Fiscal Year:	FY 2023	**Task Last Updated:**	FY 05/10/2023
PI Name:	Wang, Zheng Ph.D.		
Project Title:	Investigating the Roles of Melanin and DNA Repair on Adaptation and Survivability of Fungi in Deep Space		
Division Name:	Space Biology		
Program/Discipline:			
Program/Discipline--Element/Subdiscipline:			
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:	None		
Space Biology Special Category:	None		
PI Email:	zheng.wang@nrl.navy.mil	**Fax:**	FY
PI Organization Type:	GOVERNMENT	**Phone:**	202-404-1007
Organization Name:	Naval Research Laboratory		
PI Address 1:	4555 Overlook Ave SW		
PI Address 2:			
PI Web Page:			
City:	Washington	**State:**	DC
Zip Code:	20375-0001	**Congressional District:**	1
Comments:			
Project Type:	FLIGHT	**Solicitation / Funding Source:**	2018 Space Biology (ROSBio) NNH18ZTT001N-Artemis1 (EM1). App A: Orion (Artemis-1) (formerly Exploration Mission-1)
Start Date:	07/15/2019	**End Date:**	07/15/2023
No. of Post Docs:	2	**No. of PhD Degrees:**	
No. of PhD Candidates:		**No. of Master' Degrees:**	
No. of Master's Candidates:		**No. of Bachelor's Degrees:**	
No. of Bachelor's Candidates:		**Monitoring Center:**	NASA KSC
Contact Monitor:	Freeland, Denise	**Contact Phone:**	321-867-5878
Contact Email:	Denise.E.Freeland@nasa.gov		
Flight Program:			
Flight Assignment:	End date changed to 07/15/2023 per H. Levine/KSC (Ed., 2/27/23)		
Key Personnel Changes/Previous PI:	Dr. Jennifer Yuzon left the Naval Research Laboratory (NRL) in February, 2023. Dr. Tiffany Hennessa, Ph.D., has replaced her as Co-Investigator.		
COI Name (Institution):	Wong-Stack, Tiffany (Naval Research Laboratory)		
Grant/Contract No.:	NNK19OB09A IAA		
Performance Goal No.:			
Performance Goal Text:			

Task Description:	Spaceflight to regions beyond low Earth orbit involves exposure to unique environmental hazards, most notably radiation. Humans traveling to these regions will be exposed to radiation from cosmic rays, which will cause DNA damage and oxidative stress. The extent of this damage, however, is unknown, and more basic research into the genetic effects of combined cosmic ray and microgravity exposure is needed. We propose to study these effects using a type of organism that is almost certain to inadvertently accompany astronauts on all of their flights – fungi. Fungi are remarkably stress-resistant and have been isolated several times from spacecraft. The objective of this proposal is to use the well-studied mold Aspergillus nidulans to test two mechanisms for space adaptation – DNA repair and the production of melanin. Mutants of this organism that cannot make melanin or are defective in a type of DNA repair associated with recovering from radiation will be used. When the samples return, the spores that these strains produce will be tested for their survival, and their protein and DNA will be analyzed to find molecular signatures of adaptation to deep space. The data we collect will tell us several things: whether melanin protects from the stresses faced during spaceflight, the types of DNA damage that occur in space, and what changes occur in fungi when they are adapted to prolonged spaceflight. The results will determine characteristics of the fungi that astronauts may have to control on future missions, including pathogenic species. Because fungi share many proteins with humans, the data collected on the proteins involved in adaptation can be applicable to astronaut health. Finally, determining whether melanin assists with survival in space will provide more evidence for it to be used as a protective material for several future NASA applications.
Rationale for HRP Directed Research:	
Research Impact/Earth Benefits:	This project focuses on the tools that fungi use to be resilient against stress. In this case, the stress of deep space, which consists of a unique composition of radiation and microgravity that has rarely, if ever, been experienced by humans. However, taking astronauts to locations in space far beyond the protection of Earth's atmosphere are aspirations of all space programs, and the stress that is associated with deep space radiation exposure (for instance, free-radical damage) overlaps in many of its biological effects with more routine stresses faced on earth. Fungi, which thrive in extreme environments such as space, and possess a genetic makeup that is similar to humans but also much simpler, are also an ideal group of organisms for understanding and combating radiation and other stresses. This project addresses two components of stress resistance in the fungus Aspergillus niger – the production of melanin and DNA repair proteins, to determine the extent and nature of their importance in survival, adaptation, and damage protection during an extended flight in deep space. The data collected from the Artemis flight will inform our understanding of how cells and organisms resist, or can be made to resist, the stress of space and other damaging environments.
