Fiscal Year:	FY 2024	**Task Last Updated:**	FY 01/19/2024
PI Name:	Settles, Andrew Ph.D.		
Project Title:	Feasibility of Synthetic Biology Countermeasures for Human Exploration Beyond Low Earth Orbit		
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) Microbiology		
Space Biology Cross-Element Discipline:	None		
Space Biology Special Category:	None		
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PI Organization Type:	NASA CENTER	**Phone:**	352-283-2767
Organization Name:	NASA Ames Research Center		
PI Address 1:	AST Life Support Studies		
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PI Web Page:			
City:	Moffett Field	**State:**	CA
Zip Code:	94035-1000	**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:	12/01/2021	**End Date:**	09/30/2024
No. of Post Docs:		**No. of PhD Degrees:**	
No. of PhD Candidates:		**No. of Master' Degrees:**	2
No. of Master's Candidates:		**No. of Bachelor's Degrees:**	1
No. of Bachelor's Candidates:		**Monitoring Center:**	NASA ARC
Contact Monitor:	Griko, Yuri	**Contact Phone:**	650-604-0519
Contact Email:	Yuri.V.Griko@nasa.gov		
Flight Program:			
Flight Assignment:	NOTE: End date changed to 09/30/2024 per F. Hernandez/ARC (Ed., 12/12/23) NOTE: Project dates (POP) changed; now 12/1/2021-11/30/2023 per F. Hernandez/ARC (Ed., 1/19/22)		
Key Personnel Changes/Previous PI:	Dr. Aditya Hindupur resigned from his Science co-I position in April 2022 to take an industry position.		
COI Name (Institution):			
Grant/Contract No.:	Internal Project		
Performance Goal No.:			
Performance Goal Text:			

Task Description:	Microbial production of bioactive compounds, such as vitamins or pharmaceuticals, can reduce risks for deep-space crewed missions. Yeast are excellent chassis organisms to express countermeasure products due to their long shelf-life viability. Yeast have robust synthetic biology technology to transfer whole biosynthesis pathways for synthesis of desired products. NASA has invested in yeast production of micronutrients that are known to have a short shelf-life in prepackaged foods, with demonstrated success on the International Space Station. However, low-Earth orbit does not test yeast for resistance to higher radiation levels and the more extreme environment of deep space. The goal of this proposal is to develop methods to preserve, grow, and measure production of desired synthetic biology products from edible yeast, using the BioSensor platform. BioSensor automates yeast culture activation and monitors growth with light absorbance of specific wavelengths produced by light emitting diodes (LED). The project goal is to expand the capability of BioSensor to enable monitoring synthetic biology traits along with cell growth and metabolism. The central objectives are: 1) Develop methods to predict synthetic biology production traits, namely carotenoids and recombinant proteins, using multivariate statistical models based on three wavelength light absorbance. We anticipate that the BioSensor platform will need to be modified to replace one of the current wavelengths to a blue LED; 2) Yeast may be overly sensitive to deep space radiation, and we will engineer carotenoid producing strains to express a DNA damage protection protein from tardigrades; 3) Non-conventional, yeast species may be more efficient for recombinant protein expression in deep space conditions. We will engineer three edible species to produce a target protein that absorbs blue light, to enable monitoring of recombinant protein and carotenoids in the same BioSensor device; and 4) Determine strain and media storage conditions as well as test the multiwavelength light monitoring strategy to establish the requirements and methodology for a future lunar surface mission. The project is expected to advance the remote sensing technology in the BioSensor platform. A future flight experiment is expected to develop fundamental knowledge on the effects of deep space on protein expression and metabolite production. In addition, the project directly addresses critical gaps to advance crewed missions in deep space exploration. The specific carotenoids, beta-carotene and zeaxanthin, are important micronutrients to prevent macular degeneration and have been identified as potential countermeasures to maintain vision in astronauts during deep space missions. Recombinant protein expression in non-conventional yeast will demonstrate feasibility for production of bioactive protein therapies in deep space missions. These synthetic biology approaches are critical to provide products that are sensitive to radiation and have short shelf lives in prepackaged foods and medicines.
Rationale for HRP Directed Research:	
Research Impact/Earth Benefits:	Microbial production of bioactive compounds, such as vitamins or pharmaceuticals, can reduce risks for deep-space crewed missions. Edible yeasts are chassis organisms that are highly amenable to synthetic biology and can produce a vast array of useful compounds including vitamins and pharmaceuticals. This project is developing yeast genetic strains and remote sensing methods to study the impact of lunar surface radiation on the growth and productivity of engineered yeast. The project will be testing methods to mitigate the impact of oxidative stress in the cell on productivity of desired compounds. The fundamental knowledge developed in the project will contribute to a better understanding of cellular reactions to unfavorable environments and may reveal new approaches to improve microbial biomanufacturing when cells are experiencing environmental stress.
