Fiscal Year:	FY 2017	**Task Last Updated:**	FY 05/02/2017
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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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:**	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 7/01/2015-6/30/2018 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: 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. 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 sterility in the context of EMCS assembly, growth under EMCS-like conditions including long-term growth of 20-days or more, and optimization of media and track design. Experiments underway include final track design tests, and DNA-uptake tests. Printing and 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 multiple, extended exposures, which has resulted in minimal to no contamination. In combination with autoclave-sterilization of the seed cassette base and inserts, we developed reliable assembly procedures with sterilized hardware (excluding the seed cassette cover, which must be rendered aseptic-only). Growth and Propagation –We have subsequently determined growth under elevated ambient CO2 conditions, as found onboard ISS. CO2 appeared to have no effect on bacterial growth. We have also solved the challenge of extensive growth on very small volumes of medium by designing a highly rich, buffered medium with added nutrients to extend growth and induce catabolite repression. Stasis – We have demonstrated growth from spores after long-term storage (dried 10 months), using SBM medium dried for six months, under EMCS-like conditions. Dried medium/spore combinations, when rewetted with sterile, distilled water, after extensive periods of time in an inert/dry state, began growth in less than 24-hours post-wetting. We are now working to optimize the speed and distances capable for a given volume of medium, with growth currently continuing along the tracks for 20+ days.
Bibliography Type:	Description: (Last Updated: 06/20/2019)