6th Annual TSMC Meeting Program

TSMC
Tri-Service Microbiome Consortium

Hybrid Meeting | 27-28 Sept 2022

Lake Morey Resort, Fairlee VT

TSMC Annual Meeting 2022

Welcome!

Meeting Purpose

On behalf of the Tri-Service Microbiome Consortium (TSMC), we welcome you to the Office of the Undersecretary of Defense (Research & Engineering), Biotechnology Community of Interest, 6th Annual TSMC Meeting: TSMC 2022! We are back in person, and 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.

We encourage you all to take advantage of the interactive features of our hybrid event as much as possible to make TSMC 2022 as vibrant as usual. We also hope to see you at our no-host social on Tuesday evening! We hope you find TSMC 2022 informative and useful.

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

Please check back often for updates

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Event Resources

- TSMC Annual Meeting 2022 Website
- Download the latest version of Zoom here
- Click here to join Day 1 Meeting: Day 1 Zoom Link
- Click here to join Day 2 Meeting Link: Day 2 Zoom Link

Past TSMC Meeting Resources

- **TSMC Annual Meeting Reports**

• 1st Annual Meeting

• 2nd Annual Meeting

• 3rd Annual Meeting

• 4th 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

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PRE-MEETING ACTIVITIES:

Monday 26 Sept 2022 (all times US EST)

1530-1830 **CAC-card holder only meeting**

Organizers: Dr. Dasha Leary, NRL; Dr. Camilla Mauzy, AFRL; Mr. Kenneth Racicot, Army DEVCOM SC; Dr. Robyn Barbato, Army ERDC-CRREL

- Location: ERDC-CRREL auditorium (CAC-card required)
- In-person attendees only
- Controlled Unclassified Information (CUI)-specific

POST-MEETING ACTIVITIES:

Thursday 29 Sept 2022 (all times US EST)

0900-1100 **US Army ERDC-CRREL Facility Tour**

Organizers: Dr. Robyn Barbato, ERDC-CRREL

- Location: ERDC-CRREL main campus
- Specific Tour Details will be available at the meeting
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Tuesday 27 Sept 2022 (all times US EST) | Day 1 Zoom Link

0730-0830	**Check-in/Login/Morning Social**
0830-0840	**Opening Remarks & TSMC overview**
	Mr. Jason Soares, Chair, Army DEVCOM SC
	Dr. Michael Goodson, Vice-Chair, AFRL
0840-0900	**OUSD (R&E) Biotechnology Overview**
	Dr. Katherine Sixt, Acting Director Biotechnology
0900-0955	**Special Session – User Engagement**
0900-0920	HN Justin DeRose, NSMRL
0920-0940	LTC Andrew Farina, West Point Military Academy
0940-0955	User Representative panel discussion
0955-1025	**Morning Break**
1025-1155	**Session 1 - Human Microbiomes: Stress Response**
	Chairs: Dr. Michael Goodson, AFRL & Dr. Richard Agans, AFRL
1025-1045	Dr. Blake Stamps, AFRL: Changes in the composition of the gut microbiome during a prolonged submarine deployment
1045-1105	Dr. Scott Merrell, USUHS: Nasal microbiota evolution within the congregate setting imposed by military training
1105-1125	Dr. Nabarun Chakraborty, WRAIR (Virtual): Bioenergy Homeostasis: A Major Node in Functional Microbiome's Stress Response Model
1125-1145	Dr. Lisa Brenner, Dept. Veteran Affairs (Virtual): Medications and Gut, Oral, and Skin Microbiomes: Implications for Health and Intervention: A United States-Veteran Microbiome Project (US-VMP) Study
1145-1155	Session Panel Q&A
1155-1315	**LUNCH**
1315-1355	**Keynote Speaker: Drs. Chris Voigt, MIT**
1355-1525	**Session 2 - Microbiome Analysis & Surveillance**
	Chairs: Dr. Dasha Leary, NRL & Dr. Phil Karl, USARIEM
1355-1415	Dr. Matthew Rusling, WRAIR (Virtual): Delayed Impact of Radiation on Fecal Microbial Composition Depends on Gender

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1415-1435	Mr. Morie Alpha, NSWC Indian Head: Digital Engineering Biodefense
1435-1455	Ms. Christine Mariskanish, WRAIR: Aerobiological Surveillance using Next-Generation Sequencing and Metagenomics Analysis
1455-1515	Dr. Vijay Singh, USUHS (Virtual): Microbiome study in irradiated mice treated with BIO 300, a promising radiation countermeasure
1515-1525	Session Panel Q&A
1525-1545	**BREAK**
1545-1730	**Session 3 - Human Microbiome Enablers & Engineering (lightning talks)** Chairs: Dr. Ida Pantoja-Feliciano, DEVCOM SC & Dr. Camilla Mauzy, AFRL
1545-1555	Guest Speaker: Dr. Brandon Fields, MIT
1555-1605	Dr. Linda Chrisey, DARPA (Virtual): DARPA ReVector Program Aims to Reduce Mosquito Attraction by Engineering the Human Skin Microbiome.
1605-1615	Dr. Kristi McElmurry, US Air Force Academy (Virtual): Fighting *Pseudomonas aeruginosa* Wound Infections with an Engineered Skin Microbe
1615-1625	Dr. Karley Mahalak, USDA (Virtual): Insoluble rice bran fiber modifies gut microbial diversity in comparison to soluble fiber in vitro
1625-1635	Dr. Christopher Stamper, Dept. Veteran Affairs (Virtual): Comparison of stabilized & non-stabilized gut microbiome samples via 16S rRNA sequencing: A United States-Veteran Microbiome Project (US-VMP) Study
1635-1645	Dr. Elizabeth Wiellette, Draper: Immune Response Evaluation: A platform for evaluation of innate-adaptive immune response to microbes and antigens
1645-1655	Mr. Steven Arcidiacono, Army DEVCOM SC: In vitro Skin Model for Enhanced Testing of Antimicrobial Textiles
1655-1705	Mr. Jordan Whitman, Army DEVCOM SC: Evaluation of Probiotic Growth Dynamics Using In vitro Batch fermentation
1705-1715	Dr. Clem Fortman, BioMADE: BioMADE, the Bioindustrial Manufacturing and Design Ecosystem
1715-1730	Session Panel Q&A
1730-1735	**Closing Remarks (TSMC Chairs)**
1800-1900	**Evening Networking No-Host Social (Lake Morey Resort Lounge)** No-host dinner - optional (select at check-in for inclusion in reservation)

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Wednesday 28 Sept 2022 (all times US EST) | Day 2 Zoom Link

0730-0830	**Login/Morning Social**
0830-0835	**Welcome (TSMC Chairs)**
0835-0855	**BioTech Community of Interest - Overview** Dr. Rasha Hammamieh, WRAIR, Biotech COI - OWP Sub-area Lead
0855-1025	**Session 4 - Human Microbiomes: Countermeasures** Chairs: Dr. Blake Stamps, AFRL & Mr. Kenneth Racicot, Army DEVCOM SC
0855-0915	Dr. Phil Karl, USARIEM: Orally Ingested Probiotics, Prebiotics, and Synbiotics as Countermeasures for Respiratory and Gastrointestinal Tract Infections: A Systematic Review and Meta-analysis
0915-0935	Dr. Camilla Mauzy, AFRL: Gut-Muscle Axis Probiotic Evaluation Using In vitro Model Systems
0935-0955	Ms. Laurel Doherty, Army DEVCOM SC: Using in vitro fermentation to model the human lower GI tract microbiome
0955-1015	Dr. Adrienne Narrowe, USDA (Virtual): Linking gut microbiome and metabolome shifts following probiotic administration
1015-1025	Session Panel Q&A
1025-1055	**BREAK**
1055-1210	**Session 5 - Human Microbiome Discovery - Earth & Space (lightning talks)** Chairs: Dr. Rasha Hammamieh, WRAIR & Dr. Kristy Hentchel, ONR (Virtual)
1055-1105	Dr. Andrew Hoisington, Dept. Veteran Affairs (Virtual): Intra- and Inter- Sequencing Center Variances for 16S rRNA using MiSeq Platform: A United States-Veteran Microbiome Project (US-VMP) Study
1105-1115	Dr. Alexander Lawrence, WRAIR (Virtual): Impact of Testosterone Supplementation on the Fecal Microbiome and Metabolome During Energy Deficit
1115-1125	Dr. Johanna Lemons, USDA (Virtual): Intestinal acylcarnitines and dysbiosis: implications for inflammatory bowel disease
1125-1135	Dr. Jenni Firrman, USDA (Virtual): The effect of chlorinated drinking water on the gut microbiota: an in vivo analysis
1135-1145	Dr. Alison Hoke, WRAIR: Spaceflight Induced Stress Caused Comprehensive Alteration of Fecal Microbiome Beyond Bacteria
1145-1155	Dr. George Dimitrov, WRAIR (Virtual): Pain Management During Space Mission In Context Of Gut-Brain Axis
1155-1210	Session Panel Q&A
1210-1330	**LUNCH**

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1330-1410	**Navigating IRB/FDA for Human Use of Live Biotherapeutics - Overview** Ms. Emily Badraslioglu & Mr. Tibor Tuzson, Office of Regulated Activities (ORA), U.S. Army MRDC (Virtual)
1410-1540	**Session 6 - Environmental Micro- & Myco-biomes** Chairs: Dr. Robyn Barbato, ERDC-CRREL & TBD & Dr. Sophie Colston, NRL
1410-1430	Mr. Robert M. Jones, Army ERDC-CRREL: Whispers in the Dark: Sending Signal Waveforms Through Melanized Fungal Cultures
1430-1450	Dr. Vanessa Varaljay, AFRL: The Effect of the Joint Biological Agent Decontamination System (JBADS) on Aircraft-Associated Microbiology
1450-1510	Mr. Charles Sweet, US Naval Academy: Metagenomic and Culture-based Characterization of the Chesapeake Bay Winter and Summer Planktonic Microbiomes
1510-1530	Dr. Dominique Wagner, AFRL/UES: Microbial Communities Vary by Function and Structure on Synthetic Polymers within DoD Infrastructure
1530-1540	Session Panel Q&A
1540-1610	**BREAK**
1610-1745	**Session 7 - Environmental Microbiome Analysis & Engineering (lightning talks)** Chairs: Mr. Robert Jones, ERDC-CRREL & Dr. Vanessa Varaljay, AFRL
1610-1620	Dr. Zheng Wang, NRL: Identifying Mycobiome from Aircraft Topcoat
1620-1630	Dr. Christopher Baker, Army ERDC-CRREL: Assessing microbial threats in thawing permafrost using metagenomic sequencing
1630-1640	Ms. Elizabeth Corriveau, Army ERDC-CRREL: Permafrost Thaw and the Carbon Cycle: Comparing Green House Gas Emissions from Alaska and Abisko
1640-1650	Dr. Ryan Busby, Army ERDC-CERL: Investigating the Disturbed Soil Volatilome as a Novel Soil Sensing Tool
1650-1700	Dr. Alison Thurston, Army ERDC-CRREL: Microbial Activity in Dust Contaminated Antarctic Snow
1700-1710	Ms. Lindsay Wood, Army ERDC-CRREL: Microbial Activity in Arctic Soil: An Arctic Application of the DRTSPORE Model
1710-1720	Mr. Logan Gonzalez, Army ERDC-CRREL: Characterizing Microorganisms from Permafrost for Low-Temperature Synthetic Biology Applications
1720-1730	C1C Margaret Warner (Virtual), US Air Force Academy: Bioengineering and Optimization of Biocementation for Potential Space Applications
1730-1745	Session Panel Q&A
1745-1750	**Symposium Closing Remarks (TSMC Chairs)**

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ABSTRACTS LISTING

Session 1: Human Microbiomes: Stress Response

Changes in the composition of the gut microbiome during a prolonged submarine deployment.