Task Progress:	This project is developing engineered yeast to be assayed for performance in extreme deep-space environments using the BioSensor microfluidics system that was developed for the BioSentinel CubeSat as part of Artemis I. The BioSensor uses light emitting diodes (LED) at three wavelengths to allow measurements of cell growth and color changes in yeast cultures. The project research activities complement and directly support a NASA Payloads and Research Investigations on the Surface of the Moon project entitled, “Lunar Explorer Instrument for space biology Applications (LEIA).” The LEIA suite of instruments includes the BioSensor, a charged particle, linear energy transfer spectrometer, and fast neutron detector, which will be delivered to the lunar surface as part of a Commercial Lunar Payload Services (CLPS) mission. The two radiation detectors will allow direct correlation between radiation exposure with yeast growth and production in the BioSensor. This project has made significance progress on all four specific aims as follows. Aim 1) Develop methods to predict synthetic biology production traits, namely carotenoids and recombinant proteins, using multivariate statistical models based on three wavelength light absorbance. We determined that optical density measurements at 465 nm and 850 nm can be used to detect beta-carotene production in microtiter plate formats. To measure protein content, we engineered baker’s yeast to express Enhanced Cyan-Green Fluorescent Protein (ECGFP). Although in year 1 of the project we found that florescence of ECGFP was predictive of ECGFP levels in stationary cultures, absorbance at 465 nm is not significant enough to detect ECGFP expression. These experiments provided evidence that the spectroscopy approaches planned for carotenoid detection in LEIA BioSensor experiments are feasible. Additional experiments with desiccated yeast strains are ongoing and will be used to calibrate 465 nm absorbance to beta-carotene production levels. Aim 2) Engineer carotenoid-expressing yeast strains that enhance sensitivity or resistance to expected lunar surface environment stressors. We generated twelve yeast strains that are expected to alter sensitivity to abiotic stresses, such as reactive oxygen species (ROS) or radiation. Four strains were confirmed to be more sensitive to long-term storage of dry cells in ambient, laboratory conditions. Five of the yeast strains tested expression of damage suppressor (Dsup) or cytoplasmic abundant heat soluble (CAHS) genes from tardigrades, which have been shown to enhance radiation or desiccation tolerance in plants, animals, or yeast. 3) Engineer a non-conventional yeast species to express a blue light compatible marker for synthetic biology-enabled production. We developed a single-locus beta-carotene biosynthesis pathway construct that can be integrated into multiple yeast species. The first iteration of this construct produced low levels of beta-carotene in three baker’s yeast strains. We are currently revising the construct to improve beta-carotene production for eventual transfer into Crabtree-negative yeast. 4) Use BioSensor microfluidics cards to test inoculum, desiccation, and spectroscopy methods to measure cell growth and product formation.

	We completed initial tests with BioSensor microfluidics cards in ground support equipment (GSE). These tests showed feasibility to detect beta-carotene production using visible light absorbance at 465 nm and Near-Infrared Spectroscopy (NIR) absorbance at 850 nm.
Bibliography Type:	Description: (Last Updated: 02/07/2024)
Abstracts for Journals and Proceedings	Liddell LC, NN, Chau JW, Raj CG, Gentry D, Settles AM, Santa Maria SR. "Adapting a flexible BioSensor Platform for biological studies on the Moon." 39th Annual Meeting of the American Society for Gravitational and Space Research, Washington, DC, November 13-18, 2023. Abstracts. 39th Annual Meeting of the American Society for Gravitational and Space Research, Washington, DC, November 13-18, 2023. , Nov-2023
Abstracts for Journals and Proceedings	Raj CG, Ball NN, Chau JW, Gentry D, Gilbert R, Liddell LC, Settles AM, Santa Maria SR. "Microbial optical data processing: A key step in the metabolic assessment of Lunar Explorer Instrument for Space Biology Applications (LEIA) and Biosentinel's payload data." 39th Annual Meeting of the American Society for Gravitational and Space Research, Washington, DC, November 13-18, 2023. Abstracts. 39th Annual Meeting of the American Society for Gravitational and Space Research, Washington, DC, November 13-18, 2023. , Nov-2023
Abstracts for Journals and Proceedings	Settles AM, Ball NN, Broddrick JT, Chau JW, Ehresmann B, Gentry DM, Acevedo JG, Heffern LE, Hindupur A, James LM, Lee JA, Liddell LC, Timucin LR, Vu ST, Pletcher DL, Hassler DM, Santa Maria SR. "LEIA: An investigation of radiation risks to biology at the lunar South Pole." 39th Annual Meeting of the American Society for Gravitational and Space Research, Washington, DC, November 13-18, 2023. Abstracts. 39th Annual Meeting of the American Society for Gravitational and Space Research, Washington, DC, November 13-18, 2023. , Nov-2023