Fiscal Year:	FY 2019	**Task Last Updated:**	FY 06/20/2019
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		
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PI Organization Type:	NASA CENTER	**Phone:**	650-604-4997
Organization Name:	NASA Ames Research Center		
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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:**	2014 Space Biology Flight NNH14ZTT001N
Start Date:	07/01/2015	**End Date:**	09/30/2020
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:	0	**Monitoring Center:**	NASA ARC
Contact Monitor:	Sato, Kevin	**Contact Phone:**	650-604-1104
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Flight Program:	ISS		
Flight Assignment:	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 and Robert Bergstrom, Ph.D., Bay Area Environmental Research Institute (BAERI), is CoPI at the BAERI for grant number NNX15AM68A.		
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. Following a successful Compliance Review in April, 2018, to allow transition into the Techshot Multi-use Variable-g Platform (MVP), the Experimental Requirements Document (ERD) Review was completed on October 11, 2018, and the Science Verification Tests (SVT) began in earnest. These tasks included development of a spore protocol, selecting mutant lines, DNA types, and finalizing media composition. Upon receipt of a 3D-printed mock-up MVP module and sufficient cell cassettes from Techshot in winter 2018, we were able to perform growth runs in approximately flight-like conditions, and able to further close out tasks, including confirming growth and biocompatibility in flight-like hardware, proper materials selection (e.g., capillary mat under the PES (polyethersulfone) membrane, switch to red ink for improved hydrophobicity). We finalized sterilization and assembly protocols, and were able to do imaging from within the mock MVP module as part of a flight-like growth run. Subsequent to the conclusion of the SVT tasks May 1, 2019, the Experimental Verification Test (EVT) Readiness Review was successfully completed on May 9, and EVT began on May 15, 2019. For EVT, a full mock-up in 6 flight-like MVP modules (akin to the ground control) was performed. 42 cell cassettes were assembled and loaded with spores and media (7 per module) in a semi-randomized nature, with the full experimental matrix (2 mutants, three media types, 7 replicates per treatment). A 27-day growth experiment was initiated with each camera (2 per module) taking images every two hours, at three light intensities for the duration. In all, almost 12,000 high-resolution images of the bacterial tracks were taken as they propagated. At the completion of the EVT growth experiment, all 42 cassettes were frozen at -80°C to replicate on-station storage. After two days, 18 cassettes (n=3 for each treatment) were thawed, and used for bacterial isolation efforts and DNA extractions. All isolations were successful, and all DNA extractions produced sufficient mass and quality of DNA for downstream sequencing analysis. EVT concluded on June 12, 2019, with all acceptable success criteria, as outlined in the ERD, being met or exceeded. The Flight Readiness Review is currently scheduled for June 24, 2019. If the review is successful, this experiment is anticipated to launch aboard SpaceX-18 (no earlier than July 21, 2019) with sample return to occur no earlier than SpaceX-19 reentry (January 2020). The overall experimental framework and results from our science validation tests were presented as an oral presentation at the Joint CSA/ESA/JAXA/NASA (Canadian Space Agency/European Space Agency/Japan Aerospace Exploration Agency/NASA) Increments 59 and 60 Science Symposium in February, 2019.
Bibliography Type:	Description: (Last Updated: 06/20/2019)
Abstracts for Journals and Proceedings	Everroad RC. "Long-term multi-generational evolutionary studies of bacteria in the spaceflight environment (MVP-Cell-02). " Talk presented at the Joint CSA/ESA/JAXA/NASA Increments 59 and 60 Science Symposium, Houston, Texas, USA, February 2019. (remote participation) Joint CSA/ESA/JAXA/NASA Increments 59 and 60 Science Symposium, Houston, Texas, February 2019. , Feb-2019