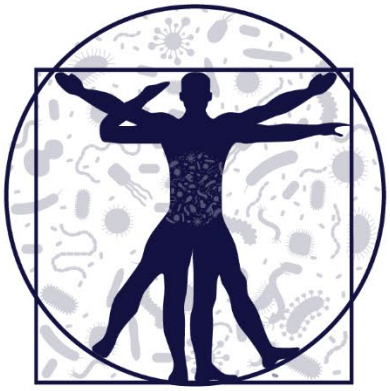

TSMC 2020

Tri-Service Microbiome Consortium

Sept. 24-25, 2020

Virtual Event

Welcome to TSMC 2020!

On behalf of the Tri-Service Microbiome Consortium (TSMC), we welcome you to the Department of Defense (DoD), Biotechnology Community of Interest, 4th Annual TSMC Meeting: TSMC 2020! We are looking forward to two days of stimulating presentations and discussions from DoD researchers and our Government, Industry, and Academic partners.

The TSMC is a forum for DoD microbiome researchers to communicate ongoing research within the Army, Navy, and Air Force to identify research and capability gaps and coordinate research, while leveraging capabilities and resources. The annual TSMC meeting is designed to enable information sharing between DoD scientists and leaders in the field of microbiome science, thereby keeping DoD consortium members informed of the latest advances within the microbiome community and facilitating the development of new collaborative research opportunities. We publish the Annual Meeting Reports, so please check them out if you are interested in learning more about microbiome research within the DoD.

The meeting is a little different this year; it was originally scheduled to be in Burlington, VT, but we felt it was prudent to make it a virtual event because of the ongoing pandemic. None-the-less, we encourage you all to take advantage of the interactive features of our virtual event as much as possible to make TSMC 2020 as vibrant as usual. We also hope to see you at our virtual social on Thursday evening!

We hope you find TSMC 2020 informative and useful. The final session of the meeting is an open forum to discuss how the TSMC can be even more useful in the future, so please plan on participating; if we have learned anything from microbiome research, it is that 'it takes a village'!

Let the symbioses begin!

Jason Soares

Chair, TSMC
Soldier Performance Optimization Directorate
US Army Combat Capabilities Development
Command - Soldier Center
Natick, MA 01760

Michael Goodson

Vice-Chair, TSMC
711th Human Performance Wing
Air Force Research Laboratory
Wright-Patterson Air Force Base, OH 45433

TSMC Meeting Reports

- 1st annual meeting
- 2nd annual meeting
- 3rd annual meeting

Other TSMC Publications

- 'Evaluation of Probiotics for Warfighter Health and Performance'
- 'Gut Microbiota-Targeted Interventions for Reducing the Incidence, Duration and Severity of Respiratory Tract Infections in Healthy Non-Elderly Adults' coming soon....

TSMC is about engaging and connecting with each other as well, and we have plenty of opportunities join the conversation and network throughout this event. Join the live Q&As and networking meetings and continue the conversation on social media engagement. To get the most out of TSMC 2020, make sure to explore your TSMC 2020 Attendee Guide and get familiar with the agenda.

This agenda is interactive! Click through the table of contents to get to that section. In the schedule, each of the speakers' names will take you to their abstract. Don't forget you can contact Christy Carson at ccarson@ues.com or Lorrie Strausbaugh at lstrausbaugh@ues.com with any questions.

Table of Contents

Schedule

24 September 2020

0730-0800	**Login**
0800-0805	**Welcome**
0805-0815	**Opening Remarks** Dr. Joseph L. Corriveau, Director U.S. Army ERDC-CRREL
0815-0820	**TSMC overview** Mr. Jason Soares, Chair, CCDC-SC Dr. Michael Goodson, Vice-Chair, AFRL
0820-0830	**TSMC 3rd annual meeting synopsis** Dr. J. Philip Karl, USARIEM
0830-0910	**Biotech Community of Interest Overview** Dr. Rajesh Naik, Chief Scientist, AFRL
0910-0955	**Keynote Speaker #1** Dr. Christopher Voigt, MIT
0955-1010	**Break**
1010-1200	**Technical Session #1: Enabling Technologies** Chair: Mr. Kenneth Racicot, CCDC-SC
1010-1040	- Dr. James Comolli, MIT-LL
1040-1110	- Mr. Robert Jones, US Army ERDC-CRREL
1110-1140	- Dr. Armand Dichosa, Los Alamos National Lab
1140-1200	- **Q&A – Session #1**
1200-1300	**Lunch**
1300-1345	**Keynote Speaker #2** Dr. Eran Elinav, Weizmann Institute of Science, Israel
1345-1535	**Technical Session #2: Microbiome for Health & Performance** Chair: Dr. Phil Karl, USARIEM
1345-1415	- Dr. Allison Hoke, ORISE Fellow, WRAIR
1415-1445	- Dr. Xiaomeng You, Post-doctoral Fellow, Brigham & Women's Hospital
1445-1515	- Dr. Blake Stamps, UES/AFRL
1515-1535	- **Q&A – Session #2**

