Fiscal Year:	FY 2024	**Task Last Updated:**	FY 11/03/2023
PI Name:	Santa Maria, Sergio Ph.D.		
Project Title:	ORGANA: Oxidation-Reduction potential and Genetic Assessments for New mission Applications		
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		
Space Biology Cross-Element Discipline:	None		
Space Biology Special Category:	None		
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Organization Name:	NASA Ames Research Center		
PI Address 1:	Space Biosciences Research Branch		
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City:	Moffett Field	**State:**	CA
Zip Code:	94035	**Congressional District:**	18
Comments:			
Project Type:	GROUND	**Solicitation / Funding Source:**	2021 Space Biology NNH21ZDA001N-LEIA E.10. Lunar Explorer Instrument for Space Biology Applications
Start Date:	01/01/2022	**End Date:**	09/30/2024
No. of Post Docs:	1	**No. of PhD Degrees:**	
No. of PhD Candidates:	1	**No. of Master' Degrees:**	
No. of Master's Candidates:		**No. of Bachelor's Degrees:**	
No. of Bachelor's Candidates:	3	**Monitoring Center:**	NASA ARC
Contact Monitor:	Griko, Yuri	**Contact Phone:**	650-604-0519
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Flight Program:			
Flight Assignment:	NOTE: End date changed to 09/30/2024 per F. Hernandez/ARC (Ed., 11/1/23).		
Key Personnel Changes/Previous PI:	No changes.		
COI Name (Institution):	Broddrick, Jared Ph.D. (NASA Ames Research Center) Gentry, Diana Ph.D. (NASA Ames Research Center) Liddell, Lauren Ph.D. (NASA Ames Research Center)		
Grant/Contract No.:	Internal Project		
Performance Goal No.:			
Performance Goal Text:			

Task Description:	Space ionizing radiation (IR) and reduced gravity pose risks to long-duration space travel and eventual non-Earth habitation. Given the difficulties in recreating these effects on Earth, developing an in-depth understanding of these risks before sending crewed missions necessitates fully autonomous biological experiments. As an example, BioSentinel is a small satellite with an integrated BioSensor that will measure metabolic and growth changes induced by deep space radiation in the model organism S. cerevisiae (budding yeast). The Lunar Explorer Instrument for Space Biology Applications (LEIA) project is leveraging the same platform to answer biological questions related to lunar exploration. We hypothesize that both metabolic (redox) and genetic (knockout) assays can distinguish specific changes due to the unique combinations of direct IR damage, indirect oxidative damage, and reduced gravity incurred by exposure to the lunar environment, low Earth orbit (LEO), interplanetary space, and ground-based simulations even before differential survival becomes apparent. These environmental perturbations are known to result in subtle changes in cell growth and activity, but the resulting data is lacking pathway and molecular specificity. By leveraging a metabolic modeling framework and a series of engineered strains, our primary goal is to perform the ground testing necessary to validate strains that yield distinct responses to specific stressors, both laying the groundwork for a potential (and successful) lunar mission to characterize the relative importance of these changes within the capabilities of the BioSensor instrument and improving the usefulness of the alamarBlue assay for past and future missions. To test our hypothesis, we will pursue the following Specific Aims: Aim 1: Derive metabolic pathways usage via modeling of a colorimetric assay. The BioSentinel platform uses a redox dye to detect changes in viability and metabolic activity. AlamarBlue changes color in the presence of cell activity and creates intricate time course data that varies as a function of environmental stress, genetic background, and metabolic activity. To date, the rich information in this data has not been connected explicitly to underlying cellular processes. We will combine the data with metabolic modeling to develop a computational tool that extracts detailed metabolic information. This predictive model will not only enable analysis of our experimental design but can be applied to detect changes caused by lunar-like conditions or any autonomous mission that utilizes this reporter dye. Aim 2: Explore biological effects of a lunar-like environment using DNA repair and stress response defective mutants. Both IR and reduced gravity induce the production of reactive oxygen species (ROS), which play a key role in the generation of cellular and DNA damage, in addition to direct IR damage (e.g., DNA breaks, membrane damage). We will generate yeast mutants defective in DNA repair and stress response pathways, including oxidative damage repair, homologous recombination, and excision repair. Given the short mission duration and the expected low cumulative IR dose, we will also generate mutants containing multiple gene knockouts to increase sensitivity. We will then perform alamarBlue assays after IR exposure in both dry form and in liquid suspension, to study their response. Aim 3: Engineer endogenous ROS scavenging capabilities in yeast. An indirect effect of IR and cellular stress is the buildup of ROS. While S. cerevisiae has several strategies for ROS removal, we will engineer ROS scavenging mechanisms and evaluate if they can mitigate the unique damage caused by spaceflight stress and IR. We will over-express a native ROS scavenging enzyme and engineer a mannitol biosynthetic pathway, which specifically targets an ROS produced by IR. This aim demonstrates a potential mitigation for future spaceflight missions, enabling insight into how engineered scavenging mechanisms could improve crew safety.