Blake Stamps[1], Mahamat Babagana[2], Brian Kupchak[2], Joseph Decicco[2], Kraig Strayer[1], Michael Goodson[1], and Joanna Halford[2]
[1]Air Force Research Laboratory, Wright-Patterson Air Force Base, OH, [2]Naval Submarine Medical Research Laboratory, Groton, CT

Disruption of the gut microbiome has been linked to ill health, particularly obesity, metabolic syndrome, impaired cognitive performance, and mental health issues. Various aspects of the submarine environment are known to disrupt the microbiome when they occur in other circumstances, including limited diet, altered circadian rhythm, poor sleep, stress, lack of exercise and lack of vitamin D. This raises the possibility that a prolonged submarine deployment could negatively impact health through adverse effects on the microbiome. The aims of this study were to 1) characterize the changes in the composition of the gut microbiome during a prolonged submarine deployment, and 2) determine whether any identified deviations were associated with changes in submariner health and performance that might impact operational readiness.

Thirty-four U.S. Navy submariners, serving on a ballistic missile submarine (SSBN), provided fecal and blood samples and completed questionnaires relating to their diet (Block Food Frequency Questionnaire) and mood (abbreviated Profile of Mood States). Samples and questionnaires were collected before, during and after an eight-week deployment. Fecal samples were analyzed metagenomically and metabolomically to identify the diversity and function of resident microbes. Blood samples were analyzed to identify metabolites and proteins of interest, focusing on those with established links to stress, neurocognitive function, immune function, and gut barrier function. The dietary and mood questionnaires were analyzed to identify any changes over the course of the deployment; and any correlations between such changes and changes in the microbiome. Preliminary results indicate that the baseline gut microbiota were highly variable between individuals. This inter-individual variability persisted throughout the study period, despite the volunteers living at close quarters for an extended period and consuming similar diets. Changes in microbiota composition within individuals over the course of the deployment were correlated with changes in metabolism, blood biomarkers for inflammation, and fatigue. Understanding whether certain microbiomes confer resiliency to deployment stressors and whether changes in the gut microbiome during a prolonged submarine deployment impact health and performance will enable us to identify targeted interventions that can improve submariners' resiliency and, therefore, mission readiness.

Disclaimer: The views expressed in this abstract reflect the results of research conducted by the authors and do not necessarily reflect the official policy or position of the Department of the Navy, Department of Defense, nor the U.S. Government.

Bioenergy Homeostasis: A Major Node in Functional Microbiome's Stress Response Model

Nabarun Chakraborty[1], Alexander B Lawrence[1,2,] Allison Hoke[1], Aarti Gautam[1] and Rasha Hammamieh[1]
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The bi-directional relationship/association between the host and its resident microbiome could be defined as the

functional microbiome that regulates a wide range of host's health, fitness and performance. Albeit limited, our current understanding is that essentially, any stress challenges this relationship; and as a consequence, the microbiota's composition and diversity of these commensal shifts, which ultimately influences the host's psycho-physiological health. Probing this host-microbiome communication profile under stress would can inform us about the disease pathophysiology and putative treatment strategies. In this context, metabolites, the typical end products of any biological function within human and microbial cells alike hold key information regarding their crosstalk. Our hypothesis is that the systems analysis of the metabolite profile is linked to host and microbiome, respectively, and integrating this information with metagenomics would help in comprehending the functional microbiome. Towards this objective, we studied both rodent and human microbiome challenged by several stressors, such as traumatic brain injury (TBI), post-traumatic stress disorder (PTSD), and radiation. Since the microbial community is most densely colonized at the colon area, we primarily investigated fecal microbiome and its metabolites. Functional analysis underlined that host and microbiome synergistically fostered certain biofunctions irrespective of the stress types. For instance, existing literature found immune response as one such functional node; holistic communication between innate and adaptive immune cells and the intestinal microbiota potentially maintains the balance between immune tolerance and host inflammation. In support, our studies with PTSD and TBI models reported elevated inflammation in host's blood and brain tissues, and a concerted shift in the abundances of those fecal commensals, such as genus *Akkermansia* under *Verrucomicrobia* phylum and *Actinobacteria* phylum, which were typically associated with inflammation. In addition, our studies of multiple stress models identified energy homeostasis as a key node fostered by host-microbiome crosstalk. Microbial contribution to the acute weight loss of PTSD mice was underlined by the concerted inhibition of starch metabolism network and activation of glycerol degradation network in fecal microbiota. In a rat TBI model, inhibited glycolysis and oxidative phosphorylation networks in the host brain were accompanied by inhibited glycolysis and activated glycogen biosynthesis networks in the fecal microbiota. Apparently, TBI caused an energy deprived condition, which had elevated the opportunistic abundance of *Deferribacteres* phylum, an anaerobic bacteria in fecal samples. In contrast, we observed a potentially asynchronized response from host and microbiome to lethal radiation. At a delayed time point post radiation exposure, fecal microbiome re-initiated bioenergy production via re-activation of lipid and amino acid metabolism networks; meanwhile, the host succumbed as bioenergy production and pertinent metabolism networks remained inhibited. This observation potentially underlined the fact that the robustness of the host-microbiome communication was susceptible to the degree of severity of stress. In conclusion, energy homeostasis or its disruption emerged as a common theme in the stress response of functional microbiome. Restoring this functional node could be a fitting purpose of next generation therapeutic strategy.

Nasal microbiota evolution within the congregate setting imposed by military training

Faith C. Blum, Jeannette M. Whitmire, Jason W. Bennett, Patrick M. Carey, Michael W. Ellis, Caroline E. English, Natasha N. Law, David R. Tribble, Eugene V. Millar, and D. Scott Merrell
Uniformed Services University of the Health Sciences, Bethesda, MD

The human microbiome is comprised of a complex and diverse community of organisms that is subject to dynamic changes over time. As such, cross-sectional studies of the microbiome provide a multitude of information for a specific body site at a particular time, but they fail to account for temporal changes in microbial constituents resulting from various factors. To address this shortcoming, longitudinal research studies of the human microbiome investigate the influence of various factors on the microbiome of individuals within a group or community setting. These studies are vital to address the effects of host and/or environmental factors on microbiome composition as well as the potential contribution of microbiome members during the course of an infection. The relationship

between microbial constituents and disease development has been previously explored for skin and soft tissue infections (SSTIs) within congregate military trainees. Accordingly, approximately 25% of the population carries *Staphylococcus aureus* within their nasal cavity, and these colonized individuals are known to be at increased risk for SSTIs. To examine the evolution of the nasal microbiota of U.S. Army Infantry trainees, individuals were sampled longitudinally from their arrival at Fort Benning, Georgia, until completion of their training 90 days later. These samples were then processed to determine *S. aureus* colonization status and to profile the nasal microbiota using 16S rRNA gene-based methods. Microbiota stability differed dramatically among the individual trainees; some subjects exhibited great stability, some subjects showed gradual temporal changes and some subjects displayed a dramatic shift in nasal microbiota composition. Further analysis utilizing the available trainee metadata suggests that the major drivers of nasal microbiota stability may be *S. aureus* colonization status and geographic origin of the trainees. Nasal microbiota evolution within the congregate setting imposed by military training is a complex process that appears to be affected by numerous factors. This finding may indicate that future campaigns to prevent *S. aureus* colonization and future SSTIs among high-risk military trainees may require a 'personalized' approach.

Medications and Gut, Oral, and Skin Microbiomes: Implications for Health and Intervention: A United States-Veteran Microbiome Project (US-VMP) Study

Andrew J. Hoisington, Christopher E. Stamper, Maggie A. Stanislawski, Joseph A. Simonetti, Kelly A. Stearns-Yoder, Teodor T. Postolache, David W. Oslin, Christopher A. Lowry, Lisa A. Brenner
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Prior studies have demonstrated that antibiotic medication use is an important modulator of the human microbiome. However, little is known about the influence of other medication classes, which may be important to account for in studies assessing the relationship between the human microbiome and health-related outcomes (e.g. physical, psychological). To address this gap, we will present findings from two studies conducted by the Military and Veterans Consortium for Research and Education (MVM-CoRE) that assessed the association between use of a variety of prescription medication classes and changes in the human microbiome among U.S. Veterans. First, we conducted a prospective study of 331 Veterans and evaluated relationships between their gut microbiomes and use of 9 classes of prescription medications identified through review of VA EMR pharmacy prescriptions and refill records, including all medications with adequate supply to cover the two weeks preceding the fecal microbiota sample collection. Topical medications were excluded. Microbial communities were significantly associated with beta-blockers, proton pump inhibitors, and psychiatric medications (including serotonin and norepinephrine reuptake inhibitors). Across all medication classes, correlations were observed with numerous taxa including *Prevotella*, *Finegoldia*, *Streptococcus*, and *Faecalibacterium*. Second, to assess potential associations between gut microbiome diversity and community structure and medication longitudinally we collected data from ten participants over 24 weeks who were enrolled in a clinical trial aimed at treating their major depressive disorder and provided microbiome samples from gut, oral, and skin locations for 16S rRNA sequencing. For a subset of 8 participants, gut samples were analyzed with metagenomics. Preliminary results showed no association between medication status and skin microbial communities, but did show changes in the fecal and oral microbial communities from the premedication state that did not stabilize within the 24 weeks of sampling. Future studies may need to determine if changes in the fecal and oral microbial communities are related to improved psychiatric outcomes. A more thorough understanding of the impact of medications across cohorts can enable better personalized health care for our Veterans, military members, and others.