24 September 2020 (Continued)

1535-1550	**Break**
1550-1700	**Lightning Talks Session #1** Moderators: Dr. Vanessa Varaljay, AFRL; Dr. Karl Indest, US Army ERDC
1550-1600	- Dr. Robyn Barbato, US Army ERDC-CRREL
1600-1610	- Dr. Alison Thurston, US Army ERDC-CRREL
1610-1620	- Ms. Flora Cullen, US Army ERDC
1620-1630	- Ms. Elizabeth Corriveau, US Army ERDC-CRELL
1630-1640	- Dr. Angelina Angelova, NRL
1640-1700	- **Q&A – Lightning #1**
1700-1705	**Closing Remarks (TSMC Chairs)**
1715-1815	**Networking Social – Talking Microbiome (BYOVB)**

25 September 2020

0730-0800	**Login**
0800-0805	**Day 2 - Opening Remarks**
0805-0850	**Keynote Speaker #3** Dr. Kristen DeAngelis, UMASS-Amherst
0850-1040	**Technical Session #3: Environmental Microbiome** Chair: Dr. Robyn Barbato, US Army ERDC-CRREL
0850-0920	- Ms. Stacey Doherty, US Army ERDC-CRREL
0920-0950	- Cadet Jackson Harris, AF Academy
0950-1020	- Dr. Vanessa Varaljay, AFRL
1020-1040	- **Q&A – Session #3**
1040-1055	**Break**

25 September 2020 (Continued)

1055-1205	**Lightning Talk Session #2** Moderators: Dr. Robert Kokoska, ARO; Dr. Nancy Kelley-Loughnane, AFRL
1055-1105	- Dr. Lisa Brenner, Rocky Mountain MIRECC
1105-1115	- Dr. Nabarun Chakraborty, US Army WRAIR
1115-1125	- Dr. Karley Mahalak, USDA-ARS
1125-1135	- Dr. Ida Pantoja-Feliciano, CCDC-SC
1135-1145	- Dr. Jordan Zambrana, US EPA
1145-1205	- **Q&A – Lightning #2**
1205-1300	**Lunch**
1300-1345	**Keynote Speaker #4** Dr. Pieter Dorrestein, UCSD
1345-1535	**Technical Session #4: Microbiome Analysis & Discovery** Chair: Dr. Camilla Mauzy, AFRL
1345-1415	- Dr. Matthew Perisin, CCDC-ARL
1415-1445	- Dr. Sophie Colston, US NRL
1445-1515	- Dr. Ritesh Kumar, DuPont Nutrition & Biosciences
1515-1535	- **Q&A Session #4**
1535-1550	**Break**
1550-1650	**Post-meeting discussion panels/groups (TSMC Chairs)** - What can TSMC do to support you? - How can you support TSMC? - Working together with government, academia, and industry - Resource sharing

Abstracts

James Comolli, MIT-LL

A Generalized Method for Engineering Non-model Microbes

Abstract

Microbiomes that inhabit the gut, lung, skin, reproductive tract, or other sites of the human body are made up of hundreds to thousands of different types of microbes that contribute to health maintenance. Manipulation of these microbiomes presents an opportunity for novel diagnostics, new approaches for therapeutics, and methods to enhance performance. Many of the key microbes that comprise these host-associated communities have been well-characterized through genomic analysis, but have not been otherwise investigated. Rather than using model microbes, we are attempting to engineer native, non-model, taxa to impact microbiome function using tools (DNA elements) developed via high-throughput combinatorial assembly of a library of genetic components, identified by previous research or through genome analysis. These are intended to be capable of delivering active genes to non-model microbes with minimal effect on off-target taxa. We initially demonstrated this approach by developing new DNA elements for modification of Bacteroides, a major constituent of the gut microbiome, then demonstrating their function in Bacteroides strains from different native human microbiomes. We are extending our efforts to identify DNA elements that efficiently modify non-model microbes in other host associated communities. Currently we are developing tools for engineering Corynebacterium strains that are prevalent in the human skin microbiome and for modifying Prevotella spp. That are abundant members of the human oral and respiratory tract microbiomes. Our goal is to further develop a pipeline that enables the rapid development of tools for engineering non-model microbes to explore and augment their native functions.

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Mr. Robert Jones, US Army ERDC-CRREL

Growing the Dark Web: Melanized Fungal Structures as a Biological Wire

Abstract

Eumelanins, a dark brown to black pigmented group of the class of bio-macromolecules collectively known as melanins, are found abundantly in nature and host many biological functions that make them prime candidates for bio-based applications. The primary purpose of eumelanin, is as a protectant from various forms of environmentally induced stress including radiation (high energy gamma to thermal), oxidative stress, and it even can protect against toxic compounds such as antimicrobials and heavy metals through broad binding and chelation. Additionally, eumelanin has been highly explored as a biological semiconductor though research has focused on extracted and synthesized eumelanin. We sought to expand on the conductive properties of eumelanin, specifically in fungus which readily produces eumelanin in or around the cell wall, further by investigating the conductivity of the melanized fungal cells themselves. And we further sought to improve the conductivity by leveraging the metal binding properties of eumelanin to dope the eumelanin sheets with copper ions by growing the fungi in copper laced media. Both copper doped and copper-less fungal cells were pelletized and conductivity and resistivity were assessed via the van der Pauw method. We discovered that though the conductivity of the melanized cells was not as conductive as synthetic melanin, the conductivity was indeed greater in the copper doped sample by an order of magnitude. In further research we hope to assess the ability of melanized fungal structures to propagate electrical signals across themselves mimicking the mycelial network utilized in symbiotic plant fungal relationships.

