of the luciferase substrate, n-decyl aldehyde, produced by LuxC, LuxD and LuxE. To overcome this limitation, a variation of the mini-CTX-*lux* plasmid has been designed which allows reduction of promoter activity via the addition of an adjacent fluoride riboswitch. While the riboswitch-controlled luminescence studies in *E.coli* SM10 and *E.coli* DH5α yielded unexpected results, the riboswitch did add a layer of regulation between the promoter and the reporter gene. This reporter system design appears to have the potential to circumvent limitations associated with reporter toxicity. If successful, this study will provide a tool which will allow the identification of previously unverifiable potent promoters in screening assays using the *lux* cassette.

MGCM81

When genetics fails to identify your antibiotic cluster, try genomics

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Increasing numbers of antibiotic resistant human pathogens have become a major concern for human health. The Gram-negative members of the *Enterobacteriaceae* constitute a group of high-risk pathogens that have been deemed serious threats by the World Health Organization. *Enterobacter* is one such pathogen that inhabits hospitals settings where it is known to cause diseases in both healthy and immunocompromised individuals. The magnitude of risk it poses because of its drug resistant nature makes the discovery of new antibiotics essential.

A survey of over one hundred strains of the bacterial group *Pantoea* identified one strain of *Pantoea agglomerans* that produces at least one natural product having antimicrobial activity against both *Enterobacter and Kosakonia.* To identify the biosynthetic gene cluster responsible for its production, the *Pantoea* strain was subjected to transposon mutagenesis, and the mutants were screened for loss of antibiotic production. Approximately 12000 mutants were screened, yielding 40 candidates. Several of the disrupted genes appeared to be within histidine, arginine, isoleucine and cysteine biosynthetic pathways. Antibiotic production in all these mutants was restored by supplementing in the corresponding amino acid, or by growing them on nutrient rich media, suggesting that these were not the antibiotic biosynthetic cluster.

A comparative genomic approach was then used to attempt to identify the cluster. EDGAR, a comparative gene content analysis tool, was used to identify all the gene clusters that were present in the antibiotic-producing strain, but not in closely related non-producing strains. Two candidate clusters were highly conserved and had fairly limited distribution in other organisms. Targeted knock-outs in one of these clusters resulted in loss of the antibiotic production in the strain. The XX-gene cluster contains predicted genes coding for essential enzymes, substrates and transporters. This study demonstrates the power of comparative genomics when traditional genetic approaches fall short.

MGCM82

Characterization of *tktB* locus associated with second site suppressors of *tktA* mutation in *Sinorhizobium meliloti*

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Rhizobia can establish a symbiotic association with legume plants in which they fix atmospheric nitrogen in return for carbon from the host plant. Due to their diverse life cycle, rhizobia can utilize a variety of carbon sources. The ability to use different carbon sources has been linked to increased competition for nodule occupancy. Therefore, the strains with inability to utilize a carbon source are used to study the role of central carbon metabolism in symbiosis. A *Sinorhizobium meliloti* Rm1021 strain with mutation in *tktA* has been isolated and shown to have severe symbiotic defects with alfalfa. *tktA* encodes a transketolase that is involved in the non-oxidative pentose phosphate pathway. Isolates containing second site suppressor mutations that partially restore symbiosis have been isolated and are being characterized to help understand the role transketolase plays during this process. Genetic localization and whole genome sequencing have revealed single nucleotide polymorphisms (SNPs) in a putative negative regulator (*SMc02340*) in two of the suppressor strains. *SMc02340* is located upstream of *tktB*, which is thought to be a minor transketolase. *tktB* is upregulated in the suppressors strains as compared to wild type and *tktA* mutation. It is likely that the suppression of the mutation in *tktA* is due to the upregulation of *tktB* caused by second-site mutations in *SMc02340*. The *tktB* locus is being characterized for its role in suppression of phenotypes associated with *tktA* mutation. In addition, a metabolomic analysis of central carbon metabolites has revealed a partial restoration of the metabolite pools in the suppressor strains as compared to the *tktA* mutation.

MGCM83

Cold-directed knockdown of a bipartite antipathogenic protein in a model cereal

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Plant pathogenic bacteria including, *Pseudomonas syringae*, express ice nucleation proteins (INPs) at high sub-zero temperatures, allowing the unrestricted growth of large ice crystals within the apoplastic space between plant cells. These ice crystals can breach cell walls and membranes from vulnerable plant species and allows *P. syringae* access to intracellular nutrients. To counter this threat from the combination of low temperatures and pathogens, certain temperate plants have evolved cold acclimation traits.

Plants that undergo cold acclimation show enhanced freeze tolerance and this phenotype can be attributed at least in part due to the presence of ice binding proteins or IBPs. False brome, *Brachypodium distachyon*, is physiologically similar to agriculturally important cereals and thus serves as a model temperate crop that can cold acclimate. As part of their response to low but non-freezing temperatures, *B. distachyon* expresses cold induced two-domain proteins, each with a N-terminal leucine rich repeat (LRR) and a C-terminal IBP. The LRR domain can be modeled most closely to a portion of the pathogen perception protein, flagellin-sensing 2 or FLS2, from the mustard cress, suggesting a putative innate immune function for this domain. Upon secretion to the apoplastic space, the protein is hydrolyzed and the LRR domain and IBP domain are thought to function independently.

A cold induced promoter was ligated to a miRNA with sequence identity to all *Brachypodium* IBP genes and used to generate transgenic transient knockdown plants using the novel “seed cut” transformation method. The

phenotype of these transgenic plants and their activities toward ice and *P. syringae* bacteria will be presented, leading to a greater understanding of the "arms race" between crops and the bacteria that prey on them.

