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Fiscal Year:	FY 2022	Task Last Updated:	FY 03/29/2022
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:	TechP	ort:	No
Human Research Program Elements:	(1) <b>HHC</b> :Human Health Countermeas	ures	
Human Research Program Risks:	(1) Food and Nutrition:Risk of Perfo (2) Microhost:Risk of Adverse Health		Crew Illness Due to Inadequate Food and Nutrition ficroorganism Interactions
Space Biology Element:	None		
Space Biology Cross-Element Discipline:	None		
Space Biology Special Category:	None		
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Zip Code:	32953 C	ongressional District:	3
Comments:	NOTE: Congressional District is for U	Iniversity of Florida (32	2611-0001)Ed., 8/17/2012; changed to 3 (Ed., 7/30/13)
Project Type:	Ground		2019 HERO 80JSC019N0001-FLAGSHIP & OMNIBUS: Human Research Program Crew Health. Appendix A&B
Start Date:	06/25/2020	End Date:	06/24/2022
No. of Post Docs:		No. of PhD Degrees:	1
No. of PhD Candidates:	2 No	o. of Master' Degrees:	
No. of Master's Candidates:	No. 0	f Bachelor's Degrees:	
No. of Bachelor's Candidates:		<b>Monitoring Center:</b>	NASA JSC
Contact Monitor:	Brocato, Becky	<b>Contact Phone:</b>	
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Flight Program:			
Flight Assignment:	NOTE: End date changed to 6/24/2022	2 per NSSC information	n (Ed., 7/13/21)
Key Personnel Changes/Previous PI:	None		
COI Name (Institution):	Fajardo-Cavazos, Patricia Ph.D. ( Uni	iversity of Florida, Gair	nesville)
Grant/Contract No.:	80NSSC20K1297		
Performance Goal No.:			

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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.

### Task Progress:

**Task Description:** 

To enhance the gastrointestinal health of astronauts, probiotic microorganisms are being considered for inclusion on long-duration human missions to the Moon and Mars. Here we tested three commercial probiotics--Bifidobacterium longum strain BB536, Lactobacillus acidophilus strain DDS-1, and spores of Bacillus subtilis (B. subtilis) strain HU58 – for their survival to some of the conditions expected to be encountered during a 3-year, round-trip voyage to Mars. All probiotics were supplied as freeze-dried cells in capsules at a titer of >10^9 colony forming units per capsule. Parameters tested were survival to: (i) long-term storage at ambient conditions, (ii) simulated Galactic Cosmic Radiation and Solar Particle Event radiation provided by the NASA Space Radiation Laboratory, (iii) exposure to simulated gastric fluid, and (iv) exposure to simulated intestinal fluid. We observed that only spores of B. subtilis were capable of surviving all conditions and maintaining a titer of >10^9 spores per capsule. The results indicate that probiotics consisting of bacterial spores could be a viable option for long-duration human space travel.

### Bibliography Type:

Description: (Last Updated: 04/17/2023)

# Abstracts for Journals and Proceedings

Fajardo-Cavazos P, Nicholson WL. "Survival of probiotics to deep space radiation and gastrointestinal passage during a simulated 3-year Mars mission." 37th Annual Meeting of the American Society for Gravitational and Space Research, Baltimore, MD, November 3-6, 2021.

Abstracts. 37th Annual Meeting of the American Society for Gravitational and Space Research, Baltimore, MD,

Abstracts. 37th Annual Meeting of the American Society for Gravitational and Space Research, Baltimore, MD, November 3-6, 2021, Abstract # 202199., Nov-2021

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Abstracts for Journals and Proceedings	Fajardo-Cavazos P, Nicholson WL. "Shelf-life and simulated gastrointestinal tract survival of probiotics during a simulated round-trip journey to Mars." 2022 NASA Human Research Program Investigators' Workshop, Virtual, February 7-10, 2022.  Abstracts. 2022 NASA Human Research Program Investigators' Workshop, Virtual, February 7-10, 2022., Feb-2022	
Articles in Peer-reviewed Journals	Fajardo-Cavazos P, Nicholson WL. "Shelf life and simulated gastrointestinal tract survival of selected commercial probiotics during a simulated round-trip journey to Mars." Front Microbiol. 2021 Oct 7;12:748950. <a href="https://doi.org/10.3389/fmicb.2021.748950">https://doi.org/10.3389/fmicb.2021.748950</a> ; <a href="https://doi.org/10.3389/fmicb.2021.748950">PMID: 34690999</a> ; <a href="https://doi.org/10.3389/fmicb.2021.748950">PMID: 3469099</a> ;	