Dr. Armand Dichosa, Los Alamos National Lab

Utilizing SCG and GMDs to advance our nation's science in public health, environmental impact, and security

Abstract

Overwhelming evidence continues to attribute microbiomes as having critical influences in human health ,national security, energy security, economy, and our global climate. Without question, genomics has provided invaluable access into these complex microbiomes to reveal its structure and function with respect to the host environment. Such findings have also revealed that there is much more to be discovered. To gain greater access to the rare and yet-to-be-identified members of the community, cultivation strategies are typically employed to provide sufficient genomic template to facilitate next-gen sequencing and bioinformatics efforts. However, > 90% of bacteria cannot be cultured. This recalcitrance to growth is due to required interactions with other microbes of the native consortium that can never be recapitulated under traditional cultivation means. To overcome this hurdle, our team integrated gel microdroplets (GMDs) with single cell genomics: Single, bacterial cells are individually captured in agarose GMDs possessing channels and pores that allow exchange of nutrients, chemical signals, gasses, wastes, etc. that the captured cell requires. Grown as a co-culture among its native microbial consortium, viable, single cells multiply as clonal microcolonies conveniently sequestered within the GMD. With high-throughput flow cytometry, millions of GMDs are rapidly screened to identify the target microcolonies and are single-sorted to undergo whole genome amplification for bacterial 16S rRNA phylotyping, genome sequencing, and bioinformatic analyses. Herein, I present the application of our SCG-GMD pipeline to various projects involving the human and environmental microbiomes to address national, energy, and public health needs, as well as visions for future applications.

Dr. Allison Hoke, ORISE Fellow, WRAIR

Longitudinal shift in fecal microbiota associated with acute and delayed effects of PTSD-eliciting stress model

Abstract

There is increased interest in the gut-brain access and alteration of the gut microbiome potentially associated with diseased states and pathophysiology. To examine the role of the microbiome on post-traumatic stress disorder (PTSD), we used a "cage-within-cage resident-intruder" model over ten days to elicit PTSD-like traits and collected feces at multiple time points in control and aggressor-exposed mice. The16S rRNA gene was assessed, alpha and beta diversity were significantly altered in response to stress, and bacterial taxa was dominated by the phyla *Firmicutes, Bacteroidetes,* and *Verrucomicrobia*. The relative abundance of the phyla *Verrucomicrobia* and *Actinobacteria* were significantly impacted longitudinally by stress and Linear Discriminant Effect Size analysis identified significant differences in *Actinobacteria* at Week 1 and *Verrucomicrobia* at Week 4, with significant alterations in lower ranking taxa. PICRUSt2 showed significant MetaCyc pathway abundances between control and aggressor-exposed mice. This longitudinal study illustrated that exposure of mice to stress does significantly alter gut microbiota and is a valuable tool in investigating the impact of PTSD.

Dr. Xiaomeng You, Post-doctoral Fellow, Brigham & Women's Hospital

Bone loss with aging is independent of age-related gut microbiome dysbiosis in mice

Abstract

Emerging evidence suggests an important role of the microbiome in bone health. However, it is unclear whether microbial dysbiosis with aging contributes to age-related bone loss. Here, age-specific microbial signatures were characterized and their roles in age-related bone loss were investigated in a murine model. 16s rRNA gene sequencing of fecal samples from 3- and 24-month old CB6F1 males indicated an age-dependent shift towards a Firmicutes-dominant community with an alteration in energy and nutrient metabolism potential. Muscipirillum, a pathobiont associated with host inflammation, was increased with age. An integrative analysis of 16s predicted metagenome and LC-MS fecal metabolome revealed enrichment of amino acid biosynthesis pathways in aged mice. Microbial methionine and S-adenosyl methionine metabolism were increased in the aged mice, which have previously been associated with the host aging process. Collectively, aging caused microbial taxonomic and functional dysbiosis in mice. Bone structure was assessed by micro-computed tomography 1 and 6 months after colonization of germ-free (GF) mice by fecal transplant from 3- or 24-month old specific pathogen-free (SPF) mice. The effect of colonization on bone phenotypes was independent of the microbiome donor age. Moreover, age related bone loss was similar in 24-month old GF mice compared to their littermates colonized at 8-week. Furthermore, bone loss from 3 to 24 months was indistinguishable in GF compared to SPF mice. Thus, GF mice were not protected from age-related bone loss. In conclusion, our study indicates age-related bone loss occurs independent of age-related gut microbial dysbiosis.

Dr. Blake Stamps, UES Fellow, AFRL

Exploring changes in the host gut microbiota during a controlled human infection model for *Campylobacter jejuni*

Abstract

Campylobacter jejuni is associated with 7.5 million disability-adjusted life years globally, and is a leading cause of foodborne disease in the United States. Additionally, it is a leading cause of travelers' diarrhea, particularly in Southeast Asia. For deployed military populations, the pathogen represents an important infectious disease threat for which primary prevention is needed. One method that offers an early assessment of potential products for *C. jejuni* prevention is the controlled human infection model (CHIM) in which a known dose of a well-characterized organism, such as *C. jejuni,* is administered to susceptible subjects in a well-controlled environment. We previously utilized a CHIM to determine if prophylactic administration of the antibiotic rifaximin prevented campylobacteriosis. This study also provided an opportunity to observe the response of the total host gut microbiome utilizing 16S rRNA gene sequencing under chemoprophylaxis and when challenged by *C. jejuni* under highly controlled conditions with multiple temporal samples. After removing an outlying sample, placebo recipients showed no difference in the relative abundance of *C. jejuni* compared to subjects given rifaximin. The relative abundance of *C. jejuni* was also not correlated with symptom presentation or severity. *C. jejuni*-treated subjects that did not meet the clinical definition of moderate to severe diarrhea had a decreased relative abundance of the *Bacteroides* and an increase in the relative abundance of the *Ruminoclostridium* prior to pathogen challenge. These results suggest a tantalizing potential protective effect of microbial communities, and point to potential targets for future probiotic study.