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Session 2: Microbiome Analysis & Surveillance

Delayed Impact of Radiation on Fecal Microbial Composition Depends on Gender

Matthew Rusling1, Nabarun Chakraborty1, Gregory Holmes-Hampton2, Vidya P. Kumar2, Allison Hoke1, Aarti Gautam1, Kevin Swift1, Rasha Hammamieh1 and Sanchita P. Ghosh2
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Recent estimation showed that the number of microbial cells exceeds the number of host cells; hence, it is essential to study this holistic host-microbiome ecosystem to fully comprehend the disease pathophysiology. Our past results showed that lethal radiation eventually disrupted the communication between the host and its resident fecal microbiota. Existing literature suggested that the fecal microbial profile is gender-specific. Therefore, our tenet was that the response dynamics to sublethal radiation could be gender specific. The current objective was to find gender-/dose-/time-specific metagenomics markers and mechanisms responding to lethal radiation.

Thirty C57BL/6J young adult mice (N=15 male, N=15 female) were exposed to total body irradiation (TBI) at doses of 7 Gy (N=10; 5M/5F) and 7.5 Gy (N=10; 5M/5F); a sham group (N=10; 5M/5F) was handled identically. Fecal samples were collected 1 month (1m) and 6 months (6m) post-TBI, and remained at -80^{0}C until DNA extraction. Subsequently, a vendor recommended set of primers was isolated, barcoded and amplified for the hyper-variable V3 and V4 regions of the 16S rRNA amplicon in the Illumina MiSeq platform. The de-multiplexed sequences were analyzed using QIIME2.

Sequencing results yielded a mean of 87k±20k reads/sample and 2,588 sequences were identified with one sample discarded due to low feature count. Accordingly the sample size/ group was 4-5 mice. Estimating the composition variabilities among the communities, PERMANOVA primarily attributed the beta-diversity to gender and radiation dose. Time since radiation (TSR) also had a smaller effect size. Since, weighing the impact of gender variance was the major objective of the present study; we now focused on the male and female subgroups separately. A gender-specific divergence of beta-diversity emerged. Among males, TSR was a significant factor to explain beta-diversity; while among females, both radiation dose and TSR emerged significant.

Likewise, alpha-diversity revealed a gender-specific shift in richness and evenness in fecal commensals. Chao1, a primary estimator of richness showed a gender-specific divergence. At 6m since 7.5Gy TBI, Chao1 was significantly increased in males, but reduced in the female group. Shannon and Simpson diversity was used to measure both evenness and richness. Interestingly, cross-gender dose-dependent alpha diversity accompanied a lower alpha-diversity in the control group.

Furthermore, we studied the major phyla to understand individual abundance across this study landscape. The abundance profile of the phyla namely *Firmicutes* and *Verrucomicrobia* varied between the genders. The shift in *Firmicutes* was primarily due to TSR and radiation dose in male and female groups, respectively. In contrast, the shift in *Verrucomicrobia* was due to the interaction between radiation dose and TSR in both the male and female groups.

In conclusion, there was a significant gender-specific diversity and commensal abundance variabilities 6m post-TBI, which was equivalent to 18 years of human life. Our overarching goal is to integrate the fecal metagenomics data with fecal metabolomics data to fully understand the perturbation of host-microbiome ecosystem and define customized countermeasure.

Disclaimer: Material has been reviewed by the Walter Reed Army Institute of Research. There is no objection to its

presentation and/or publication. The opinions or assertions contained herein are the private views of the author, and are not to be construed as official, or as reflecting true views of the Department of the Army or the Department of Defense.

Research was conducted under an approved animal use protocol in an AAALAC International-accredited facility in compliance with the Animal Welfare Act and all other federal statutes and regulations relating to animals and experiments involving animals, and adheres to principles stated in the Guide for Care and Use of Laboratory Animals, NRC Publication, 2011 edition. The opinions and assertions expressed herein are those of the author(s) and do not reflect the official policy or position of the Uniformed Services University of the Health Sciences or the Department of Defense. Neither the authors nor their family members have a financial interest in any commercial product, service, or organization providing financial support for this research.

Microbiome study in irradiated mice treated with BIO 300, a promising radiation countermeasure

Amrita K Cheema,[1,2] Yaoxiang,[1] Jatinder Singh,[3,4] Ryan Johnson,[5] Michael Girgis,[1] Stephen Y. Wise,[3,4] Oluseyi O. Fatanmi,[3,4] Michael D. Kaytor,[6] Vijay K. Singh[3,4]
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Background: The mammalian gut harbors a very complex and diverse microbiota that play an important role in intestinal homeostasis and host health. Exposure to radiation results in dysbiosis of the gut microbiota leading to detrimental pathophysiological changes to the host. BIO 300, containing synthetic genistein formulated as an amorphous solid dispersion or as an aqueous suspension of nanoparticles, is a promising candidate under advanced development. The aim of this study was to investigate the effects of BIO 300 on the gut microbiome and metabolome of mice exposed to ^{60}Co gamma-radiation.

Methods: To alleviate the effects of irradiation, several candidate countermeasures are under investigation. The gut microbiota and metabolome of control and drug-treated mice exposed to radiation was characterized by bacterial 16S rRNA amplicon sequencing and untargeted metabolomics.

Results: We found that irradiation altered the *Firmicutes/Bacteroidetes* ratio and significantly decreased the relative abundance of *Lactobacillus*, both in BIO 300-treated and control mice; however, the ratio returned to near normal levels in BIO 300-treated mice by day 14 post-irradiation. Concomitantly, we also observed corrective shifts in metabolic pathways that were perturbed after irradiation.

Conclusions: Overall, the data presented show that radiation exposure led to a relative depletion of commensals like *Lactobacillus* leading to an inflammatory metabolic phenotype while the majority of the drug-treated mice showed alleviation of this condition primarily by restoration of normal gut microbiota. These results indicate that the radioprotective effects of BIO 300, at least in part, may involve correction of the host-microbiome metabolic axis.

Digital Engineering Biodefense

Morie Alpha
Chemical Biological Radiological (CBR) Defense Division R22 NSWC Indian Head Division

The United States Navy and Marine Corps Digital Systems Engineering Transformation Strategy guides the integration of a network of technological innovations across the lifecycle, based on modeling and authoritative sources for subsystem data. Emerging infectious diseases and potential malicious use of biology present a complex challenge to sailor health and ship performance; and to address this challenge, intentional biodefense is a necessary attribute of new ship classes, theoretically accessible to digital engineering. However, effective digital engineering requires detailed, authoritative subsystem data and interface control; but for biodefense, the most significant subsystem is the ship's crew. Crew members are notoriously complex and variable from an engineering perspective. Currently, Navy Surface Warfare Center Indian Head (NSWC IHD) is developing a first spiral of digital engineering for CBR defense called the Navy Shipboard CBRN Performance Model. It begins the process of explicitly modeling the crew under biological or chemical threat to assess the impact of CBRN exposure on human performance and mission functions at the scale of the ship. The model integrates QUIC (a dispersion model system) and CONTAM (a multizone indoor air quality and ventilation analysis software) into an explicit model of how the ship and crew interact, differentiating crew members primarily by billet.

The Navy Shipboard CBRN Performance Model simulates the interaction of contaminants from CBRN exposure with a Navy ship's exterior surfaces, interior surfaces, along with its detection, monitoring and protection systems. A model such as the Shipboard Performance Model theoretically enables CBRN digital engineering and is likely to greatly enhance the engineering of robust ship performance in a chemically contaminated environment. However, for infectious disease, additional considerations around crew member variability may dominate model performance. As a result, a more complex model of the human subsystem is anticipated for future iterations of the Shipboard Performance Model. One aspect of this subsystem model is the human microbiome, including transmission of non-pathogenic organisms among crew members, particularly as these organisms traverse the shipboard abiotic environment. Another aspect of this subsystem model are the body's vital functions. Data from vital signs devices could be used to simulate human performance at various microbiota state and physical environmental conditions. Incorporating these considerations into digital engineering for biodefense is in its infancy but the theoretical work required has been initiated; anticipating a more holistic approach that applies the principles of biology and behavioral analysis to minimize the spread of infection.

Aerobiological Surveillance using Next-Generation Sequencing and Metagenomics Analysis

Christine A. Mariskanish, Adam R. Pollio, Susan E. Kosisky, Jun Hang
US Army Centralized Allergen Extract Lab US Army Garrison-Forest Glen, Silver Spring, Maryland
Viral Diseases Branch, Walter Reed Army Institute of Research, Silver Spring, Maryland

Enhanced surveillance of respiratory infections and disease outbreaks are vital for informed control of airborne pathogen transmission and effective pandemic mitigation, preparedness and response. The standard surveillance of collection and testing of human respiratory specimens is time-consuming, often limited to symptomatic subjects, and challenging to trace exact time and location of transmission. In this study, we explored the feasibility of an airborne biota monitoring system through a metagenomics approach to detect early warning of emerging threats to respiratory health.

The United States Army Centralized Allergen Extract Laboratory (USACAEL) collects and analyzes aerobiological data for predominant area pollen and mold spores year-round. Pollen and spore reports are provided to media networks, public health organizations, scientific agencies and the community at large. Reports advise of seasonal

allergy conditions to facilitate patient treatment and prevention strategies. In this study, we developed methods for nucleic acid extraction from the USACAEL Rotorod collector rods, metagenome next-generation sequencing (mNGS), and bioinformatics analysis to generate comprehensive aerobiological data. A volumetric rotating-arm impaction sampler (Model 40 Rotorod Sampler, SDI Company, Plymouth Meeting, PA) was installed on the roof of a two-story building in the campus of Walter Reed Army Institute of Research (WRAIR). Silicone grease was applied on the glass collector rods to form an adhesive surface for volumetric harvest of particles in the air. Each polystyrene 'I' rod was set to 2,400 rpm for 60 secs every 10 min for 24 hrs. The rods were stained with Calberla's solution and analyzed under light microscopy in order to identify pollen and fungal spores. Following microscopy, silicone grease from each rod was dissolved and used for extraction and purification of nucleic acids using Direct-zol RNA Miniprep (Zymo Research, Irvine, CA). The DNA/RNA extracts were analyzed using unbiased mNGS and bioinformatics analysis for sequence-based taxonomic identification of biological contents on the rods, i.e. captured air samples.

In the samples collected from May to July 2022, pollen aeroallergens including oak, mulberry and pine identified through mNGS, were also present in microscopy. Relative abundance of oak identified through mNGS was also similar to light microscopy. Moreover, mNGS analysis identified environmental bacteria, fungi and bacteriophage. Overall, the results suggested that the mNGS aerobiological survey is a technically feasible approach for monitoring aeroallergens and pathogens in atmospheric environments. Further development and proof-of-performance study in a controlled lab setting and in the field are needed to achieve the sensitivity, specificity, and speed desired for a successful field-deployable air quality surveillance system.

Session 3: Human Microbiome Enablers & Engineering (lightning talks)

DARPA ReVector Program Aims to Reduce Mosquito Attraction by Engineering the Human Skin Microbiome.

