Fiscal Year:	FY 2023	**Task Last Updated:**	FY 05/26/2023
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:**	09/30/2024
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		
Flight Assignment:	NOTE: Extended to 09/30/2024 per F. Hernandez/ARC (Ed., 9/12/23) NOTE: Extended to 09/30/2023 per F. Hernandez/ARC (Ed., 9/6/22) NOTE: Extended to 12/31/2022 per F. Hernandez/ARC (Ed., 9/23/21) NOTE: Extended to 9/30/2021 per F. Hernandez/ARC (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/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 Research Center and Robert Bergstrom, Ph.D., Bay Area Environmental Research Institute (BAERI), is CoPI at the BAERI for grant number NNX15AM68A. September 2021 report: An Ames Space Biology Biology Space Life Sciences Training Program (SLSTP) Research Associate joined the project in June 2021. May 2022 report: The SLSTP internship ended in September 2021. Co-I Brad Bebout retired from NASA civil service in October 2021, but remains as a named collaborator. Mike Lee has been added as a collaborator for his bioinformatics expertise. Robert Bergstrom, Ph.D., Bay Area Environmental Research Institute (BAERI), is no longer a CoPI as the BAERI cooperative agreement for grant number NNX15AM68A ended in 2020 (should have been reported for the previous report). May 2023 report: Brad Bebout (Formerly of NASA, now retired, an original Co-I and current collaborator on the project) will assist with flight build.
COI Name (Institution):	Koehne, Jessica Ph.D. (NASA Ames Research Center) Ricco, Antonio Ph.D. (NASA Ames Research Center)
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 Science Verification Testing (SVT) was completed in the summer of 2022, with cassette designs, media preparation protocols, volumes, and genomic DNA types finalized for the project. The Experiment Verification Testing (EVT) readiness review was completed on September 6, 2022. The Principal Investigator (PI) traveled to Techshot/Redwire on January 9, 2023, and the EVT build commenced the next day. Test cassettes were successfully built and activated without issue. Witness samples were also prepared and activated successfully. The full EVT assembly was completed on January 11, and after the requisite 3-day media rest, no contamination was observed. EVT cassettes were activated by Techshot/Redwire on January 17, 2023. The EVT commenced with all 42 cassettes germinating, with good growth, and no contamination observed. The EVT was terminated on February 3, 2023, after 18 days of growth. Preliminary image analyses and post-experiment genomic extractions confirmed that the success criteria for the investigation were met. With the successful completion of the EVT, the Mission Readiness Review was conducted on March 8, 2023, and the investigation is now preparing for flight to the International Space Station (ISS), with an anticipated launch on NG-19 no earlier than August 2, 2023. An oral presentation titled, "Microbial Evolution in the Spaceflight Environment" was given at the 38th ASGSR annual meeting in Houston, TX, in November 2022. See the Bibliography for full citation (Ed., 6/1/23).
Bibliography Type:	Description: (Last Updated: 06/01/2023)
Abstracts for Journals and Proceedings	Everroad RC, Koehne J, Cornejal N, Lee MD, Ricco A. "Microbial evolution in the spaceflight environment." 38th Annual Meeting of the American Society for Gravitational and Space Research, Houston, TX, November 9-12, 2022. Abstracts. 38th Annual Meeting of the American Society for Gravitational and Space Research, Houston, TX, November 9-12, 2022. , Nov-2022