5th Annual TriService Microbiome Consortium Meeting
13th-15th September 2021 | AGENDA (all times US Eastern)

Monday 13th September 2021

1145-1200 Login
1200-1210 **Opening Remarks & TSMC overview:** Mr. Jason Soares, Chair, DEVCOM SC, Dr. Michael Goodson, Vice-Chair, AFRL
1210-1235 **Biotech Community of Interest Overview:** Dr. Patrick Mason, ONR, Steering Committee Chair
1235-1255 **User Engagement Session #1**
1235-1245 Navy user rep #1: LCDR Joseph Decicco, NSMRL
1245-1255 AF user rep #2: Dr. Jimmie Jacobs, Air Mobility Command/A10N
1255-1430 **Technical Session #1: Microbiome analysis and discovery – Part 1**
   Chair: Dr. J. Philip Karl, USARIEM
   1255-1320 Speaker #1: Dr. Armand Dichosa, LANL
   1320-1345 Speaker #2: Dr. Sara Campbell, Rutgers
   1345-1410 Speaker #3: Dr. Tonya White, USAF
   1410-1430 Session Panel Q&A
1430-1440 BREAK
1440-1615 **Technical Session #2: Microbiome analysis and discovery – Part 2**
   Chair: Dr. Vanessa Varaljay, AFRL
   1440-1505 Speaker #1: Dr. Wanchang Cui, USUHS
   1505-1530 Speaker #2: Dr. Jasmohan Bajaj, VA
   1530-1555 Speaker #3: Dr. Scott Merrell, USUHS
   1555-1615 Session Panel Q&A
1615-1620 BREAK
1620-1750 **Lightning Talks Session #1: Human microbiome theme**
   Chair: Dr. Blake Stamps, UES/AFRL
   1620-1630 Speaker #1: Dr. Phil Karl, USARIEM
   1630-1640 Speaker #2: Dr. Allison Hoke, WRAIR
   1640-1650 Speaker #3: Dr. Charles Budinoff, IFF Health
   1650-1700 Speaker #4: Mr. Patrick Radcliffe, USARIEM
   1700-1710 Speaker #5: Dr. Diana Brostow, VA
   1710-1720 Speaker #6: Dr. Grace Giles, DEVCOM SC
   1720-1730 Speaker #7: Dr. Linda Chrisey, DARPA
   1730-1750 Session Panel Q&A
1750-1755 **Closing Remarks (TSMC Chairs)**
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<td>Speaker #1: Dr. Elaine Merrill, AFRL</td>
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<td>Speaker #5: Ms. Laurel Doherty, DEVCOM-SC</td>
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<td>1720-1800</td>
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# Abstracts of the 5th Annual Meeting

**Tri-Service Microbiome Consortium**

**Wednesday 15th September 2021**

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<td>Army user rep #2: Mr. George Matook, DEVCOM SC, PM MASTR-E</td>
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<td>1225-1400</td>
<td>Special Session: Regulatory Framework for Microbiome Solutions</td>
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<td>Speaker #1: Dr. Gautam Dantas, Washington University/NMRC</td>
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<td>Speaker #2: Ms. Stacy-Ann Miller, WRAIR</td>
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<td>Speaker #6: Mr. Jordan Zambrana, EPA</td>
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Armand Dichosa

Genomics investigation of the human gut microbiome in microgravity using gel microdroplets and community cultivation strategies

Long term space travel for scientific and defense related missions is inevitable. Our women and men who serve our nation under either of these operations are exposed to various environmental factors that are arguably considered extreme. Even in relatively short-term flight missions, changes in diet, sleep patterns / altered Circadian rhythms, enclosed / artificial environments, and increased exposure to radiation and microgravity have resulted in observed physiological changes to the space traveler.

Surprising evidence has shown that commonly nascent bacterial species – isolates that are known members of the human gut and skin microbiomes – increased their mutation rates and virulence due to exposure to microgravity conditions. Thus, it is conceivable that certain members of the normal human microbiota may become pathogenic to the human host during long-term space travel. While this scenario entails an enrichment of pathogens due to microgravity exposure, we also postulate that certain key bacterial species that normally place the opportunistic pathogens in check may become depleted, thereby creating a suitable environment that permits pathogenicity. Because of challenges to study such processes in humans in space, our understanding of the mechanisms that lead to pathogenicity of such microbes in gut is completely lacking.

Herein, Los Alamos National Laboratory (LANL) Bioscience Division and Rhodium Scientific seek to understand the effects of microgravity exposure to the normal human gut microbiome at the community level. By avoiding an isolate level approach, our premise was to investigate the effects of microgravity on the near-native microbial environment by preserving natural cell-to-cell interactions. Our team utilized a novel, dual in vitro cultivation strategy purposed to genomically identify changes in the human gut microbial community structure over time, when comparing flight (microgravity) to ground (control) cultures. The first are bulk cultures, whereby viable bacteria extracted from therapeutic human fecal material was aliquoted into defined growth medium shown to preserve the gut microbiome at the taxonomic family level. The second are gel microdroplets (GMDs) cultures, whereby single bacterial cells of the same extract are randomly single-captured in micron-sized agarose beads and co-cultivated with the native consortium to form sequestered, clonal microcolonies. While the bulk cultures allow changes in community composition that will be detected with genomics, the GMDs offer the viable recovery of bacterial isolates and subsequent genomics.

To execute this study, our team prepared three replicates of each bulk and GMD cultures to represent 6, 12, and 48 hr incubation periods at 37ºC for flight and ground cultivation. SpaceX-20 delivered the flight bulk and GMD cultures to the ISS NL, whose team executed the cultivation at the designated time points and immediately stored at -80ºC. Incubation of the ground bulk and
GMD cultures at the identical times points was executed at LANL and immediately stored at -80°C. All post-flight samples were returned frozen to LANL for genomics and bioinformatics analyses. DNA from all the bulk cultures were extracted, QC’d, and processed for amplicon sequencing of the V3-V4 regions of the bacterial 16S rRNA gene, the universal marker to identify bacteria at various taxonomic levels. The V3-V4 amplicons of every bulk culture sample were uniquely barcoded for multiplex sequencing on the Illumina MiSeq sequencing platform at the LANL Genomics Facility. The GMD cultures were initially analyzed via cytometry to detect changes in growth over time, and further anaerobically cultivated to recover viable isolates. All recovered isolates were phylotyped using whole-cell PCR and subsequent sequencing of the 16S rRNA gene. All bioinformatics analyses of the bulk cultures were done using Qiime2 on LANL’s EDGE Bioinformatics platform, while the GMD cultures were analyzed using CLC Bio and RDP.

Extensive analyses of the bulk community revealed potential trends of bacterial growth – increasing or decreasing in relative abundance – which permitted the identification and further investigation of specific bacterial taxa exhibiting such trends. Our findings, among others, revealed a notable enrichment of bacteria associated with human gut diseases, including Dialister, Enterococcus, and Dorea spp. Our findings also identified trends in bacteria suggested to be of benefit to the human host, including Roseburia and Faecalibacterium spp. Furthermore, our bulk community analyses revealed the presence novel human gut bacteria that have yet to be identified at various taxonomic levels. For the GMD cultures, our team viably recovered several bacterial isolates from the flight cultures, including Enterococcus, Staphylococcus, Bacillus, and putative Catellicoccus spp. Based on phylogenetic analysis of their respective 16S rDNA sequences, these post-flight recovered bacterial isolates represent novel Genus and species in the bacterial Domain, and particularly for the Enterococcus, Bacillus, and Catellicoccus isolates, have direct ties to the human gut microbiome.

Our dual cultivation approach was designed as a low-cost, rapid means to interrogate the complex human gut microbiome amenable to genomic analyses to infer how microgravity may affect the gut microbiome at the community level. Our bulk cultures revealed potential growth trends of certain bacterial taxa that could be used as biomarkers to monitor the health of the long-term space traveler, as well as the identification of novel bacterial taxa that must be identified. Our GMD cultures successfully and viably recovered several, novel bacterial isolates that will permit further whole-genome assemblies for functional gene annotation and, if significant to human health, could be used for in vivo studies for multifaceted studies in microgravity environments.

Our study demonstrates the simultaneous utility of both cultivation strategies as the foundation for expanded, in-depth, ‘omics-based investigations (e.g., transcriptome, proteome, metabolome) that will, undoubtedly, provide revealing insights to the dynamics of gut microbiome interactions in the human gut, and how these interactions will affect the space traveling human host.
Sara Campbell

Unique Microbes and Metabolites are Associated with Brown Adipose Tissue

Sara C. Campbell, PhD, FACSM1, Olufunmilola Ibironke, PhD1, Candace R. Longoria1, Lee J. Kerkhof, PhD2, Marko Oydanich3, Xiaoyang Su, PhD4, Eric Chiles4, Stephen F. Vatner, MD3

1Department of Kinesiology and Health, 2Department of Marine and Coastal, 3Cardiovascular Research Institute, 4Cancer Institute of NJ, Metabolomics Share Resource Center, Rutgers, The State University of New Jersey

Background: The gut microbiota is linked to brown adipose tissue (BAT), but the mechanisms and microbes facilitating BAT production are largely unknown. A novel mouse model containing a gene knock-out of regulator of G protein signaling 14 (RGS14KO) has increased BAT. Our proposed studies seek to identify the key active gut microbial species involved in the gut of RGS14KO mice and link these findings to BAT.

Methods: Twenty-two mice (N=13 RGS14KO, N=9 Wild type (WT)) were used to identify predominant microbes and metabolites. Bacterial ribosomal operons were sequenced using the Oxford Nanopore MinION to obtain the gut microbiota profiles. Metabolomics used HPLC to evaluate polar and nonpolar positive and negative untargeted metabolites in fecal, cecal, brain, and BAT samples. For microbiome data Kulczynski distance was used to compare WT reads to RGS14KO reads in two-dimensional non-metric multidimensional scaling (NMDS) plots. Two-tailed t-tests were used to compare WT and RGS14KO metabolite means with a p-value<0.05 considered statistically significant.

Results: Approximately 500k rRNA reads post QA/QC ((84% identify; >1000 bp alignment) were obtained from all samples by MegaBlast. NMDS plots showed significant bacterial community differences (Genus: p=0.035; Species: p=0.028; Strain: p=0.037) between WT and RGS14KO mice. Specifically, RGS14KO mice housed two unique strains of Akkermansia muciniphila (A. muciniphila BIOML-A22 and A. muciniphila AN78) that are not seen in WT animals. Overall metabolomics revealed 47 positive and 65 negative metabolites that are significantly different between WT and RGS14KO mice in one of the four tissue samples. Of note, RGS14KO animals had significantly higher levels of G6P, glycy1-l-proline, glycerophosphocholine, isoleucine, pipercolic acid, thymidine, UDP-D-glucose, malate, leucic acid, NADP+, and guanosine in BAT compared to WT animals. Many of these metabolites have not only been linked physiologically to each other, similar pathways, they also have been linked to BAT activation and function.