MGCM84

Designer *Sinorhizobium meliloti* strains and multi-functional vectors for direct inter-kingdom transfer of DNA

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Storage and manipulation of large DNA fragments is crucial for synthetic biology applications, yet DNA with high G+C content can be unstable in many host organisms. Here, we report the development of *Sinorhizobium meliloti* as a new host that can store DNA, including high G+C content, and mobilize DNA to *Escherichia coli*, *Saccharomyces cerevisiae*, and the eukaryotic microalgae *Phaeodactylum tricornutum*. We deleted the *S. meliloti hsdR* restriction-system to enable DNA transformation with up to 1.4 x 10^5 efficiency. Multi-host and multi-functional shuttle vectors (MHS) were constructed and shown to stably replicate in *S. meliloti*, *E. coli*, *S. cerevisiae*, and *P. tricornutum*, with a copy-number inducible *E. coli* origin for isolating plasmid DNA. Crucially, we demonstrated that *S. meliloti* can act as a conjugative donor for MHS plasmids with a cargo of at least 62 kb of G+C rich DNA derived from *Deinococcus radiodurans*.

MGCM85

Exploiting polymicrobial interactions to thwart antimicrobial resistance

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Microbes rarely exist in solitude but rather live in multispecies communities that are rich with ecological interactions. Although mutualistic interactions between diverse groups of microorganisms can enable co-habitation of certain niches, competition is the dominant form of interspecies interaction. Competition originates from nutrient limitation and space restriction. During interference competition, microbes can use antimicrobial compounds to damage adjacent cells, and therefore, natural antimicrobial resistance may have a role in allowing some bacteria to co-colonize the same spaces as antibiotic producers. Unsurprisingly, some antibiotic-producing organisms have evolved the capacity to subvert the antimicrobial resistance of competitors via the secretion of small molecules that act as antibiotic potentiators. For example, *Streptomyces clavuligerus* co-regulates production of cephamycin C, which is a beta-lactam antibiotic, with clavulanic acid, which is a beta-lactamase inhibitor. Nevertheless, the prevalence of these chemical interactions among microbes is unknown. We propose that polymicrobial interactions may be a source for natural antibiotic adjuvants with the capacity to thwart antimicrobial resistance of pathogens that top the WHO global priority list of antibiotic resistant pathogens to guide research and development of new antibiotics. I have worked as part of team to develop a natural product pipeline for the discovery of microbial metabolites that can potentiate the activity of meropenem against

carbapenem-resistant *Pseudomonas aeruginosa*. Carbapenems are antibiotics of last resort. This work was enabled by first helping to build the Alberta Microbiota Repository, which is a collection of bacteria and fungi cultivated from a diverse range of clinical, animal and environmental sources. Using a high-throughput method, the spent media from 400 of the repository isolates were assayed in the presence and absence of meropenem. A well-defined mathematical criterion was used to classify organisms producing soluble molecules that alter antibiotic resistance. In collaboration with natural product chemists, we used activity-guided fractionation, mass spectrometry and nuclear magnetic resonance (NMR) spectroscopy to isolate and identify the active component from the spent medium. Our initial efforts revealed that spent media from 11 strains reduced growth of meropenem-resistant *P. aeruginosa* by 2.5- to 40-fold. Here, I will describe the progress we've made with one of our top candidates from the meropenem adjuvant screens. The bacteria that produces our compound of interest, called strain 171, comes from the oropharyngeal tract of a healthy human male adult volunteer. Spent medium from strain 171, when combined with meropenem, increased killing of *P. aeruginosa* by up to 1000-fold. Extraction into hexanes, fractionation, and NMR led to the identification of a derivative of a human essential dietary fatty acid (which we term B2D-1A) that can alter carbapenem sensitivity of *P. aeruginosa*. We found that B2D-1A can work synergistically with meropenem to reduce the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) by 4-fold. Taken together, our results not only identify a group of fatty acid derivatives that affect antimicrobial sensitivity, but also suggest that microbe-microbe interactions determining antimicrobial sensitivity have been grossly underestimated.

MGCM86

Inter-kingdom transfer of conjugative plasmid from *Escherichia coli* to *Saccharomyces cerevisiae* within solid media

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Many prokaryotic species use conjugation as a means of contact-dependent DNA transfer which can be involved in the spread of antibiotic resistance and virulence. Conjugation can also be used as an alternative method for DNA transfer in biotechnology applications. Other methods for moving DNA into cells include electroporation and chemical transformation, however, these need to be adapted to new species and after optimization, may still suffer from poor efficiency. Through recombinant DNA strategies, designer plasmids can be easily created and then transferred via conjugation from donor bacterial cells to recipient eukaryotic or prokaryotic cells. Development of conjugation protocols require less overall optimization and can be used to transfer large pieces of DNA that would not be possible with other standard transformation techniques. Differences in conjugative protein complex formation, structure, and function will determine if a conjugation system is stable enough for conjugation in liquid media or if it will require a solid surface. A protocol where conjugation occurs within solid media could potentially improve stability and increase conjugation efficiency. The prokaryote *Escherichia coli* is a commonly used conjugative donor strain with an extensive genetic toolbox available and has been previously shown to conjugate to eukaryotic species such as the model yeast *Saccharomyces cerevisiae* and the algae *Phaeodactylum tricornutum*. Conjugation from *E. coli* to *S. cerevisiae* can be used as a model for conjugation to other eukaryotic species. We proposed to create a conjugation protocol for transfer from donor *E. coli* to recipient *S. cerevisiae* within solid media. The donor *E. coli* strain contained a conjugative plasmid titled pTA-Mob 2.0, a derivative of the conjugative plasmid pTA-Mob, that contains selection markers and replication sequences for both donor and recipient strains, the genes required for conjugative proteins, and an origin of transfer sequence. The two cultures were then mixed within a molten agar auxotrophic selection media, poured onto a plate, and successful *S. cerevisiae* with the pTA-Mob 2.0 colonies grew within the top agar layer after three to four days.

