Fiscal Year:	FY 2023	**Task Last Updated:**	FY 01/08/2023
PI Name:	Lee, Jessica Ph.D.		
Project Title:	Microbial Eco-evolutionary Dynamics in Simulated Microgravity and Space Radiation		
Division Name:	Space Biology		
Program/Discipline:			
Program/Discipline--Element/Subdiscipline:			
Joint Agency Name:		**TechPort:**	No
Human Research Program Elements:	None		
Human Research Program Risks:	None		
Space Biology Element:	(1) Cell & Molecular Biology (2) Microbiology		
Space Biology Cross-Element Discipline:	None		
Space Biology Special Category:	None		
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Zip Code:	94035	**Congressional District:**	18
Comments:			
Project Type:	Ground	**Solicitation / Funding Source:**	2020 Space Biology NNH20ZDA001N-SB E.12. Flight/Ground Research
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No. of Bachelor's Candidates:		**Monitoring Center:**	NASA ARC
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Flight Program:			
Flight Assignment:	NOTE: End date changed to 9/30/23 per F. Hernandez/ARC (Ed., 3/30/23)		
Key Personnel Changes/Previous PI:	No personnel changes.		
COI Name (Institution):	Broddrick, Jared Ph.D. (NASA Ames Research Center)		
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Task Description:	While there is substantial evidence that microorganisms behave differently in space than in terrestrial environments, much still remains unknown about why they do, and how their behavior might be connected to two primary stressors of spaceflight, microgravity and radiation. A dominant hypothesis is that the lack of density-driven convection in microgravity makes mixing diffusion-limited and therefore slower, which could lead microbes growing in liquid media to experience both starvation from substrate limitation and pH stress from waste buildup [1–4]. This would affect the both the physiology of individual microbes and the dynamics of communities, as many interspecies interactions involve the exchange of soluble metabolites (cross-feeding). We aim to study this hypothesis in the context of multispecies microbial communities. Ground-based research on microbial communities has shown that environments with poor mixing lead to localized interactions among community members, which in turn leads to lead to slower growth when organisms depend on cross-feeding (exchange of metabolites) but also greater stability of cooperative relationships [5]. We also aim to investigate the effect of deep-space radiation, primarily galactic cosmic rays (GCR), on microbial communities. While little is known about the effect of GCR on actively metabolizing microorganisms, work on comparable spatially heterogeneous stressors in microbial communities frequently support a "weakest link" hypothesis: in a community where organisms are mutually interdependent, direct damage to one organism indirectly affects its dependent partner. In the face of stress, therefore, the growth of the entire community is reduced in a manner predictable by the resistance of the weaker partner [6,7]. This study aims to test hypotheses based on these observations using a well-characterized model microbial cross-feeding community consisting of an Escherichia coli and a Salmonella enterica strain, in which members are mutually dependent on exchanged growth substrates. The relationship between metabolite mass transfer and growth of this community has previously been quantitatively predicted using genome-scale metabolic modeling in diverse environments [5]. We will culture the community in simulated microgravity in Rotating Wall Vessels (RWVs), where the degree of mixing, and thus the fidelity of the microgravity simulation, can be adjusted by adjusting rotation rate. GCR simulation will be carried out at the NASA Space Radiation Laboratory. To test the stability of the partnership, we will conduct experiments in which the cooperating S. enterica strain competes with a non-cooperating S. enterica strain. Growth assays will be paired with metabolic modeling, as well as RNA sequencing for gene expression analysis. We will test the following hypotheses: Hypothesis 1) On short (ecological) timescales, metabolic exchange between cells is reduced in simulated microgravity relative to a well-mixed environment, slowing the growth of a cross-feeding community. Hypothesis 2) On longer (evolutionary) timescales, simulated microgravity creates a spatially-structured environment in which a cooperating strain of S. enterica is favored, whereas well-mixed environments favor the non-cooperating strain. Hypothesis 3) Exposure to simulated GCR causes cell damage or stress, and the effect on the community is greater if cells are metabolically interdependent. This is exacerbated in simulated microgravity. Results of this study will further our understanding of how microorganisms in communities experience deep-space radiation and the microgravity fluid environment.
Rationale for HRP Directed Research:	
Research Impact/Earth Benefits:	This project aims to build an understanding of how microbial cells interact with their physical and chemical environments when growing in fluid media, and how those interactions affect the ecological and evolutionary functioning of microbial communities. These are fundamental concepts that apply not just to space microbiology but also to all terrestrial ecosystems where microbes of different species live together and communicate with each other in a liquid environment-- from oceans to laboratory bioreactors. With this early-career grant I hope to lay the foundation for a future body of work in this area. In addition, this project will ultimately produce: - computational models providing microbiologists with the ability to visualize cells' chemical environments and plan experiments using that knowledge; - outreach materials to help learners understand the basics of space microbiology and how microbes experience the fluid environment; - straightforward methods for monitoring microbial growth and activity in microgravity simulation, in real time.