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Mosquitoes are vectors for a wide array of diseases impacting both warfighters and civilians living and working in endemic environments. Although many approaches exist to slow the spread of vector-borne disease (e.g., bed nets, chemical repellents, anti-malarial drugs), they each have logistical burdens or side effects that make them impractical for use during military deployments. For example, the need to frequently reapply repellents and inconsistent use of bed nets together with repellent-treated uniforms often results in insufficient protection. Mosquitoes are initially attracted to humans by exhaled carbon dioxide and body heat. Subsequently, the human skin volatilome (comprised of both human- and microbially-generated small molecules) may also influence mosquito landing and biting. Researchers on the DARPA ReVector program are working to develop safe and efficacious technologies to modulate the skin's volatilome by changing the organisms in the skin microbiome and/or altering their metabolic processes. In contrast to available vector control measures, it is hoped that ReVector treatments could be applied once, produce no detectable scent, and last for up to two weeks without reapplication, thus offering improved, sustained protection against bites from disease vectors.

The initial approach for the program involved literature-driven identification of candidate mosquito-attracting and repellent volatile compounds. Mosquito behavioral responses to the volatile chemicals (both single-molecule and mixtures) were assessed using an olfactometer and used to guide subsequent microbial engineering strategies. Metabolic pathways were designed to either knock out production or rapidly degrade attractant molecules (such as lactate). Additional synthetic biology approaches aimed to up-regulate production of or add genes for synthesis of repellents (e.g., terpenes). Finally, 'ecological' strategies to develop consortia from skin microbe strains that naturally modulated the production of attractants/repellents has also been explored.

Methods for genomic insertions or deletions were successfully developed and tested in different genera of commensal human skin microbes (e.g., *Staphylococcus* and *Corynebacterium*). Pathways were verified both genetically and analytically (e.g., using gas chromatography/mass spectrometry) before proceeding to mosquito behavior studies to assess efficacy. High-throughput olfactometry, coupled with flight-tracking imaging systems, were performed with *Aedes*, *Culex,* and *Anopheles* mosquitoes to ascertain their response to individual wild-type or modified bacterial strains *in vitro*. Strains with the metabolic pathway for lactate production deleted, or those that expressed certain terpenes, resulted in a reduction of mosquitos attracted to the samples, versus the wild-type strains.

Animal studies (in mice) have initially focused on determining persistence of human skin microbe consortia after application. Engraftment of a four-member community was followed for up to 3 months (depending on the skin preparation method used). Studies are currently in progress to ascertain if the engineered microbial strains reduce mosquito landings on mice to which they've been applied.

Fighting *Pseudomonas aeruginosa* Wound Infections with an Engineered Skin Microbe

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Despite remarkable advances in prevention, wound infections continue to threaten U.S. military members in healthcare and combat settings, especially as bacteria gain increasing resistance to antibiotics and drug treatments. *Pseudomonas aeruginosa* (*P. aeruginosa*) is a particularly resistant strain of bacteria commonly found in the environment. It can cause infections and even death, which makes finding alternatives to *P. aeruginosa* antibiotics an urgent matter.

Prescribed preventive measures against *P. aeruginosa* infections are not always feasible in military settings, and they are becoming less effective as the bacteria builds drug resistance. Antibiotic resistance and constrained access to care during military operations necessitate development of *P. aeruginosa* infection prevention and treatment alternatives. Therefore, this project aims to engineer a skin microbe to prevent infections caused by *P. aeruginosa* during wound healing. This novel approach to combating *P. aeruginosa* will also aid in preserving the efficacy of existing antibiotics.

We intend to provide a microbial countermeasure to *P. aeruginosa* to help prevent and treat wound infections in combat and hospital settings via a three-pronged approach. (1) Identify a *P. aeruginosa* target protein and genetically engineer *E. coli* to secrete a nanobody to for it. (2) Demonstrate nanobody excretion from *E. coli* and determine nanobody-*P. aeruginosa* interactions using classical molecular biology techniques. (3) Evaluate nanobody capability to inhibit *P. aeruginosa* growth and activity by killing and biofilm eradication concentration assays.

This project has the potential to deliver a microbial method for fighting *P. aeruginosa* to improve wound healing in combat and health care environments. It could also set the foundation for producing antibiotic alternatives for other pathogens.

Comparison of stabilized and non-stabilized gut microbiome samples via 16S rRNA sequencing: A United States-Veteran Microbiome Project (US-VMP) Study

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The microbiome field has experience unprecedented growth over the past decade and research on the impact of collection and shipping protocols for fecal microbiome has garnered increased attention. For the convenience of participants and enable larger prospective studies, fecal sampling at home followed by shipping to a research facility is a simple and effective method. Although the home sampling is beneficial, there are unintended consequences for the microbial communities when shipped. For example, the American Gut Project determined that fecal samples shipped back to the research facility had select aerotolerant anaerobes that bloomed after collection, representing an artificial relative abundance difference from the actual sample populations. Attempting to retroactively remove bloomed taxa with bioinformatics is problematic because many of the taxa are associated with disease states, increased inflammation, and dysbiosis. Many sample stabilization methods have become available to prevent samples from deviating from their original state. In the present study we examined microbial changes in the same fecal samples, either stabilized or non-stabilized. Initial results show that samples that were stabilized and non-stabilized differed in beta diversity and specific genera of Proteobacteria and Bacilli. The magnitude of deviation varied by participant and time spent in transit. Furthermore, metagenomic sequencing from a subset of 20 samples showed key differences related to species and KEGG pathways. In conclusion, careful study design is recommended for fecal microbiome projects to optimally ship samples with chemical stabilization or frozen.

Insoluble rice bran fiber modifies gut microbial diversity in comparison to soluble fiber in vitro.

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Research has demonstrated that a high fiber diet has a positive influence on human health. Insoluble fibers, a type of prebiotic, are fermented in the colon by the gut microbiota. This process allows the microbiome to produce metabolites that are related to positive health outcomes such as improved blood glucose control and lower inflammation in the gut. To understand how different types of fiber may affect the gut microbiome, we cultivated the gut microbiota of 3 different donors in the Simulator of the Human Intestinal Microbial Ecology (SHIME®) system with treatments of either insoluble or soluble rice bran fiber. We used metagenomics and GC-MS to perform a comparative analysis of the microbial community structure and function. Our results demonstrate that insoluble rice fiber causes a larger shift in the gut microbiome compared with soluble rice fiber, specifically in the proximal colon.

Immune Response Evaluation: A platform for evaluation of innate-adaptive immune response to microbes and antigens

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The microbiome and the human immune system are tightly linked, where each modulates the status and function of the other. This interdependence can be studied in the context of animal models, but studies of human microbiome:host co-regulation are hampered by the challenge of disentangling causation from correlation. Therefore, characterization of human immune and microbe interactions will benefit from *ex vivo* models that provide co-culture of microbes and host cells, thereby allowing controlled experiments. To meet this need, Draper is developing a novel platform that integrates key human immune cell types with relevant epithelial cells, which provide both the barrier and the permissive interface between the host and microbiome. Two tissues of interest are being developed: ileum and skin. The ileum in the small intestine is a crucial site of exposure and a training

ground for the immune system, where Peyer's patches of the epithelial lining provide a permeable interface for luminal content sampling by sentinel Dendritic cells and Macrophages. The skin and its microbiome are accessible and mostly aerobic, there is growing interest to study and leverage this microbiota for human health and performance. Our approach is to build a custom three-dimensional matrix similar to the lamina propria and designed to support Dendritic and T cells, in immediate contact with a relevant epithelial monolayer. This structure enables an in vitro model where potential antigens and tolerogens, including bacterial strains and consortia, can be introduced to the epithelium, and the innate and adaptive immune response can be evaluated. This presentation will summarize our combined culture of primary human epithelial cells, Dendritic cells, and T cells within the context of an electrospun matrix custom-designed to support appropriate cell interactions. In addition, preliminary experiments that integrate bacterial consortia are underway. Future work will include development of a platform that will house the material and provide reproducible and resilient assay conditions.

In vitro Skin Model for Enhanced Testing of Antimicrobial Textiles

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Deployed Warfighters in austere environments lack access to shower and laundry facilities and may wear the same garments for extended periods of time. As such, a number of antimicrobial technologies have been incorporated into textiles for odor control. Numerous standard laboratory test methods for antimicrobial textiles are used to measure evaluate activity; many use a single specific test microorganism is applied to the textile and grown under optimal conditions, then recovered for quantitation to determine activity. However, these conditions do not reflect the actual performance environment that consists of polymicrobial communities in sub-optimal conditions. Lab testing often does not translate to performance of treated textiles when worn by individuals. Here we propose development of *in vitro* skin model method to bridge the gap between lab testing and wear studies. The model consists of a defined polymicrobial community of 5-7 commensal microbes simulating the skin microbiome, seeded onto a solid substrate platform to represent the skin. The protocol would entail adding a non-commensal test organism of interest to the defined commensal community and applying a textile sample to the solid substrate. Bacteria will then be recovered to quantitatively measure organism growth or reduction by qPCR. Parameters involved the model build includes choice of defined microbial communities, as well as growth conditions (culture media, concentration, pH, temperature), which have been shown to affect community growth profiles. Solid substrates evaluated include agar plates and surrogate skin tissue. This model could begin to answer the following questions: 1) is the treated textile effective against the target organism; and 2) how the defined community is affected; and 3) does the textile cause unwanted effects toward the skin simulant. The proposed model would determine activity under conditions more comparable to the intended application and provide expanded knowledge relative to current standard test methods. This model can also be used for testing skin pre- and probiotics, textiles treated for other functionalities and engineered organisms.

Evaluation of Probiotic Growth Dynamics Using *In vitro* Batch fermentation

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Probiotics have been used to help support positive health states in the body by aiding digestion and protecting from the damage of acute inflammation. Postbiotics are of particular interest as cell-free products from fermented probiotics that contains a wealth of beneficial metabolites and byproducts. Here, select commercial probiotics from

three vendors, Probiotical S.p.A, Deerland Probiotics, and Lonza Biologics, were grown to generate postbiotic fermentates for use in *in vitro* muscle models to investigate the ability of probiotics to accelerate recovery from acute muscle damage. Utilizing our *in vitro* fermentation system, each organism was grown in conditions simulating the human colon under ideal pH, media, and atmospheric conditions. qPCR results confirmed that each probiotic reched stationary phase after 24-48hrs of fermentation. Postbiotic metabolite profiles were generated using UPLC-MS. In Bacillus subtilis DE111, provided by Deerland Probiotics, the most prevalent metabolites produced were amino acids, sugar acids and peptides. In Lactobacillus plantarum TWK10, provided by Lonza, the most prevalent metabolites produced were amino acids, bile acids and sugar acids. In Bifidobacterium bifidum BB10 and Bifidobacterium breve BR03, provided by Probiotical S.p.A, the foremost metabolites produced were amino acids, peptides, sugar acids and nucleic acid derivatives. An analysis of short-chain fatty acids using GC-FID was conducted in-house and results will also be presented. The data generated herein will facilitate interpretations of outcomes from our colleagues at US Air Force Research Labs and University of Massachusetts Lowell, who are both employing unique in vitro muscle models for subsequent studies. The views and opinions presented herein are those of the author(s) and do not necessarily represent the views of DoD or its Components.