Conclusion: Gut microbiome analysis identified specific differences between RGS14KO and WT
mice at genus to strain level. Unique metabolites found in RGS14KO BAT are directly linked to BAT function. Our early studies show that BAT function is linked to specific microbes and metabolites known to activate BAT. Follow up studies will link what microbes are most active and if this activity is linked to specific metabolite production.

**Navy Relevance:** An Office of Naval Research priority is to understand the functional link between the gut microbiome and BAT. This priority hopes to enable warfighters to better tolerate cold, and to provide a source of energy for long missions where eating/drinking is not feasible. ONR Award # 826640

**Disclaimer:** The authors declare no conflict of interest and have nothing to declare.

**Learning Objectives:**
1. Describe the difference between a WT and RGS14KO mouse.
2. Discuss the importance of microbiome differences between WT and RGS14KO mice.
3. Discuss the importance of metabolite differences between WT and RGS14KO mice.
4. Understand how this is relevant to Naval warfighters.
The Influence of Diet Quality on Vaginal Microbiota Composition in Pregnant Women

Molecular bacterial vaginosis (BV), a microbial dysbiosis, contributes to adverse pregnancy outcomes and affects 30% of reproductive age women. It is unclear whether diet shapes the vaginal microbiota. This is important knowledge because diet is readily modifiable and may be leveraged to reduce adverse pregnancy outcomes. This study sought to fill the gap on whether diet influences the vaginal microbiota, thus BV, by examining the association between longitudinal diet quality and molecular BV. A subsample of 55 out of 66 women from a large federally funded project (BEAM; NR014826) met the inclusion criteria.

This study had two specific aims: (1) to assess the influence of diet quality on the vaginal communities to determine if differences in diet quality, based on the Healthy Eating Index-2015 (HEI-2015) total, adequacy, and moderation scores were associated with molecular BV; and, (2) to analyze the relationship of HEI-2015 adequacy and moderation diet quality scores and molecular BV using diet data at six, seven, and eight months gestation. Binary logistic regression and adjusted multivariate mixed models were used adjusting for race, obesity, age, gestational age at delivery, marital status, education, and household income. No significant association between diet and molecular BV were observed. Gestation length, age, and presence of molecular BV at six months gestation were noted to be significant predictors of molecular BV.

This study substantiated findings that molecular BV contributes to early birth. A surprising finding was that obese women trended toward lower odds of molecular BV. Diet does not play a notable role in shaping the vaginal microbiota. The findings from this study suggest that age and marital status may be contributing factors to the occurrence of molecular BV. This is particularly important when considering young recruits. However, age and marital status are not modifiable factors thus future exploration is needed to determine the modifiable risk factors of molecular BV in young, unmarried service members.

Keywords: Diet, women's health, vaginal microbiota, pregnancy, bacterial vaginosis

Disclaimer: The views expressed are those of the [author(s)] [presenter(s)] and do not reflect the official views or policy of the Department of Defense or its Components. The voluntary, fully informed consent of the subjects used in this research was obtained as required by 32 CFR 219 and DODI 3216.02_AFI 40-402.
Wanchang Cui

The gut microbiome change in wild type and IL-18 knockout mice after 9.0 Gy total body irradiation

Wanchang Cui\textsuperscript{1, 2}, Xianghong Li\textsuperscript{1}, Lisa Hull\textsuperscript{1, 2}, Alex Zizzo\textsuperscript{1}, Li Wang\textsuperscript{1, 2}, Bin Lin\textsuperscript{1, 2}, Min Zhai\textsuperscript{1, 2}, Mang Xiao\textsuperscript{1}.

\textsuperscript{1}Armed Forces Radiobiology Research Institute, Uniformed Services University of the Health Sciences, Bethesda, MD, USA.
\textsuperscript{2}The Henry M. Jackson Foundation for the Advancement of Military Medicine, Bethesda, MD, USA

The gut microbiome plays essential roles in different pathophysiological conditions, such as obesity, cancer, and bowel disorders. Recent studies have shown that gut microbiome may play important roles in response to radiation exposure. IL-18, an inflammatory cytokine, is highly elevated in mice, mini-pigs and nonhuman primates after radiation exposure. Blocking IL-18 using its endogenous binding protein (IL-18BP) increases mice survival after radiation exposure by decreasing bone marrow interferon-gamma levels. To further characterize the roles of IL-18 in response to radiation, both wild type and IL-18 knockout (IL-18KO) mice were exposed to 9.0 Gy total body irradiation. Our 30-day survival data demonstrated that IL-18KO mice were significantly more resistant to radiation compared to the wild type mice (p<0.0001). Mouse fecal samples were collected at pre-radiation, d1, d3, d7, d14 and d21 after radiation exposure. Microbiome profiling was performed on the fecal samples using 16S sequencing technology. Preliminary data analysis showed that there was significant difference in the microbiome between wild type and IL-18KO mice. There were 32 significantly different genus between the wild type and IL-18KO mice at baseline. Cohousing of wild type and IL18-KO mice decreased the microbiome difference between the two genotypes. Radiation was associated with very few significantly changed bacteria genus in wild type mice. However, there were much more significantly changed bacteria genus in the IL-18KO mice after radiation exposure. The significantly different bacteria genus between wild type and IL-18KO mice at different time points after radiation was also studied. The current study helps understand the gut microbiome in different genetic backgrounds and its temporal changes after radiation exposure. Our data provides insight into the mechanisms underlying radiation-induced toxicity and helps develop effective radiation countermeasures.

(This study is supported by AFRRI, NIAID and JPC to MX; the views expressed here do not represent those of HJF, AFRRI, USUHS, or US DoD. The authors declare no conflict of interests.)
Jasmohan Bajaj

Interaction of gut metagenomes in veterans with cirrhosis and post-traumatic stress disorder

Jasmohan S. Bajaj¹, Christopher Stamper², Amirhossein Shamsaddini³, Andrew Fagan⁴, Kelly A Stearns-Yoder⁵, Ms. Edith A Gavis⁶, Patrick M Gilleve⁵ and Lisa Brenner⁷

(¹)Virginia Commonwealth University and Richmond VA Medical Center, (²)Rocky Mountain Regional VA Medical Center, (³)George Mason University, (⁴)Mcguire VA Medical Center, (⁵)2. Rocky Mountain Mirecc, Rocky Mountain Regional VA Medical Center, (⁶)Mcguire VAMC, (⁷)2. Rocky Mountain Mirecc, Rocky Mountain Regional VA Medical Center

Background: Cirrhosis and PTSD often co-exist in Veterans and their impact on the gut-brain axis could have implications for addiction therapy and alcohol use disorder. The unique microbial signature of PTSD vis-à-vis cirrhosis needs to be determined to focus on the appropriate management strategy in patients with both conditions. Aim: Determine the metagenomic gut microbial signature of PTSD and cirrhosis individually compared to PTSD+Cirrhosis in Veterans with cirrhosis.

Methods: Outpatients (controls, PTSD only, Cirrhosis only and PTSD+Cirrhosis) were enrolled from two large VA Medical Centers. Demographics, concomitant medications were recorded, and stool metagenomics were performed. Bacterial species and gut-brain modules (GBMs) were compared between the groups. α/β-diversity and individual taxa/GBMs were analyzed. Finally, we adjusted for age, gender, education, race, PPI use and diabetes in multi-variable models (MAAslin2) for species and GBMs.

Results: 150 subjects (46 controls, 15 PTSD, 75 cirrhosis & 14 PTSD+Cirrhosis) were included. Cirrhotics were older and had more PPI use and diabetes than the rest. Most patients were men regardless of group. Microbiota:

Bacterial species: There was a higher α/β-diversity (Fig A/B) due to lower α-diversity in PTSD. Short-chain fatty acid (SCFA) producers (Alistipes, Eubacterium) were higher in controls and only PTSD patients, while Veillonella spp were higher in both cirrhosis groups. PTSD+Cirrhosis had higher Bifidobacterium & Clostridial spp. Streptococcus and Rothia were only higher in Cirrhosis only patients (Fig A).

GBMs: Patterns of GBM α/β-diversity followed bacterial species(Fig A/C). SCFA metabolism and glutamate synthesis was higher in controls, while NO degradation was highest in PTSD+Cirrhosis (Fig A). Glutamate degradation was highest in cirrhosis alone.
**Multi-variable analysis** for species and GBMs show that despite controlling for age, PPI, gender, group distribution and diabetes, both species and GBMs that were different above remained statistically significant in differentiating the groups.

### Table

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<th>Demographics</th>
<th>Controls (n=46)</th>
<th>PTSD (n=15)</th>
<th>Cirrhosis (n=75)</th>
<th>PTSD+Cirrhosis (n=14)</th>
<th>P value all groups</th>
<th>Significant on MAASlin2 Species</th>
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### Conclusion

Metagenomic profiling shows that short-chain fatty acid producing taxa and their metabolism differed between patients with PTSD and cirrhosis. Since PTSD and cirrhosis often coexist, these distinct microbial profiles could guide appropriate management.
Nasal Microbiome Evolution Within the Congregate Setting Imposed by Military Training

Faith C. Blum¹, Jeannette M. Whitmire¹, Jason W. Bennett², Patrick M. Carey², Michael W. Ellis², Natasha N. Law², Sean Conlan³, Clayton Deming³, Julie A. Segre³, David R. Tribble², Eugene V. Millar² and D. Scott Merrell¹

¹ Uniformed Services University of the Health Sciences, Bethesda, MD
² Infectious Disease Clinical Research Program, Bethesda, MD
³ National Institutes of Health, Bethesda, MD

While a great deal of information has been gained from ‘snapshot’ studies that define the microbiome of a particular body site at a particular time, the composition and relative abundance of various species within the microbiome of an individual can change over time. Thus, dynamic changes within the human microbiome are being examined via longitudinal research studies that seek to examine the impact of environmental factors and/or personal interactions on the microbiome of individuals. These studies are important since the particular composition of an individual's microbiome, in combination with host and environmental factors, can precipitate pathogenesis of commensal microorganisms and can result in infections. Furthermore, resident microbes can influence the ability of an encountered pathogen to colonize and cause disease.

The relationship between the presence of particular microbes and disease development has been previously examined for skin and soft tissue infections (SSTIs), which are frequently encountered maladies within the Military Health System. To this end, even though Staphylococcus aureus benignly colonizes the nares of approximately 25% of the population at any one time, colonized individuals are known to be at increased risk for development of subsequent SSTIs. As such, S. aureus is the most frequently identified infectious agent within cutaneous abscesses.