Task Progress:	Our progress on this project so far has consisted of the following: 1. Demonstrated measurement of growth rate and species ratio in mixed culture using fluorescence. This study uses a two-species community consisting of an E. coli strain that can metabolize lactose to acetate but is auxotrophic for methionine; and an S. enterica strain that cannot metabolize lactose but excretes methionine. In lactose minimal medium, the two strains are mutually dependent on each other for growth. Both constitutively express fluorescent proteins: E. coli is labeled with Cyan Fluorescent Protein (CFP); S. enterica is labeled with Yellow Fluorescent Protein (YFP). In prior studies using this community, species ratios were assayed using flow cytometry or colony counts on selective media. In this study, we have demonstrated that species ratios can be quantified using just the fluorescence spectrum of the mixed culture. We grew cells separately and then mixed them in known ratios, and measured their fluorescence spectra when illuminated at 450 nm. We found that the two species had emission peaks at different wavelengths, and that by comparing ratios of the height of these two peaks, the proportion of each strain can be determined. We may still conduct further work to refine this analysis by averaging across several spectra or several peaks to reduce noise. 2. Demonstrated automated measurement of fluorescent S. enterica in simulated microgravity over time. A common method of simulating microgravity for microorganisms is to grow them in a Rotating Wall Vessel (RWV). Traditional RWV systems require destructive sampling, making it difficult to measure growth rate or track other properties over time. This study requires making growth rate measurements. We are therefore developing a system to monitor the growth of bacteria in simulated microgravity in real time, using fluorescence as a proxy for growth (Schlechter et al. 2021). [Ed. Note. See References below.] The system consists of Cell Spinpods (Hammond et al. 2021) on a laboratory bottle roller in a dark incubator. Cell Spinpods are optically clear. Fluorescence is measured in the spinpods as they are rolling using a visible-light spectrometer with a fiber-optic backscatter probe. The roller, light source, and spectrometer are enclosed in a dark incubator at 35 °C; the light source and spectrometer are programmed to acquire spectra at set time intervals. Initial experiments have demonstrated that, after a few minutes of equilibration, the signal from YFP-expressing S. enterica is easily distinguished from background and is stable for at least an hour. 3. Determined optimum rotation rate for microgravity simulation. An RWV simulates the quiescent fluid environment of microgravity by keeping cells suspended within a solid body of fluid, ideally ensuring that cells travel only within their "zone of depletion" where solute mixing is diffusion-limited. The rate of rotation of an RWV is the primary factor experiments can use to determine the fidelity of the microgravity simulation: if rotation is too fast, cells will be centrifuged out of their zone of depletion; if it is too slow, they will sediment out of it (Allen et al. 2022). We will use the calculations by Allen and colleagues to determine the optimal rotation rate for E. coli cells in defined mineral medium: 5 RPM. Furthermore, we will vary rotation rate as an experimental parameter: in addition to the optimal rotation rate, we

	will also test faster and slower rates as well-mixed controls. 4. Mentored student in computational modeling work and generation of public outreach/education materials to support this study. In Summer 2022, I hosted a Research Assistant through the NASA Ames Space Life Sciences Training Program (SLSTP) who worked on a project related to this grant. She wrote code using the finite difference method to calculate the extent of the substrate depletion zones surrounding individual E. coli cells in diffusion-limited conditions, and is now finishing a manuscript for submission for publication. She has also produced some educational videos on how microbes experience microgravity, which are currently being reviewed by the Office of Communication for potential release on the NASA YouTube channel. We are requesting a no-cost extension on this grant to accommodate our schedule for beam time at NASA Space Radiation Laboratory (NSRL). In future months, we will continue methods optimization and then carry out the experiments to test Hypotheses 1, 2, and 3 as proposed, measuring growth rates in cell spinpods, measuring species ratios in cooperator / non-cooperator competition experiments, and sequencing RNA to assess gene expression responses. References Schlechter RO, Kear EJ, Remus DM, Remus-Emsermann MNP. Fluorescent Protein Expression as a Proxy for Bacterial Fitness in a High-Throughput Assay. Appl Environ Microbiol. 2021;87: e00982-21. doi:10.1128/AEM.00982-21 Hammond TG, Nislow C, Christov IC, Batuman V, Nagrani PP, Barazandeh M, et al. Cell spinpods are a simple inexpensive suspension culture device to deliver fluid shear stress to renal proximal tubular cells. Sci Rep. 2021;11: 1–19. doi:10.1038/s41598-021-00304-8 Allen LA, Kalani AH, Estante F, Rosengren AJ, Stodieck L, Klaus D, et al. Simulated Micro-,Lunar, and Martian Gravities on Earth—Effects on Escherichia coli Growth, Phenotype, and Sensitivity to Antibiotics. Life. 2022;12: 1399. doi:10.3390/life12091399
Bibliography Type:	Description: (Last Updated: 03/04/2024)
Articles in Peer-reviewed Journals	Gesztesi J, Broddrick JT, Lannin T, Lee JA. "The chemical neighborhood of cells in a diffusion-limited system." Front Microbiol. 2023 Apr 18;14:1155726. https://doi.org/10.3389/fmicb.2023.1155726 ; PMID: 37143535; PMCID: PMC10151505 , Apr-2023