Fiscal Year:	FY 2017	Task Last Updated:	FY 05/02/2017
PI Name:	Everroad, Craig Ph.D.		
Project Title:	Experimental Evolution of Bacillus subtilis Populat	tions in Space; Mutation, Selecti	on and Population Dynamics
Division Name:	Space Biology		
Program/Discipline:			
Program/Discipline Element/Subdiscipline:	SPACE BIOLOGYCellular and molecular biolog	у	
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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Zip Code:	94035-0001	Congressional District:	18
Comments:	NOTE: PI previously at Bay Area Environmental R	Research Institute until 2018	
Project Type:	Flight	Solicitation / Funding Source:	2014 Space Biology Flight NNH14ZTT001N
Start Date:	07/01/2015	End Date:	06/30/2018
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: Period of performance changed to 7/01/201 NOTE: End date change to 6/30/2018 per A. Chu/A (Ed., 9/23/15)	5-6/30/2018 per NSSC (Ed., 9/1 ARC and NSSC; start date to rem	4/16) Iaain at 11/1/2014 per A. Chu/ARC
Key Personnel Changes/Previous PI:	Ed. Note 8/8/18: PI Craig Everroad is now civil ser Environmental Research Institute (BAERI), is CoP	vant at NASA Ames and Robert I at the BAERI for grant number	Bergstrom, Ph.D., Bay Area 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 Environ	er) enter) nter) nmental Research Institute, gran	t 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 generational experimental evolution on bacteria on the International Space Station (ISS), the work proposed here aims to advan
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 barires. 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. This year's progress has been primarily related to advancing and refining experimental conditions and protocols to sustain long-term growth, and to adapt our experimental system to the flight hardware of the European Modular Cultivation System (EMCS) onboard the ISS, including into five non-flight seed cassettes provided to the science team. For the current reporting period, our primary objectives have been to finalize appropriate experimental procedures with respect to conditions onboard ISS, including growth under elevated CO2, testing for background DNA contamination, maintaining sterilization – We have advanced our printing protocols to extend bacterial growth, based on the 'dumbbell pattern' previously reported, by narrowing the path for growth, removing the starting circle for growth, and adding a grid pattern to the wax to assist with imaging analysis. Some final experiments are underway to optimize growth duration and propagation speed. One of the challenges of adapting the seed cassettes to heterotrophic bacteria and complex media is the risk of contamination and a need for sterilization of the hardware pre-flight (excepting the bacterial spores). We have solved this challenge with an ultraviolet light (UV) protocol, with mu
Bibliography Type:	Description: (Last Updated: 06/01/2023)