To understand the evolution of the nasal microbiome of U.S. Army Infantry trainees stationed at Fort Benning, Georgia, individuals were sampled longitudinally from their arrival at Fort Benning until completion of their training 90 days later. These samples were then used for 16S-based microbiome profiling. Given the congregate setting encountered by these military trainees, we originally hypothesized that we would observe an overall convergence of the individuals' nasal microbiomes to a more similar composition. However, microbiome stability varied dramatically on an individual basis; some subjects showed great stability, some subjects showed gradual temporal changes and some subjects showed dramatic shift in nasal microbiome composition.

Analysis of available patient metadata suggests that S. aureus colonization status and geographic origin of the trainees may be key drivers of nasal microbiome stability within this population.
Thus, nasal microbiome 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 within the military population may require a ‘personalized’ approach.
J. Philip Karl

Inter-individual variability in gut microbiota composition is associated with changes in the fecal metabolome of individuals consuming a military ration diet

J. Philip Karl, Nicholes J. Armstrong, Patrick N. Radcliffe, Holly L. McClung

Objective: The fecal metabolome provides a functional readout of interactions between host, diet and the gut microbiota that may help identify gut microbiota-derived compounds associated with health outcomes. This study aimed to determine associations between inter-individual variability in gut microbiota composition, diet-induced changes in the fecal metabolome and gastrointestinal symptoms in adults consuming a diet consisting solely of military rations.

Methods: Secondary analysis of a randomized-controlled trial in which 54 healthy adults (32 ± 14 yr, BMI 26 ± 3 kg/m²) were randomly assigned to consume their usual diet (Control) or a provided diet of Meal, Ready-to-Eat military rations (MRE) for 3wk. Fecal microbiota composition was measured by 16S rRNA sequencing and the fecal metabolome by untargeted UPLC-MS/MS at baseline and post-intervention. Self-reported gastrointestinal symptoms were measured weekly using the Irritable Bowel Severity Scoring System (IBSSS).

Results: Principal coordinates analysis of baseline gut microbiota composition separated MRE participants into two clusters determined primarily by ratio of Bacteroides to Prevotella (HIGH-BP (n=17) or LOW-BP (n=10)). Random Forest classification of changes in the fecal metabolome within Control, HIGH-BP, and LOW-BP produced error rates of 7%, 18% and 100%, respectively, suggesting a more discriminant metabolome response in HIGH-BP than LOW-BP. Between-group differences in 153 metabolites were detected by ANOVA (FDR < 0.20). Among those, 39 identified and 20 unidentified metabolites demonstrated an association with the gut microbiota (HIGH-BP vs. LOW-BP, P < 0.05). Compounds within xenobiotic, peptide/amino acid, and lipid metabolism pathways comprised 29 of the microbiota-associated metabolites. Changes in microbiota-associated metabolites were not correlated with changes in IBSSS scores.

Conclusions: Changes in the fecal metabolome of individuals consuming a short-term military ration diet are associated with inter-individual variability in gut microbiota composition, but changes in microbiota-associated fecal metabolites were not associated with gastrointestinal symptoms.

Disclaimer: Authors’ views do not reflect official DoD or Army policy.
Allison Hoke

Longitudinal effects of anti-inflammatory diet on gut-brain axis in rat model of traumatic brain injury

Allison Hoke1,2, Nabarun M Chakraborty2, Aarti Gautam2, Stacy Ann-Miller1,2, Lalith Naidu1,2, Michelle L. Condlint3, Rasha Hammamieh2, Angus G. Scrimgeour3

1ORISE, Oak Ridge Institute of Science and Education, Oak Ridge, TN 37830
2Medical Readiness Systems Biology, Walter Reed Army Institute of Research, Silver Spring, MD 20910
3Military Nutrition Division, U.S. Army Research Institute of Environmental Medicine, Natick, MA 01760

The disruption of microbial composition (dysbiosis) is typically associated with diseased states, such as traumatic brain injury (TBI). The hypothesis is that the dysbiosis harbingers the clinical manifestation of illness; hence, fecal microbiome is a potential non-invasive source as an early marker of host response to TBI. Since TBI triggers an inflammatory surge, and according to some records, resident microbiome plays a significant role in host immune response, we postulate that anti-inflammatory intervention could ameliorate the symptoms of TBI via restoring eubiosis. This proof of principle study investigated the impact of anti-inflammatory diet mix (AIDM) on the fecal metagenomics of a rodent model exposed to mild TBI (mTBI).

Adult, male Wistar rats were exposed to mTBI using a modified Marmarou closed head weight drop protocol. During the 30 days post-injury, rats were fed an AIDM diet supplemented with omega-3 polyunsaturated fatty acids and vitamin D3 or regular house chow (CON). Sham rats were not exposed to TBI, but fed AIDM or CON diet. Rats were euthanized 48h, 14d and 30d post-injury, and brain frontal cortex (FCx) and the descending colon contents were collected 48h and 30d post-injury. Whole rat genome microarrays were performed on FCx and 16S rDNA metagenomics of colon contents were run on Illumina MiSeq.

Behavioral tests post-injury showed no cognitive deficits typically associated with mTBI. In Injured-CON, plasma NF-L levels were significantly elevated at 48h post-injury ($p<0.005$) but returned to normal by 14d post-injury, and at 30d post-injury, T-tau, GFAP and UCH-L1 concentrations were significantly increased ($p<0.005$).

Metabolic dysfunction is a major comorbidity of TBI, and in the FCx there were time and diet-related changes in the molecular networks associated with cell death, biogenesis, inflammation, and bioenergetics. The glycolysis network in FCx remained inactivated at 30d post-injury in the Injured-CON group, which possibly supported that TBI induced long-term energy deprivation. For metagenomic alpha and beta-diversity, time was the most significant factor, followed by diet
and time interaction. The host had an energy-deprived state reflected in the microbiota as anaerobic bacteria, primarily *Deferrribacteres*, was increased in the Injured-CON group at 48h post-injury. There was a disruption in the microbiome bioenergy-related networks in the Injured-CON group, as glycolysis II signaling was inhibited and NAD biosynthesis I from aspartate signaling was activated 48h post-injury.

The shift in phylum *Firmicutes* in Injured-AIDM captured the AIDM effect. The beneficial effects of the AIDM intervention were revealed in the Injured-AIDM group where the regular energy biosynthesis networks, including the TCA cycle I were activated at 30d post-injury in the microbiome and there were simultaneous shifts in networks linked protein biosynthesis, biogenesis of energy products, and ion channel maintenance. In conclusion, this study demonstrates that a strategic intervention, using select nutrients, was able to ameliorate the bioenergy deprivation caused by TBI.

**DISCLAIMER:** Research was conducted in compliance with the Animal Welfare Act, and all other Federal requirements. The views expressed are those of the authors and do not reflect the official policy of the Department of Army, Department of Defense, or the U.S. Government. Funded by USAMRDC.
Probiotic consortia improve anti-viral immunity to SARS-CoV-2 in Ferrets

Lehtinen Markus J¹, Kumar Ritesh², Zabel Bryan³, Mäkelä Sanna M¹, Nedveck Derek³, Tang Peipei³, Latvala Sinikka¹, Guey Sebastien⁴, Budinoff Charles R³

¹ Health & Biosciences, IFF, Kantvik 02460, Finland;
² Health & Biosciences, IFF, Wilmington, DE 19803, USA;
³ Health & Biosciences, IFF, Madison, WI 53716, USA;
⁴ Health & Biosciences, IFF, Niebull 25899, Germany

Probiotics have been suggested as one solution to counter detrimental health effects by SARS-CoV-2, however, data so far is scarce. To investigate the effect and mechanism of action of probiotics against SARS-CoV-2 we designed two probiotic consortia to stimulate innate immune function. Our studies tested the effect of the consortia in a ferret SARS-CoV-2 challenge model and in a human monocyte-derived macrophage (Mf) and dendritic cell (DC) model. In the ferret model we measured viral loads along with gene expression in tissue samples using qPCR and in the in vitro monocyte model we measured cytokines along with gene expression using transcriptomics. Our results showed that the consortia significantly reduced the viral load, modulated immune response, and regulated viral receptor expression in ferrets compared to placebo. In human Mf and DC model, OL-1 and OL-2 induced cytokine production and genes related to SARS-CoV-2 anti-viral immunity. These results indicate that probiotic stimulation of the ferret immune system leads to improved anti-viral immunity against SARS-COV-2 and that critical genes and cytokines for anti-SARS-CoV-2 immunity are stimulated in human immune cells in vitro. The effect of the consortia against SARS-CoV-2 warrants further investigations in human clinical trials.
Patrick N Radcliffe

Severe, short-term sleep restriction marginally affects gut microbiota composition and does not impact intestinal permeability in healthy young men

Patrick N. Radcliffe\textsuperscript{a,b}, Claire C. Whitney\textsuperscript{a}, Marques A. Wilson\textsuperscript{a}, Heather S. Fagnant\textsuperscript{a}, Nabarun Chakraborty\textsuperscript{c}, Ross Campbell\textsuperscript{c}, Allison Hoke\textsuperscript{c}, Aarti Gautam\textsuperscript{c}, Rasha Hammamieh\textsuperscript{c}, Tracey J. Smith\textsuperscript{a}, J. Philip Karl\textsuperscript{a}

\textsuperscript{a}U.S. Army Research Institute of Environmental Medicine, Natick, MA
\textsuperscript{b}Oak Ridge Institute of Science and Education, Oak Ridge, TN
\textsuperscript{c}Walter Reed Army Institute of Research, Silver Spring, MD

Objective: Sleep deprivation is one of multiple stressors that may underlie changes in gut microbiota composition and concomitant increases intestinal permeability previously reported during arduous military training. However, the effects of reduced sleep on gut microbiota composition and intestinal permeability are inconsistent across studies and not well characterized. This study aimed to determine the effects of severe, short-term sleep restriction on gut microbiota composition and intestinal permeability.

Methods: Crossover study wherein 19 healthy men (mean ± SD; BMI 24.4 ± 2.3 kg/m\textsuperscript{2}, 20 ± 2 yr) were assigned in random order to three consecutive nights of adequate sleep (AS; 7-9 hr sleep/night) and three consecutive nights of restricted sleep (SR; 2 hr sleep/night) with a ≥4 d washout between phases. Sleep restriction was induced by delaying bedtimes and slightly delaying wake times. Diet and physical activity were prescribed and controlled during both phases. Study outcomes assessed at the end of each phase included gut microbiota composition measured by 16S rRNA gene sequencing and small intestinal permeability measured by dual-sugar absorption test.

