Fiscal Year:	FY 2021	**Task Last Updated:**	FY 04/12/2021
PI Name:	Nicholson, Wayne Ph.D.		
Project Title:	Bacillus Spore Probiotics: Evaluation of Survival and Efficacy After Exposure to Deep-Space Radiation Simulating Long-Duration Human Exploration Missions		
Division Name:	Human Research		
Program/Discipline:			
Program/Discipline--Element/Subdiscipline:			
Joint Agency Name:		**TechPort:**	No
Human Research Program Elements:	(1) **HHC**:Human Health Countermeasures		
Human Research Program Risks:	(1) **Food and Nutrition**:Risk of Performance Decrement and Crew Illness Due to Inadequate Food and Nutrition (2) **Microhost**:Risk of Adverse Health Effects Due to Host-Microorganism Interactions		
Space Biology Element:	None		
Space Biology Cross-Element Discipline:	None		
Space Biology Special Category:	None		
PI Email:	WLN@ufl.edu	**Fax:**	FY
PI Organization Type:	UNIVERSITY	**Phone:**	321-261-3773
Organization Name:	University of Florida		
PI Address 1:	Space Life Sciences Laboratory		
PI Address 2:	505 Odyssey Way, Room 201-B		
PI Web Page:			
City:	Merritt Island	**State:**	FL
Zip Code:	32953	**Congressional District:**	3
Comments:	NOTE: Congressional District is for University of Florida (32611-0001)--Ed., 8/17/2012; changed to 3 (Ed., 7/30/13)		
Project Type:	GROUND	**Solicitation / Funding Source:**	2019 HERO 80JSC019N0001-FLAGSHIP & OMNIBUS: Human Research Program Crew Health. Appendix A&B
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No. of PhD Candidates:	2	**No. of Master' Degrees:**	
No. of Master's Candidates:		**No. of Bachelor's Degrees:**	
No. of Bachelor's Candidates:		**Monitoring Center:**	NASA JSC
Contact Monitor:	Stenger, Michael	**Contact Phone:**	281-483-1311
Contact Email:	michael.b.stenger@nasa.gov		
Flight Program:			
Flight Assignment:	NOTE: End date changed to 6/24/2022 per NSSC information (Ed., 7/13/21)		
Key Personnel Changes/Previous PI:	None		
COI Name (Institution):	Fajardo-Cavazos, Patricia Ph.D. (University of Florida, Gainesville)		
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Task Description:

Background: Probiotics are defined by the World Health Organization as "live microorganisms that confer a health benefit on the host when ingested in adequate amounts." Acquisition of diverse microbes from the environment is important for maintaining a healthy gastrointestinal (GI) microbiome, but astronauts' GI microbiomes change during long-term spaceflight missions. Addition of probiotics to the astronaut diet could provide an effective countermeasure. Current data is limited regarding the shelf life of probiotics during long-duration space exploration, particularly to the radiation environment of interplanetary space. Due to limited onboard refrigerator/freezer space, it would be desirable to store probiotics at ambient temperature as freeze-dried capsules. However, freeze-dried formulations of common probiotic species (e.g., Lactobacillus, Bifidobacterium) rapidly lose viability at ambient temperatures. In contrast, probiotic formulations containing spores of various Bacillus species have very long shelf lives and survive passage through the acidic environment of the stomach and small intestine with high potency. Moreover, recent clinical trials have shown that Bacillus spore probiotics are safe, can significantly improve GI symptoms, and stimulate the immune system.
Hypothesis: Exposure of probiotic bacteria to simulated space radiation will result in a decrease in survival and potency, at a rate which can be empirically measured. Probiotics containing Bacillus spores will demonstrate enhanced long-term stability and potency compared to traditional Lactobacillus- or Bifidobacterium-containing probiotics.

Aims: Using the NASA Space Radiation Laboratory (NSRL) at Brookhaven, NY, we propose to evaluate the survival and potency of Bacillus spore probiotics vs. traditional probiotic formulations following exposure to simulated Galactic Cosmic Rays (GCRSim) and Solar Particle Events (SPESim) expected to be encountered during NASA Design Reference Missions (DRMs) lasting up to 3 years. Specific aims include: A. Select appropriate freeze-dried, prepackaged probiotic formulations to test. B. Expose samples at NSRL to GCRSim and SPESim at dosages representative of a 3-year DRM, in parallel with matched lab controls and transport controls. C. Measure viability of all samples vs. exposure dose and compare data from exposed vs. control samples.

Methods: DRM-appropriate GCRSim and SPESim exposures will be performed at NSRL. Survival to radiation exposure will be measured by viable counts and live/dead staining. Survival to simulated gastric and intestinal juices will be measured by standard procedures. Comparison of datasets between traditional probiotics and Bacillus spore formulations will be performed using appropriate statistical methods.

Deliverables: The proposed study will provide data on survival kinetics of probiotic formulations to GCRSim and SPESim exposures, as well as survival to simulated passage through the upper GI tract before and after irradiation. These results will help mission planners in decisions regarding probiotic inclusion in the food system of upcoming long-duration missions.

Significance: The gut microbiome influences virtually all aspects of human health. Microbiome health and dysfunction have been associated with myriad human health conditions ranging from digestive to cardiovascular, immunological, and psychological. Thus, a healthy GI microbiome is of prime importance to astronaut health during long-duration missions. Inclusion of probiotic supplements to the astronaut diet will promote health and mitigate detrimental effects of chronic exposure to long-term spaceflight.