Optimization of the cell densities, ratios of cells used, the temperature of molten media, and the concentration of agar used in the top layer, yielded a final protocol for conjugation to eukaryotic cells within solid media. Future research will compare conjugation efficiencies to other conjugation protocols and can be adapted for use with other conjugative systems, donor, and recipient species.

MGCM87

Investigating the biofilm stimulation response to sub-MIC antibiotics

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There is an urgent need for new antibacterial therapies for gram-negative pathogens *Pseudomonas aeruginosa* and *Escherichia coli*. Their propensity to form biofilms adds to their ability to withstand antibiotic insults. Biofilms increase tolerance of antibiotics through production of a protective extracellular matrix and metabolic heterogeneity. High concentrations of antibiotics effectively inhibit bacterial growth, but sub-minimal inhibitory concentrations (sub-MIC) of antibiotics perversely stimulate biofilm formation. In a clinical setting, heterogenous penetration of antibiotics to various body sites may lead to interaction of sub-MIC levels of antibiotics with pathogens, increasing biofilm formation and complicating treatment. While sub-MIC antibiotic stimulation of biofilms is well documented, the mechanism behind this phenomenon is unclear.

Since bacteria form biofilms in response to a range of antibiotics of diverse chemistry and mode of action, we hypothesize biofilm stimulation is a response to the stress these compounds impose. To uncover components of this response, our lab created a transposon mutant library of *P. aeruginosa* PAO1 and screened it for mutants unable to respond to 3 different sub-MIC antibiotics. We identified the genes *oprF*, *sigX*, *dsbA*, and PA2200 as hits. OprF is a major outer membrane porin whose expression depends on the sigma factor SigX. PA2200 is a membrane bound cyclic-di-GMP phosphodiesterase whose activity may be regulated by its periplasmic disulphide-bond containing domain, connecting its function to that of the disulphide bond isomerase DsbA. Cyclic-di-GMP is a key metabolite that controls biofilm formation and associated phenotypes. We also screened the Keio Collection – a single gene deletion library in *E. coli* – using the dye Congo Red, which binds the biofilm matrix as a reporter of biofilm stimulation by sub-MIC antibiotics. Understanding the genes involved in responding to sub-MIC antibiotics could lead to potential targets for compounds that inhibit this response. Further, we can exploit the biofilm stimulation response to detect sub-MIC antimicrobial activity in high-throughput drug screens, which reduces the concentrations that can be detected compared to monitoring growth inhibition alone. Here, we show how the Congo Red assay can be used to detect antibiotic activity well below the MIC. In the future, we will leverage this assay to screen for novel compounds that stimulate biofilm formation, and thus have potential antibiotic activity.

MGCM88

Modulation of bacterial multicellularity via differential polysaccharide secretion

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The development of multicellularity is a key evolutionary transition allowing for differentiation of physiological functions across a cell population that confers survival benefits; among unicellular bacteria, this can lead to

complex developmental behaviours and the formation of higher-order community structures. However, knowledge concerning the determinants of bacterial multicellularity is limited. Herein, we demonstrate that in the social δ-proteobacterium *Myxococcus xanthus*, the secretion of a novel secreted biosurfactant polysaccharide (BPS) mediates swarm migration and predation of other bacteria, as well as the formation of multicellular swarm biofilms and fruiting bodies. The biosynthesis of BPS is shown to titrate the adhesiveness of exopolysaccharide (EPS) on the surface of vegetative cells via increased hydrophobicity, with both BPS and EPS produced via dedicated Wzx/Wzy-dependent polysaccharide assembly pathways distinct from that responsible for spore coat assembly. Together, these data reveal the central role of secreted polysaccharides in the intricate behaviours coordinating bacterial multicellularity.

MGCM89

Natural antisense transcripts of *Toxoplasma gondii*

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Natural **a**ntisense **t**ranscripts (NATs) are non-protein coding RNAs that can regulate the expression of their counterpart protein encoding transcript. While NATs are widespread in eukaryotic genomes, very little is known about their mechanism. Our study focuses on gaining a better understanding of the function of NATs in *Toxoplasma gondii,* a pathogenic unicellular eukaryote. We recently characterized the gene encoding the first committed enzyme in SUMOylation, named ubiquitin-like protease 1 (TgUlp1), and showed that the expression of TgUlp1 is vital to the life cycle of *T. gondii.* Interestingly, the locus of *TgUlp1* also transcribes a NAT species. Using a dual luciferase assay, we identified the promoter of *TgUlp1* NAT to be located within the 3'-region of its counterpart coding sequence. The identified promoters of NAT and mRNA are more active in tachyzoites (the rapidly growing stage) than bradyzoites (the slowly growing stage). RT-qPCR showed *TgUlp1* mRNA was lower, but *TgUlp1* NAT higher in bradyzoites compared to tachyzoites. Taken together, the data suggests that the expression of *TgUlp1* NAT and mRNA are differentially regulated at the transcriptional level, *via* promoter activity and transcript turnover. Furthermore, when *TgUlp1* NAT was *in vitro* processed by RNase III, the products retain the ability to lower the expression of engineered reporters carrying *TgUlp1* mRNA sequences, suggesting the involvement of RNA interference pathway. To evaluate whether the RNase III activity of DICER is required for the processing and function of *TgUlp1* NAT *in vivo*, RT-qPCR analysis was performed in DICER knockdown strain (TgDICER-KD). Compared to its parental strain, TgDICER-KD has a similar level of *TgUlp1* NAT, but a much lower level of *TgUlp1* mRNA, indicating that DICER-RNase III activity is involved in maintaining *TgUlp1* mRNA. Underlying mechanisms remain to be investigated.

