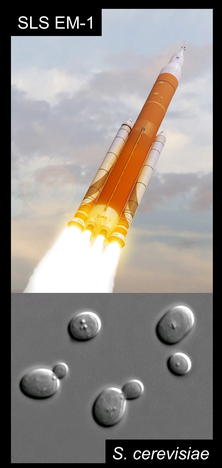
National Aeronautics and Space Administration

Identification of Novel Desiccation-Tolerant *S. cerevisiae* Strains for Deep Space Biosensors

Sofia Massaro Tieze[1,2,3], Sergio R. Santa Maria[4], Lauren Liddell[5], Sharmila Bhattacharya[6]

[1]Blue Marble Space Institute of Science, [2] Haverford College, [3]Pennsylvania Space Grant Consortium, [4]University of New Mexico, [5]Logyx LLC, [6]NASA Ames Research Center

Abstract

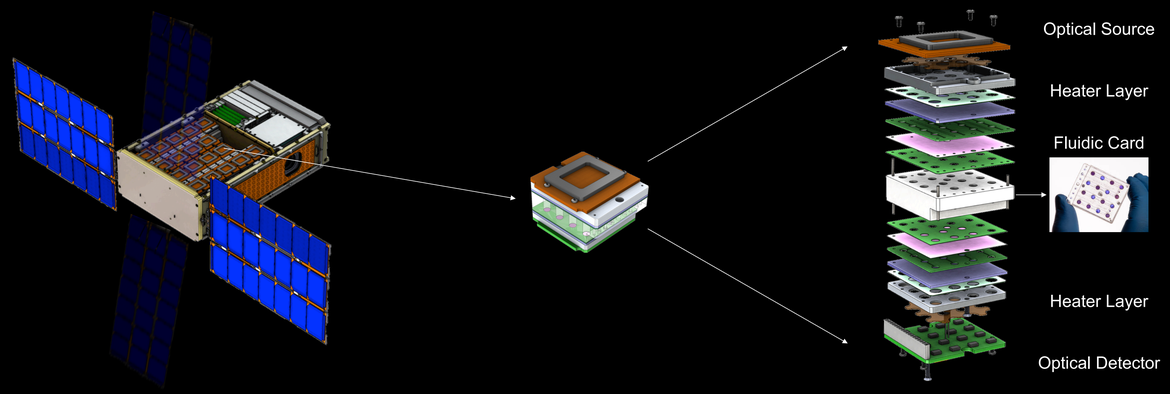


NASA's BioSentinel mission, a secondary payload that will fly on the Space Launch System's first Exploration Mission (EM-1), utilizes the budding yeast *S. cerevisiae* to study the biological response to the deep space radiation environment. Yeast samples are desiccated prior to launch to suspend growth and metabolism while the spacecraft travels to its target heliocentric orbit beyond Low Earth Orbit. Each sample is then rehydrated at the desired time points to reactivate the cells. A major risk in this mission is the loss of cell viability that occurs in the recovery period following the desiccation and rehydration process. Cell survival is essential for the detection of the biological response to features in the deep space environment, including ionizing radiation.

The aim of this study is to mitigate viable cell loss in future biosensors by identifying mutations and genes that confer tolerance to desiccation stress in *rad51*, a radiation-sensitive yeast strain. We initiated a screen for desiccation-tolerance after rehydrating cells that were desiccated for three years, and selected various clones exhibiting robust growth. To verify retention of radiation sensitivity in the isolated clones—a crucial feature for a successful biosensor—we exposed them to ionizing radiation. Finally, to elucidate the genetic and molecular bases for observed desiccation-tolerance, we will perform whole-genome sequencing of those *rad51* clones that exhibit both robust growth and radiation sensitivity following desiccation. The identification and characterization of desiccation-tolerant strains will allow us to engineer a biological model that will be resilient in face of the challenges of the deep space environment, and will thus ensure the experimental success of future biosensor missions.

The BioSentinel Mission

Primary Objective: Develop a biosensor with autonomous life support technology to study and compare the biological effects of space radiation in different orbital environments, including interplanetary space and the International Space Station.



BioSentinel nanosatellite displaying 18 internal fluidic cards

Multilayer 16-well fluidic card containing desiccated yeast

Value to NASA: Deep space radiation cannot be replicated in test facilities on Earth due to its unique flux and composition. For biological systems, the implications of prolonged exposure have yet to be described. This mission will fill a critical knowledge gap by examining the deleterious effects of ionizing radiation in space. The deep space radiation environment is a formidable challenge facing future missions that will send astronauts to Mars. The data collected by BioSentinel will arm NASA with knowledge in preparation for long-term human exploration and residence in deep space.

