Fiscal Year:	FY 2022	**Task Last Updated:**	FY 05/11/2022
PI Name:	Everroad, Craig Ph.D.		
Project Title:	Experimental Evolution of Bacillus subtilis Populations in Space; Mutation, Selection and Population Dynamics		
Division Name:	Space Biology		
Program/Discipline:			
Program/Discipline--Element/Subdiscipline:	SPACE BIOLOGY--Cellular and molecular biology		
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:	(1) Reproductive Biology		
Space Biology Special Category:	None		
PI Email:	craig.everroad@nasa.gov	**Fax:**	FY
PI Organization Type:	NASA CENTER	**Phone:**	650-604-4997
Organization Name:	NASA Ames Research Center		
PI Address 1:	Exobiology Branch		
PI Address 2:	Mail Stop 239-4; Bldg 239/ Room 367		
PI Web Page:			
City:	Moffett Field	**State:**	CA
Zip Code:	94035-0001	**Congressional District:**	18
Comments:	NOTE: PI previously at Bay Area Environmental Research Institute until 2018		
Project Type:	FLIGHT	**Solicitation / Funding Source:**	2014 Space Biology Flight NNH14ZTT001N
Start Date:	07/01/2015	**End Date:**	09/30/2023
No. of Post Docs:	0	**No. of PhD Degrees:**	0
No. of PhD Candidates:	0	**No. of Master' Degrees:**	0
No. of Master's Candidates:	0	**No. of Bachelor's Degrees:**	0
No. of Bachelor's Candidates:	1	**Monitoring Center:**	NASA ARC
Contact Monitor:	Griko, Yuri	**Contact Phone:**	650-604-0519
Contact Email:	Yuri.V.Griko@nasa.gov		
Flight Program:	ISS		
Flight Assignment:	NOTE: Extended to 09/30/2023 per F. Hernandez/ARC (Ed., 9/6/22) NOTE: Extended to 12/31/2022 per F. Hernandez/ARC (Ed., 9/23/21) NOTE: Extended to 9/30/2021 per F. Hernandez/ARC (Ed., 9/11/20) NOTE: Extended to 9/30/2020 per F. Hernandez/ARC (Ed., 7/23/19) NOTE: Extended to 9/30/2019 per F. Hernandez/ARC (Ed., 4/2/19) NOTE: Extended to 6/30/2019 per F. Hernandez/ARC and NSSC information (Ed., 8/8/18) NOTE: Period of performance changed to 7/01/2015-6/30/2018 per NSSC (Ed., 9/14/16) NOTE: End date change to 6/30/2018 per A. Chu/ARC and NSSC; start date to remain at 11/1/2014 per A. Chu/ARC (Ed., 9/23/15)		

Key Personnel Changes/Previous PI:	Ed. note 8/8/18: Principal Investigator (PI) Craig Everroad is now civil servant at NASA Ames Research Center and Robert Bergstrom, Ph.D., Bay Area Environmental Research Institute (BAERI), is CoPI at the BAERI for grant number NNX15AM68A. September 2021 report: An Ames Space Biology Biology Space Life Sciences Training Program (SLSTP) Research Associate joined the project in June 2021. May 2022 report: The SLSTP internship ended in September 2021. Co-I Brad Bebout retired from NASA civil service in October 2021, but remains as a named collaborator. Mike Lee has been added as a collaborator for his bioinformatics expertise. Robert Bergstrom, Ph.D., Bay Area Environmental Research Institute (BAERI), is no longer a CoPI as the BAERI cooperative agreement for grant number NNX15AM68A ended in 2020 (should have been reported for the previous report).