MGCM90

Old drug, new trick: thiostrepton inhibits growth of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* in low-iron conditions

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Pseudomonas aeruginosa is a biofilm-forming opportunistic pathogen and intrinsically resistant to many antibiotics. In a high-throughput screen for molecules that modulate biofilm formation, we discovered that the thiopeptide antibiotic, thiostrepton (TS) - considered inactive against Gram-negative bacteria - stimulated *P. aeruginosa* biofilm formation in a dose-dependent manner. This phenotype is characteristic of exposure to

antimicrobial compounds at sub-inhibitory concentrations, suggesting that TS was active against *P. aeruginosa.* Supporting this observation, TS inhibited growth of a panel of 96 multidrug-resistant (MDR) *P. aeruginosa* clinical isolates at low micromolar concentrations. TS also had activity against *Acinetobacter baumannii* clinical isolates. Expression of Tsr - a 23S rRNA-modifying methyltransferase - in trans conferred TS resistance, confirming that the drug acted via its canonical mode of action, inhibition of ribosome function. Deletion of oligopeptide permease systems used by other peptide antibiotics for uptake failed confer TS resistance. TS susceptibility was inversely proportional to iron availability, suggesting that TS exploits uptake pathways whose expression is increased under iron starvation. Consistent with this finding, TS activity against *P. aeruginosa* and *A. baumannii* was potentiated by FDA-approved iron chelators deferiprone and deferasirox. Screening of *P. aeruginosa* mutants for TS resistance revealed that it exploits pyoverdine receptors FpvA and FpvB to cross the outer membrane. Our data show that the biofilm stimulation phenotype can reveal cryptic sub-inhibitory antibiotic activity, and that TS has activity against select multidrug resistant Gram-negative pathogens under iron-limited growth conditions, similar to those encountered at sites of infection.

MGCM91

Phage moron JBD30-4 mediates twitching and swimming motility in *Pseudomonas aeruginosa*

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Bacteriophages (phages), viruses that infect bacteria, can integrate their genomes into bacterial chromosomes and exist as prophages. In this state, most phage genes are silenced; however, a small number of genes known as 'morons' are actively expressed from the prophage. These genes are not required for the life cycle of the phage itself but provide some selective advantage to the phages that carry them. Some of these phage morons have been shown to increase bacterial virulence by mediating toxin production, increasing antibiotic resistance, and immune evasion. I discovered that the expression of moron JBD30-4 in *P. aeruginosa* abrogates swimming and twitching motility, two key forms of bacterial motility. While twitching motility is abrogated, the bacteria remain susceptible to phages that require the type IV pilus for infection. This lack of phage resistance suggested the pili responsible for twitching motility are present on the surface of the cells but are defective, rendering the bacteria nonmotile. As swimming motility is driven by a single polar flagellum in liquid environments, and twitching motility is mediated by type IV pili upon adherence to solid surfaces, I examined the production of these bacterial structures in the presence of JBD30-4 expression. Bacterial surface protein-shearing assays showed that expression of JBD30-4 downregulates localization of FliC, an essential flagellar component, to the surface of the cell. In addition, I observed an increase in PilA, the type IV pilus monomer, relative to wild type PAO1, potentially suggesting a hyperpilliated phenotype. Hyperpiliated *P. aeruginosa* is unable to twitch and still retains sensitivity to pilus-specific phage infection, as observed in the Δ*pilU* strains of *P. aeruginosa*. Recently, I found PilH, a Che-Y like response regulator, interacts with JBD30-4 through the bacterial two hybrid system. PilH is a Che-Y like response regulator and links PA chemotaxis to twitching motility by modulating the activity of the type IV pili retraction ATPases, PilT and PilU, which are responsible for retraction of the type IV pili from the bacterial cell surface. I hypothesize that JBD30-4 inhibits PilH, subsequently leading to a loss of type IV pilus retraction via PilU. Furthermore, PilH also controls levels of cAMP, which modulates gene expression via binding to the virulence factor regulator (Vfr), a transcription factor that regulates more than 200 genes involved in virulence responses, including those for type IV pilus and flagellar biosynthesis. Vfr is known to repress transcription of FleQ, the master regulator of flagellar gene expression. Thus, an interaction between JBD30-4 and PilH may also explain the loss of surface flagellin observed via shearing assays in PA upon JBD30-4 expression. JBD30-4 is a nonessential phage gene, but its conservation among many prophages in PA clinical isolates suggests it confers a fitness advantage. This fitness advantage is likely increased resistance to phages - the single biggest

danger to Pseudomonas survival. However, since flagella are highly immunogenic, I believe that JBD30-4 may also play an important role in human disease by decreasing the immune reactivity of the strains expressing the moron, while allowing for persistence within the human host. Understanding the role of JBD30-4 in P. aeruginosa will further our understanding of phage-host interactions and ultimately how these interactions affect the course of bacterial infections.