BioMADE

Clem Fortman, Senior Technology Program Manager BioMADE

BioMADE, the Bioindustrial Manufacturing and Design Ecosystem, is a public private partnership established by the Department of Defense in 2021 with a vision for a sustainable, domestic, end-to-end bioindustrial manufacturing ecosystem. BioMADE's mission is to help build a sustainable, domestic, end-to-end bioindustrial manufacturing ecosystem; foster and sustain biotechnology and biomanufacturing in the U.S.; and propel new biotechnology products from the laboratory to the commercial market. In addition to supporting the development of innovative new products and technologies, BioMADE is building the workforce of the future by partnering with K-12 schools, community colleges, universities, and professional development organizations.

To achieve these goals, BioMADE has established a member ecosystem composed of stakeholders from across the bioindustrial manufacturing ecosystem (industry, academia, and government). BioMADE works with the members to identify specific issues hampering the transition of emerging bioindustrial products from the bench to the marketplace. Project calls are developed to find solutions to these problems. Teams of members apply to these project calls and a subset of those teams are funded to develop solutions. BioMADE also works closely with government stakeholders to develop and execute mission-specific projects to advance agency objectives.

The BioMADE mission space shares a large overlap with the mission space of the Triservice Microbiome Community. We hope you'll engage us to see where we can work on our mutual problems together.

Session 4: Human Microbiomes: Countermeasures

Orally Ingested Probiotics, Prebiotics, and Synbiotics as Countermeasures for Respiratory and Gastrointestinal Tract Infections: A Systematic Review and Meta-analysis

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Background: Respiratory (RTI) and gastrointestinal (GTI) tract infections pose a significant burden to military healthcare systems and force readiness. Orally ingested probiotics, prebiotics, and synbiotics reduce RTI and GTI burden in some populations. However, the extent to which these gut microbiota-targeted interventions impact the incidence, duration, and severity of RTI and GTI in non-elderly adults, and the factors moderating any such effects, are unclear.
Objective: Two systematic reviews and meta-analyses were conducted to determine the effects of orally ingested probiotics, prebiotics, and synbiotics versus placebo on RTI and GTI incidence, duration, and severity in non-elderly adults, and to identify potential sources of heterogeneity in study results.
Methods: Eligible studies were identified by searching CENTRAL, PubMed, Scopus, and Web of Science. English-language, peer-reviewed publications of randomized, placebo-controlled studies that tested an orally ingested probiotic, prebiotic, or synbiotic intervention at any dose for ≥1 week duration in adults aged 18-65 years were included. Results were synthesized using random-effects meta-analyses. Heterogeneity was explored by subgroup meta-analyses and meta-regression. Risk of bias (RoB) was assessed using the Cochrane RoB2 tool for randomized trials.
Results: Forty-two manuscripts reporting effects of orally ingested probiotics (n=38), prebiotics (n=2), synbiotics (n=1), or multiple -biotic types (n=1) on RTI incidence, duration, or severity were identified. Probiotics reduced the risk of experiencing ≥1 RTI (risk ratio=0.91 [95% CI: 0.84, 0.98]; p=0.01), and total days (rate ratio=0.77 [95% CI: 0.71, 0.83]; p<0.001), duration (Hedges' g=-0.23 [95% CI: -0.39, -0.08]; p=0.004), and severity (Hedges' g=-0.16 [95% CI: -0.29, -0.03]; p=0.02) of RTI. Effects were relatively consistent across different strain combinations, doses, and durations, though reductions in RTI duration were larger when fermented dairy was the delivery matrix, and beneficial effects of probiotics were not observed in physically active populations. Eighteen manuscripts reporting effects of probiotics (n=14), prebiotics (n=3), or synbiotics (n=1) on GTI incidence or duration were identified. Probiotics (risk ratio=0.84 [95% CI: 0.71, 1.00]; p=0.05) and prebiotics (risk ratio=0.79 [95% CI: 0.63, 0.97]; p=0.03) both reduced the risk of experiencing ≥1 GTI. Duration of GTI was not affected by probiotics and reported in only two studies on prebiotics. Overall risk of bias was rated as "some concerns" and "high" for the majority of RTI and GTI studies, respectively.
Conclusions: Orally ingested probiotics, relative to matched placebo, modestly reduced the incidence, duration, and severity of RTI and the incidence of GTI in non-elderly adults. Physical activity and delivery matrix may moderate some of the effects of probiotics on RTI. Whether prebiotic and synbiotic interventions confer similar protection remains unclear due to a lack of relevant studies.

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Gut-Muscle Axis Probiotic Evaluation Using *In vitro* Model Systems

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Military training with minimal recovery time can induce muscle damage, resulting in muscle fatigue and soreness with decreased muscle strength and function. Recent evidence supports the existence of a "*gut-muscle axis*" (GMA) by which gut microbes can influence muscle growth and repair through multiple mechanisms. To examine GMA mechanistic ability to enhance muscle repair and/or recovery, we have evaluated commercially-developed FDA-approved and/or GRAS (generally accepted as safe) probiotics using *in vitro* muscle models. These bacterial strains have published or internal data supporting GMA modulation to potentially improve muscle healing or alleviate muscle fatigue. Individual probiotic strains were grown in colonic-specific conditions using a HEL BioXplorer 100 fermentation system (Soares, CCDC) to create microbe-unique fermentates containing secreted compounds that would normally be available *in situ* for host interactions. Clarified fermentates were added to differentiated C2C12 mouse myotubes (fused, multinucleated myoblasts) to examine the ability of the secreted compounds to effect muscle damage or fatigue. Using an automated scratch device (BioTek Autoscratch), wounded C2C12 myotubes/myoblasts were monitored over a 96-hour period using a high-content imager to quantitate wound closure (Molecular Devices, IX-M confocal microscope). A muscle myotube fatigue model was developed using electrical pulse stimulation (EPS, Ionoptix, C-Pace). Fatigue response was examined with respect to different parameters (magnitude, frequency, polarity) to mimic exercise or fatigue profiles. In both phenotype analyses, multiple evaluations were conducted to determine viability, function, and structural alterations to the cells upon treatment with the probiotic fermentate. We are using the *in vitro* data to down-select the most promising single species and transitioning into a human trial (USARIEM) to determine its effectiveness as a dietary additive to provide muscle support for military performance sustainment.

Using *in vitro* fermentation to model the human lower GI tract microbiome

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The human gastrointestinal tract (GI) microbiome is a key modulator of human health, metabolism, and immune function. Models of the GI tract enable more detailed characterization of microbiome dynamics as well as study of regions, such as the small intestine microbiome, which are relatively inaccessible *in vivo*. Here, we present recent progress on GI-jA2COB, an *in vitro* fermentation model of the lower gastrointestinal tract including both small and large intestine model microbiomes. Use of an automated, parallel bioreactor platform enabled real-time monitoring and control of fermentation parameters including pH, anaerobicity, and volume. Bioreactors representing the ileum and three domains of the colon were cultured either separately under batch conditions for 24-48 hours or in sequence for up to five weeks. Large intestine incoula were prepared from fresh fecal donations pooled from multiple individuals and grown anaerobically. To model the small intestine microbiome, a polymicrobial community representing major small intestine phyla and functions was cultured in a microaerophilic environment representative of the ileum; small intestine nutrient absorption was simulated via filtration. Recent findings utilizing the large intestine batch model with supplementation by a polyphenol, cranberry proanthocyanidin, under different colonic domain conditions indicated a domain-dependent effect on microbial metabolism. Initial results from long-term validation of the sequential lower GI tract model, including the small intestine component, will also be presented. Use of *in vitro* fermentation to model the human intestinal microbiota will enable broader investigation of the effect of Soldier-relevant stressors on gut health and may act as a precursor to human or animal studies. Insight gleaned from these models, alone or in concert with *in vivo* studies, can inform nutritional strategies to restore and maintain Soldier gut homeostasis.

Linking gut microbiome and metabolome shifts following probiotic administration

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Lacticaseibacillus (formerly *Lactobacillus*) *rhamnosus* GG (LGG) is among the best-known probiotic strains. While LGG's benefits for human health are well-established, the mechanisms underlying these benefits are still being explored. However, efforts to isolate the impact of LGG on real gut microbial communities are complicated by individual host effects. Using the SHIME (Simulator of the Human Intestinal Microbial Ecosystem), a human-based *ex vivo* incubation system which closely mimics human gut conditions, we can assess the effects of LGG administration in a human gut microbial community independent of the effects of the host's metabolism and immune system. With data collected prior to, during and following LGG administration, we evaluated the fecal metabolome of LGG vs. untreated samples and paired this metabolomic profile with microbial community profiling (16S rRNA sequencing) and microbial functional profiling (shotgun metagenomic sequencing.) Combined analysis of these datasets allows for inference of both direct and indirect (community mediated) effects of LGG on the fecal metabolome with implications for human nutrition and gut health. The host-independent SHIME data provides important information on the expected effects of LGG supplementation, which we can use to better understand how individuals may respond differently to this probiotic, offering an opportunity for the optimization of probiotic consortia and delivery methods.

Session 5: Human Microbiome Discovery - Earth & Space (lightning talks)

Intra- and Inter- Sequencing Center Variances for 16S rRNA using MiSeq Platform: A United States-Veteran Microbiome Project (US-VMP) Study

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Recently expanding interests regarding associations between microbiomes and human health and reduced cost of microbiome 16S rRNA sequencing has led to a proliferation of microbiome studies and sequences. One major limitation to comparing results across studies has been previously reported in variability in sequencing technologies. However, far less is understood is the variability of the same sequencing machine over different sequencing runs (intra-variance) or difference sequencing facilities (inter-variance). With larger sample sizes and increased use of longitudinal study design, it can be advantageous to split sampling runs. In this post-hoc study, we compared two sampling events at the same sequence center and two different sequencing centers using the same model of MiSeq (Illumina) sequencer with v3 chemistry. To gain a broad understanding of differences, we compared human microbiome samples from the gut (intra 188 samples each, inter 387 samples each), skin (intra 63 samples each, inter 188 samples each), and oral (intra 188 samples each, inter 387 samples each). Initial results show significant differences of microbial communities detected between sequencing centers for gut, skin, and oral samples (inter-variance). Within the same sequencing center, higher microbial community differences were observed between skin and oral samples compared to fecal samples, however, the intra-variance was not as high as the inter-variance for all three sampling locations. Based on these results, it is suggested include previously sequenced samples in new sequencing runs to check for variability. The increased use of positive control samples of known communities should also assist in recognizing variability between sequencing attempts, especially as those controls become more robust with additional species. The results highlight challenges associated with comparing and contrasting samples run overtime and at different facilities.