COI Name (Institution):	Koehne, Jessica Ph.D. (NASA Ames Research Center) Ricco, Antonio Ph.D. (NASA Ames Research Center)
Grant/Contract No.:	Internal Project ; NNX15AM68A
Performance Goal No.:	
Performance Goal Text:	
Task Description:	The proposed research aims to understand the effects of the space environment on evolutionary processes in the bacterium Bacillus subtilis. Different mutant lines will be 'raced' along solid surfaces to allow continuous selection in the cultures and to maximize the number of generations possible. Deep sequencing of winners will identify evolutionary rates, mechanisms, and targets of selection. We propose printing wax barriers to make paths along a growth surface (agar, membranes) and spotting each starting position of each path with dormant spores of the experimental bacteria to 'race' different mutants. Once on orbit, the material is wetted with growth medium, allowing the individual spots of B. subtilis to grow along their determined paths. This approach provides an opportunity for exponential growth only along the propagating edges, generating continuous bottlenecking thus amplifying selective pressures on the experimental populations. By monitoring the respective growth rate of different mutant lines maintained in each of these experimental conditions, we can estimate relative fitness of the lines. Long-term changes in relative growth rate indicate adaptation. Deep-sequencing of DNA from adapted cells ('winners' at the end of runs) will identify genetic changes within the respective populations. We expect that rates of mutation will differ between microgravity, 1-g, and ground controls, and that the targets of these mutations will differ as the different populations of bacteria adapt to their respective conditions. This research will also utilize the native ability of B. subtilis to uptake foreign DNA. Information-rich environmental DNA is added into the growth medium, and the populations are raced as above. By sampling the winners, and identifying if/what foreign genes are assimilated in each treatment, this experiment will identify potential genes of interest for future studies of genetic adaptation to the space environment. Our approach maximizes the number of generations possible in the 60-day window for this call, and maximizes the potential for evolutionary processes to occur. By performing multi-generational experimental evolution on bacteria on the International Space Station (ISS), the work proposed here aims to advance understanding of the evolutionary processes and challenges facing biological systems in long-term space exploration and habitation.
Rationale for HRP Directed Research:	
Research Impact/Earth Benefits:	Improved understanding of the evolutionary process and in the dynamics of adaptive evolution in a model bacterium.
Task Progress:	The objective of this study is to ascertain how evolutionary processes in bacteria change in response to the spaceflight environment, and specifically to microgravity. We propose to use growth rate as a proxy for fitness, and to 'race' a non-motile mutant of Bacillus subtilis along a membrane wetted with growth media and bounded by impassable printed wax barriers. As cells grow into the fresh media, they will create a front of newly divided cells. These 'racetracks' will be imaged as the cells propagate, and we will be able to observe changes in growth rate over time for treatments in microgravity, 1g on board the International Space Station (ISS), and 1g on the ground. Deep-sequencing of winning lines will identify what genetic changes occurred with respect to the ancestral cells. Due to the COVID-19 pandemic, laboratory access and research capabilities were severely limited for a part of the reporting period. Research activities have focused on redesign of the cell cassettes, with iterative modifications to the design. Additional testing of fully assembled high-fidelity hardware was possible; also possible was needed biocompatibility testing on new materials used in the updated design. Preliminary growth tests with new spore and media preps were performed. A new racetrack insert design was finalized and printing of new tracks is ongoing for both Science Verification Tests (SVT) and Experiment Verification Tests (EVT). The SVT is currently underway. The Experimental Requirements Document (ERD) was updated (Revision A), signed, and released on January 31, 2022, with success criteria refined to incorporate lessons learned from the original flight and hardware. A draft Return to Work Plan was originally submitted to Exobiology Branch line management on April 6, 2021, as previously reported. Several iterations and revisions were made prior to formal submission to the NASA Ames Reserch Center (ARC) Space Science and Astrobiology Division on June 1, 2021, and submission to the ARC Science Directorate for approval on June 12, 2021. The work plan was approved on August 24, 2021, which allowed for laboratory access for the project. In June 2021, an ARC Space Biology Space Life Sciences Training Program (SLSTP) Research Associate joined the project and began a remote internship working to improve the flight growth media based on data collected from the original flight. The project used a genomics approach to improve the selected genomic DNA used for the DNA-amended growth media, in order to make the overall experiment more reproducible and more relevant to the Space Biology and Astrobiology community. The results were presented at the American Society for Gravitational and Space Research (ASGSR) annual meeting in November 2021.
Bibliography Type:	Description: (Last Updated: 06/01/2023)
Abstracts for Journals and Proceedings	Cornejal N, Lee MD, Everroad RC. "Microbial evolution in the spaceflight environment." 37th Annual Meeting of the American Society for Gravitational and Space Research, Baltimore, MD, November 3-6, 2021. Abstracts. 37th Annual Meeting of the American Society for Gravitational and Space Research, Baltimore, MD, November 3-6, 2021. , Nov-2021