Pre-Launch SLS-Integration at KSC 6-9 mo. → Science Operations 6-12 mo.

Mission Risk: Viable cell loss following long term desiccation and acute rehydration stress. Previous viability studies indicate that 40% of wild type cells and just 4% of *rad51* cells survive the desiccation and rehydration process. Yeast samples must survive 13-22 months of desiccation under variable temperature conditions to ensure the success of the mission.

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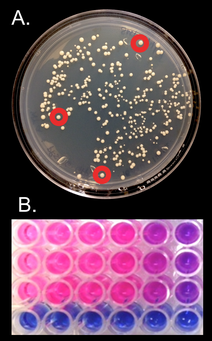
Desiccation-Tolerance Screen

Goal: Increase desiccation-tolerance of radiation-sensitive *rad51* yeast strain, and consequently improve cell viability, to mitigate risk in future biosensor missions.

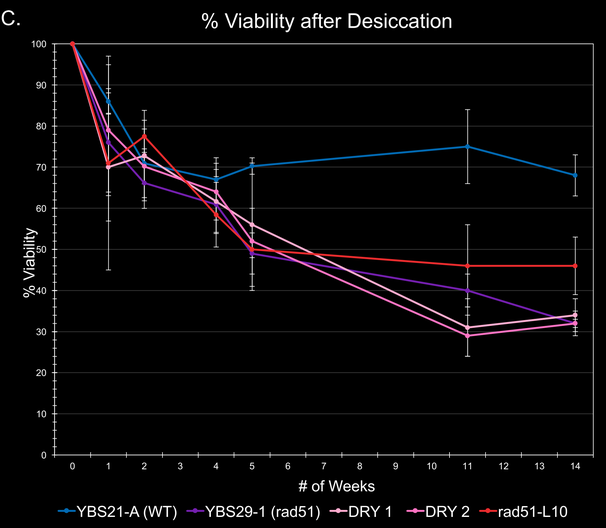


Methods:

1. *rad51* yeast samples (previously in a desiccated state for three years) were rehydrated and grown along with wild type and *rad51* controls and desiccation-tolerant *rad51* clones (A).
2. The largest colonies were selected (A), cultured, and desiccated by air drying in 10% trehalose for seven days.
3. Following the initial desiccation period, strains were rehydrated at various time points over several months. Viability was measured with viable cell counts, and growth, metabolism and radiation sensitivity were assessed with an alamarBlue dye reduction assay (B) (dye turns pink when cells are metabolically active).
4. Cells with an improvement in viability over *rad51* controls will be sent for whole-genome sequencing to identify mutations that may be responsible for conferring desiccation-tolerance. qPCR and RNA-seq will also be performed to analyze the adaptive response to desiccation stress.

Results: A sharp decrease in % cell survival was observed for all strains following the initial seven-day air-drying process, as expected (C). DRY1 and DRY2 (shown in pink) have similar desiccation-tolerance compared to the previously undesiccated control, YBS29-1 (*rad51*) (shown in purple). A previous screen had found DRY1 and DRY2 to have greater desiccation-tolerance than YBS29-1 (*rad51*). These clones were frozen and stored at -80C as glycerol stocks. However, upon regrowing the clones from the frozen stocks, they did not maintain their previously observed desiccation-tolerance. This disparity likely indicates that the observed desiccation-tolerance of DRY1 and DRY2 may be due to a sustained adaptive response rather than heritable genetic mutation. Following 10 weeks of desiccation, L10 (shown in red) exhibits greater viability than YBS29-1 (*rad51*), indicating superior desiccation-tolerance. This strain was also found to remain sensitive to gamma radiation, and to have growth and metabolism comparable to YBS29-1 (*rad51*), implicating it as a potential strain for use in future biosensor missions.

C.



Future Directions: The observed increase in cell viability of strain L10 will be confirmed under long-term "flight-like" conditions, and by repeating the screening procedure by regrowing the strain from the frozen stocks to look for heritability of this phenotype. If the phenotype persists, L10 will be subjected to whole genome sequencing to identify whether there are any mutations that may be responsible for conferring desiccation-tolerance. Transcriptomic changes of known desiccation-tolerance genes (e.g. hydrophilins, trehalose biosynthesis) will be tested in strain L10 by RNA-seq and qPCR experiments.

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Acknowledgements

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