Rationale for HRP Directed Research:	
Research Impact/Earth Benefits:	The ORGANA project (and the investigator team) was the basis for a new mission to the lunar surface, LEIA, which was recently awarded a ~$20M grant as part of NASA's PRISM program. Both projects are trying to understand the effects of the lunar surface environment on biological organisms, both to provide valuable information for future human missions to the Moon and to improve bio production of chemicals of interest like medicine or food. Thus, our project aims to provide lessons on how to improve instrumentation for future missions beyond low Earth orbit (LEO) as well as valuable data for future missions including biology.
Task Progress:	Aim 1: Derive metabolic pathways usage via modeling of a colorimetric assay. We have continued to investigate the kinetics of the alamarBlue colorimetric assay using a variety of nutrient media and sensors during cell growth. We have developed a new custom sensor-embedded instrument to measure metabolic parameters, including redox state, conductivity, pH, oxygen content, absorbance, etc. For this purpose, we have 3D-printed vessels using a variety of polymers and tested them for optimal sterilization and biocompatibility. We are currently performing tests to ensure the different components are ready to initiate validation bio experiments. This Aim will continue into 2024. Aim 2: Explore biological effects of a lunar-like environment using DNA repair and stress response defective mutants. Last year, we initiated a variety of experiments with 40+ yeast strains, including mutants defective in DNA damage repair and stress response. So far, we have completed 17 months of long-term desiccation and gamma radiation experiments and have down-selected to approx. 12 candidate strains as part of the Lunar Explorer Instrument for Space Biology Applications (LEIA) PRISM mission to the lunar surface. The next step is to initiate biocompatibility experiments using flight-like hardware (fluidic cards, optical ground support equipment, etc.). We will also test their sensitivity to simulated galactic cosmic radiation (GCRsim) at the NASA Space Radiation Laboratory in the Spring 2024. This Aim will continue as part of the LEIA PRISM project. Aim 3: Engineer endogenous reactive oxygen species (ROS) scavenging capabilities in yeast. Like Aim 2, we have generated a series of strains containing genetic countermeasures to improve long-term desiccation and viability, including expression of genes involved in ROS mitigation and exogenous genes from tardigrades. We are also testing better methods to improve long-term desiccation and viability by modifying our current protocols. Even though expression of tardigrade genes was not originally proposed in this study, it is one strategy we are using in support of the LEIA lunar mission, and we are already testing them for desiccation and ionizing radiation tolerance.
Bibliography Type:	Description: (Last Updated: 11/24/2023)
Articles in Peer-reviewed Journals	Rahmanian S, Slaba TC, Braby LA, Santa Maria SR, Bhattacharya S, Straume T. "Galactic cosmic ray environment predictions for the NASA BioSentinel mission." Life Sci Space Res. 2023 Aug 1;38:19-28. https://doi.org/10.1016/j.lssr.2023.05.001 ; PMID: 37481304 , Aug-2023

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