MGCM92

Physical, physiological, and molecular characterization of the near-minimal bacterium *Mesoplasma florum*

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Synthetic biology promises to address some of the most difficult challenges of the 21st century by programming cells to accomplish specific tasks. However, our current ability to engineer predictable outcomes from a cellular chassis remains relatively poor, principally due to our incomplete comprehension of fundamental rules governing global cell functioning. In this context, minimal or simplified cells represent powerful tools for which the complete characterization and modelling of all the molecular processes is more practical. The near-minimal bacterium *Mesoplasma florum* constitutes a prime candidate to undertake such a characterization due to its small genome (<800 kb), simple metabolism, lack of pathogenic potential, and fast growth rate. In addition, genetic manipulation tools as well as whole-genome cloning and transplantations procedures have also been developed for this organism, and a genome-scale model (GEM) is currently being reconstructed. Here, we present the procedures and experimental data used to obtain a deep physical, physiological, and molecular characterization of *M. florum*. Briefly, the macromolecular composition of the cell was evaluated by quantifying the total amount of proteins, RNA, lipids, DNA, and carbohydrates per cell, accounting for ~47%, ~22%, ~18%, ~8%, and ~4% of the cell dry mass (~22 fg), respectively. We also measured the buoyant cell density of *M. florum* by discontinuous Percoll density gradient centrifugation (1.05-1.08 g/ml), and used different cell mass equations to infer the most probable cellular mass (~88-103 fg) as well as refine the average cell diameter evaluated using transmission electron microscopy (TEM) and stimulated emission depletion (STED) microscopy (~538-570 nm). We investigated the growth kinetics of *M. florum* in rich medium and observed a doubling time of ~32 min at an optimal temperature of 34°C. We also performed the first transcriptome and proteome analyses of *M. florum* using a combination of genome-wide 5'-rapid amplification of cDNA ends (5'-RACE), RNA sequencing (RNAseq), and two-dimensional liquid chromatography-tandem mass spectrometry (2D LC-MS/MS). The individual as well as combinatorial analysis of these datasets notably revealed more than 400 active promoters in *M. florum* characterized by a conserved -10 box typical of the σ^{70} family, the transcription and expression levels of all annotated genes, the first experimental cartography of transcription units and untranslated regions (UTRs), as well as evidences suggesting the presence of antisens promoters and alternative open reading frames (AltORFs). Finally, we used the biomass quantification data to convert gene transcription and expression levels into molecular species abundance and generate an unprecedented view of the intracellular composition of the *M. florum* cell.These efforts will help acquiring a detailed understanding of global cell functioning and facilitate future genome engineering efforts for this bacterium.

MGCM93

Plasmid-based tools for Cas9 genome editing and stable maintenance of synthetic pathways in *Phaeodactylum tricornutum*

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Diatoms are a diverse and widespread group of eukaryotic microalgae that play an important role in many aquatic ecosystems through their capacity to fix atmospheric CO2. With the completion of the genome sequence, and development of an efficient conjugation-based transformation system allowing the introduction of stable episomes, the model diatom *Phaeodactylum tricornutum* has become an ideal platform for the study of diatom biology and synthetic biology applications. The development of plasmid-based genetic tools is the next step to improve manipulation of this species. We report the identification of endogenous *P. tricornutum* promoters and terminators allowing selective expression of antibiotic resistance markers from stably replicating plasmids in *P. tricornutum*. Significantly, we developed a protocol for sequential conjugation of plasmids from *Escherichia coli* to *P. tricornutum* and demonstrated simultaneous replication of two plasmids in *P. tricornutum*. We developed a simple and robust conjugative system for Cas9 editing that yielded up to 60% editing efficiency of the urease gene. Finally, we constructed a plasmid encoding eight genes involved in vanillin biosynthesis that was propagated in *P. tricornutum* over four months with no evidence of rearrangements, with whole-plasmid sequencing indicating that most mutations occurred after plasmid assembly and initial conjugation rather than during long-term propagation. The plasmid-based tools described here will facilitate investigation of the basic biology of *P. tricornutum* and enable synthetic biology applications.

MGCM94

Rapid multicellular cell shape evolution: lesson form *Neisseriaceae* family

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The bacterial cell shape is a conserved trait that directs the adaptation and colonisation of bacteria to different environments. Members of the *Neisseriaceae* family have a great diversity of forms, ranging from bacilli, cocci, and some with a multicellular arrangement. Using an evolutionary approach, previously demonstrated that the cocci form in two major pathogens, *N. meningitidis* and *N. gonorrhoeae,* emerged from a bacilli ancestor upon the loss of *yacF* gene implicated in bacterial cell division. Through a similar approach we explored the molecular and evolutionary mechanisms that allowed the cell shape transition from bacilli to multicellular organisation. We employed PacBio long read sequencing to obtain closed genomes for different cocci, bacilli, and multicellular *Neisseriaceae*. Comparative genomic analysis using MycoHIT showed that some conserved genes were lost in multicellular *Neisseriaceae* such as *S. muelleri* . Interestingly we found that 50% of these genes encode proteins that are important in determining the bacterial cell shape such as; the conserved cell division protein MraZ, the peptidoglycan transglycosylase MtgA and the RNAse adaptor protein RapZ. Here, we focus on determining the role of the MraZ regulator in the *Neisseriaceae* family. We constructed MraZ null and MraZ over expressing strains from a bacilli shaped model *Neisseria elongata.* We also used 9 different *Neisseriaceae* species, three for each morphology (bacilli, cocci and multicellular). RNA was extracted from these strains and transcriptomic

analysis done by RNA sequencing. The first arm of analysis was done on wild type *Neisseria elongata,* the *MraZ* mutant and over expressing strains, whereas the second set of analysis compared the 9 different *Neisseriaceae* species based on morphology. Altogether, our results show that MraZ is an activator of the *dcw* cluster (a locus that encodes genes for division and cell wall). Genes such as *mraW, penA* and *murE* were significantly downregulated in the absence of *mraZ* gene and upregulated in its presence. Its over-expression led to shorter cells. Therefore, we think that the loss of this gene might contribute to the irregular and incomplete cell division in multicellular *Neisseriaceae*. We are confident that this panoramic view of the evolution of the cellular form of *Neisseriaceae* will help to better understand the bacterial multi-cellularity and how some species adapt preferentially to certain hosts or regions of the body but also will help to reveal common features of cell cycle in the *Neisseriaceae* family.