Impact of Testosterone Supplementation on the Fecal Microbiome and Metabolome During Energy Deficit

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Background: Arduous military training is often characterized by energy deficits that result from high physical activity and inadequate dietary intake, along with endocrine disruptions that reduce circulating testosterone. Changes in gut microbiome composition and metabolic activity have also been reported, though underlying mechanisms are unclear. Emerging evidence suggests a bidirectional relationship between testosterone and the gut microbiome. However, whether testosterone mediates changes in the gut microbiome during arduous military is unknown. This study aimed to determine the effects of testosterone supplementation on gut microbiome structure, function and metabolic activity during sustained exercise- and diet-induced energy deficit.
Methods: Fifty healthy, active men participated in this randomized, double-blind, placebo-controlled study. Participants were provided individualized weight-maintaining diets for 14 days, and then randomly assigned to receive weekly injections of testosterone enanthate (TEST; 200 mg/wk) or placebo (PLA; sesame oil) while living in a controlled environment wherein exercise was increased and energy intake was reduced to create a 55% energy deficit for 28 days. Fecal samples were collected at the beginning (PRE), midpoint and end (POST) of the 28 day period. Fecal microbiome structure and function were assessed by shotgun metagenomics and using the HUMAnN2 pipeline, and the fecal metabolome was measured by Q-TOF mass spectrometry.
Results: Free and total testosterone concentrations decreased and were unchanged from PRE to POST, respectively, in PLA while both free and total testosterone concentrations increased from PRE to POST in TEST and were higher than PLA. Changes in fecal microbiome alpha- and beta-diversity did not differ between treatment groups (diet-by-time interaction, $P > 0.05$). Effects of testosterone were observed at the species level with relative abundances of five bacteria and one virus demonstrating time-by-treatment interactions ($q < 0.10$). However, those effects did not translate into between-group differences in any gene pathway (time-by-treatment interaction, $q > 0.10$). Changes in fecal metabolomes and individual metabolites did not differ by treatment (time-by-treatment interaction, $q > 0.10$). In contrast, 142 species, 3 gene pathways and 893 metabolites demonstrated changes over time ($q < 0.10$).
Conclusion: Findings suggest that testosterone supplementation has minimal impact on changes in gut microbiome structure, function and metabolic activity during sustained exercise- and diet-induced energy deficit.

Disclaimer: Material has been reviewed by the Walter Reed Army Institute of Research and US Army Research Institute of Environmental Medicine. There is no objection to its presentation and/or publication. The opinions or assertions contained herein are the private views of the authors, and are not to be construed as official, or as reflecting true views of the Department of the Army or the Department of Defense.

Intestinal acylcarnitines and dysbiosis: implications for inflammatory bowel disease

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Background: Altered plasma acylcarnitine levels are well known biomarkers for a variety of mitochondrial fatty acid oxidation disorders. These membrane permeable fatty acid intermediates are excreted into the gut lumen via bile and are increased in the feces of patients with inflammatory bowel disease (IBD). We have previously shown that long chain acylcarnitines can be utilized as an alternative energy source for the intestinal epithelium when short chain fatty acids are low. Herein, based on data from human subject, animal model, and bacterial culture studies, we show that the gut microbiota can also consume host-derived acylcarnitines.
Methods and Results: A new analysis of fecal samples collected in a study examining recovery of the gut microbiome following an antibiotics-polyethylene glycol purge in healthy human adults consuming different diets revealed that fecal acylcarnitines were significantly increased concurrent with the reduction in bacterial load immediately after gut purge. Levels decreased upon recovery of the microbiota in a diet dependent fashion. We also determined that acylcarnitines throughout the intestinal tract were significantly greater in germ-free than conventionally housed mice despite no difference in levels in bile. Together, these results suggest that the gut microbiota can consume acylcarnitines. A new analysis of data collected from a pediatric IBD cohort shows that bacteria belonging to the Bacteroidetes and Firmicutes Phyla, were negatively correlated with fecal acylcarnitine levels. By contrast Proteobacteria, specifically Enterobacteriaceae such as E. coli, were positively correlated with fecal acylcarnitine levels. We were able to show that under anaerobic conditions E. coli and other common gut residents can utilize the carnitine and acylcarnitines that are naturally present in brain heart infusion (BHI) broth in taxon specific ways.
Conclusions: Our results provide evidence that luminal acylcarnitines can be consumed by the gut microbiota and that there is a strong positive correlation between the abundance of Enterobacteriaceae and a wide variety of acylcarnitines. Further investigation into the relationship between elevated luminal acylcarnitines and their effect on the gut microbiota may provide novel insights into IBD progression.

The effect of chlorinated drinking water on the gut microbiota: an *in vivo* analysis

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Safe drinking water is often achieved through the use of chlorination, in which a low level of chlorine is added to deter growth of disease-causing microbes. Water chlorination has been a standard process in the US for over a century, and chlorinated drinking water (<4ppm) is considered as safe for human consumption. However, the gut microbiota that resides within the gastrointestinal tract is also subject to chlorinated drinking water, yet whether or not the presence of chlorine will affect this community, and to what extent, was unclear. In this study, the effect of chlorinated drinking water on the gut microbiota was evaluated *in vivo*. Post weaning, male and female B6 mice were separated into two groups, one of which was provided chlorine-free water and one was provided water containing 4 ppm chlorine for four weeks. Each week, feces was harvested from both groups of mice, and at the end of the experiment, all mice were euthanized and their cecal content removed. The results found no overall significant difference between the control mice and experimental mice in terms of alpha diversity, assessed in terms of richness, Shannon's index, and Faith's phylogenetic diversity. In terms of taxonomic structure, there was a significant decrease in *Turicibacter* at week 1 for females and a significant increase to *Ileibacterium* at week 3 for males, between the control and experimental groups. However, these alterations only occurred at these time points, and were not apparent throughout the course of the experiment. These results show that the addition of

chlorine to drinking water did not significantly impact the gut microbiota community structure of post-weaned mice.

Pain Management During Space Mission In Context Of Gut-Brain Axis

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Spaceflight presents a number of environmental challenges to astronauts such as microgravity, small doses of radiation, chronic musculoskeletal unloading, disrupted circadian rhythm, metabolic disorder, and immunosuppression. This polytrauma condition affects the entire host-microbiome ecosystem. Emerging knowledge suggested a bi-directional relationship between the host and its resident microbiome that controls an array of physiological and psychological stress responses. In this context, we probed the role of host-microbiome ecosystem in pain perception in spaceflight. To note, osteoporosis is a major health problem for astronauts, which coupled with other comorbidities, such as immunosuppression, delays wound healing and possibly shifts pain perception in spaceflight.

Pain is an intricate experience that encompasses extensive biological networks associated with nociception, neurotransmission, neuroreception, somatosensation and inflammation. The aim of this research is to examine the distinct effects of microgravity on the processing of pain resulted from inflicted segmental bone defect (SBD) *in vivo*. Male C57BL/6j mice of 6-8w of age were used in this NASA/RR4 program. Ten mice underwent a surgical SBD resection filled with bioabsorbable scaffold imbued with saline. Five of these mice were housed on the International Space Station (ISS) for 28 days and euthanized thereupon (FLT-Saline). Rest of the five mice were housed on ground and mirrored the spaceflight protocol to generate the ground control (G-Saline). In addition, 5 healthy mice were housed on the ISS (FLT-Sham) and ground (G-Sham), respectively for 28 days to create baseline.
Image analysis revealed that the spaceflight mice suffered a higher rate of failed bone union. The transcriptomic analysis was performed on the brain tissues obtained from these four groups. Dual dye microarray analysis found more genes significantly altered in Saline than Sham group (2,212 vs. 657), where >95% of genes were exclusively altered in Saline group due to microgravity. These Saline-exclusive genes were seeded to Ingenuity Pathway Analysis (IPA) for functional analysis. The neuropathic pain network was significantly altered in microgravity, along with a cluster of signals linked to neuromodulators such as GABA, glutamate, corticotrophin, and cortisol. An activated synaptic transmission and potentiation was coupled with inhibited sensation and nociception in spaceflight. This network analysis potentially outlined an adaptive pain sensitization process under a stressful condition. Brain region specific information is recommended to capture a comprehensive picture.

Metagenomics shotgun sequencing and global metabolomics of colon contents revealed that gut resident microflora was significantly involved in neurotransmission and bioenergetics. Together, we found an impaired gut-brain axis that potentially contributed in neuroinflammation, active synaptic transmission but reduced pain sensation, a characteristics of chronic pain paradigm. Our research is poised to understand the impact of microgravity on pain perception. Further understanding of how the brain modulates pain will serve as a platform for future studies on optimizing the management of pain during space missions.

Disclaimer: Research was conducted in compliance with the Animal Welfare Act, and all other Federal requirements. The views expressed are those of the author(s) and do not constitute endorsement by the U.S. Army.

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Spaceflight Induced Stress Caused Comprehensive Alteration of Fecal Microbiome Beyond Bacteria

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Spaceflight induces a unique type of polytrauma, which encompasses chronic unloading, microgravity, radiation (typically low grade), disrupted circadian rhythm, and a novel environment. Emerging studies including our own reports found that spaceflight caused a wide range of physiological deficiencies, such as energy deprivation, immunocompromisation, osteoporosis, and delayed wound healing. Since the resident microbiome is known to contribute to host immunity and energy biosynthesis, we assumed spaceflight could alter the function and abundance of microbial community, termed 'dysbiosis.' In this context, dysbiosis could influence osteoporosis and/or wound healing, two well-acknowledged health challenges facing astronauts and are poised to be a great hindrance for future deep-space exploration. By recent estimation, the number of bacterial cells are nearly equal to that of host cells, while other microbiota such as virus, fungi, protozoa, and archaea are less abundant. Nevertheless, each of these domains have unique features and functional contributions towards host's health and performance. Therefore, it is important to probe the entire host-microbiome ecosystem to fully comprehend the bi-directional relationship between host and its resident microbiome. The aim of this research is to examine the distinct effects of spaceflight and bone defects upon the entire fecal microbiome. Segmental bone defect (SBD) model was used to create a 4mm wound in mouse tibia to explore the dynamics of the healing mechanism. In the present study, male C57BL/6j mice were used for the NASA/Rodent Research 4 (RR4) program. Ten mice underwent surgical SBD four days before launch, and five of these mice were housed on the International Space Station (ISS) for 4 weeks (FLT-Surgery), whereas the other five mice were housed on ground under identical conditions (G-Surgery). In addition, 5 healthy mice were housed in the ISS (FLT-Sham) and ground (G-Sham), respectively, for 4 weeks. The mice were euthanized and the colon contents from the carcasses were processed for shotgun sequencing following the Illumina TruSeq DNA Nano protocol on the Illumina HiSeq4000 platform. The sequenced reads were trimmed, normalized, and mapped using kraken2, providing the taxonomic information of kingdoms namely bacteria, virus, fungi, protozoa, and archaea. As expected, the commensal percent abundances revealed that bacteria encompassed the largest portion, e.g. 81.9%, followed by eukaryota 15.6%, archaea 2.4%, and viruses 0.06%. Further multivariate analysis (Spaceflight/Ground vs. Sham/Surgery) found that spaceflight induced-stress caused the most significant alteration in the percentage abundance of viruses (p value = 0.01). The beta diversity estimation of the compositional variabilities among the communities and alpha diversity estimation of richness and evenness in fecal commensals underscored variations among different microbial kingdoms. For instance, overall bacterial and viral beta-diversity was attributed to both spaceflight and surgery, but their evenness and richness did not shift much across the taxa. On the other hand, there were significant shifts in both alpha and beta diversity in the microbial kingdoms of fungi and archaea. The current research gives insight into the gut microbiome as it relates to ground versus spaceflight, while also delving into not just the bacteria involved but the full commensal profile of the microbiome.

