Fiscal Year:	FY 2022	**Task Last Updated:**	FY 05/05/2022
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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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
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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. Jillian Romsdahl left the Naval Research Laboratory (NRL) in July, 2021. Since January 2022, Tiffany Wong-Sack, Ph.D., has joined this project as CoInvestigator.		
COI Name (Institution):	Yuzon, Jennifer Ph.D. (Naval Research Laboratory) Wong-Stack, Tiffany (American Society for Engineering Education/Naval Research Laboratory)		
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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.
	Background As NASA looks to send personnel farther into space, an understanding of the effects of spaceflight on biological systems, particularly for the environment beyond low Earth orbit, needs to be more fully developed. Some of this knowledge will come from human research. However, such studies are inherently limited due to the necessarily small sample sizes and the types of data that can be collected. There is value, therefore, in conducting studies on other organisms from which relevant data can be generated on a large scale. We have proposed that such experiments should make use of the organisms that are already suited and have the potential to "hitchhike" as contaminants inside spacecraft. Fungi are an excellent candidate for these studies, as they possess genetic similarity to humans but also have the benefits of microorganisms such as rapid and simple growth conditions, and easy genetic manipulation. The first Artemis flight will extend beyond the Moon and experience conditions of deep space, with a unique radiological profile. It also allows for a passive biological cargo to be flown as long as it maintains viability over an extended time through large temperature fluctuations, and to take advantage of this mission and produce the most valuable biological data possible, we are using the fungus Aspergillus niger (A. niger) to examine how certain cellular mechanisms, one unique to fungi (the production of melanized spores), and one conserved throughout higher organisms (DNA repair) can assist in survival, protection, and adaptation to deep space. Overview of the Project Deep space is characterized by a lack of gravity as well as the presence of high levels of cosmic ionizing radiation (IR), of which the major biological target is DNA. Fungi such as A. niger have recently been used as models for studying the biological effects of cosmic IR, as they deal with DNA damage in a manner similar to that of animals. Specifically, when IR produces double strand breaks in DNA, fungi and animals use one of two general mechanisms to repair them: non-homologous end-joining (NHEJ), a rapid process which can theoretically fuse any two pieces of DNA together; and homologous recombination (HR), which makes use of homologous chromosomes as templates to accurately achieve repair and reconstitution of a double strand break. Another reason for using fungi such as A. niger in radiobiological experiments is their production of melanin, which is currently being explored for its ability to protect against detrimental effects of IR. In this experiment, we will observe how NHEJ, HR, and melanin affect survival and adaptation of A. niger to the Artemis flight. Three mutant strains of A. niger will be developed: kusA, deficient in NHEJ; uvsC, deficient in HR; and fwnA, deficient in melanin production. Five cultures of each of these strains, along with the parental strain (wild type, WT), will be included in the growth chamber contained within the Artemis capsule, with a combined sample size of 20 cultures. The experimental design will be set up to achieve three things: delaying the start of growth so that it does not occur prior to launch (up to 2 weeks after handing over samples), allowing the samples to grow as long as possible during the flight (up to 6 weeks between launch and landing), and obtaining as much tissue as possible after recovering the samples. To delay growth, we will inoculate cells in slowly dissolving capsules and place these capsules in the bottom of the growth chambers, which will be 50 mL centrifuge tubes. The capsules will slowly dissolve and the cells will not start growing until the capsules are infiltrated with the medium, delaying the initiation growth during the pre-flight period. Next, the centrifuge tubes will be filled with medium, such that cultures will be able to grow to the top of the chambers (~110 cm). Finally, we will use growth medium with Pluronic F-127 as the gelling agent. This compound is solid at room temperature but is liquid near freezing, which will allow us to liquefy and collect the tissue embedded within for biomolecule analysis, without heating up and damaging them (i.e., through melting). In this way, nearly all of the tissue produced during flight will be available for scrutiny. When molds such as A. niger sense oxygen, they produce spores for wind-dispersal. This means that our samples, after growing through the medium up the tube, will produce spores in the space between the tube cap and the medium . Each of these spores contains one nucleus, and therefore represents one genome which was formed in deep space and adapted, to some extent, to the conditions there. Upon obtaining the samples after the flight, then, these spores will be collected, plated sparsely on fresh medium, and observations of individual spores will be used to measure: • Cellular survival (i.e., the percent of spores able to form colonies) and phenotypes (e.g., resistance to ionizing and ultraviolet radiation, hydrogen peroxide) • Mutation rates (i.e., the distribution, kind, and amount of mutations induced in several individual nuclei due to cosmic radiation or another aspect of flight) • The space-adapted transcriptomic signature (i.e., the gene expression

Task Progress:

differences occurring in spores during outgrowth on new medium after spaceflight). Additionally, the rest of the culture will be cooled to liquefy the medium, and the tissue will be collected through centrifugation.

This tissue will then be used for: • Proteomic analysis – to determine the identity and nature of proteins produced by tissue responding to spaceflight • Metabolomic analysis - to determine the identity and nature of other metabolic compounds produced by tissues responding to spaceflight. With these measurements, we will be testing three hypotheses. The first is that disrupting one or both of the DNA repair pathways will result in specific mutations occurring in the offspring of the mutant strains (kusA and uvsC) as a result of IR exposure. Genome sequencing will allow us to identify these mutations, if they occur, and understand the relative contribution each gene has in contributing to the protection against these mutations. Second, we hypothesize that adaption to the spaceflight conditions will cause changes in gene expression, and protein and metabolite production in A. niger that will provide information on what the space environment is doing to this organism. Additionally, we will be able to detect any of these changes through proteomic and metabolomic analysis of the tissue collected from the tubes, as well as the gene expression changes occurring in colonies subsequently grown from spores collected from each experimental sample, through RNA-seq. Third, we hypothesize that the disruption of melanin biosynthesis or DNA repair pathways will contribute to a decrease in survival under the conditions experienced in the Artemis capsule. This will be tested by measuring the biomass produced by each strain and the ability of spores from each strain to grow after obtaining the samples post-flight.