Results: Principal coordinates analysis of Bray-Curtis, UniFrac, and unweighted UniFrac distances revealed strong individual biases but no measurable shifts in community composition between AS and SR (PERMANOVA, P=1.0 for all). In contrast, \(\alpha\)-diversity measured by the Chao1 index was 21% lower during SR relative to AS (P=0.03) while Shannon and Simpson diversity indexes did not differ, suggesting that community richness was lower during SR but evenness was not affected. Between-condition differences in relative abundances of 32 ASVs were identified (Q<0.05). Differences included several ASVs assigned to taxa involved in metabolism of carbohydrates and production of beneficial short chain fatty acids, including multiple ASVs within the genus...
*Prevotella* and the family *Ruminococcaceae*, which were generally less abundant during SR relative to AS, and multiple ASVs within the genus *Clostridium* which demonstrated more variable responses. At the genus level, the relative abundance of *Bacillus* and an unclassified member of the family *F16* were lower during SR relative to AS, while the relative abundance of *Anaerostipes* was higher (Q<0.05). No between-condition differences in the relative abundances of any phyla or in intestinal permeability were detected.

**Conclusion:** Severe, short-term sleep restriction induced marginal, but potentially undesirable, changes in gut microbiota composition and did not increase small intestinal permeability. Sleep deprivation may therefore contribute to changes in gut microbiota composition previously reported during arduous military training, but neither sleep deprivation nor resulting changes in gut microbiota composition appear to impact intestinal permeability. Further work is needed to characterize the effects of more prolonged and/or less severe sleep restriction, especially in the context of circadian misalignment.

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Diana Brostow

Dietary habits and the gut microbiota in military Veterans: Results from the United States-Veteran Microbiome Project (US-VMP)

Diana Brostow, Dept of Veterans Affairs, Denver, CO

Dietary patterns influence gut microbiota composition. To date, there has not been an assessment of diet and gut microbiota in Veterans, who have a history of unique environmental exposures, including military deployment, that may influence associations between diet and gut microbiota. Our aim was to characterize Veteran habitual dietary intake and quality, and to evaluate correlations between diet and gut microbiota.

We administered food frequency questionnaires (FFQs) and collected stool samples from 330 Veterans. FFQ data were used to generate Healthy Eating Indices (HEI) of dietary quality. Exploratory factor analysis was used to identify two dietary patterns we defined as “Western” and “Prudent”. Stool samples underwent 16S rRNA gene sequencing, and the resulting data were used to evaluate associations with dietary variables/indices. Analyses included linear regression of α-diversity, constrained analysis of principal coordinates of β-diversity, and MaAsLin and ANCOM analyses of dietary factors and phylum- and genus-level taxa.

There were no significant associations between dietary patterns or factors and α-or β-diversity. At the phylum level, increasing HEI scores were inversely associated with relative abundance of Actinobacteria, and added sugar was inversely associated with abundance of Verrucomicrobia. Veterans largely consumed a Western-style diet, characterized by poor adherence to nutritional guidelines.
Characterizing combinatorial stress impacts impact on the human gut-brain axis

Giles, G.E.¹, J. Philip Karl², Kenneth Racicot¹, and Jason W. Soares¹

¹US Army Combat Capabilities Development Command (DEVCOM) Soldier Center; ²U.S. Army Research Institute of Environmental Medicine (USARIEM)

Warfighters commonly engage in complex, emotionally demanding, and physically challenging operations which degrade cognition, emotion, and behavior in complex, dynamic, and underspecified ways. An emerging scientific literature suggests a bidirectional relationship between gut microbiota and human brain function, that is the gut microbiota-gut-brain axis, indicating that gastrointestinal homeostasis influences emotion, motivation, and higher cognitive functions, and conversely, that ongoing psychological states (e.g. stress, anxiety) may alter gastrointestinal homeostasis.

However, the gut microbiota’s role in mediating stressor-induced changes in human cognitive performance is largely unexplored. Thus, our ongoing research aims to relate multiple levels of the gut microbiota-gut-brain axis response to stress. Toward that aim, two studies are underway.

First, in a cross-over study, volunteers undergo two days of continuous exposure to hypobaric hypoxia and normoxia following two weeks of consuming a prebiotic intervention or placebo. Gut microbiota composition and metabolic activity, intestinal permeability, cognitive function, emotion, and biomarkers of gut barrier damage, immunity and inflammation are assessed before and during hypobaric hypoxia and normoxia.

Second, in a double-blind, placebo-controlled, parallel arm study, volunteers consume probiotic or prebiotic supplements to increase gut Lactobacillus spp. and Bifidobacterium spp. populations. At the beginning and end of the intervention gut microbiota composition, gut microbiota metabolic activity, GI permeability, inflammation, HPA axis activity, mood, and cognitive performance are measured before, during and/or after acute physical and psychological stress.

Together the research will characterize the extent to which gut microbiota-targeted interventions reduce GI permeability, inflammation, HPA-axis reactivity, and cognitive decrements in response to military-relevant physical and psychological stress.

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Linda Chrisey

ReVector

Linda Chrisey, DARPA Biological Technologies Office, Arlington VA

Mosquitoes transmit pathogens that cause dengue, malaria, and other diseases that present significant risks to the readiness and resilience of military personnel, and public health more generally. The ReVector program aims to maintain the health of military personnel operating in disease-endemic regions by reducing attraction and feeding by mosquitoes.

Mosquitoes are attracted to the general area of humans by volatiles emitted in human breath. However, it is the heat and volatile molecules from human skin that direct mosquitoes to the specific sites on the body where they feed. Many of those volatile molecules are produced by the metabolism of organisms in the skin microbiome. Researchers on the ReVector program are working to develop precise, safe, and efficacious technologies to modulate the profile of skin-associated volatile molecules by changing the organisms that are present in the skin microbiome and/or their metabolic processes.

Although other approaches already exist to slow the spread of vector-borne disease (e.g., bed nets, chemical repellants, anti-malarial drugs), they each have logistical burdens or side effects that make them impractical for use during military deployments. For example, the requirements for frequent reapplication of repellants or repeat dosing of drugs often result in inconsistent protection. In contrast, the envisioned ReVector treatments could be applied just hours before a mission with minimal equipment or training, would produce no detectable odor, and would last for up to two weeks without reapplication, offering improved, sustained protection against disease vectors.
M. Tyler Nelson

Evaluating Function and Biocompatibility of Syn-Bio Constructs in a Human Gut-on-a-Chip

M. Tyler Nelson, 711th Human Performance Wing, Air Force Research Laboratory, Wright-Patterson AFB, OH 45433

The development of novel microbially-derived, live therapeutics is currently limited by available screening technologies. Synbiotics, a term to describe live bacterial therapeutics, have the potential to fill the gaps in clinical care, by meeting the unmet needs of pharmacologics. Proving the efficacy and translation potential of synbiotics requires robust human relevant test beds capturing both strain functionality and host tissue responses. Herein, we discuss the use of organ-on-a-chip technology to test and screen synbiotics in a human relevant platform. We were able to show that the gut-chip is able to simulate non-human primate and human responses of a well characterized strain for the treatment of phenylketonuria (PKU), as well as show efficacy of a novel sense and respond synbiotic to alter cognitive state. Taken together organ-on-a-chip technology is poised to aid in rapidly identifying high potential therapeutics and translating them for pre-clinical assessment.
Robyn A. Barbato

Using ecological theory to understand the survival of exogenous microorganisms in complex matrices


Synthetic biology hinges on the survival of the whole cell or cell-free system in nature such that it can perform its intended function before it dies. Currently, the design, build, test cycle of synthetic biology lacks emphasis on the test portion, particularly in nature. Soil systems have complex ecologies such that simply adding more of an organism to soil is often not effective. Though the soil system is dynamic and complex, the microorganisms within adhere to laws of nature. Here, we describe important features of soil systems and microbial trajectories following disturbance that could be used as guidelines when adding genetically modified microorganisms to complex matrices such as soil. We focus specifically on disturbance events which change the dynamics of the interactions within the soil community, offering an opportunity for an invading member to colonize. The outcome of aligning synthetic biology with microbial ecology will be the development of genetic constructs that have a higher propensity to survive in the environment.
Stephanie Servetas

Engineering Complex Whole Cell Microbial Reference Materials

Stephanie L. Servetas, Jennifer N. Dootz, Monique E. Hunter, Samuel P. Forry, Scott A. Jackson

National Institute of Standards and Technology, Biosystems and Biomaterials Division, Complex Microbial Systems Group, Gaithersburg, MD, USA

Microbiome analyses rely on complex workflows that include sample collection, sample storage, analyte extraction, analyte measurement (detection and/or quantification), data processing, data analysis, and reporting. As a result of the complex workflow and biases introduced at each step, comparison of data can be a challenge. While we are unlikely to find the perfect workflow to eliminate bias, developing standards that can help to characterize and understand the bias at each step will improve confidence in the results and comparability of the data. To this end, NIST is working to develop complex whole cell microbial mixtures to serve as reference materials for microbiome workflows. While not as complex as many real-world microbiome samples, these engineered consortia have the advantage of ground-truth. Working as the Independent Verification and Validation partner in DARPA's Friend or Foe program, NIST has developed a method to design, construct, and characterize homogeneous microbial mixtures. To-date we have generated mixtures containing up to 80 strains and characterized for composition and viability by metagenomics and culture. In addition to the development of microbial consortia, NIST now has a tunable method that can be used to target specific microbiomes, species, or traits (e.g. antimicrobial resistance) ensuring the material is fit-for-purpose. Furthermore, these mixtures can be added to different matrices such as soil or serum for added complexity. Since these mixtures contain live microbes, they can be used to evaluate metagenomic workflows such as microbiome characterization or pathogen detection but are also suitable for phenotypic assays including metabolomic analyses and cultivation. With the framework developed for designing complex microbial consortia, future work is focused on improving the characterization of the engineered communities by developing new microbial measurement tools using flow-cytometry, metabolomics, and additional molecular analyses.
Seid Muhie

Differential effect of classes of chemical pollutants on microbiota populations: a shotgun metagenomic study

Seid Muhie\textsuperscript{1,2}, Ross Campbell\textsuperscript{1}, Austin K. Baldwin\textsuperscript{3}, Erik Mylroie\textsuperscript{4}, Bintu Sowe\textsuperscript{1,5}, Steven R. Corsi\textsuperscript{6}, Aarti Gautam\textsuperscript{1}, Rasha Hammamieh\textsuperscript{1}, Edward Perkins\textsuperscript{4} and Natalia Vinas\textsuperscript{4}

\textsuperscript{1}Medical Readiness Systems Biology, CMPN, Walter Reed Army Instt. of Research, Silver Spring, MD
\textsuperscript{2} The Geneva Foundation, Tacoma, WA
\textsuperscript{3} U.S. Geological Survey, Idaho Water Science Center, Boise, Idaho
\textsuperscript{4} U.S. Army Engineer Research and Development Center Environmental Lab., Vicksburg, MS
\textsuperscript{5} Oak Ridge Institute of Science and Education, Oak Ridge, TN
\textsuperscript{6} U.S. Geological Survey, Idaho Water Science Center, Middleton, WI

Environmental pollutants are known to adversely affect the biodiversity of living organisms. Particularly, chemical pollutants tend to accumulate in the environment over time, distorting the natural balance of biodiversity of microbial populations. In this study, we collected sediment and water samples from eight sites within the Great Lakes (USA). Samples were characterized using shotgun metagenomic sequencing and chemical analysis for more than 200 chemicals belonging to ~16 broad classes of chemicals (pesticides, industrial products, personal care products and pharmaceuticals). We carried out integrative and differential comparative and correlation analyses on the bimodal datasets. Microbiota density (as approximated by adjusted total counts of sequence reads) decreased with an increased total concentration of chemical pollutants.