Dr. Robyn Barbato, US Army ERDC-CRREL

DRTSPORE: A model that predicts soil activity across the landscape

Abstract:

Environmental properties influence biological activity and collectively define the state of soils, yet these properties are typically described through time-intensive, ground-based sampling efforts. Techniques have been developed to assess microbial community structure and function in soils; however, questions remain regarding which environmental data are most relevant and how and to what degree their fluxes would impact the system. Our aim was to investigate the soil microbiome's response to soil particle size distribution, temperature, and water potential because they are well-established factors that influence microbial activity and could be predicted using remote sensing data and weather forecasts. We conducted an extensive investigation subjecting four soils to a range of temperatures and moistures and measured their activity through CO_2 efflux and resolved microbial community shifts through amplicon sequencing. Our results showed changes in CO_2 efflux rates according to organic matter content and dynamic conditions as well as bacterial community shifts that were more distinct in soils with limited nutrients, suggesting a buffering capacity inherent in high nutrient soils. The fungal patterns differed in that their response to temperature and moisture was similar regardless of the soil physiochemical properties. This information will feed into Geospatial Repository and Data Management System products for mission planning focused primarily at the tactical level for mobility and maneuverability.

Disclaimer: The views expressed in this abstract are those of the authors and do not reflect the official policy of the Department of Army, Department of Defense, or the U.S. Government.

Dr. Alison Thurston, US Army ERDC-CRREL

Changes in Permafrost Microbial Community Composition after Thaw

Abstract

Arctic and Subarctic permafrost is thawing at an unprecedented rate, significantly altering the ecosystem. Microbial blooms triggered by permafrost thaw accelerate global warming, change permafrost structure and impact the vegetation. Where and when these blooms will occur is poorly understood. Our study examined the microbial communities in permafrost over a controlled thaw regime. Samples consisted of six different permafrost soils collected in interior and northern Alaska. Both abiotic and biotic factors were examined in to investigate drivers of the microbiome and associated processes. We seek to determine whether the initial microbial and biochemical compositions of permafrost soils reflect the final compositions to better understand succession of the microbial community over thaw.

Ms. Flora Cullen, US Army ERDC

Abstract

Bacteria are remarkable organisms capable of carrying out complex and highly specific processes. Their uses range from the manufacture of food and medicines to enzymes, fuels, and solvents. Psychrophiles are an even more specialized category of bacteria including those that grow optimally at 15 ° C or below. These incredible organisms produce modified proteins, lipids, and polysaccharides to protect against cold temperatures and are capable of growth at temperatures as low as -18 ° C (Clarke et al. 2013). These adaptions make psychrophiles organisms of interest for various cold-region applications.
In this poster I outline the methods we have used to create a catalog of psychrophiles isolated from a variety of cold sources including arctic seawater, the Greenland ice sheet, Alaskan permafrost, and even Antarctica in order to create a library of phychrophilc and psychrotolerant organisms at the Cold Regions Research and Engineering Laboratory. I will also discuss potential psychrophiles of interest and give an overview of the various projects these organisms are already being used for as well as potential future applications.

Ms. Elizabeth Corriveau, US Army ERDC-CRELL

UV-Resistant Psychrotolerant Microorganisms: Understanding the Role of Pigmentation for Survival

Abstract

Microorganisms that survive in icy environments with long daylight hours (e.g. Greenland ice sheet) also possess biological pigments (e.g. chlorophyll, phycobilins, and carotenoids) to survive the extreme Ultraviolet (UV) light irradiation (Cockell et al., 1999; Dieser et al., 2010). Carotenoid pigments in particular enable survival of psychrotolerant bacteria (Dieser et al., 2010). We developed the CRREL Psychrophile Library, which is a library of bacterial isolates obtained from cold regions. Of these, 23 bacterial isolates from Greenland ice are strong candidates for further analysis because of their pigmentation. After using the MinION sequencer (Oxford Nanopore) to obtain the whole genome sequences of our organisms, the goal of this project is to investigate the genes associated with carotenoid pigments and cold adaptations in the genome. We adapted a bioinformatics pipeline to BLAST whole genome sequences of our organisms against existing carotenoid databases (Nupur, 2016). These existing databases also provide data which play a major role in the identification of carotenoids, such as NMR, UV-vis absorption, IR, MS and HPLC. In future research, we plan to use this process to screen for potential UV-resistant bacteria from our CRREL Psychrophile Library.

