Fiscal Year:	FY 2021	**Task Last Updated:**	FY 09/10/2021
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:**	12/31/2022
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 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.		

COI Name (Institution):	Bebout, Brad Ph.D. (NASA Ames Research Center) Koehne, Jessica Ph.D. (NASA Ames Research Center) Ricco, Antonio Ph.D. (NASA Ames Research Center) Bergstrom, Robert Ph.D. (CoPI: Bay Area Environmental Research Institute, grant NNX15AM68A)
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, 1-g onboard the International Space Station (ISS), and 1-g 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. The ground control samples were successfully shipped to Ames Research Center (ARC) on September 21, 2020. Similarly, the flight samples were successfully shipped to ARC from Johnson Space Center on October 6, 2020. All experimental samples (flight and ground) are accounted for, safely stowed at -80°C at ARC, and were maintained in a frozen state during all shipments and transfers and are in good condition. Unfortunately, it is not currently possible to analyze these samples. Formal approval for a re-flight was completed February 10, 2021. With no lab access, research activities have focused on redesign of the cell cassettes, and limited testing of hardware (volumes, camera angles, valve tests). Additionally, the Experimental Requirements Document (ERD) was updated, and success criteria refined to incorporate lessons learned from the original flight and hardware. This process was ongoing at the end of this reporting period. A draft Return to Work Plan was originally submitted to ARC management on April 6, 2021, with several iterations and revisions made prior to formal submission for approval on June 12, 2021. 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. This project has just begun. The overall experimental framework and preliminary results from our flight and ground experiments were presented as an invited virtual oral presentation to the Department of Biological Sciences, Tokyo Metropolitan University, Tokyo Japan, in June, 2020 (Note: Talk was given before the current reporting period but after submission of the previous year's report so is included here).
Bibliography Type:	Description: (Last Updated: 06/01/2023)
Abstracts for Journals and Proceedings	Everroad RC. "Experimental evolution of Bacillus subtilis 168 in the spaceflight environment." Invited talk given as a virtual Departmental Seminar, Department of Biological Sciences, Tokyo Metropolitan University, Tokyo, Japan. June 1, 2020. Tokyo Metropolitan University, Tokyo, Japan, invited talk, Virtual, June 1, 2020. , Jun-2020