Protozoan, metazoan, and fungal populations were negatively correlated with concentrations of chemical pollutants whereas some bacterial (proteobacteria) and archaeal populations were positively correlated with increasing concentrations of chemical pollutants. As expected, concentrations of chemical pollutants from sediment samples were at much higher concentration and had larger dynamic range compared to concentrations of the same class of chemicals in water samples. Among other factors, differential concentration of pollutants may be the reason why we see that some bacterial, metazoan, and protozoan population showed detectable abundance only at certain sites or sample types (water or sediment). Our preliminary finding shows that microbial diversity could potentially be correlated with the type and concentration of chemical pollutants.

Dietary patterns influence gut microbiota composition. To date, there has not been an assessment of diet and gut microbiota in Veterans, who have a history of unique environmental exposures, including military deployment, that may influence associations between diet and gut microbiota. Our aim was to characterize Veteran habitual dietary intake and quality, and to evaluate correlations between diet and gut microbiota.
We administered food frequency questionnaires (FFQs) and collected stool samples from 330 Veterans. FFQ data were used to generate Healthy Eating Indices (HEI) of dietary quality. Exploratory factor analysis was used to identify two dietary patterns we defined as “Western” and “Prudent”. Stool samples underwent 16S rRNA gene sequencing, and the resulting data were used to evaluate associations with dietary variables/indices. Analyses included linear regression of α-diversity, constrained analysis of principal coordinates of β-diversity, and MaAsLin and ANCOM analyses of dietary factors and phylum- and genus-level taxa.

There were no significant associations between dietary patterns or factors and α-or β-diversity. At the phylum level, increasing HEI scores were inversely associated with relative abundance of Actinobacteria, and added sugar was inversely associated with abundance of Verrucomicrobia. Veterans largely consumed a Western-style diet, characterized by poor adherence to nutritional guidelines.
Differential composition and gene expression among microbiomes of military aircraft and vehicles potentially associated with variable biocorrosion and biodeterioration

Dominique N. Wagner$^{1,3}$, Vanessa A. Varaljay$^{1}$, Blake W. Stamps$^{2,3}$, Caitlin Bojanowski$^{1}$, Audra Crouch$^{1,3}$, Carrie Drake$^{1,3}$, Christopher Ecker$^{1,3}$, Bradley S. Stevenson$^{4}$, and Wendy J. Crookes-Goodson$^{1}$

$^1$Soft Matter Materials Branch, Materials and Manufacturing Directorate, Air Force Research Laboratory, Wright-Patterson AFB, Ohio; $^2$711th Human Performance Wing, Airman Systems Directorate, Air Force Research Laboratory, Wright-Patterson AFB, Ohio, $^3$UES, Inc., Dayton, Ohio, USA, $^4$Department of Microbiology and Plant Biology, University of Oklahoma, Norman, OK

How microbes interact with one another and their environment is a central, yet challenging question in biology. These interactions are especially relevant when microbial communities facilitate the degradation of polymers in surface coatings and insulation of 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 from military aircraft and 3 from military trucks.

A combined ultra-deep DNA and RNA sequencing-based approach was used to capture both the genetic capability and expression profiles of the aircraft and trucks sampled. Transcriptionally active microbiomes, quantitated based on small subunit ribosomal RNA expression data extracted from aircraft metatranscriptomes, were dominated by fungi while the trucks were dominated by bacteria. The data were then mined for hydrolase enzymes such as lipases, esterases, cutinases, peptidases, and proteases, known to be key players in polyurethane polymer degradation. Thousands of putative hydrolase enzymes were recovered for further verification and validation, with selected candidates to be expressed and tested for polymer-degrading activities in high-throughput in cell-free assays with near-infrared carbon nanotube probe hydrolytic detection. Yet, hundreds of these putative hydrolase sequences were unable to be annotated using classic methods such as BLAST.

We have implemented DeepGoPlus, a convolutional neural network machine learning approach to characterize novel hydrolases and investigate species-species and species-environment interactions among members of the microbial community through gene ontology biological
process analyses. Additionally, we are investigating differential expression among aircraft sampling locations and between aircraft and trucks to determine if hydrolase expression and other enzymes vary in their expression patterns among aircraft sampling locations and between aircraft and trucks. These data will ultimately be used for the assessment of the biodegradation potential in microbial communities on polyurethane polymer surfaces and for environmental enzyme bioprospecting for industrial and bioremediation purposes.
Chris Baker

Seasonal variation in microbial community depth profiles: implications for understanding nutrient movements


Climate warming is resulting in permafrost thaw and the deepening of the seasonally-thawed soil active layer. These changes are likely to be accompanied by changes to soil biogeochemistry and nutrient cycling, mediated by shifts in the permafrost microbiome, with implications including the potential for feedback to climate change. To date, however, research has largely focused on changes to the permafrost microbiome from spring snowmelt through summer, when cold regions are most accessible. In this study, we explore active layer, transition zone and permafrost bacterial and fungal communities from summer through fall, using 16S rRNA gene and ITS amplicon sequencing of samples collected from Imnavait Creek, Alaska in June, August and October 2019. Our preliminary sampling suggests changes in the depth profile of the microbial communities between these time points, potentially reflecting processes such as movement of pore water, or microbial dispersal. Our findings suggest that summer sampling alone may not generate a complete picture of the linkages between microbial communities and nutrient movements through groundwater, highlighting the importance of capturing seasonal variation by sampling throughout the year.
Elaine Merrill

In silico Modeling of a Gut-on-a-Chip: Optimizing Dosimetry of an engineered Bacterial and Predicting Individualized Microbiomic Outcome

Elaine Merrill¹, Peter Robinson¹,², M. Tyler Nelson¹, Mark Charbonneau³, Heidi Coia¹,⁴, Camilla Mauzy¹

¹Applied Biotechnology Branch, Airman Bioengineering Division, Airman Systems Directorate, 711th Human Performance Wing, Air Force Research Laboratory (AFRL), Wright-Patterson AFB, ²Henry M. Jackson Foundation for the Advancement of Medicine (HJF), ³Synlogic, Inc., ⁴National Research Council, National Academy of Sciences, Engineering and Medicine, Washington, DC

The human microbiome is incredibly complex and dynamic, with individual variation based on multiple parameters. As beneficial probiotics or novel engineered bacteria are developed for therapeutic or performance enhancement purposes, the potential impact(s) of commensal-based interventions cannot be experimentally evaluated for each unique permutation of host genetics, basal microbiome, stressor exposure, diet, etc. Therefore, there is a strong need for a quantitative understanding of key biological processes and driving principles to allow prediction of individual outcomes. In order to accomplish this, both in vitro and in vivo data are needed together with computational (in silico) models to interpret these data in terms of underlying mechanisms, including molecular signaling between bacterial species, as well as bacterial-host communication.

We have developed computational models of both the in vitro gut-on-chip microfluidic system and the in vivo system, with a focus on the function of E. coli Nissle (ECN) engineered to metabolize phenylalanine, as constructed by Synlogic, Inc., to use as a treatment for phenylketonuria. In vivo (mouse and primate) data were available to validate the in vitro gut microbiome microfluidics output. Our in silico model successfully predicts the uptake of Phe from the gut into systemic circulation and the impact of varying doses of the synthetic bacteria.

This in silico modeling approach provides analyses of in vitro to in vivo extrapolation (IVIVE) to leverage current understanding of underlying mechanisms to predict the performance enhancement effect of probiotic and synbio interventions tailored to specific individuals. AFRL 711 HPW is leading USAF efforts uniquely focused on engineering commensal bacteria that could be used as probiotics to enhance warfighter readiness. The results from this effort provide algorithms that will allow prediction of probiotic/engineered commensal behavior in more complex and variable in situ conditions within the human gut. This combinational approach of in silico and in vitro modeling will speed outcome analyses of probiotic and/or engineered commensals by limiting the need for extensive in vivo examination.
Disclaimer: The views presented here are those of the authors and do not represent those of the Department of Defense (DoD)

Key Words: Organ-on-chip, gut-on-chip in silico, extrapolation, probiotics, engineered bacteria, in vitro, in vivo, extrapolation, clinical
Towards the development of an ex vivo human colonic tissue model to study the pathogenesis of Shiga toxin (Stx)-producing Escherichia coli in the presence of gut flora

Gregory J. Weber¹, Sarah C. Pearce, Laurel A. Doherty², and Jason W. Soares²

¹Soldier Sustainment Directorate U.S. Army Combat Capabilities Development Command Soldier Center, Natick, MA
²Soldier Effectiveness Directorate, U.S. Army Combat Capabilities Development Command Soldier Center, Natick, MA

The host-microbe interaction is critical for intestinal homeostasis. By-products from microbial metabolism of unabsorbed dietary components have been studied increasingly as potential contributors to health and disease. In vitro fermentation systems provide a way to simulate microbial activity and by-product production of the colon using human fecal samples. Objectives of the study were to determine how clarified supernatants from two different fermentation conditions affect markers of cell proliferation, differentiation, barrier function, and immune function in a human induced pluripotent (iPSC) colon organoid model. Short-chain and branch-chain fatty acid concentrations of the supernatants were analyzed and were similar to known in vivo concentrations. Molecular results showed 25% of the clarified supernatant from batch fermentation led to a more physiological intestinal phenotype including increased markers of differentiation, including alkaline phosphatase, chromogranin-A, SCFA transport monocarboxylate transporter-1, (6.2-fold, 2.1-fold, 1.8-fold, respectively; P<0.05). Mucin production (mucin-2, mucin-4) was increased in cells treated with 25% supernatant, as observed by confocal microscopy. In addition, increased tight junction expression (claudin-3) was noted by immunofluorescence in 25% supernatant treated cells. A dose-response increase in barrier function was observed over the 72-hour time course, with a 2-fold increase in transepithelial electrical resistance (TER) in the 25% group compared to the control group (P<0.05). To further investigate host effects, clarified supernatants from a continuous multi-stage fermentation representing the ascending (AC), transverse (TC), and descending (DC) colonic domains were utilized and some regional differences were observed including increased markers of inflammation (IL-1ß, 6.15pg/mL; IL-6, 27.58pg/mL; TNFα, 4.49pg/mL; P < 0.05) in DC treated samples only. Overall, clarified supernatants represent a valuable model to examine effects of microbial by-products on host intestinal development and function and future efforts will be designed to further understand microbial communities and metabolites, along with additional host response measures.
**Rebecca Bova**

**Human iPSC Colonoid Function is Improved by Exposure to Fecal Fermentates**

Rebecca A. Bova¹,²,³, Theodore J. Picou²,³, Vincent B. Ho¹,², and Angela Melton-Celsa¹

¹Uniformed Services University, Bethesda, Maryland.
²4D Bio³ Center, Uniformed Services University, Bethesda, Maryland.

