Fiscal Year:	FY 2021	**Task Last Updated:**	FY 05/28/2021
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		
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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:**	07/15/2022
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
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Flight Program:			
Flight Assignment:			
Key Personnel Changes/Previous PI:	May 2021 report: Jennifer Yuzon, Ph.D., is now CoInvestigator, taking over Dr. Zachary Schultzhaus's task for this project.		
COI Name (Institution):	Romsdahl, Jillian Ph.D. (Naval Research Laboratory) Yuzon, Jennifer Ph.D. (Naval Research Laboratory)		
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Performance Goal No.:			
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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:	In Year 2 of the Artemis project, our research group focused on successful completion of the Experiment Verification Test (EVT). This involved simulating the full-length flight experiment at Kennedy Space Center with a predetermined temperature profile that fell between the minimum and maximum temperature range, followed by collection of the appropriate biomass and biomolecules for downstream analyses. A detailed summary of the EVT results based on the Success Criteria are described in the following sections. • Loss of some samples due to floating gelatin capsules: As described in the Background section, fungal samples were cultured in gelatin capsules using media containing "reverse agar" (pluronic polyol F-127) as the gelling agent. This substance becomes liquid when cooled to temperatures lower than 10oC, a property that allows for the collection of tissue embedded within solid medium while avoiding agar contamination, which can affect the collection of macromolecules. The Mission Temperature Profiles described in Attachment 1 of Appendix A of the Orion EM-1 Research Announcement indicated that the temperature of the samples would vary between 54oF/12oC and 107oF/42oC for up to 72 h. Therefore, we anticipated that the Pluronic media would not liquefy during the mission. The purpose of the gelatin capsules is to delay the growth of the samples prior to deep space exposure. As the capsules dissolve, spores are released into the nutrient media and growth is initiated. For the EVT, we used 3 capsules embedded in one another in an effort to delay growth for a maximum time period. Ideally, growth should not be initiated until samples reach space. During the EVT, the samples were exposed to temperatures of 6-8oC approximately 16 days after experiment initiation. We had anticipated that by this point the capsules would have dissolved. However, the EVT made it apparent that 16 days is not a sufficient duration for 3 embedded capsules to dissolve and release spores, especially at lower temperatures. Therefore, the capsules floated to the surface when the Pluronic media liquefied, resulting in a complete loss of science for those samples. Specifically, it resulted in a loss of 0 WT samples, 3 kusA samples, 1 uvsC sample, and 2 fwnA samples (from an original total of 5 samples per strain). Since achieving an "Acceptable" result from the Success Criteria relied on our ability to retrieve biomaterial from at least 3 WT samples (and not from any of the mutant strains), the EVT was deemed successful. However, the science team believes that it is critical that any loss of science be mitigated for the flight mission. Therefore, in order to prevent the floating of the capsules in the event of cool liquefying temperatures, it is crucial that we modify the experiment configuration to ensure that the spore-containing capsules remain at the bottom of the tube throughout the entirety of the mission (see section on "Post-EVT Testing" for more information). • Viability, spore and tissue separation, and biomass: Spores were collected from the surface of each tube using 1 mL sterile water. To determine viability, spores were diluted 100x and 10 ul of the spore suspension were plated onto YPD plates and germination (i.e., cell viability) was observed under a microscope at 400x magnification. The results indicated that for the samples that were not lost due to floating capsules, spore viability ranged from 95-100%. Tubes were then incubated at 4oC for 3 h, which resulted in liquefying of the Pluronic media, and tissue was successfully collected by centrifugation at 10,000 x g for 10 min at 4oC. Tissue pellets were washed three times with 50 mL of sterile water and freeze-dried for 24 h. Dry tissue was weighed to determine biomass. Over 100 mg of biomass was obtained for all but one of the recovered samples. No sample contamination was observed. Based on these data, the EVT achieved "Acceptable" results for the criteria of "Culture Viability," "Spore and Tissue Separation," "Sample Contamination," "Biomass Measurements," and "Phenotypic Analysis." • DNA and RNA Extraction: To collect DNA and RNA, each experimental sample was purified by isolating colonies from individual spores, which were regrown on YPD agar plates. DNA was isolated using the G-Biosciences OmniPrep for Yeast kit and RNA was isolated using the Invitrogen RiboPure RNA Purification kit. The concentration and purity of isolated DNA were evaluated using a NanoDrop. Isolated RNA was evaluated using a Bioanalyzer, which revealed RNA concentration and RNA integrity. These data revealed that the DNA and RNA isolated from the EVT experiment were of sufficient quality and quantity for whole genome sequencing, RNA-sequencing, respectively. Therefore, the