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Session 6: Environmental Micro- & Myco-biomes

Whispers in the Dark: Sending Signal Waveforms Through Melanized Fungal Cultures

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Melanin is a naturally occurring black-brown pigmented molecule that has the unique ability to interact with ionic and electrical charge, making it of potential high value to bioelectronic applications. While the facets of electricity have been explored in synthetic melanin, many gaps on the electrical properties of melanin in its native state. Melanized fungi incorporate melanin into and around their cell wall and an understanding of the propagation of electrical current through the melanized structures could inform as to how the fungi could be used to replicate common circuit elements. This could be of value in the creation of bioelectronics if it is demonstrated that many of the electrical properties found in synthetic melanin are exhibited in native state melanin, allowing melanized bioelectrical components to be grown instead of manufactured. Here we sought to understand how electrical signals propagate through melanized fungal structures and whether the fungal melanin is conducive to the propagation of specific frequencies which could allude to signal transmission applications. To investigate the aspects of signal propagation in melanized fungi, we propagated sinusoidal signal waveforms from 1Hz to 20MHz across cultures of the melanized fungus *Curvularia lunata* using a signal generator and oscilloscope. The melanized fungus propagated signals in the range of 1Hz to 80kHz with a minimal loss in power (0 to -.5 dB). From 80kHz to 20MHz, the signal quality decayed rapidly and was attenuated across the range. However, increases in the capacitance and resistance in this range suggest that at these frequencies the current is experiencing dynamic electrical processes that warrant further study. Given the propagatable range of the fungus (1Hz to 80kHz) encompasses the audio frequency range, a test was conducted attempting to propagate an audio waveform. It was revealed that the melanized fungus could successfully propagate the audio waveform, however, at a reduction in signal power. Further studies will dive deeper into the dynamic processes of the high frequencies specifically the impedance which can inform as to how the current is interacting with the melanized fungus.

The Effect of the Joint Biological Agent Decontamination System (JBADS) on Aircraft-Associated Microbiology

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Microbiological contamination of aircraft can take many forms, including contamination by biothreat agents, pathogens, and corrosion-causing microorganisms. Most approved decontamination methods require surface application of disinfectants and biocides, a labor intensive process. The efficacy of surface treatments is limited, particularly in areas that are difficult to access and/or heavily contaminated. Therefore, there is a requirement for a decontamination system that is rapid, efficacious, and limits exposure of maintenance crews to biological hazards. The Joint Biological Agent Decontamination System (JBADS) was developed specifically for biothreat agent (anthrax) decontamination and relies upon heat and humidity for non-contact, total aircraft decontamination. In multiple studies, JBADS was assessed for its ability to reduce naturally occurring populations of aircraft-associated microorganisms on cargo aircraft. Twenty-nine locations on aircraft were assessed for levels and types of

microorganisms pre- vs. post-decontamination. Three assessment methods were used: adenosine triphosphate (ATP) measurements, cultivation, and/or amplification and sequencing of 16S/18S small subunit ribosomal genes (microbiomes). Following exposure of the aircraft to JBADS (170 +/-5°F; 90 +/- 5% relative humidity; 72h), the microbial community was re-assessed by the same methods. ATP measurements in highly contaminated areas were reduced to baseline and the number of cultivable microorganisms in contaminated areas was reduced 99.99%, with most locations having ≤1 log growth/in^2 or no growth. Correspondingly, the number of cultivable, potentially corrosion-causing microorganisms was significantly reduced. There was also a significant shift in the relative abundance of both the total bacterial and eukaryotic microbiomes after decontamination. These results demonstrate that deploying JBADS to reduce levels of aircraft-associated microbiota may be a viable option for remediating aircraft following detection of pathogens, biothreat agents, and fungal and bacterial biofilms.

Metagenomic and Culture-based Characterization of the Chesapeake Bay Winter and Summer Planktonic Microbiomes

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Background: The Chesapeake Bay is the largest estuary in the United States and is of ecological, economic, and strategic importance. We are conducting a multi-year effort to determine the structure of the planktonic bacterial microbiome in both the Middle Bay (Annapolis region) and the Severn River. These experiments will determine not just the composition of the microbiome but also the seasonal, microecological, and year-over-year variability in these populations. It will also support investigation of stability of this microbiome over time, as well as the effects of anthropogenic and/or climatic forces in this important watershed.
Methods: We have sampled the planktonic bacterial microbiome from the water column in summer and winter of 2020-2022 by direct isolation of strains on environmental bacterial media, as well as by purification of whole-community genomic DNA from water samples. Genetic identification was performed by 16S Sanger sequencing of culturable bacteria and by full shotgun (HiSeq) metagenomic sequencing of the isolated DNA.
Objectives: These data were characterized by identification through homology and by bioinformatic analysis and were used to assess two hypotheses: 1) That variation exists in the microbiome structure of the Chesapeake Bay watershed based on location, season, and depth and 2) That these planktonic bacterial communities are consistent over time, repopulated year-over-year with the same organisms (likely from headwater reservoirs of biological diversity).
Results: The analysis presented here shows high similarity between the Severn River and Mid-Bay surface waters with differences in taxonomic distribution by depth and season. Repopulation of the watershed with a similar microbiome year-over-year occurs at higher taxonomic levels; however, there was a greater than expected variation in cultured species and of some clades in the metagenomic phylogeny. The source of these variations is not yet known; possible contributions include complex hydrological mixing and variation of Chesapeake inputs, tidal influence in the estuary, anthropogenic factors, and/or climatic effects. Extension of this work is planned in future years to more fully establish the core microbiome of the estuary and include additional rivers and locations.

Microbial Communities Vary by Function and Structure on Synthetic Polymers within DoD Infrastructure

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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 vehicles. 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 vehicles sampled. Transcriptionally active microbiomes, extracted from aircraft metatranscriptomes, were dominated by filamentous fungi and yeasts while the vehicles were dominated by bacteria, particularly cyanobacteria. Metagenomic assembled genomes (MAGs) with >80% completion were constructed for 26 bacteria and two fungi from the samples. The two fungal MAGS were classified to be *Rachicladosporium sp.* (mold) and *Meira* (black yeast) which are organisms typically characterized as saprophytes and/or plant pathogens. The metatranscriptomics data were mined for hydrolase enzymes such as lipases, esterases, cutinases, and proteases, known to be key players in polyurethane polymer degradation. Annotated fungal cutinase enzymes, highly expressed by *Rachicladosporium*, were predominant in the aircraft interior locations and were in the top 99 percentile of all expressed transcripts. Whereas these cutinases were not highly expressed in sunlight exposed locations, we found instead that genes for photosynthesis were in the top 99 percentile of all expressed transcripts. Based on our meta-omics sequencing, we hypothesize that exposure to sunlight and community composition may influence polymer degradation on contaminated aircraft and vehicle surfaces. These data will ultimately be used for the assessment of the biodegradation potential by microbial communities on polyurethane polymer surfaces and further characterization of microorganisms on aircraft could influence maintenance practices, including deployment of antifouling materials in locations prone to microbiological growth.

Session 7: Environmental Microbiome Analysis & Engineering (lightning talks)

Identifying Mycobiome from Aircraft Topcoat

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Microbiological attack of polyurethane topcoat in aircraft interiors creates two problems: (1) Degradation of the topcoat and production of corrosive metabolic byproducts or use of corrosive cleaners can lead to damage in the underlying aluminum alloy; (2) Exposure of personnel to the microbes and their volatile metabolic byproducts is a health hazard. Preliminary development of a test method has begun with examination and sampling of an existing airframe interior to obtain relevant inoculum. Gross examination of a decommissioned UH-60 airframe coated with MIL-PRF 85285 and chromate primer suggested little or no growth on the outside of the aircraft, but copious growth unevenly distributed throughout the interior. UV and drying appear to protect the exterior effectively, but interior inaccessible or concealed moist sites housed complex assemblages of microbes. Dry interior sites had patchy black fungal disfigurement; sunlit interior sites had green co-colonization with photosynthetic organisms; and wet sites had complex mixtures of organisms. Samples were taken for culture and molecular characterization. Culturing the interior samples from different coating areas in Czapek agar revealed that fungal colony morphologies were quite distinct, indicating that diverse fungal species were enriched by specific materials. Sequencing the amplified ITS regions from eight interior samples revealed overwhelmingly fungal species Toxicocladosporium, Cladosporium, and Erythrobasidium. Cladosporium sp. are recovered from patients with atopic dermatitis but not

from healthy skin are a prominent cause of respiratory symptoms (Sharpe et al 2015), and are also capable of corroding aircraft aluminum (Videla 1986, as cited by Lavoie and Little 1996). Multiple Cladosporium were evident from distinct samples within the helicopter. However, Erythrobasidium and other fungi, rather than Cladosporium, were recovered in a higher frequency from fluid contaminated topcoat. Visibly clean interior surfaces were measurably contaminated with spores from other interior sites, but the exterior helicopter topcoat had relatively little fungal DNA and grew comparatively few fungal colonies on media. These preliminary data will lead to design of laboratory test methods which enables evaluation of microbial degradation of aircraft interior topcoat and can motivate the development of more resilient topcoats and effective cleansers.