We established a human colonic tissue model that consists of human primary colonic epithelial cells, hAD-MSC-derived myofibroblasts, and human primary colonic microvascular endothelial cells with the ultimate goal of using the model to study enteric pathogens. Because the gut microbiota serves as a barrier against infection, we first tested the system with either a diarrheal pathogen, Shiga toxin (Stx)-producing *Escherichia coli* (STEC), or the purified Stx(s) in the absence of commensal flora. The most virulent STEC have the capacity to adhere to intestinal cells in an intimate, localized manner that requires the locus of enterocyte effacement (LEE). The LEE encodes a type-III secretion system that injects effectors and the bacterial adhesin receptor into the host cell. To cause severe disease, STEC must not only adhere, but also make Stx. There are two immunologically distinct Stxs: Stx1 and Stx2. These toxins have the capacity to enter the body from the intestine by a mechanism that is not well understood. Once the toxin reaches the kidney, renal damage and renal failure may occur.

For our preliminary studies, we exposed polarized human primary intestinal epithelial cells to STEC strains and found that the transepithelial electrical resistance (TEER) was greatly reduced by 24 hr post-exposure. Unexpectedly, measurable toxin was only present on the basolateral side of the transwells when the infecting strain had the LEE. We next exposed the polarized primary intestinal cells to purified Stxs and measured translocation. We found that the Stxs translocated across the monolayer when toxin was applied to either the apical or the basolateral side of the epithelial cells, without a disruption in the TEER across the monolayer. After we found that the primary epithelial cells could translocate the toxins, we challenged the human intestinal tissue model with the Stxs. We found effective translocation of both Stx1 and Stx2 across the multi-layer tissue model. We are currently testing co-infection of STEC with known commensal *E. coli* strains. Successful deployment of this model will allow for the study of pathogenesis in an *ex vivo* system similar to the human colon.
Using Cloud Based Jupyter Notebooks to Effectively Analyze and Share Microbiome Data

Maj. Preston Dihle, Department of Chemistry and Life Science, USMA, West Point, NY

A common issue faced by microbiome researchers are the challenges surrounding effectively analyzing and sharing the vast amounts of data generated by their experiments. A glaring omission in most microbiome papers is a detailed discussion of the computational analysis that led to their conclusions. Within the DOD this problem is compounded by lack of access to appropriate computational tools. Often, researchers within the DOD do not have ready access to the Unix/Linux machines required to run microbiome analysis or if those machines are available, they are prohibited from accessing the network. One solution to this problem is the use of cloud based Jupyter Notebooks. Cloud based notebooks allow researchers to conduct analysis in multiple programing languages including command line languages, allowing users to use Linux based programs in their analysis. Additionally, using notebooks allows researchers to share their full analysis pipeline with collaborators, reviewers, and journals. Herein, I present the initial steps necessary to implement cloud based Jupyter Notebooks in your microbiome analysis.
Laurel Doherty

An in vitro fermentation model of the human lower gastrointestinal tract microbiomeing

Laurel A. Doherty, U.S. Army DEVCOM Soldier Center, Natick, MA

The gut microbiome is a key modulator of human health, metabolism, and immune function. Extensive study on the role of the large intestine microbiome has taken place; however, detailed characterization of the small intestine with regard to impact of the resident microbiome is limited due to the relative inaccessibility of the organ. Here, we present our efforts to utilize in vitro fermentation as a modelling tool to simulate the microbial ecosystem of the human lower gastrointestinal tract by augmenting our current large intestine fermentation model with a small intestine component. Initial efforts consisted of development of a single-stage model of the ileum with resident microbiome, simulated via selection a consortium of organisms was selected to represent major phyla, functions and competitive growth dynamics representing “fed-state” ileum conditions. Passive nutrient absorption was simulated using hollow-fiber columns and optimized using small molecules to mimic dietary digestion byproducts. More recent efforts to integrate the small intestine model with the existing large intestine model will also be discussed. The completed model represents the microbial ecology of the entire lower gastrointestinal tract and will enable better characterization of the relationship between microbiome and dietary inputs. Insight gleaned from this model, alone or in concert with in vivo studies, can inform nutritional strategies to restore and maintain microbiome homeostasis.
Isabel Smokelin

MIT-Lincoln Laboratory Artificial Gut (ArtGut) System

Isabel Smokelin, MIT Lincoln Laboratory, Lexington MA

No-fail missions executed by small teams of Special Operations Forces (SOF) require all members to be at peak performance throughout mission lifetime. Continuous, low-level chemical, biological, radiological, nuclear and explosive (CBRNE) exposures (e.g. organophosphate nerve agents) are a significant threat to these missions as they are difficult to detect and host symptoms may not be obvious but are still life threatening (e.g. tiredness). To prevent decrements in performance due to these CBRNE exposure threats, we propose monitoring the sensitive and dynamic gut microbiome for early-warning diagnostic biomarkers. MIT-Lincoln Laboratory has previously developed the Artificial Gut (ArtGut) system that uniquely replicates the gut environment via an oxygen gradient which allows co-culture of facultative and strict anaerobic bacteria in a high-throughput fashion. In collaboration with Massachusetts General Hospital (MGH), we intent to replicate low-level organophosphate nerve agent exposure to human gut microbiome cultures in the ArtGut system and then use MGH’s specialty bioinformatic tools to identify microbial feature (e.g. composition, metabolism) diagnostic biomarkers. In later years of the program, we then aim to further investigate these biomarkers as potential therapeutic targets for both exogenous and endogenously engineered countermeasures. The overarching goal of our program is to develop a resilient modulatory microbiome that enables warfighters to maintain peak health and performance even when exposed to low-level CBRNE threats.
Aria McLauchlan

Soil Health in Agricultural Systems

Aria McLauchlan, Land Core, Grass Valley, CA

Soil health in agricultural systems is a proxy for soil biology and a soil microbiome that is healthy and results in fully functioning ecosystems and landscapes. Soil health is significantly impacted by agricultural management practices, which in turn are influenced by agricultural policy. This talk provides an overview of the political landscape of soil health, and opportunities in the federal policy sphere to advance soil health and protect the soil microbiome. While I don’t provide an in-depth overview of soil science, I cover several key means by which building soil health increases soil microbiome and ecosystem function, including by restoring biodiversity, increasing water infiltration and holding capacity, improving water quality, supporting crop resilience, and sequestering carbon. Making the resulting benefits - in terms of agricultural productivity, resilience and profitability - attainable for producers is a key goal of Land Core's federal policy work. In advancing these opportunities, Land Core does not focus on the regulatory aspects but rather on legislative and agency-level policies and actions that can advance our understanding of soil health and make its rapid adoption and scalability possible in American agriculture. It is worth noting that soil health policies that avoid new regulations and focus instead on voluntary initiatives and incentives tend to be well received by both farmers and policymakers. Major policy opportunities covered include the infrastructure and reconciliation packages, the upcoming 2023 Farm Bill, other key pieces of legislation, and new initiatives at USDA. Learn more at landcore.org.
Richard Murray

Preparing for Future Products of Biotechnology

Richard M. Murray, Control & Dynamical Systems and Bioengineering, California Institute of Technology, Pasadena, CA 91125 USA

With continuing advances in genome editing and synthetic biology, the number and types of products available to consumers through use of biotechnology will likely increase substantially in the next decade. A committee convened by the National Academies of Sciences, Engineering, and Medicine explored what these products may be in its 2017 report, Preparing for Future Products of Biotechnology. The committee identified plant, animal, microbial, and synthetic biotechnology products designed for nonmedical uses likely to be on the market in the next five to ten years. Some products are likely to use genome-editing techniques like CRISPR for familiar applications, such as modifying agricultural crops. Other future products are expected to be entirely new: plants that serve as sentinels of environmental contamination, for example. The committee also identified advances needed to adequately assess risks of products that are more complex and less familiar than those currently available, particularly for engineered microbes, plants, and insects designed to thrive in the environment with no human management.
Scott Jackson

Measurement Assurance for Innovation in Microbiome Science

Scott Jackson, Complex Microbial Systems Group, NIST, Gaithersburg MD

Appreciation for the role of microbes in our lives has been growing rapidly, but the measurement science needed to understand and fully exploit microbial systems has developed at a much slower pace. In all applications involving complex microbial communities, the research is hampered by the lack of standards, protocols, and technical infrastructure to allow confidence in the data and comparability. At NIST, we are developing the tools necessary to enable measurement assurance of complex microbial systems for applications in clinical diagnostics, biothreat detection, agriculture, and the environment.
Dynamics of gut microbiome and resistome changes during international travel and travelers’ diarrhea

Manish Boolchandani¹,², Kevin S. Blake¹,², Drake H. Tilley³, Miguel M. Cabada³,⁴, Drew J. Schwartz¹,²,⁵, Sanket Patel¹,², María Luisa Morales⁶, Rina Meza³, Giselle Soto³, Sandra D. Isidean⁷,⁸, Chad K. Porter⁷, Mark P. Simons³,⁶,⁷#, Gautam Dantas¹,²,⁹,¹⁰

¹ The Edison Family Center for Genome Sciences and Systems Biology, Washington University School of Medicine, St. Louis, MO, USA
² Department of Pathology and Immunology, Washington University School of Medicine, St. Louis, MO, USA
³ Naval Medical Research Unit No. 6, Callao, Lima, Peru
⁴ Department of Internal Medicine, Division of Infectious Diseases, University of Texas Medical Branch, Galveston, TX, USA
⁵ Department of Pediatrics, Division of Infectious Diseases, Washington University School of Medicine, St. Louis, MO, USA
⁶ Cusco Branch – Tropical Medicine Institute, Universidad Peruana Cayetano Heredia, Lima, Peru
⁷ Naval Medical Research Center, Silver Spring, MD, USA
⁸ Henry M. Jackson Foundation for the Advancement of Military Medicine, Bethesda, MD, USA
⁹ Department of Molecular Microbiology, Washington University School of Medicine, St. Louis, MO, USA
¹⁰ Department of Biomedical Engineering, Washington University in St. Louis, St. Louis, MO, USA