Human Research Roadmap Gaps addressed: MICRO-01 (We need to determine the efficacy of current countermeasures and the need for countermeasure development based on changes in microbial populations and characteristics), MICRO-02 (We need to determine if spaceflight induces changes in diversity, concentration, and/or characteristics of medically significant microorganisms associated with the crew and environment aboard the International Space Station that could affect crew health), MICRO-03 (We need to determine which medically significant microorganisms display changes in the dose-response profiles in response to the spaceflight environment that could affect crew health), MICRO-04 (We need to determine how physical stimuli specific to the spaceflight environment, such as microgravity, induce unique changes in the dose-response profiles of expected medically significant microorganisms), FOOD-01 (We need to determine how processing and storage affect the nutritional content of the food system), and FOOD-03 (We need to identify the methods, technologies, and requirements that will deliver a food system that provides adequate safety, nutrition, and acceptability for proposed long-duration Design Reference Mission operations).

Rationale for HRP Directed Research:

Research Impact/Earth Benefits:

Probiotics are broadly defined as living microorganisms that when consumed exert positive health effects. This research is to determine the shelf-life of three common probiotic bacteria: Lactobacillus acidophilus, Bifidobacterium longum, and spores of Bacillus subtilis under radiation conditions simulating a 3-year round-trip journey to Mars without access to refrigeration. Aside from the obvious implications for astronaut health, understanding the survival of living probiotics during long-term storage under ambient conditions has implications for probiotic efficacy in communities with limited access to refrigeration.

The Specific Aims of this project are to:
A. Select appropriate freeze-dried, prepackaged probiotic formulations to test.

B. Expose samples at NSRL to GCRSim and SPESim at dosages representative of a 3-year DRM, in parallel with matched lab controls and transport controls.

C. Measure viability of all samples vs. exposure dose and compare data from exposed vs. control samples.

Despite delays due to COVID-19, we have completed Specific Aims A and B. We are currently completing Specific Aim C. Details follow.

Specific Aim A. Select appropriate freeze-dried, prepackaged probiotic formulations to test. We have completed Specific Aim A. From the vast collection of commercial probiotic preparations we chose three common probiotics for further testing: freeze-dried vegetative cells of Lactobacillus acidophilus strain DDS-1 and Bifidobacterium longum strain BB536, and freeze-dried spores of Bacillus subtilis strain HU58. Strains DDS-1 and BB536 are cultivated anaerobically on MRS medium containing 0.05% L-cysteine, and HU58 is cultivated aerobically on Miller LB medium. The identities of the three strains were verified by amplification and sequencing of their 16S rRNA genes.

Specific Aim B. Expose samples at NSRL to GCRSim and SPESim at dosages representative of 1, 2, and 3-year DRMs, in parallel with matched lab controls and transport controls. Specific Aim B has been accomplished. Probiotic capsules

Task Progress:	were color-coded (black = B. longum, red = L. acidophilus, green = B. subtilis) and loaded into 31- place blister cards (Apothecary Products, Burnsville MN) in random positions determined by use of an online random number generator (https://). Cards were shipped by Fedex to the NASA Space Radiation Laboratory (NSRL), Brookhaven National Laboratories (BNL), Upton, NY. Five cards were exposed to GCRSim at a total dose of 0.75 Gy, and another 5 cards were exposed to SPESim at a total dose of 1.0 Gy. Included in the package was an extra unexposed card to serve as a shipping control and a TLD dosimeter to monitor the radiation dose received during shipping. The Fedex courier was instructed to avoid exposure of the package to X-ray or e-beam scanning during shipping. The cards were mounted vertically on the exposure platform. The beam area was 60 cm x 60 cm. Detailed explanations of simplified 5-ion GCRSim and SPESim can be found on the NSRL web site at https:// and https:// , respectively. After exposure, cards were shipped back to the SLS Lab for assay of survival in the beams relative to the laboratory and shipping controls (Specific Aim C). Specific Aim C. Measure viability of all samples vs. exposure dose and compare data from exposed vs. control samples. Viability of B. longum, L. acidophilus, and B. subtilis probiotics was assayed and compared. Capsules containing B. longum poorly survived shipping, and no NSRL data could be obtained. Capsules containing L. acidophilus suffered ~3-log reduction but we were able to measure survival nonetheless. No significant differences were seen between the Shipping control capsules and the GCRSim-exposed or SPESim-exposed capsules. Capsules containing B. subtilis spores maintained full viability for the duration of the experiment, and also showed no significant differences in viability among the shipping controls, GCRSim-exposed and SPESim-exposed capsules. We are currently assaying survival of probiotics to simulated gastric and intestinal fluids, results of which will be presented in the next report.
Bibliography Type:	Description: (Last Updated: 04/17/2023)
Abstracts for Journals and Proceedings	Fajardo-Cavazos P, Nicholson WL. "Lactobacillus, Bifidobacterium, and Bacillus probiotics: evaluation of survival and efficacy after exposure to radiation simulating a 3-year Mars Design Reference Mission." Presented at the 2021 NASA Human Research Program Investigators' Workshop, Virtual, February 1-4, 2021. Online abstracts. 2021 NASA Human Research Program Investigators' Workshop, Virtual, February 1-4, 2021. , Feb-2021