Task Progress:	Our research team prepared fungal samples, which were successfully launched to the Moon orbit by the Orion spacecraft as part of the Artemis I mission. Before the first scheduled launch date (August 29, 2022), spores of Aspergillus niger and its three mutants (kusA, uvsC, and fwnA) were inoculated into 40 ml newly configured growth media in 50 ml Falcon tubes. Five replicates were prepared from each fungal strain (twenty samples in total). Since launches were slipped a couple of times due to technical issues, we had to prepare fresh samples for the November launch. Because the flight duration of Artemis I was shortened from the original 40 days to 25 days in the new mission, we accommodated it by reducing the volume of each growth medium from 40 ml to 25 ml in order to allow mycelia to grow to the top of the medium in shorter time. Artemis I was successfully launched on November 16 and returned to Earth on December 11. We received the returned samples from NASA Kennedy Space Center (KSC) on December 20. 1. All of the twenty samples showed growth of mycelia throughout the tubes, and spores were observed on the top of each growth medium. No sample contamination was observed. 2. To determine viability, spores were collected from the surface of each tube and then were diluted 100x in sterile water. 10 ul of the spore suspension were plated onto yeast peptone dextrose (YPD) plates, and germination (i.e., cell viability) of ~100 spores from each sample was observed under a microscope at 400x magnification. In all of the twenty samples, spore viability ranged 99% - 100%, suggesting sustainability of those fungal strains in Deep Space. 3. Tubes were then incubated at 4°C for 3h, which resulted in liquefying of the Pluronic media, and tissue was successfully collected by centrifugation at 10,000 x g for 10 min at 4°C. Tissue pellets were washed three times with 50 mL of sterile water and freeze-dried for 24h. Dry tissue was weighed to determine biomass. Average biomass per strain ranged from 10 to 95 mg. Notably, average biomass of DNA repair mutants (kusA and uvsC) are higher than that of the wild type and melanin deficient mutant (fwnA). Based on these data, the flight experiment achieved "Excellent" results for the criteria of "Culture Viability", "Spore and Tissue Separation", "Sample Contamination", "Biomass Measurements", and "Phenotypic Analysis". 4. All of the spores, mycelia, and culture supernatants were collected and stored at -80°C for further analyses (DNA, RNA, protein and metabolites). Ground control experiment: 20 samples similar to those for the Artemis I mission were prepared for the ground control experiment at KSC, which mimics the flight profile. The experiment started on April 4, 2023 and will conclude on May 18, 2023. After receiving the control samples, we will process them similarly to the flight samples. We plan to conduct –omics analyses of both sets of samples in parallel in the summer. In summary, we have successfully launched twenty fungal samples to the Moon orbit in the Artemis I mission and started the ground experiment at KSC that closely mimics the duration and temperature profiles of the flight. Following experiment termination, the appropriate biomass and biomolecules required for downstream analyses was collected and evaluated against the Success Criteria. The new configuration of growth media prevented the floating of capsules when Pluronic liquefied and each of the fungal strains grew during the mission. Therefore, we achieved an "Excellent" result based on the Experiment Success Criteria.
Bibliography Type:	Description: (Last Updated: 06/06/2023)
Significant Media Coverage	Patten M. "NRL fungal experiment launches as Artemis I payload." NRL News, August 26, 2022. https://www.nrl.navy.mil/Media/News/Article/3140516/nrl-fungal-experiment-launches-as-artemis-i-payload/ , Aug-2022

Significant Media Coverage	Roza D. "Why the Navy is shooting fungus into space." Task and Purpose News, November 2, 2022. https://taskandpurpose.com/news/navy-nasa-artemis-i-fungus/ , Nov-2022
Significant Media Coverage	NASA. "Space Experiments: Why is NASA studying fungi?" NASA Science News and Media. https://science.nasa.gov/biological-physical/why-is-nasa-studying-fungi , Aug-2022
Significant Media Coverage	NASA. "Space Experiments: Investigating the roles of melanin and DNA repair on adaptation and survivability of fungi in Deep Space." NASA Science Investigations. https://science.nasa.gov/biological-physical/investigations/melanin-and-dna-repair , Aug-2022
Significant Media Coverage	Seck H. "The US military just sent its first biological experiment to space." SANDBOXX, December 14, 2022. https://www.sandboxx.us/blog/the-us-military-just-sent-its-first-biological-experiment-to-space/ , Dec-2022