MGCM95

The fidelity of DNA polymerases in microgravity

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Current space missions involving low earth orbit, in addition to prospective interplanetary exploration missions, will expose participants and their associated microbiota, to extended periods of microgravity and radiation, environmental stressors for which earth-based life is ill-equipped to survive. The impact of exposure to these phenomena on DNA replication machinery, however, has not been fully elucidated. DNA polymerases play a major role in both DNA replication and repair, and thus can be considered central to the maintenance of genomic integrity, especially in the presence of radiation-induced DNA lesions. The “Polymerase Error-Rate in Space (PolERIS) experiment” was designed to assess whether a differential in DNA polymerase fidelity and replication rate exists under conditions of microgravity generated by parabolic flight when compared to earth gravity (1g). Upon initiation of a parabolic arc, DNA polymerization reactions on a primed synthetic single-stranded DNA template were initiated by introduction of an enzyme mixture (*E. coli*-derived Klenow Fragment +/-; with and without proofreading exonuclease activity). The reactions were quenched after ~20 sec using a divalent cation chelator and heat inactivated following the completion of a parabola at 70˚C. Sequencing to determine replication fidelity utilized the Illumina NovaSeq 6000™ system. While initial results do not indicate a significant change in the *E. coli* DNA polymerase replication product length in flight, assessment of replication fidelity is still underway. When coupled with the novel single-molecule combinatorial DNA tag mechanism developed for this experiment, we posit that an extremely accurate determination of any differential in DNA polymerase error rate will be achieved. As such, the PolERIS experiment represents a new approach to enzymology research in microgravity. Furthermore, it is our hope that the engineering, genetics, and bioinformatics techniques developed here may prove invaluable to future space health and microbiology research.

MGCM96

Towards a mechanistic understanding of bacterial social networking via outer-membrane extrusion

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Remodelling of the bacterial cell surface is a ubiquitous phenomenon for maintaining connections with the substratum and/or other cells, thus facilitating communication, behavioural coordination, and long-term residence. In Gram-negative bacteria, the cell surface is composed largely of lipopolysaccharide (LPS) in the

outer leaflet of the outer membrane (OM). With the model Gram-negative δ-proteobacterium *Myxococcus xanthus*, cell aggregates become connected to each other via networks of OM-derived tubes (OMTs) and vesicle (OMV) chains composed of LPS. OMVs are ubiquitous in Gram-negative bacteria; however, the mechanism of OM extrusion in general is poorly understood. To study this phenomenon, we fluorescently labelled the *M. xanthus* OM via incorporation of Kdo(8)-N_3 (that we synthesized) into *M. xanthus* LPS, followed by click chemistry attachment of DBCO-sulforhodamine to the -N_3 moiety. This allowed for real-time fluorescence imaging of *de novo* OMT formation and attachment in bacteria. Pixel measurements of OMT vs cell length over time from various cells indicates that the cell body retains a fixed length as the OMT is extended indicating that OMT extrusion is not simply a result of senescence and shortening. Though OMT-forming cells can vary in length, the first "burst" of OMT extrusion results in a constrained initial OMT length, suggesting OMT initiation is a regulated process. In the native *M. xanthus* LPS structure, a certain proportion of Kdo molecules are modified with a phosphoethanolamine (*P*EtN) moiety, a modification which cannot be introduced on Kdo(8)-N_3 due to the position of the -N_3 azide group; we have also synthesized a Kdo variant with the -N_3 at carbon 7 of the sugar, Kdo(7)-N_3, to promote *P*EtN incorporation into the modified LPS molecule. Testing of *M. xanthus* physiology with respect to single-cell and group motility, as well as fruiting body formation will be carried to probe community-wide differences due to the various surface modifications.