Dr. Angelina Angelova, NRL

Compositional variability and biodegradative potential of the plastisphere in aquatic environments

Abstract

Microbial communities have been known to play a significant role in degradation of xenobiotic contaminants in the environment and have been implicated for their ability to affect plastic contaminants. Despite this, very few studies have utilized '-omic' technologies to explore the biodegradative capacity of naturally formed plastic-associated microbial communities. In this study, we utilized shotgun sequencing to explore the metagenomic content of natural microbial biofilm communities accumulating on three common plastics (PE, PP and PET) in both marine and estuarine environments. The results indicated that factors influencing the taxonomic composition of the plastic-associated communities did not include the type of plastic and that despite variability in the chemical composition of the plastic, maturation of the microbial communities did not increase their potential for plastic biodegradation. Generally, the communities showed abundant capacity for plastic degradation, however, no selective pressure, niche partitioning or genetic directionality were observed in response to the chemical backbone of the different plastics, even after long term exposure. We conclude that floating plastic litter play the role of mere substrata and are not of metabolic interest to microbial communities within aquatic environments, despite their metabolic capacity for such function. Any microbially mediated plastic degradation, was therefore postulated to occur as a side effect of other microbial processes.

Ms. Stacey Doherty, US Army ERDC-CRREL

Determination of the ecological processes structuring microbial communities during permafrost thaw using a phylogenetic null modeling approach

Abstract

Phylogenetic and functional beta diversity are closely related in soil microbes. Given this relationship, phylogenetic approaches can be used to understand functional traits of microbial populations and the ecological processes that structure their identity and abundance (i.e. assembly) in a community. Phylogenetic null modeling can be used to differentiate between deterministic and stochastic processes in community assembly. Deterministic processes are driven by abiotic and biotic selection pressures and stochastic processes, which include inherent randomness, are more unpredictable. Permafrost thaw results in shifts in community composition due to the dramatic environmental change, however the ecological processes that shape the final composition are less understood. We characterized microbial community assembly during permafrost thaw using *in situ* observation and a laboratory incubation of soils from the Storflaket Mire in Abisko, Sweden where permafrost thaw has occurred over the past decade. The dominant assembly processes were determined at eight depths encompassing active layer, transition zone, and permafrost soils. We found permafrost thaw increased the relative contribution of stochastic assembly immediately after thaw. Ecological drift became particularly important after laboratory-induced thaw of deeper soil layers. Our results suggest the observed increase in stochastic assembly with thaw may result in increased carbon emissions due to poor fitness and low efficiency of the final community. Identification of the dominant microbial community assembly processes can to improve our ability to predict the functional implications of permafrost thaw.

Cadet Jackson Harris, AF Academy

PFAS-Degrading Microorganism Mining

Abstract

Per- and polyfluoroalkyl substances (PFAS) concentrations in some contaminated public ground and surface waters are orders of magnitude higher than EPA limits for drinking water. Such a high level of PFAS pose serious danger to wild life and human health. Conventional methods, such as incineration, for remediating PFASs are hazardous, costly, or currently inefficient at large scales. Thus, there is an urgent need for developing novel technologies for mitigating PFAS contaminations. Here, we explore biological methods for PFAS degradation by isolating microorganisms with PFAS degradation activity and mining their genomes to identify potential enzymes and pathways involved in the process. Soil isolated from a previously contaminated PFAS site was added to Winogradsky columns containing either PFAS (100ppm or 666ppm) or Perflourooctanoic Acid (PFOA; 100ppm or 750 ppm) artificially contaminated water. These samples sat undisturbed for 6 weeks, prior to removing a sample from the aerobic layer for 16S rRNA gene amplicon sequencing analysis. A subsequent aerobic layer and soil layer was sent for similar analysis after three months. A comparison between these contaminated columns and the control columns displays a shift in the microbiome communities suggesting there is a population of microorganisms enriched for PFAS degradation. Using this information, we isolated microorganisms from these contaminated soil sites and identified both bacterial and fungal isolates with the capability to grow on and degrade PFAS using a fluorine detection assay. These isolates can grow on PFOA as their sole C source over the course of weeks. Bioinformatics approaches were used to identify dehalogenases associated with one of the isolates (*Delftia acidovorans*), which will be cloned and expressed in a heterologous host to test if these enzymes can degrade PFAS in the laboratory. The genomes of this and other isolates will be sequenced as well as metagenomes directly from the contaminated soil samples to mine for (e.g., using Hidden Markov Modeling) the suite of potential degradation mechanisms and the taxa which contribute to this process. Ultimately we aim to predict the classes of PFAS-degrading microorganisms and enzymes to use for optimized PFAS degradation.

Dr. Vanessa Varaljay, AFRL

Microbiomes of military aircraft and vehicles and their connection to biocorrosion and biodeterioration

Abstract

Microbial communities can contribute to biodegradation of coatings and insulation within the built environment. However, no studies have addressed the composition and effects of environmental microbial communities on military aircraft and vehicles. Therefore, our laboratories characterized the total (fungal and bacterial) microbiomes of surface-contaminated samples including 4 military aircraft and 3 military trucks. A combined ultra-deep DNA and RNA sequencing based approach was used to capture both the genetic capability and expression profiles of the aircraft and trucks sampled. Transcriptionally active microbiomes, quantitated based on small subunit ribosomal RNA expression data extracted from aircraft metatranscriptomes, were dominated by fungi while the trucks were dominated by bacteria. The data were then mined for hydrolase enzymes such as lipases, esterases, cutinases, and proteases, known to be key players in polyurethane polymer degradation. Hundreds of putative hydrolase enzymes were recovered for further verification and validation, with selected candidates to be expressed and tested for polymer-degrading activity in a heterologous chassis in the lab. These data will ultimately be used for the assessment of the biodegradation potential in microbial communities on polyurethane polymer surfaces and for environmental enzyme bioprospecting for industrial and bioremediation purposes.