Assessing microbial threats in thawing permafrost using metagenomic sequencing

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Climate warming is causing rapid and widespread permafrost thaw. Biogeochemical processes in these thawed soils have not been well studied. Viable microbes are present in permafrost and some have been shown to become more biologically active following thaw, with implications for microbially-mediated ecosystem processes such as biogeochemical cycling. Emerging microbes may also act as pathogens to humans, crops or livestock; or carry genetic content (e.g. antibiotic resistance genes) that facilitates pathogenicity. A 2016 anthrax outbreak in Arctic Russian Siberia was a high-profile example of the risk posed by viable bacterial spores emerging from infected animal carcasses embedded in permafrost, leading the US National Academies of Science, Engineering and Medicine to host a workshop on the potential threat of emerging Arctic microorganisms in response to climate change. However, our understanding of the scope of the pathogen risk remains limited. In this study, we explore the pathogen risk in thawing permafrost using metagenomic sequencing of samples collected from the CRREL Permafrost Tunnel in Fox, Alaska. A laboratory incubation was used to simulate thaw of samples from the Tunnel, with shotgun sequencing capturing changes in metagenomic content. Read-based analysis of the sequencing data confirms that thaw causes shifts in community composition, with the trajectory varying between sampling sites within the Tunnel. Assembly-based analysis yielded 97 genome bins of varying quality from the shotgun data. We are presently using both read- and assembly-based approaches to examine changes in pathogenicity and virulence factors, along with linked genomic content. We discuss preliminary results, and the potential for shotgun metagenomics to facilitate high-throughput surveys of pathogen risk in rapidly changing Arctic environments.

Permafrost Thaw and the Carbon Cycle: Comparing Green House Gas Emissions from Alaska and Abisko

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Ancient carbon-rich organic material in permafrost regions represent a large carbon reservoir vulnerable to change in a warming climate. Over tens of thousands of years cold and frozen conditions have protected these carbon-rich organic materials from microbial decomposition. However, warming and thawing of permafrost makes a substantial fraction of this organic material susceptible to microbial breakdown. Current climate change models do not include these permafrost carbon emissions, and there is a need for additional modeling to more accurately assess this climate feedback. While laboratory work is needed for identifying the key mechanisms for potential greenhouse gas release from permafrost, some important processes are difficult to address with incubation experiments. Herein we focus on comparing CH_4 and CO_2 data we collected during field respiration studies from both Fox, Alaska (64° N, -147° E) and Abisko, Sweden (68° N, 18° E) which will be used to validate larger modeling. Preliminary results from

Abisko, Sweden show a trend of methane having the highest flux from locations consisting of wetter conditions with some pulse events occurring early in the mornings. Carbon dioxide showed a trend of increasing with soil wetness and temperature. Alaska data analysis is still ongoing, but thus far exhibits similar trends.

Investigating the Disturbed Soil Volatilome as a Novel Soil Sensing Tool

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Objective: Our goal is to identify key relationships between emitted soil volatile compounds, the producers of those compounds, and environmental properties including soils, soil disturbance attributes, and climate variation.
Methods: We developed an optimized solid-phase microextraction procedure to detect very low concentrations of volatile compounds in the field. This method is now being coupled with collection of field samples using a simplified, repeatable soil disturbance procedure (bulb planter). Background concentrations are collected, soils are disturbed, and additional concentrations are collected. Additionally, soil samples are collected for 16S and ITS sequencing to compare composition and for quantification of geosmin and 2-methylisoborneol synthase genes. A number of growth chamber studies are in progress with the same method using a single soil to individually study the influence of biological, chemical, and physical soil properties on emissions, microbial composition, and synthase gene densities.
Results: Our optimized solid-phase microextraction procedure proved the existence of biogenic volatile emissions from disturbed and undisturbed soils, consisting primarily of alcohols geosmin and 2-methylisoborneol emitted by diverse soil microbes. Further, initial trials identified differences in background and disturbed soil volatile concentrations over time and space, with strong relationships detected with regional agricultural tillage activities and soil temperatures and moisture. Vegetation and soil texture also influenced disturbed soil volatile emissions. To date, field samples have been collected from IL, UT, NH, and Finland. All locations and samples have indicated a low background level of these compounds in the air and much higher volatile emissions with low levels of soil disturbance. The initial growth chamber study indicated that plant functional group associations influence the volatile compounds in different ways. Additional results will be presented from data collected over the next 6 weeks.
Discussion/Conclusions: This research is the first to ever quantify and characterize these biogenic volatile compounds in situ and document increased emission from disturbed soils. The most prevalent of these compounds (geosmin and 2-methylisoborneol) are emitted by ubiquitous soil microbes, including actinobacteria, cyanobacteria, and fungi. Pervasive background concentrations were also confirmed that fluctuate over time and occur at much lower concentrations compared to low level soil disturbance. As soil moisture, texture, and temperature all influence emission of these compounds, further understanding environmental relationships could yield an important new tool for environmental interrogation.

Microbial Activity in Dust Contaminated Antarctic Snow

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Microorganisms can survive in extreme environments, including the coldest regions of the earth. Using airborne dust as a transport mechanism, microorganisms are deposited on snow and can proliferate when growth needs such as UV radiation, increased temperature, water availability, and nutrient availability are met. In this study, we examined the dust particulate and associated microbial deposition on snow samples near the Pegasus and Phoenix Ice Runways at the U.S. McMurdo Station in Antarctica. The deposited particulate matter melts into the surface initially forming steep-sided holes, which can widen to form large patches of weak and rotten snow and ice. These

changes negatively impact the ice and snow runways and snow roads trafficked by vehicles. Snow was collected and shipped frozen to the Cold Regions and Research Laboratory (CRREL) where we performed a respiration study to measure the microbial activity during a simulated snow melt, isolated microorganisms, examined particle grain size, and performed 16S rRNA gene sequencing. We measured higher levels of carbon dioxide production in dust-containing samples compared to clean snow samples, indicating viable dust-associated microbial communities. Additionally, 11 microorganisms were isolated and cultured from snow samples containing dust particles. Wind patterns and satellite images suggest that the deposited particles could derive from nearby Black Island, interestingly the particle size and chemical composition did not match soil particles collected from Black Island, indicating another origin source. This study aims to help understand how these particles negatively impact snow runway strength and how to more effectively mitigate their impact.

Microbial Activity in Arctic Soil: An Arctic Application of the DRTSPORE Model

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The Dynamic Representation of Terrestrial Soil Predictions of Organisms' Response to the Environment (DRTSPORE) model was established to characterize soil activity in remote and inaccessible areas of US Army concern and predict biological impacted processes in soil. DRTSPORE uses empirical data collected from laboratory incubation studies such as soil respiration, soil moisture, and soil temperature in order to make soil activity predictions across various soil types. Here we focus on arctic soils in order to expand the model's capabilities into the global arctic region. The arctic soil environment is experiencing rapid changes as the climate warms; undergoing longer periods of permafrost thaw and active layer expansion each summer, causing wetter soil conditions and changes in greenhouse gas fluxes and microbial extracellular enzyme activity. It is critical to model soil respiration during these thaw events to understand how soil activity is changing in the arctic and expand the DRTSPORE model's capability into arctic regions. In order to mimic wetter and warmer conditions due to climate change and permafrost thaw, active layer soils from three locations in Alaska were sampled and experimentally wetted to 5 different matric potentials and incubated in microcosms at 4 different temperatures. Carbon dioxide (CO_2) flux rates were measured and potential enzyme extracellular activity was flourometrically measured once the CO_2 rates stabilized. At the warmer temperatures, we saw higher rates of CO_2 flux across two of the three Alaskan soils. The CO_2 rates were highest at -14 kPa to -33 kPa for the colder temperatures, but were highest in the most saturated soils for warmer temperatures. Extracellular enzyme activity was most influenced by increasing temperature across the three soils. β-Glucosidase activity was greatest out of the six enzymes assayed, indicating these soils have a propensity to degrade sugars. Overall, we saw greater CO_2 emissions and extracellular enzyme activity with warmer and wetted soils, providing new parameters to increase the scope of the DRTSPORE platform.

Characterizing Microorganisms from Permafrost for Low-Temperature Synthetic Biology Applications

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Operations in the Artic and other cold regions require technologies that can perform reliably under extreme cold conditions. Permafrost and frozen soils harbor a wide range of bacteria, archaea, and fungi that have adapted to extremely low temperatures with unique metabolic capabilities relevant to military operations and which could be exploited to develop biotechnologies optimized for cold environments. Since synthetic biology constructs will only

perform as well as their chassis, it is critical that circuits expected to perform under extreme cold conditions are housed in chassis that are adapted to those conditions. Cold-tolerant bacteria (psychrophiles and psychrotrophs) are critical in the development of synthetic biology technologies meant to work in cold environments like the Arctic. Using bacteria isolated from Alaskan permafrost, we apply an experimental pipeline to identify the best candidates for use as biological platforms, or chassis, for low temperature synthetic biology. So far, we have engineered two permafrost isolates related to *Rhodococcus fascians* and *Sphingomonas faeni* using the broad host range plasmids pBTK519 and pBAV1K-T5-gfp, respectively. These isolates are capable of growing at subzero temperatures, suggesting them as potential candidates for use as novel cold-adapted chassis for synthetic biology.

Bioengineering and Optimization of Biocementation for Potential Space Applications

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Objective: Biocementation is the natural process of using calcium carbonate precipitation as a way to bond particles of sand together into a solidified material. Ureolytic bacteria have the ability to break down urea to form carbonate that can react with calcium in microbially induced calcite precipitation (MICP). Biocementation is not a new technique, but we are seeking to bioengineer *E. coli* to be capable of ureolytic activity and to make use of silica present in martian regolith in a process called biosilicification, which allows for the production of silicate. These modifications are intended to increase biocement strength and provide protection against UV radiation. The use of *E. coli* with alterations for biocementation could make the process a valid option for possible space applications, as they maintain the potential for further engineering to tolerate more extreme conditions. These space applications include dust stabilization for landing spacecraft on the lunar and martian surfaces, increasing regolith particle size for better gas exchange in plant roots, and producing construction material in space that can be grown from just a few cells and/or isolated proteins.

Methods: We used three silicatein genes from *Latrunculia operinae, Suberites domuncula,* and *Ephydatia fluviatilis* – marine and freshwater sponges that form crystalline structures through silica polymerization. These gene sequences were engineered in pET-28 plasmids for transformation into *E. coli* BL21 DE3 cells for protein expression. We also built upon a construct provided by AFRL, containing *S. pasteurii ureABC* but showing no urease activity, by attempting to amplify a larger region of the urease gene cluster from *S. pastueurii* to include genes *ureABCEFGD* and hope to effectively create urease activity in our *E. coli* strain.

Results: Protein expression from all three silicatein genes may have been detected in the transformed *E. coli*. Further testing is ongoing to determine their ability to protect against UV radiation and strength improvement in biocement. Similarly, once verified that the entire urease construct of *ureABCEFGD* is present, it may have the ability to increase the effectiveness of the biocementation process.

Discussion/Conclusion: Bioengineered bacteria capable of biocementation and biosilicification may possess the ability to improve the current biocementation process and make it more feasible for space applications. Biosilicification has the potential to help initial nucleation of calcium carbonate during the biocementation process and improve resistance to compressive stress, as well as protect against high levels of UV radiation outside of the earth's atmosphere.

TSMC Annual Meeting 2022

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

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