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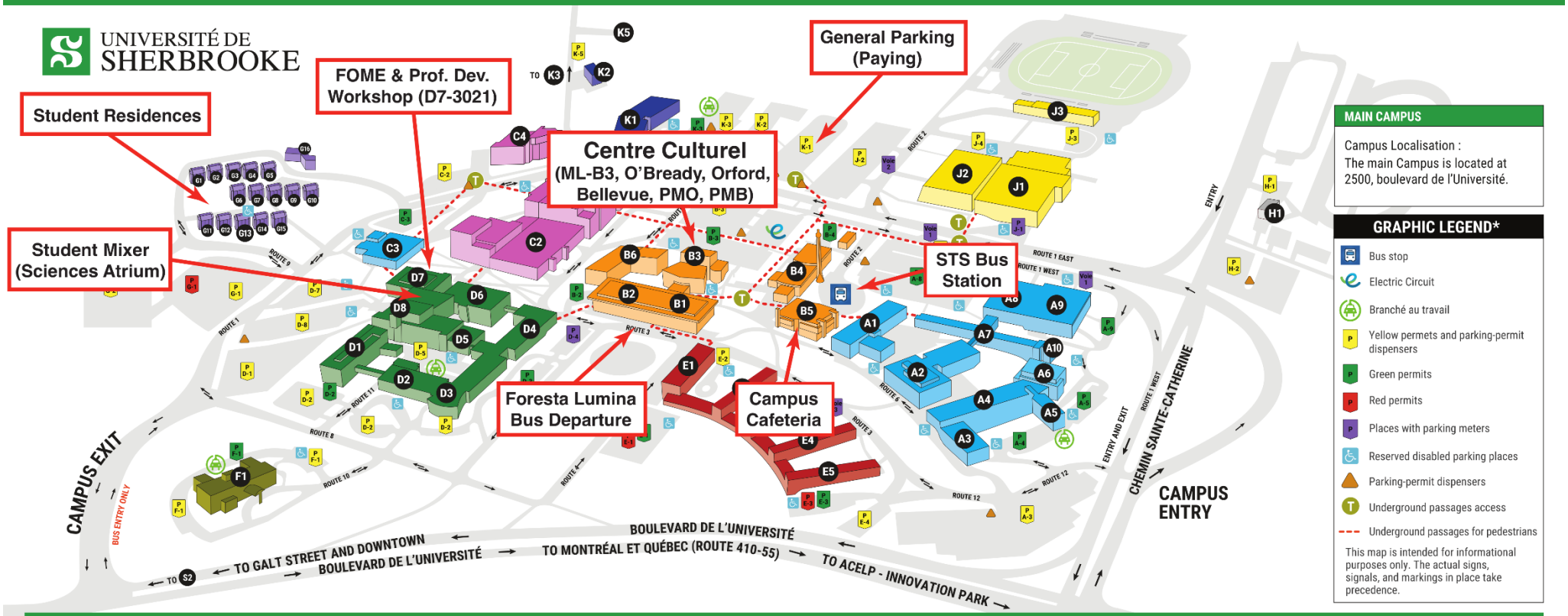
Scheffer Gabrielle	University of Calgary
Sciortino Josephine	Canadian Science Publishing
Scott Andrew	Agriculture & Agri-Food Canada
Seidel Laura	University of Guelph
Sellam Adnane	Université Laval
Shannon Blake	Western University
Shapiro Rebecca	University of Guelph

Shareefdeen Hiba	McMaster University
Sharma Aditi	University of Regina
Shehata Hanan	University of Guelph
Shekarriz Shahrokh	McMaster University
Shepherdson Evan	McMaster University
Shetty Deeksha	Mcgill University
Short Steven	University of Toronto Mississauga
Sidibe Amadou	Université de Sherbrooke
Silue Navoun	University of Ottawa
Singh Mandeep	University Institute of Pharmaceutical Sciences, Panjab University
Singh Bhavya	McMaster University
Sinha Anshul	McGill University
Sit Clarissa	Saint Mary's University
Slater Cole	University of Manitoba
Slattery Sam	Western University
Smith-Roberge Julien	University of Waterloo
Soltysiak Maximillian	University of Western Ontario
Sorout Naveen	University of Regina
Soutar Craig	University of Regina
Staples Greg	Olympus
Stephens Colton	Thompson Rivers University
Stogios Peter	University of Toronto
Subirats Medina Jessica	Agriculture and Agri-Food Canada
Sukdeo Nicole	Concentric Agriculture Inc.
Sukumaran Arjun	University of Guelph
Sule Akshay	University Of Western Ontario
Sutcliffe Steven	McGill University
Szymanski Christine	University of Georgia
Taguer Mariia	McGill University
Tahlan Kapil	Memorial University of Newfoundland
Talbot Guylaine	Agriculture and Agri-Fooda Canada
Tan Stephanie	University of Toronto
Tanaka Takuji	University of Saskatchewan
Taylor Nicole	University of Calgary
Tchagang Caetanie	Université d'Ottawa
Telhig Soufiane	Laval University
Thomas Nikhil	Dalhousie University
Tiller Lauren	McMaster University
Tong Madeline	McMaster University
Torres Paniagua Adriana	McGill University
Touchette David	McGill University
Touchette Benoit	Directeur Infors Canada
Trahan Olivier	Université de Sherbrooke
Tran Jason	McMaster University
Tremblay Lisa	Illumina, Inc.
Tsuji Jackson	University of Waterloo
Tsviklist Valeria	University of Alberta

Tuffs Stephen	University of Western Ontario
Uche-Okereafor Nkemdinma	University of Johannesburg
Uddin Muhammed Salah	University of Alberta
Uhland Carl	Veterinary consultant
Umansky Andrew	Université de Sherbrooke
Van Dyk Adrian	University of Waterloo
Van Hamme Jonathan	Thompson Rivers University
Vanessa Yoon Calvelo	University of Toronto
VanMensel Danielle	University of Windsor
Vasquez Alexandra	INRS-Institut Armand-Frappier
Venkatesan Meenakshi	University of Toronto
Veyrier Frederic	INRS-Institut Armand-Frappier
Villemur Richard	INRS-Institut Armand-Frappier
Vincent Antony	INRS-Institut Armand-Frappier
Voordouw Maarten	University of Saskatchewan
Vucetic Andrea	McGill University
Walker Virginia	Queen's University
Warmoota Rahul	Panjab University Chandigarh, India
Weber Brent	McMaster University
Weisener Christopher	University of Windsor
Wen Grace	University of Windsor
Whelan Fiona Jane	University of Nottingham
Wiedemeyer Alyssa	University of Alberta
Wilde Jacob	University of Guelph
Williams Ashley	University of Regina
Williams Danielle	University of Guelph
Wright Madison	University of Guelph
Wushke Scott	Agriculture and Agri-Food Canada
Xue Kevin	University of Toronto
Yaeger Luke	McMaster University
Yassine Hadi	Qatar University
Yergeau Etienne	INRS-Institut Armand-Frappier
Yost Chris	University of Regina
Yousef Ahmed	Khalifa University
Yusuf Farhan	Ryerson University
Zaheer Rahat	Agriculture and Agri-Food Canada
Zambri Matthew	McMaster University
Zhang Xiafei	McMaster University
Zitouni Mina	Université de Sherbrooke

MAIN CAMPUS



MAIN CAMPUS

Campus Localisation :
The main Campus is located at 2500, boulevard de l'Université.