Travelers’ diarrhea is exceedingly common among international travelers and deployed military personnel, resulting in significant operational costs and leading to persistent gastrointestinal disturbance post-travel. Furthermore, travelers’ diarrhea exacerbates the risk of acquiring multidrug-resistant organisms, which can then be spread globally upon return home. Little is known about the impact of diarrhea on travelers’ gut microbiomes and resistomes, and the dynamics of these changes throughout travel and during specific diarrheal episodes. Here, we assembled a cohort of 159 international students visiting the Andean city of Cusco, Peru and applied next-generation sequencing techniques to 718 longitudinally-collected stool samples. We found that gut microbiome composition changed significantly throughout travel, but taxonomic diversity remained stable. However, diarrhea disrupted this stability and resulted in an increased abundance of antimicrobial resistance genes that can remain high for weeks. We also identified taxa differentially abundant between diarrheal and non-diarrheal samples, which were used to develop a classification model that distinguishes between these disease states with high accuracy (84.2%) and precision-recall (89.4%). Additionally, we sequenced the genomes of 212 diarrheagenic Escherichia coli isolates, and found those from travelers who experienced diarrhea
encoded more antimicrobial resistance genes than those from travelers who did not. In summary, we find the gut microbiomes of international travelers to be resilient to dysbiosis; however, they are susceptible to colonization by multidrug-resistant bacteria, a risk that is more pronounced in travelers with diarrhea.
Stacy-Ann Miller

Time sensitive influences of sub-lethal radiation on fecal microbiome

Stacy-Ann Miller¹⁴, Nabarun Chakraborty¹, Ross Campbell², Neel Sharma³, Vidya P. Kumar³, Gregory Holmes-Hampton³, Candace Moyler⁴, Aarti Gautam¹, Rasha Hammamieh¹, Sanchita P. Ghosh¹

¹Medical Readiness Systems Biology, CMPN, Walter Reed Army Institute of Research, Silver Spring, MD
²Geneva Foundation, Medical Readiness Systems Biology, CMPN, Walter Reed Army Institute of Research, Silver Spring, MD
³Armed Forces Radiobiology Research Institute, Uniformed Services University of the Health Sciences (USUHS), Bethesda, Maryland 20889-5603, United States
⁴ORISE, Oak Ridge Institute of Science and Education, Oak Ridge, TN 37830

Radiation exposure even with low dose can cause chronic illness with various clinical symptoms that are typically referred as acute radiation syndrome (ARS). ARS manifests different symptoms depending on the exposure dose resulting in a high degree of mortality, making it imperative to understand the ARS-induced changes in the biological processes.

Our recent study using mouse radiation model showed an early elevation of host inflammatory networks caused by ARS. Since, the gut microbial community plays a key role in mediating the host’s immunological fitness, we now focus on inspecting the fecal microbiota composition and its potential relationship with host in the context of ARS. Our hypothesis is that ARS negatively influences the host-microbiome relationship. Therefore, identifying an strategic intervention to enrich the host-microbiome relationship can help in mitigating ARS.

To meet this objective, twelve to fourteen weeks old mice were irradiated at a dose rate of ~0.6 Gy/min to total midline doses of 9.5 Gy and 11 Gy, respectively. Fecal samples were collected before irradiation (baseline control), and day 1, day 3 and day 9 post irradiation. For metagenomics assay, DNA was extracted from the snap frozen fecal samples and V3-V4 hyper-variable regions of the 16s ribosomal DNA were amplified. The quantified libraries were sequenced on MiSeq platform (Illumina, Inc.); the operational taxonomic units (OTUs) was assigned by clustering sequence reads at 97% similarity and the most abundant sequences with a minimum sequence length of 150 bp was aligned to determine the taxonomic profile. Another aliquot of same fecal samples were screened for untargeted metabolomics profiling by a Q-TOF Premier mass spectrometer and significantly different peaks were annotated.

Post-TBI temporal delay irrespective of radiation dose emerged as the primary factor explaining
the alpha and beta diversity of the fecal microbiota. Probing the abundances of individual taxa found Firmicutes significantly varied due to both radiation doses and time. The ratio of Bacteroidates and Firmicutes (B/F) emerged significantly susceptible to the interaction of radiation doses and post-TBI temporal delay. The metabolite profile also showed a dominant temporal bias. Functional analysis predicted a panel of metabolites potentially secreted from fecal microbiota and subsequent data dimension reduction approach identified how the radiation dose regulated the functional relationship between host and resident microbiota. Together, our result showed a chronic impact of sub-lethal radiation dose on fecal microbiome. Strategic intervention on microbial composition might help the host to combat ARS.
Christopher Stamper

Characterization of the gut microbiota among Veterans with unique military-related exposures and high prevalence of chronic health conditions: A United States-Veteran Microbiome Project (US-VMP) Study

Christopher E. Stamper1,2,3, Maggie A. Stanislawski4, Kelly A. Stearns-Yoder1,2,3, Andrew J. Hoisington1,2,5,6, Diana P. Brostow1,2,3, Jeri E. Forster1,3, Teodor T. Postolache1,2,7,8, Christopher A. Lowry1,2,3,9,10,11, Lisa A. Brenner1,2,3,12

1Rocky Mountain Mental Illness Research Education and Clinical Center (MIRECC), Rocky Mountain Regional VA Medical Center (RMRVAMC), Aurora, CO, USA
2Military and Veteran Microbiome: Consortium for Research and Education, Aurora, CO, USA
3Department of Physical Medicine & Rehabilitation, University of Colorado Anschutz Medical Campus, Aurora, CO, USA
4Division of Biomedical Informatics and Personalized Medicine, School of Medicine, University of Colorado Anschutz Medical Campus, Aurora, CO, USA
5Tyndall AFB Reconstruction Project Management Office, Tyndall AFB, FL, USA
6Department of Systems Engineering & Management, Air Force Institute of Technology, Wright-Patterson AFB, OH, USA
7Mood and Anxiety Program, University of Maryland School of Medicine, Baltimore, MD, USA
8VISN 5 MIRECC, Department of Veterans Affairs, Baltimore, MD, USA
9Department of Integrative Physiology, University of Colorado Boulder, Boulder, CO, USA
10Center for Neuroscience, University of Colorado Boulder, Boulder, CO, USA
11Center for Neuroscience, University of Colorado Anschutz Medical Campus, Aurora, CO, USA
12Departments of Psychiatry and Neurology, University of Colorado Anschutz Medical Campus, Aurora, CO, USA

The gut microbiome is impacted by environmental exposures and has been implicated in many health conditions. United States (US) military Veterans are a unique population in that their military-related exposures can have consequences for both physical and mental health. Nevertheless, the gut microbiome of this population has been understudied. In this study, we describe the exposures, health conditions, and medication use of Veterans in the US-Veteran Microbiome Project (US-VMP), and examine the associations between these characteristics and the gut microbiota. This cohort included 331 US Veterans seeking care in the Veterans Health Administration that were 83% male with an average age of 47.6 ± 13.4 years. The cohort displayed a high prevalence of posttraumatic stress disorder (49.8%), traumatic brain injuries (76.1%), multiple chronic diseases, and high use of prescription medications (74.9%). We report significant associations between the gut microbiota composition and gastroenteritis, peripheral vascular disease (PVD), bipolar disorders, beta-blockers, serotonin and norepinephrine reuptake
inhibitors (SNRI) antidepressants, diabetes medications, opioids, and proton pump inhibitors. Many of the Veteran characteristics examined were also associated with shifts in specific taxa. We also found that PVD and cardiovascular disease (CVD) were associated with lower gut microbiota α-diversity, while vitamin use was associated with higher α-diversity. Our study contributes novel insights into how the unique exposures of Veterans correlate with the gut microbiome and, in line with previous findings with other population-level studies of the microbiome, confirms that medication use has important effects that should be considered in studies of the human gut microbiome.
Investigating the Structure of Cold Microbiomes: The Chesapeake Bay in Winter and Arctic North Slope in Summer

Caitlyn J. Koo¹*, Logan M. Treaster¹*, Amanda J. Barker², Thomas A. Douglas², Joseph P. Smith³, Sophie M. Colston⁴, Shawn G. Gallaher³, Charles R. Sweet¹, †

*these authors contributed equally to this work
† to whom correspondence should be addressed: sweet@usna.edu

1 – U.S. Naval Academy (USNA) Chemistry Department, Annapolis, MD 21402, 2 – U.S. Army Corps of Engineers (USACE) Engineering Research & Development Center (ERDC) Cold Regions Research & Engineering Laboratory (CRREL), Fort Wainwright, AK 99703, 3 – U.S. Naval Academy (USNA) Oceanography Department, Annapolis, MD 21402, 4 – Center for BioMolecular Science & Engineering, US Naval Research Laboratory, Washington, DC, 20375

Background: The bacterial community of Arctic rivers (the planktonic microbiome) and of temperate rivers during winter is of interest to ecologists and microbiologists as unique collections of organisms adapted to psychrophilic (cold-growth) conditions.

Methods: We characterized these microbiomes by both culture-based and DNA extraction water sampling, identification, and taxonomic analysis in both the Kuparuk River and Sagavanirktok River of North Slope Borough (AK) in summer 2019 and the Chesapeake Bay main stem and Severn River (MD) in winter of 2020.

Objectives: 16S identification of cultured bacteria and shotgun metagenomic characterization of the whole microbial community were used to assess two hypotheses: 1.) That there is commonality in microbiome structure and composition between the summer arctic river watersheds and the winter Chesapeake/Severn River and 2.) That the headwaters-origin hypothesis of river microbial communities is representative of the microbiome structure in arctic riverine watersheds.

Results: Our preliminary data do not support the commonality hypothesis between the arctic summer and Chesapeake winter microbiomes, and suggest that the headwater-origin hypothesis for river microbial populations discussed in other studies only partially explains development and structure of the arctic riverine microbiome. The analysis presented here shows differences in taxonomic distribution by location independent of headwater origin, and potential correlation to the hydrology and/or geochemistry of the arctic watersheds. We are working to establish
sampling and analysis programs for vertical year-over-year characterization of these microbiomes as well as further horizontal comparison between these watersheds.