Dr. Lisa Brenner, Rocky Mountain MIRECC

Evaluation of an Immunomodulatory Probiotic Intervention for Veterans with Co-Occurring Mild TBI and PTSD: a Pilot Study

Abstract

Background: United States military Veterans from recent conflicts are coping with symptoms related to signature injuries of the conflicts (mild traumatic brain injury [mTBI] and persistent post concussive [PPC] symptoms, and posttraumatic stress disorder [PTSD]). One potential common underlying feature of both mTBI and PTSD is exaggerated inflammation; peripherally and in the central nervous system, which is thought to play an important role in the vulnerability to, aggravation of, and perpetuation of adverse consequences of these co-occurring conditions.

Objective: To assess the feasibility, acceptability, and safety of a probiotic intervention. Efforts were also aimed at beginning to evaluate potential biological outcomes

Design: Pilot randomized controlled trial with US military Veterans from recent conflicts in Iraq and Afghanistan. Participants were randomized to intervention (*Lactobacillus reuteri* DSM 17938) or placebo supplementation at a 1:1 ratio, stratified irritable bowel syndrome status.

Results: Thresholds for feasibility, acceptability, and safety were met. Probiotic supplementation resulted in a decrease in plasma C-reactive protein (CRP) concentrations that approached statistical significance ($p = 0.056$). Although during the Trier Social Stress Task (TSST; administered post-supplementation) no between-group differences were found on a subjective measure of stress responsivity (Visual Analogue Scale), there was a significantly larger increase in mean heart beats per minute between Baseline and the Math task for the control group as compared to the probiotic group (Estimated Change, Probiotic 5.3 [-0.55, 11.0], Control 16.9 [11.0, 22.7], $p = 0.006$).

Findings: Results suggest that CRP may be a viable inflammatory marker of interest. Further research is required.

Dr. Nabarun Chakraborty, US Army WRAIR

Impact of radiation dose and temporal delay on host-biome relationship and fecal metagenomics

Abstract

Radiation exposure even with low doses can cause several health effects with various clinical symptoms referred as acute radiation syndrome (ARS). Even a low dose of ARS causes hematopoietic sub-syndrome and higher doses of ARS damages the gastro-intestinal system and central nervous system. Our recent mouse total body radiation (TBI) study showed early elevation of inflammatory networks caused by ARS, which justified our present investigation on the gut microbiota, since the microbial community plays key role in mediating the host's immunological fitness. Mice of 12-14w ages were irradiated bilaterally in the AFRRI's cobalt-60 gamma radiation facility to total midline doses of 9.5Gy and 11Gy, respectively. Fecal samples were collected before irradiation, and 1-day, 3-day and 9-day post-TBI. The V3-V4 hyper-variable regions of the 16s DNA was sequenced in MiSeq platform (Illumina, Inc.) The second aliquot of fecal samples was probed for untargeted metabolomics profiling. The Principal coordinate analysis depicted a time resolved increment of α-diversity in microbial landscape; dose independent 9-day post-TBI clustered furthest from the baseline- a sign of increased variations of fecal microbial richness. In contrast, β-diversity showed gradual reduction of evenness from its pre-irradiation state. Further, 11Gy caused a significant longitudinal increment of Firmicutes' percentage abundance and the ratio of Firmicutes and Bacteroidates. A synchronized temporal bias was also evident in the metabolite profile. In conclusion, the lethal radiation dose temporally increased the richness but diminished the evenness of fecal microbiota.

Dr. Karley Mahalak, USDA-ARS

The *in vitro* human gut microbiome system

Abstract

The human gut microbiome is a complex ecosystem that has a great influence on human performance, health, and disease. This bacterial community can change rapidly with variations in environmental conditions, such as nutrition, pH, and other stressors. Our group uses batch-culture *in vitro* experiments to understand how these changes occur in an effort to provide guidance on optimizing gut health. Our Center hosts the only TWINSHIME® in the USA, a unique, *in vitro* system that can include both lumenal and mucosal phases of the gut microbial population along with the three regions of the large intestine. Using this system, we are able to establish a stable gut microbial community *in vitro*, this stability of 5 weeks and is long enough to implement adequate and advance evaluation of most food and food components. To date, we have worked with multiple collaborators, including researchers at ProDigest, the University of Massachusetts, and the University of Pennsylvania, to explore the effects of the antimicrobial triclosan, the alternative natural sweetener stevia, and the effects of pH on the gut microbial community. Our future work includes projects regarding multiple fiber types, milk, a food supplement bar, and the development of a small intestine model.

