Fiscal Year:	FY 2020	**Task Last Updated:**	FY 06/05/2020
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:**	09/30/2021
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:	Griko, Yuri	**Contact Phone:**	650-604-0519
Contact Email:	Yuri.V.Griko@nasa.gov		
Flight Program:	ISS		
Flight Assignment:	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.		
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. As previously reported, the Experimental Verification Test (EVT) Readiness Review was successfully completed on May 9, 2019. EVT began on May 15, 2019 and was completed on June 12, 2019, with all acceptable success criteria being met or exceeded. The Flight Readiness Review (FRR) was successfully presented and approved at NASA Ames Research Center (ARC) on June 24, 2019. Members of the Science team first arrived at Kennedy Space Center (KSC), Merritt Island, FL, on July 12, 2019 to assemble the flight and ground control experiments. All of the work was performed in the Space Station Processing Facility (SSPF) at KSC. The first 3 days involved laboratory set-up, safety training, inventory and assembly of the 84 flight cassettes (2 strains, 3 media types, 7 replicates per treatment, 2 gravity treatments (microgravity, 1-g)) for the flight experiment. On day 4 the different media types were prepared with the associated foreign DNA (high complexity, low complexity, DNA-free), loaded into syringes, and integrated into the 84 flight cassettes (Figure 1). On day 5, 42 cassettes for the ground control experiment were assembled and the different media types were loaded into syringes and integrated to the cassettes, as per the flight experiment set-up. QA tests were performed on all of the cassettes, before and after integration of the media. Serial numbers and other metadata associated with cassette assembly and media loading operations were recorded. Loaded cassettes were placed in the 25°C incubator and checked at 24, 48, and 62 hours to assess potential contamination of the media. No contamination was observed on any of the flight or ground control media. Flight and control cassettes were delivered to payload developer (PD) for integration on July 17 and 20, respectively. Handover of the integrated flight hardware for flight occurred on July 18, 2019. The flight experiment launched aboard SpaceX CRS-18 on July 25, 2019 and was activated onboard the ISS on August 27, 2019. The experiment ended on September 15 after 19 days of growth, and the samples were moved into cold stowage at 4°C. ISS crew imaged each cassette as they were transferred to cold stowage. Samples were subsequently transferred to -80°C on September 24, 2019. The ground control experiment was activated by the PD on August 30, 2019 and the ground control experiment ended on September 24, 2019. No contamination occurred in the flight or ground control samples, and all but 8 of the 126 replicates germinated (94%). Some challenges occurred during the experiment, causing loss of some replicates as well as imaging data. However, a sample size of at least n=3 was preserved for all treatments. Flight samples returned to Earth on SpaceX CRS-20 on April 7, 2020. Ground control samples are currently stored at -80°C by the PD, and flight samples are currently stored at -80°C at NASA Johnson Space Center (JSC), awaiting return to Ames Research Center (ARC) for isolation and genomics work to proceed. Preliminary imaging results from the ground control experiment indicate by-strain differences in growth, as well as a treatment effect from the different DNA types in the growth media. The overall experimental framework and preliminary results from our flight and ground experiments were presented as an oral presentation at the American Society for Gravitational and Space Research 35th Annual meeting, Denver, CO, USA, Nov. 20-23, 2019.
Bibliography Type:	Description: (Last Updated: 06/01/2023)

Abstracts for Journals and Proceedings	Everroad RC, Bebout B, Chang C, Detweiler AM, Harshfield N, Karouia F, Koehne J, Kost D, Logan S, Martin KR, Ricco AJ, Thomas N. "Experimental evolution of Bacillus subtilis 168 in the spaceflight environment." Oral presentation presented at the 35th Annual Meeting of the American Society for Gravitational and Space Research, Denver, CO, November 20-23, 2019. Abstracts. 35th Annual Meeting of the American Society for Gravitational and Space Research, Denver, CO, November 20-23, 2019. , Nov-2019