This research was supported by SERDP (USNA Polar Science and Technology Program Alaskan North Slope Material Flux Study grant to SG), DTRA (CBT Service Academy Research Initiative grant to CS), and the Office of Naval Research (ONR).
Rhizosphere community structure is dependent on plant type and differs under altered snowpack conditions

Stacey Doherty¹, Alison Thurston¹, Chris Baker¹, Robert Jones¹, Ryan Busby², and Robyn Barbato¹

¹Engineer Research Development Center, Cold Regions Research and Engineering Laboratory, Hanover, NH
²Engineer Research Development Center, Construction Engineering Research Laboratory, Champaign, IL

Army operations rely on accurate predictions of the battlespace terrain, particularly under future climate scenarios. Climate change in Arctic regions is resulting in less precipitation, reduced snowpack, and earlier spring snowmelt. Snow cover insulates soils and, in its absence, soils undergo more frequent freeze-thaw cycles. Both temperature and plant composition affect microbial dynamics in soils, however the impact of loss of snowpack on plant-microbe interactions is largely unknown. We conducted a laboratory microcosm study using a sub-Arctic soil to investigate the influence of simulated snowpack conditions on bulk and rhizosphere soils under three plant types. We found a strong relationship between simulated snowpack treatments and plant type in structuring prokaryotic rhizosphere communities. Specifically, prokaryotic rhizosphere community structure varied for the same plant type depending on whether it experienced constant cold temperature (i.e., insulated by snowpack) or freeze-thaw conditions (i.e., no snowpack), indicating that changing climate conditions could influence primary producers in sub-Arctic soils. In contrast, fungal community structure was most strongly influenced by plant type, supporting findings that fungi are less susceptible to freeze-thaw cycles compared to bacteria. Our results highlight the intimate relationship between plants and microbes under future climate scenarios. Dominant microbial community structure will be an important consideration when forecasting plant dynamics with altered snowpack conditions.
Biodegradation of Per- and Polyfluoroalkyl Substances (PFAS) utilizing Delftia acidovorans Enzymes

Megan Doherty, Alex Perminov, Ryann Ramey, Nikolas Schwendeman, Marie Gross, David Hatfield, Owen O'Connor, Gary Kedziora, Hao-Bo Guo, Pat Dennis, Nina Lombardo, Eric Harper, Sanaz Farajollahi, Rajiv Berry, John Sitko, J. Jordan Steel, Erin Almand, Chia Hung, Vanessa Varaljay, Nancy Kelley-Loughnane

Per- and polyfluoroalkyl substances (PFAS) have recently gained substantial attention due to their threat toward environmental and human health. More than 4,000 PFAS chemicals have been manufactured for water repellency, heat resistance, and high durability needs. In addition to firefighting foams, PFAS have been incorporated into textiles, electroplating, ammunition, climbing ropes, artificial turf, and more. Due to their common use, PFAS discharge from manufacturing facilities travels through runoff and seeps into ground water, resulting in human consumption via drinking water and seafood. PFAS exposure has been associated with a myriad of human health issues, including liver disease, kidney disease, insulin, and lipid dysregulation, altered immune and thyroid function, reduced infant birth weight, and cancer. The prevalence of these compounds means there are many highly PFAS-contaminated sites around the world. We sought to identify microorganisms capable of surviving in high PFAS contamination areas and assess the ability of isolates to metabolize or breakdown these harmful chemicals. Our research isolated the aerobic bacterial species Delftia acidovorans in a PFAS-contaminated soil sample that proved capable of growth despite months of high PFAS exposure. Sequencing and analysis of the D. acidovorans genome identified several genes that encode potential dehalogenases, Dehalogenase 1-5 (DeHa 1-5). Protein modeling and molecular dynamics simulations are currently ongoing to determine enzymatic active sites and substrate binding. The five dehalogenases genes were cloned individually into E. coli expression vectors, and the enzymes were expressed and purified. Assays of these purified enzymes revealed monodefluorination activity for DeHa 2 and 4, as determined by release of fluoride ions. By contrast, preliminary data suggests DeHa 1 and DeHa 5 may prove capable of degrading perfluorooctanoic acid (PFOA). Our data indicate that these enzymes may have the ability to partially break down PFAS chemicals. These D. acidovorans enzymes could be part of a scalable PFAS biodegradation solution that would provide an alternative to current expensive and inefficient treatment protocols. This enzymatic bioremediation method may contribute to the degradation of PFAS and decrease the negative health effects experienced by communities with PFAS-contaminated drinking water.
Robert Jones

A Tale of Soil and Volts: Soil Microbial Fuel Cells as a Method of Contaminant Detection

Robert M Jones¹, Michael J. Musty¹, Scott Michael Slone¹, Molly Creagar², Robyn A. Barbato¹

¹US Army Corps of Engineers Engineering Research and Development Center Cold Regions Research and Engineering Laboratory
²University of Nebraska Lincoln

Microbial fuel cells (MFCs) are contained reactors that utilize the metabolism of electrogenic microbes to produce electrical current. Electrogenic microbes are so called because they have specialized mechanisms and structures, such as protein nanowires, that allow them to transfer electrons produced during the redox reactions of various substrates onto conductive surfaces thus creating the gradient of charge necessary to produce current. MFCs can be constructed using a variety of media and substrates that influence the performance and reactions; for example, MFCs are commonly made using wastewater as a media and produce energy from the breakdown of the wastewater components while also naturally processing the wastewater effluent. While renewable energy is of great interest in the future of MFCs, there is also the potential for the MFCs to function as living sensors. Soil based MFCs have particular utility as they can be embedded in the terrain and exposed to a variety of environmental perturbations. Because the activity of the electrogenic microbes (voltage) is a function of their environment, we hypothesize that they may respond in a sensitive and characteristic manner to environmental disturbances, such as the contamination by exogenous material, in a way that can be modelled and serve as a diagnostic. In our preliminary study we created a simulated acute contamination event by exposing soil based MFCs to a high concentration of urea after they had achieved a steady state. We monitored the voltage output continuously throughout the study to track how the voltage changed in response to the influx of urea and the change in environment (decrease in pH). Our data suggests that while there is an inherent voltage resiliency to change in the MFC, the contaminant does result in a new voltage level within a week of exposure that could be characteristic of the contaminant type. While this will require further study, there are great implications for the utility of soil based MFCs as sensors.
Eamon McHugh

Per- and Polyfluorinated Alkyl Substances alter fungal morphologies in Penicillium sp. and Fusarium sp.

Eamon McHugh, Nikolas Schwendeman, Andrii Gryganskyi, Chia Hung, Nancy Kelley-Loughnane, Vanessa Varaljay, J. Jordan Steel, Erin Almand

Poly-and perfluorinated alkyl substances (PFAS) have been used in Aqueous Film-Forming Foams (AFFFs) to quench fires starting in the 1960's and were still used around the world until recent regulation in 2016 when the CDC issued a health advisory on PFAS. PFAS are highly thermodynamically stable compounds and do not currently have an effective degradation solution. Due to the widespread use of PFAS containing products, 1,244 sites in the United States have been flagged for the contaminant. Recent studies and investigations have shown increased human health risks in correlation to PFAS exposure such as increased cholesterol levels, decreased vaccine response in children, changes in liver enzymes, increased risk of high blood pressure or pre-eclampsia in pregnant women, small decreases in infant birth weights, and increased risk of kidney or testicular cancer. While current investigations focus on the adverse effect of PFAS on humans, this study analyzed the response of Penicillium sp. and Fusarium sp. to varying PFAS contaminated environments. Both of these fungal genera are found across the world and have a significant effect on the ecosystems in which they are present. Penicillium participate in organic matter decomposition, nutrient cycling, pathogen protection for plants, and symbiosis with roots of crop plants. Fusarium are known for their impact on a variety of subjects including ecosystems, agriculture, food production, biotechnology, and human and animal health. Our results show that fungal morphologies were significantly altered when exposed to PFAS contamination. Mycelial pellet size and pigment intensity are the main visible morphological changes in liquid culture for Penicillium and Fusarium respectively. Fungal morphology and phenotypes are critical for the life cycle of the fungus and play a large role in the overall function of the health in the surrounding microbial communities. This study demonstrates that while the effects PFAS has on humans are seriously dangerous, the effect they have on our ecosystems pose an equally critical threat.
Jordan Zambrana

The Contribution of Biological Particulate Matter to Indoor PM: EPA Literature Survey

Jordan Zambrana¹, Nicole K. Scharko², Daniel Malashock¹ and Vito Ilacqua¹

¹U.S. Environmental Protection Agency (EPA), Office of Radiation and Indoor Air, Indoor Environments Division
²American Association for the Advancement of Science (AAAS) STP Fellow at U.S. EPA, Office of Radiation and Indoor Air, Indoor Environments Division

The health effects of exposure to particulate matter (PM) are well established and supported by decades of research (1). Most of the exposure to PM typically happens indoors (2,3) particularly in residential settings, where people spend most of their time (4,5). Yet, no comprehensive record of PM levels indoors exists that is comparable to that of PM of outdoor origin. Understanding typical indoor levels of PM is particularly important given that chronic exposure to high levels of PM may increase vulnerability to respiratory disease (7,8). Moreover, an important source of indoor PM is from biological materials. Biological PM has been shown in several studies to contribute between 5% and 34% of indoor pollution, making it a potential serious contributor to the indoor air quality issues caused by PM in general (6). Biological PM includes larger particles such as allergens from pet dander and pests, and smaller particles originating from fungi, bacteria, and viruses, including endotoxins and mycotoxins. PM settled as house dust may also serve as a medium for microbial growth indoors. While studies of indoor PM levels don’t often differentiate PM of biological origin from other sources, a survey of measured indoor PM levels would offer insight into what is typically encountered overall. EPA recently completed a literature survey of indoor PM measurements from 1990-2019 in residential spaces worldwide and a new comprehensive database of literature on indoor PM measurements was established. The search results of the literature survey will be used to identify the number of articles reporting biological PM. The findings of this study could provide insights into how substantial the literature is regarding residential biological PM indoors, and it could help guide inquiries regarding future research activities and needs.

References


Logan Gonzalez

Characterizing Organisms from Low-Temperature Environments for Biotechnological Applications

Logan Gonzalez, Alison Thurston, Flora Cullen, Elizabeth Corriveau, Robyn Barbato

Psychrophilic (cold-loving) and psychrotrophic (cold-tolerant) microorganisms provide exciting opportunities for developing biotechnological tools specialized for low-temperature function. Currently, the number of microorganisms adapted to cold environments that have been identified as good candidates for biotechnology remains relatively small, limiting the breadth of low-temperature applications. Our goal is to mine the unique microbial diversity found in cold environments such as Alaskan permafrost and Greenland ice sheets for developing new biotechnological platforms. We will discuss the organisms we have isolated from various cold regions as well as methods for screening our catalog of isolates to identify those best suited for low-temperature biotechnology.