Fiscal Year:	FY 2016	**Task Last Updated:**	FY 09/02/2015
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:**	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
Contact Email:	kevin.y.sato@nasa.gov		
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:	None		
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)		
Grant/Contract No.:	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 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 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 on-board 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 defining experimental conditions and protocols to be used for our proposed flight experiment using the European Modular Cultivation System (EMCS) hardware on-board the ISS. Our primary objectives thus far in this science flight definition phase have been to select appropriate experimental mutants of Bacillus subtilis strain 168, determine biocompatibility and types of growth media, growth surfaces, and materials/patterns to be used for track printing. Efforts are underway towards developing suitable sterilization protocols of growth surfaces post-printing. We have begun work to determine growth rates, and establish reliable 'wake-up' of Bacillus spores, and propagation along printed paths. We have also worked to develop methods for the image analysis under EMCS-like conditions. Printing and Sterilization – Preliminary printing trials revealed no problems with either chromatography paper, or the polyethersulfone (PES) membranes similar to the materials used in the EMCS seed cassette containers. We have tested several track patterns, and are currently optimizing track width. Post printing sterilization has also been demonstrated successfully with ethanol washes of the printed filters. Ethanol causes minimal distortion to the wax, or membrane materials, and currently is superior to other approached such as autoclaving, which deform/melt the materials. Growth and Propagation – Biocompatibility tests have shown that Bacillus appears to grow well on the PES membranes, as well as on chromatography paper, and glass fiber filter paper, although the rates do vary between materials. There does not seem to be any inhibition of growth due to the wax, though tests continue to determine this. Initial media tests are underway, with as are procedures for sporulation / wake-up protocols for preparing inert cells for spaceflight. Growth rate experiments are underway for our preliminary mutant lines to determine optimal growth temperature, as well as effects of materials on overall growth rate. We are also working to optimize track widths, media concentrations and experimental conditions for consistent propagation at reliable speeds. Imaging – We have developed methods for correlating biomass with image data as can be collected with the EMCS hardware. Using open source software for pixel analysis, we have successfully demonstrated that correlations between image pixel value, and total biomass, can be made. It is our hope that such data will be valuable, in conjunction with growth speed along the printed paths, for determining number of generations and growth rate.
Bibliography Type:	Description: (Last Updated: 06/01/2023)