EVT achieved “Excellent” results for the criteria of “Whole Genome Sequencing” and “Transcriptomic Analysis.”

• Protein and metabolite extraction: Lastly, the freeze-dried biomass was used to extract proteins and metabolites. Proteins were isolated by bead-beating, and protein concentration was determined using a Bovine Serum Albumin assay. Metabolites were extracted by sonicating with methanol and 1:1 methanol-dichloromethane, followed by drying using a SpeedVac. Metabolites were analyzed using an UltiMate 3000 HPLC system, which revealed that metabolites could be observed all analyzed samples. Both of these extractions revealed that sufficient biomolecules can be obtained from the freeze-dried tissue for downstream protein and metabolite analyses. Therefore, the EVT achieved “Excellent” results of the criteria of “Protein and Metabolite Analysis.”

Post-EVT Testing: In order to achieve ideal sample growth during the Artemis space mission, it is critical that the spore-containing gelatin capsules remain at the bottom of the tube, even in the event of the Pluronic liquefying. This configuration enables fungal growth to initiate from the tube bottom and grow towards the surface of the medium throughout the mission. Therefore, we hypothesized that by using a small amount of glue (1-2 µl) to secure the capsule to the bottom of the tube, we could achieve a configuration that mitigates the possibility of floating capsules and thereby optimizes science recovery following the mission. In order for the glue to remain cured throughout the mission, it should 1) be waterproof, so that it does not disassociate if the Pluronic liquefies, and 2) bind to polypropylene, which the Falcon tubes are composed of (notably, the majority of glues do not effectively bind to polypropylene). Based on these criteria, we identified and tested a few glues and determined that DAP Auto/Marine Sealant, which cures at room temperature to form a flexible silicone rubber capable of resisting water and vibration, is best suited for the mission. Since this sealant provides waterproof and weatherproof seal, minimal leaching is expected to occur and therefore we do not anticipate any toxicity issues, although we are testing this just to be certain. In the modified configuration we also plan to include approximately 50 µl of sterile glass beads in the outer capsule, which provides a buffer between the glue and fungal spores.

We are currently conducting an “EVT repeat” experiment in our laboratory at the Naval Research Laboratory that closely mimics that duration and temperature profile of the EVT. Following experiment termination, the appropriate biomass and biomolecules required for downstream analyses will be collected and evaluated against the Success Criteria. The experiment will be considered successful if we are able to prevent the floating of capsules in the event of Pluronic liquefying, and if we are able to achieve either “Excellent” or “Acceptable” results based on the EVT Success Criteria.

Bibliography Type:	Description: (Last Updated: 06/06/2023)
Significant Media Coverage	Eichner C. "Researchers prepare to send fungi for a ride around the moon. Article on PI's upcoming flight experiment." Naval Research Laboratory press release, May 28, 2021. https://www.nrl.navy.mil/Media/News/Article/2638434/researchers-prepare-to-send-fungi-for-a-ride-around-the-moon/ , May-2021
Significant Media Coverage	Astrobiology Web. "'Researchers Prepare To Send Fungi For A Ride Around The Moon.' Article about PI's upcoming flight experiment." Astrobiology website, June 1, 2021. http://astrobiology.com/2021/06/researchers-prepare-to-send-fungi-for-a-ride-around-the-moon.html , Jun-2021