Dr. Ida Pantoja-Feliciano, CCDC-SC

In vitro fermentation as a complement to human studies to explore gut microbial composition and metabolic capacity

Abstract

Gut microbiome homeostasis *in vivo* is due to complex microbial interactions which can be perturbed by acute stress-induced changes in the gastrointestinal environment, potentially leading to dysbiosis. Diet in particular, can greatly influence the dynamics of gut microbiome composition and metabolism. In our laboratory we used an in vitro gut model to investigate the influence of a sudden change in diet, namely 21 days sole sustenance on the Meal, Ready-to-Eat (MRE) U.S. military combat ration relative to volunteers on habitual diet (HAB), on population dynamics within the gut microbiome using fermentable fiber and protein substrates. Fecal samples collected from individuals before and after consuming their habitual diet or only MREs for 21 days underwent 24hr *in vitro* fermentation in nutrient-rich media supplemented with saccharolytic and proteolytic substrates under ascending colon domain-specific conditions. 16S rRNA amplicon and Whole Genome Sequencing (WGS) were used to measure community composition and functional potential. Specific statistical analysis were implemented to detect changes in relative abundance from taxa and genes and pathways. These findings will reveal how gut microbial metabolic capacity is altered as a function of a sudden change in diet. Additionally, this study begins to identify candidate nutritional intervention substrates for modulating the gut microbiome to impact on health and performance.

Disclaimer: The views expressed in this abstract are those of the authors and do not reflect the official policy of the Department of Army, Department of Defense, or the U.S. Government.

Dr. Jordan Zambrana, US EPA

EPA Activities Related to Indoor Microbiomes and Human Health

Abstract

People spend most of their time indoors, however limited knowledge is available on the influence of human activities on indoor microbial ecology, and the impacts changes to this microbiome may have on human health and well-being. The U.S. Environmental Protection Agency's (EPA) Indoor Environments Division (IED) develops voluntary strategies for reducing or eliminating indoor exposures to airborne contaminants that contribute to health risks of occupants of homes, schools and other non-industrial buildings. IED also supports and promotes research to address identified gaps in knowledge and allow for greater dissemination of information related to the indoor environment, microbiomes and human health. This includes several activities EPA sponsored through the National Academies of Sciences, Engineering and Medicine to convene scientific experts and develop summary or consensus reports. Furthermore, IED hosts scientific and informational webinars to highlight research and generate discussion related to the indoor microbiome. IED has also developed practical strategies for improving indoor air quality to protect public health, including the recently updated guidance on the use of residential air cleaners. Research shows that filtration can be an effective supplement to source control and ventilation in improving indoor air quality, however little is known about its relationship with indoor microbiomes. IED is interested in the potential implications of the use of more efficient portable air cleaners and HVAC filters, among other factors affecting indoor air quality, for built environment microbiomes.

Dr. Matthew Perisin, CCDC-ARL

Metagenomics and genome-scale metabolic modeling of microbes sampled from corroded Army equipment

Abstract

Corroding and degrading equipment costs the DoD over $20 billion dollars each year for repairs and downtime. Microbes are pervasive on this equipment and can form dense biofilms that induce and accelerate corrosion. These biofilms commonly involve multiple species that metabolically interact but identifying these interactions and their roles in corrosion is difficult *in situ*. Understanding these ecological interactions will inform intervention strategies to prevent corrosion. To first identify microbes that have colonized corroding equipment, we sampled biofilms from equipment located in a boneyard at Aberdeen Proving Ground. We extracted DNA, performed whole genome sequencing, and classified taxa to define the community composition. To understand the functional capacity of these communities, we assembled metagenomes and binned contigs into metagenome assembled genomes (MAGs). We used these MAGs to create genome-scale metabolic models to predict complex metabolic interactions between microbes. The models further informed possible metabolisms that influence corrosion. Overall, these results will identify intervention points to prevent corrosion.

Dr. Sophie Colston, US NRL

Multiomic analysis of the gut microbiome of US Navy divers

Abstract

Purpose. The human gut microbiota is a complex and diverse ecosystem with implications in various components of host health. Gut microbes may also play a role in physical performance and endurance, which can significantly impact or be influenced by occupational activities. As there are relatively few studies investigating the impact of diving on the gut microbiome, the Naval Research Laboratory (NRL) and the Navy Experimental Diving Unit (NEDU) have partnered to collect samples from human subjects involved in diving-centric operations and employ a multi-level analysis to ascertain the impact on the microbiome, and ultimately to warfighter physiological and pathophysiological functions.

Methods. Stool samples and metadata were collected from subjects in a time series that included four timepoints: pre-dive/baseline (1), between dives (2), and post-dive (1). DNA was extracted from preserved samples for targeted deep sequencing (16S/18S rRNA) and metagenomic sequencing and analysis. A portion of each sample was also used to test protein extraction methods which included mechanical, chemical, or a combination of both for subsequent metaproteomic analysis.

Conclusions. 16S/18S rRNA analysis provided a general perspective of the microbial diversity found in the gut microbiome as well as potential trends inferred from comparisons between different timepoints and subjects. Metagenomics will provide further characterization of the community structure as well as the genetic diversity and potential ecological framework therein. The optimization of protein extraction techniques will allow for more robust metaproteomic methodologies and, together with the corresponding metagenome, may give us deeper insight into the physiological functions of the microbial community.