Brief Summary of Year 1 Progress

In Year 1, our research group successfully generated mutant stains with the kusA, uvsC, and fwnA genes individually deleted. Characterization of the deletion strains revealed that deletion of fwnA resulted in increased susceptibility to UV-C radiation and deletion of kusA resulted in increased susceptibility to both UV-C and gamma-radiation. We validated Pluronic F-127 as a culture medium gelling agent suitable for A. niger, and confirmed that the medium could be liquefied within 2 hours at 4 degrees Celsius, followed by tissue separation via centrifugation. Finally, we completed the Science Verification Test (SVT), which involved exposing samples to temperatures fluctuating from 18 degrees Celsius to 37 degrees Celsius over a 71-day time period, followed by collection of the appropriate biomass for downstream analyses. The SVT was deemed to be successful based on the predetermined Success Criteria.

Brief Summary of Year 2 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 NASA 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. 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 10 degrees Celsius, a property that allows for the collection of tissue embedded within a solid medium while avoiding agar contamination. During the EVT, the samples were exposed to temperatures of 6-8 degrees Celsius approximately 16 days after experiment initiation. Therefore, the capsules floated to the surface when the Pluronic media liquefied, resulting in a complete loss of science for those samples. However, we achieved an “Acceptable” result from the Success Criteria. In order to prevent the floating of the capsules in the event of cool liquefying temperatures, we modified the experiment configuration to ensure that the spore-containing capsules remained at the bottom of the tube throughout the entirety of the mission.

Year 3 Goals and Progress Towards These Goals

Experiment Verification Test 2: In Year 3, our research group modified the original configuration for the fungal spaceflight experiment. With the new configuration, we were able to successfully complete a second Experiment Verification Test (EVT-2) that followed the temperature profile of the first EVT. In the new configuration, we used a small amount of glue (1-2 µl) to secure the capsule to the bottom of the tube, to mitigate the possibility of floating capsules and thereby optimize science recovery following the mission. We determined that DAP Auto/Marine Sealant is best suited for the mission for the following reasons: 1) the glue binds to the polypropylene material of the Falcon tubes, and 2) cures at room temperature to form a flexible silicone rubber capable of resisting water and vibration. Since this sealant provides waterproof and weatherproof seal, minimal leaching is expected to occur and we did not identify any toxicity issues. In the modified configuration we also included approximately 50 µl of sterile glass beads in the outer capsule, which provides a buffer between the glue and fungal spores.

• Viability, spore, and tissue separation, and biomass: After the EVT test, none of the capsules floated to the top and all tubes showed growth throughout the tube. Spores were collected from the surface of each tube using a sterile inoculation loop. To determine viability, spores were diluted 100x and 10 µl of the spore suspensions were plated onto Yeast Extract–Peptone–Dextrose (YPD) plates and germination (i.e., cell viability) was observed under a microscope at 400x magnification. The results indicated that for all the samples, spore viability ranged from 96.1 - 99.0%. Tubes were then incubated at 4 degrees Celsius 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 degrees Celsius. 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 6.2 to 18.75 mg. No sample contamination was observed. Based on these data, the EVT achieved “Excellent” 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 second EVT experiment was of sufficient quality and quantity for whole genome sequencing, and RNA-sequencing. 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 High Performance Liquid Chromatography (HPLC) system, which revealed that metabolites could be observed in 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 for the criteria of “Protein and Metabolite Analysis”.

In summary, we have conducted an “EVT repeat” experiment in our laboratory at NRL that closely mimics that duration and temperature profile of the EVT. Following experiment termination, the appropriate biomass and biomolecules required for

	downstream analyses were collected and evaluated against the Success Criteria. The experiment was successful because the new configuration prevented the floating of capsules when Pluronic liquefied. Therefore, we achieved an “Excellent” result based on the EVT Success Criteria. Additional Group Achievements / Publications: Romsdahl J, Schultzhaus Z, Cuomo CA, Dong H, Abeyratne-Perera H, Hervey WJ, Wang Z. "Phenotypic characterization and comparative genomics of the melanin-producing yeast exophiala lecanii-corni reveals a distinct stress tolerance profile and reduced ribosomal genetic content." J Fungi. 2021 Dec 15;7(12):1078. https:// ; PMID: 34947060; PMCID: PMC8709033 (Supported by NASA) Goals and Plans for Year 4 In the third year of this project, we plan to complete the following task: Prepare for Flight. Currently, the Artemis launch is scheduled to launch sometime after June 2022, although this is subject to change. In any case, we have ordered materials and prepared strains for the flight. This will not be a difficult goal to achieve, as the sample setup is minimal and all the strains have been stored to maintain viability indefinitely and can be prepared for flight within approximately one week.
Bibliography Type:	Description: (Last Updated: 06/06/2023)
Articles in Peer-reviewed Journals	Romsdahl J, Schultzhaus Z, Cuomo CA, Dong H, Abeyratne-Perera H, Hervey WJ, Wang Z. "Phenotypic characterization and comparative genomics of the melanin-producing yeast exophiala lecanii-corni reveals a distinct stress tolerance profile and reduced ribosomal genetic content." J Fungi. 2021 Dec 15;7(12):1078. https://doi.org/10.3390/jof7121078 ; PMID: 34947060; PMCID: PMC8709033 , Dec-2021