GRAPHIC LEGEND*

- Bus stop
- Electric Circuit
- Branché au travail
- Yellow permets and parking-permit dispensers
- Green permits
- Red permits
- Places with parking meters
- Reserved disabled parking places
- Parking-permit dispensers
- Underground passages access
- Underground passages for pedestrians

This map is intended for informational purposes only. The actual signs, signals, and markings in place take precedence.

BUILDINGS

FACULTIES

Arts, Humanities and Social Sciences - School of Music	A3 à A6, C3
Business School	K1
Education	A1, A2, A7
Engineering	C1, C2, C4
Law	A9
Science	D1 à D8
Physical Activity Sciences	A8

SERVICES

Alumni Relations -Foundation	S2
Building Services	B4, F1
Cultural Centre	B3
Communications	F1
Coop Work Term and Placement	B6
Education Support	A1
Human and Financial Ressources	B1
Information Hub	B1
Information Technologies	F1
International Relations Agency	E5
Libraries and Archives	B2
Office of the President	B1
Office of the Registrar	B1
Research, Innovation and Creation Support	B6
Sport and Physical Activity	J1, J2
Student Services - Main Campus Service Centre	E1, B1

CENTRE

University Centre for Environmental Sustainable Development Studies	D7

LIBRARIES

Archives	A9
Frère-Théode (Science and Engineering)	D6
Law and Government -Publications	A9
Music	B2
Roger-Maltais (Humanities)	B2

PAVILLIONS

Albert-Leblanc	A8, A9
Georges-Cabana	B1, B2
Irénée-Pinard	B6
J.-Armand-Bombardier	C1, C2
John-S.-Bourque	F1
Life Sciences	D8
Marie-Victorin	D6, D7
Multifonctional	B5
Student Services	E1
Centre sportif Yvon-Lamarche	J1, J2
Humanities and Social Sciences Research	A10

OTHERS

Animal Supply Facility	D5
Art Gallery	B6
Cafeteria	B5
Creative Studio	C4
Day-care Centre	G16
Environment and Sustainable Development Observatory (OEDD)	A6
Indoor Stadium	J2
Maintenance Garage	K2
Maurice-O'Bready Hall	B3
Office oh the Ombudsperson	E3
Outdoor Stadium	J3
Parking Permits	B5
Pool-Gymnasium	J1
Power Plant	B4
Remote Sensing Sation	K3
Residences	E2 to E4, G1 to G15
Secretariat for the Periodic Assessment of Programs	A9
Security	B5, F1
Service Building	H1, C4


Chemin des Écossais
C.H.U.S.
Rue Bank
Rue Dufferin
Rue Wellington Nord
Banquet Théâtre Granada
Rue Frontenac
Rue Marquette
Rue Belvédère Nord
King Ouest
Rue Wellington Sud
Rue du Dépôt
Rue Alexandre
Place de la Gare
Grand Hôtel Times
Rue Brûlotte
Rue King Est
Rue Galt Est
Boulevard Lavigerie
Rue Bowen Sud
Rue Queen
Rue St. Francis
Rue College
Rue Bel-Horizon
Chemin d'Haskell Hill
Rue Dunant
Rue Thibault
Boulevard de l'Université
Chemin de Sainte-Catherine
Résidences étudiants
Student residences
Université de Sherbrooke
Rue Denault
Rue Galt Ouest
Lac des Nations
OTL Gouverneur Sherbrooke
Comfort Inn
Hôtel Le Président
Centre d'achats Carrefour de l'Estrie
Shopping Mall
Boulevard de Portland
Boulevard Industriel
Rue King Ouest
Boul. Lionel-Groulx
Boulevard Jacques-Cartier N.
Boul. Queen-Victoria
Rue Saint-François Nord
Boulevard Queen-Victoria
12e Avenue Nord
13e Avenue Nord
Rue Terrill
Rue du Cégep
Rue Pépin
Chemin Dion
Boulevard René-Lévesque
Boulevard Bourque
Boulevard des Vétérans
Rue du Président-Kennedy
Boul. de Monseigneur-Fortier
autoroute de Québec
autoroute de Montréal
Microbrasseries
Microbreweries
1 Le refuge des brasseurs
2 Siboire Jacques-Cartier + Dépôt
3 La Mare au Diable
4 Le Boq
5 Pub Le Lion d'Or
Restaurants
1 OMG Resto
2 Table du Chef
3 Da Toni
4 Antidote Food Lab
5 Persépolis
6 LoRé, fine cuisine du marché
7 Masala Café
8 Méchant Steak
9 La Rose des Sables
10 Le Classyco
11 Le Bouchon
12 Le Baladi
13 Auguste
14 O'Chevreuil
15 Pizzicato
16 Sballo
17 Baumann Smokehouse
18 L'Empreinte - cuisine soignée
10-13 juin 2019
June 10-13, 2019
destination SHERBROOKE.com
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