Dr. Ritesh Kumar, DuPont Nutrition & Biosciences

Comparative microbiome study in lean vs. obese population: Discovery of novel probiotics for metabolic health

Abstract

Metabolic disorders have become a major health problem of the modern world. The economic burden from these disorders is significant, not only in the Western world, but globally with the spread of the Western lifestyle. The costs are from heath care and also from a loss of economic activity. There is persuasive evidence that gut microbiota extends effects beyond gut health. The role of gut microbiota is very well established in metabolic disorders, as it affects the host's energy balance, nutrient availability, and immunity (inflammation and autoimmunity), and can lead to metabolic dysfunction (e.g., insulin resistance and deficiency, obesity, cardiovascular disorders). In this study, we compared the microbiome of lean vs. obese cohort and identified the OTUs associated with the lean subjects. Lean/lean healthy-associated OTUs were further correlated to relevant clinical metabolic markers to evaluate their potential influence on beneficial health outcomes. We isolated and characterized around 5000 strains from lean individuals. We tested the efficacy of four bacterial species (*Eubacterium eligens, Intestinimonas massiliensis, Prevotella copri, Akkermansia* sp and *Akkermansia* sp in combination with *I. massiliensis*) in a diet-induced obesity (DIO) mice model. These bacterial strains were also evaluated for antibiotic susceptibility and stability. *P. copri, Akkermansia* sp, and *Akkermansia* sp in combination with *I. massiliensis* have demonstrated improvements in insulin resistance, glucose tolerance and decrease in leptin levels in DIO mice. Our findings demonstrate that the novel strains identified in this study have potential as safe next generation probiotics and modulate the severity of the host's metabolic dysfunctions.

Acknowledgements

We would like to thank all of those who have contributed to the planning and success of TSMC2020! We feel this long and distinguished list conveys the interest and importance of this meeting to the Department of Defense and to the microbiome field in general.

Specifically, we would like to thank:

- Keynotes, Speakers, and the TSMC 2020 Participants for their interest and commitment
- Session Chairs for moderating their sessions: Dr. Robyn Barbato; Dr. Karl Indest; Dr. J. Philip Karl; Dr. Camilla Mauzy; Dr. Robert Kokoska; Dr. Nancy Kelley-Loughnane; Mr. Kenneth Racicot; and Dr. Vanessa Varaljay
- TSMC 2020 Annual Meeting Planning Committee for their dedication and enthusiasm: Mr. Jason Soares (TSMC Chair), Dr. Michael Goodson (TSMC Vice Chair); Dr. Robyn Barbato; Dr. J. Philip Karl; Dr. Robert Kokoska; Dr. Camilla Mauzy; and Mr. Kenneth Racicot
- The Office of Naval Research for their generous support of the meeting.
- And finally, we would *especially* like to thank Ms. Erin Ruef, Ms. Christy Carson, Ms. Cassidy Wright, Ms. Lorrie Strausbaugh, Dr. Stephaney Shanks, and team at UES, Inc., for handling the logistics surrounding TSMC2020, including pivoting to a virtual event, with such patience and enthusiasm

If you are interested in learning more about the TSMC, please reach out to your TSMC working group representative:

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Dr. J. Philip Karl (USARIEM)
Dr. Robert Kokoska (ARO)
Mr. Kenneth Racicot (CCDC SC)
Dr. Chris Sund (ARL)

Look out for the upcoming TSMC meetings!

TSMC Topical meeting on Bioinformatics for Microbiome Science – 9-10 November 2020

Sessions:

- Amplicon sequencing
- Genomics/transcriptomics
- Proteomics
- Metabolomics
- Multi-omics integration
- Statistical analyses and visualization
- Predictive approaches

17 Confirmed speakers:

Pat Schloss (Michigan)
Ashley Shade (Michigan State)
Greg Caporaso (Northern Arizona)
Adam Arkin (UC-Berkeley)
Adam Phillipy (NIH)
Harris Wang (Columbia)
Mark Saito (Woods Hole OI)
Robert Hettich (Oak Ridge NL)
Jennifer Pett-Ridge (LLNL)
Ali Rahnavard (George Washington)
Ben Bowen (JGI)
Sean Gibbons (UWashington)
Susan Holmes (Stanford)
Rita Colwell (UMaryland)
Amy Willis (UWashington)
Daniel Segre (BostonU)
Yana Bromberg (Rutgers)

…and TSMC 5th Annual Meeting Fall 2021. More info to come….

Attendees

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Mr. David Ahlberg	Student
Dr. Erin Almand	USAFA
Ms. Danielle Anderson	US Army Combat Feeding
Mr. Steven Arcidiacono	Army
Dr. Lucia Arsintescu	SJSU/NASA
Dr. Deepika Awasthi	Lawrence Berkeley Lab
Dr. Mahamat Babagana	USN
Dr. Robyn Barbato	US Army CRREL
Mr. Nicholas Bathurst	San Jose State University
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Dr. Alexander Burdette	59th MDW Office of Science and Technology
Dr. Tina Burke	WRAIR
Dr. David Butler	National Academies of Sciences, Engineering, and Medicine
Dr. David Butler	National Academies of Sciences, Engineering, and Medicine
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Dr. Julie Caruana	Naval Research Lab
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Dr. Eran Elinav	Weizmann Institute of Science
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Ms. Heather Fagnant	Employee
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Ms. Jeanette Frey	Henry M. Jackson Foundation
Dr. Anthony Fries	USAF
Dr. Justin Gallivan	AFRL
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Dr. Jo Halford	Navy / NSMRL
Dr. Svetlana Harbaugh	AFRL
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