Task Book Report Generated on: 05/08/2024

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Program/Discipline- Element/Subdiscipline- Subdiscipline-	Division Name:	Space Biology			
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Human Research Program None					
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Enterococci are gram-positive bacteria that originated when our ancient animal ancestors emerged from the oceans to live on land, and brought their gut flora with them. Enterococcus faecalis (EF) and Enterococcus faecium are common human commensals and can harbor multidrug resistance. Both have been previously isolated on the International Space Station (ISS). Likely as a consequence of their evolutionary origins, enterococci show remarkable stress resistance within, but also outside, their human hosts. Their antibiotic resistance, coupled with tolerance to desiccation, starvation, and disinfection, make some EF strains potent pathogens in the built environment (e.g., hospitals), and a potential risk to crew health during space missions. The proposed study includes flight components to:

- 1) Characterize the frequency and genomic identity of antibiotic resistant organisms, including enterococci, on the ISS;
- 2) Assess the evolutionary selective pressure of the space environment (microgravity, space radiation) using EF as a model system;
- 3) Characterize the "natural" evolutionary history of EF on Earth and in space to reveal mechanisms of microbial adaption including natural selection.

Task Description:

The CS-05A: Genomic Enumeration of Antibiotic Resistance in Space (GEARS) payload is designed to fulfill specific aim 1, the characterization of the frequency and genomic identity of antibiotic resistant organisms on the ISS. The Co-Principal Investigators propose to carry out longitudinal sampling of ISS surfaces in a repeated measures design.

The CS-05B: Enterococcus Growth Advantage on ISS via Tn-seq (EnteroGAIT) payload is designed to fulfill specific aim 2: to assess the evolutionary selective pressure of the space environment. The flight experiment will utilize on-board long-duration microbial growth to measure the selective pressure of the space environment on a defined microbial population: Enterococcus faecalis mutants are created by transposon insertional mutagenesis; selection is measured by sequencing (Tn-Seq) and occurs on timescales far shorter than natural or experimental evolution.

The third study, Adaptation & Evolution of Resilient Enterococcus in Space (AERES) will generate complete whole genomes of Enterococcus isolates from the ISS and ground, combined with follow up characterization, to seek evidence of persistence and/or evolution in the space environment.

Rationale for HRP Directed Research:

Research Impact/Earth Benefits:

Antibiotic resistance is a growing threat to human health on Earth, resulting in infections in 2.8 million people, and causing 35,000 deaths annually (CDC data). Overuse or improper use of antibiotics is also contributing to this growing threat. Bacteria are evolving in response to the usage of antibiotics: for example, some strains of Staphylococcus aureus have acquired resistance to vancomycin from Enterococcus. Staphylococci and enterococci are the first and second leading causes of hospital-acquired infections, respectively. By studying the distribution of antibiotic resistant microbes on the International Space Station (ISS), a built environment similar in some ways to hospitals, we can also gain insight into how antibiotic resistant organisms survive, adapt, and evolve in response to their environment. Thus, this study will result in data that could also be relevant to human health on Earth.

This award started on 11/4/2020. Progress to date has largely focused on project tasks required to support flight definition for CS05A Genomic Enumeration of Antibiotic Resistance in Space (GEARS) and CS05B Enterococcus Growth Advantage on ISS via Tn-seq (EnteroGAIT), including completion of Investigative Summary Forms (ISFs) for both projects and the Science Requirements Document (SRD) for CS05B. This SRD was used by NASA to solicit proposals from implementation partners to support the CS05B investigation. Proposals are currently under review.

A formal project kickoff was conducted on March 1, 2021, involving a NASA-PI interchange meeting with a seminar given by PI Carr. Carr is a new faculty member at the Georgia Institute of Technology (Georgia Tech) and so initial project activities involved lab set up for microbiology, including incubator in biosafety cabinet (for later BSL-2 Entercoccus cultures), smoke testing, certification, PPE (personal protective equipment) provisioning. Jordan McKaig, a graduate student in the Georgia Tech School of Earth and Atmospheric Sciences, joined the project. We also re-established our nanopore sequencing pipeline in the new lab (hardware, software, reagents, personnel, training). Nanopore sequencing is used in GEARS and Adaptation & Evolution of Resilient Enterococcus in Space (AERES) to obtain complete whole genome information from colonies and isolates, respectively.

Task Progress:

In support of the GEARS project, initial testing of the protocol to be used for on-orbit culturing and sequencing was evaluated in the lab of GEARS Co-PI Sarah Wallace (NASA Johnson Space Center). This initial data was analyzed and used to assemble two microbial genomes, simulating one potential outcome of the GEARS on-orbit culturing and sequencing experiments.

We submitted an abstract to the American Society for Gravitational and Space Research (ASGSR) Annual Meeting, scheduled for November 3-6, 2021, to share details about these investigations including our progress to date.

Note that the supporting work behind this publication was completed during Postdoc Noelle Bryan's Space Biology Fellowship and provides important background for the project: Bryan NC, Lebreton F, Gilmore M, Ruvkun G, Zuber MT, Carr CE. Genomic and Functional Characterization of Enterococcus faecalis Isolates Recovered From the International Space Station and Their Potential for Pathogenicity. Front Microbiol. 2021 Jan 11;11:515319. https://doi.org/10.3389/fmicb.2020.515319">https://doi.org/10.3389/fmicb.2020.515319">https://doi.org/10.3389/fmicb.2020.515319">https://doi.org/10.3389/fmicb.2020.515319">https://doi.org/10.3389/fmicb.2020.515319">https://doi.org/10.3389/fmicb.2020.515319">https://doi.org/10.3389/fmicb.2020.515319">https://doi.org/10.3389/fmicb.2020.515319">https://doi.org/10.3389/fmicb.2020.515319">https://doi.org/10.3389/fmicb.2020.515319">https://doi.org/10.3389/fmicb.2020.515319">https://doi.org/10.3389/fmicb.2020.515319">https://doi.org/10.3389/fmicb.2020.515319">https://doi.org/10.3389/fmicb.2020.515319">https://doi.org/10.3389/fmicb.2020.515319">https://doi.org/10.3389/fmicb.2020.515319

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Bibliography Type:

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