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 BIOLOGYCellular 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			
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PI Web Page:				
City:	Moffett Field	State:	CA	
Zip Code:	94035-0001	Congressional District:	18	
Comments:	NOTE: PI previously at Bay Area Environmental R	esearch 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			
	NOTE: Extended to 12/31/2022 per F. Hernandez/A NOTE: Extended to 9/30/2021 per F. Hernandez/AI	ARC (Ed., 9/23/21) RC (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/AI	RC (Ed., 4/2/19)		
Flight Assignment:	NOTE: Extended to 6/30/2019 per F. Hernandez/ARC and NSSC information (Ed., 8/8/18)			
	NOTE: Period of performance changed to 7/01/201	5-6/30/2018 per NSSC (Ed., 9/1	4/16)	
	NOTE: End date change to 6/30/2018 per A. Chu/A (Ed., 9/23/15)	RC and NSSC; start date to ren	nain at 11/1/2014 per A. Chu/ARC	
Key Personnel Changes/Previous PI:	Ed. note 8/8/18: Principal Investigator (PI) Craig Ev Robert Bergstrom, Ph.D., Bay Area Environmental NNX15AM68A. September 2021 report: An Ames (SLSTP) Research Associate joined the project in Ju	verroad is now civil servant at N Research Institute (BAERI), is Space Biology Biology Space I une 2021.	ASA Ames Research Center and CoPI at the BAERI for grant number Life Sciences Training Program	

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 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 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 an	
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 ARC from Johnson Space Center on October 6, 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 B	
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	