BioSentinel: Improving desiccation tolerance of yeast biosensors for deep-space missions.

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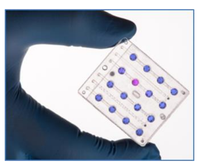
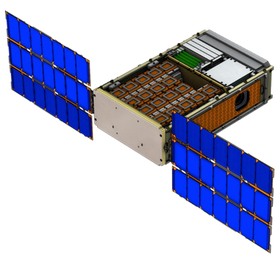
Introduction

Within a few decades, astronauts will be venturing past lower earth orbit (LEO) to asteroids and eventually Mars. One issue that astronauts will face is space radiation exposure. Space radiation consists of ionizing radiation from galactic cosmic rays and solar particle events, which eject heavy ion particles through space. Heavy ionizing radiation can split DNA and cause double-stranded breaks (DSB's), which increases mutagenesis and cancer risk. The purpose of the BioSentinel mission is to determine the biological effect of the radiation beyond LEO by measuring DNA damage and repair response to space radiation. We will use the budding yeast *Saccharomyces cerevisiae* (baker's yeast) as a biosensor to determine how deep-space radiation affects living organisms and to quantify the biological change induced by deep space radiation.

BioSentinel

- Satellite: 6U CubeSat
- Flight: Space Launch System Exploration Mission 1 (2019)
- Science payload: microfluidic cards with *S. cerevisiae*
- Yeast strains: wild-type and *rad51*
- *rad51:* Cannot efficiently repair double stranded DNA breaks from radiation exposure





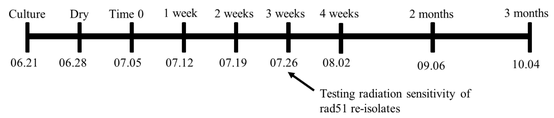
Credit: NASA

Desiccation Tolerance

- Desiccation can damage yeast cell and decrease viability
- Trehalose: a disaccharide found in many desiccation tolerant strains of *S. cerevisiae*
- Goal: identify genes potentially responsible for desiccation tolerance through screens of yeast cells and measuring intracellular trehalose content over time

Experimental Methods and Approach

- **Project 1**: Screening twenty *rad51* strains for higher desiccation tolerance mutants
- **Project 2:** Using intracellular trehalose measurements to screen for higher desiccation tolerant mutants
  Trehalose assay (Parrou and Francois, 1997; Tapia et al., 2015)



Preliminary Results

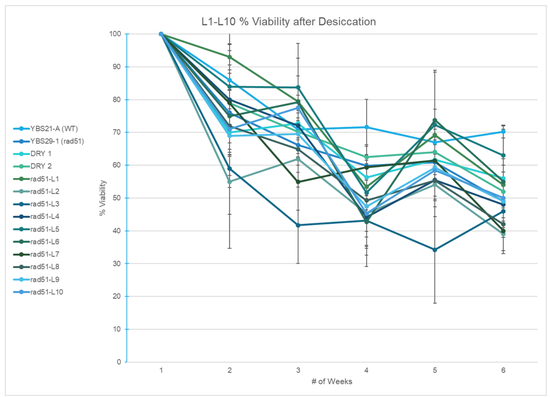


Figure 1. L1-L10 viability analyses of cell counts after desiccation. Percent viability was calculated by dividing average cell counts from each strain/isolate during each respective time point compared to average cell counts before desiccation. Fluctuations in percent viability are evident during this 6 week time period.

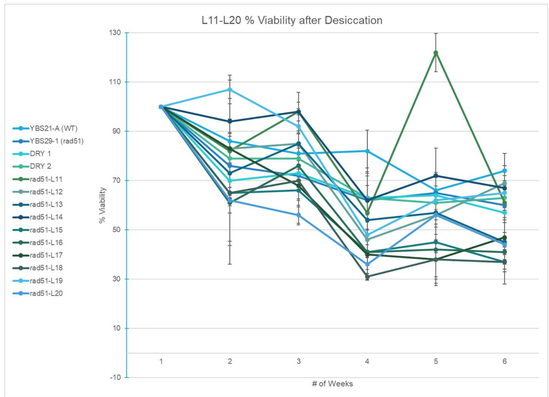


Figure 2. L11-L20 viability analyses of cell counts after desiccation. Percent viability was calculated by dividing average cell counts from each strain/isolate during each respective time point compared to average cell counts before desiccation. L14 is a potential candidate for desiccation tolerance due to its consistently high percent of survival. The peak during week 5 for isolate L11 is potentially due to human error through plating methodology.

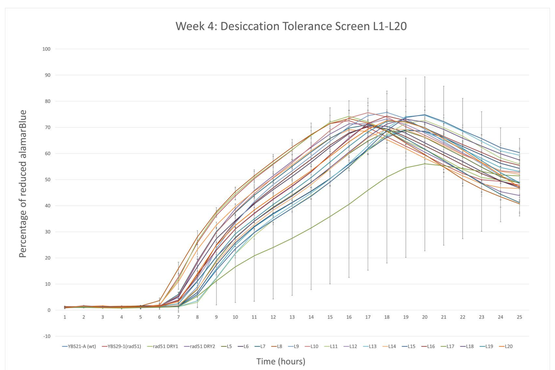


Figure 3. Percent reduction of alamarBlue for "Week 4". The absorbance data presented is for the control strains and 20 re-isolates of *rad51*. The slope corresponds to metabolic rate of reduction of alamarBlue. Our slope analyses show that only 25% of the strains (L4, L7, L8, L9, L10) were not significantly different ($p > 0.05$) than the rad51 control. The other 15 re-isolates had significantly different average metabolic rates than the rad51 control.

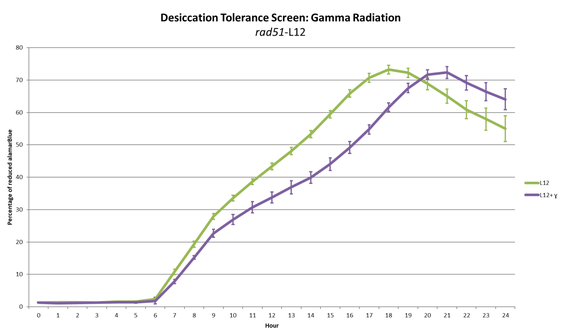


Figure 4. Percent reduction of alamarBlue for rad51-L12 with gamma radiation. Irradiating all cells with 10 Gy of Cs-137 gamma radiation for 13.33 minutes generated significantly different absorbance curves. For re-isolate L12, the irradiated cells required more time to metabolize alamarBlue, indicating that L12 is still sensitive to radiation.

Preliminary Results (cont.)

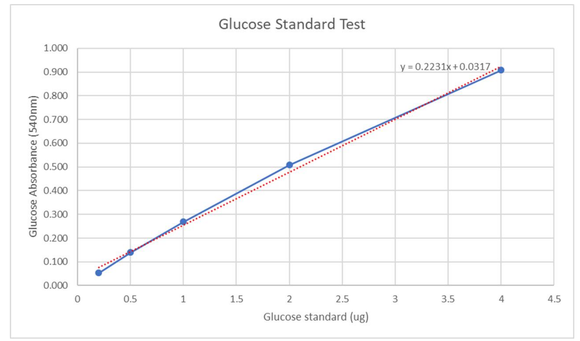


Figure 5. Glucose standard curve. Repeating the glucose standard tests with higher concentrations of glucose standard produced the curve in Figure 7. The linear regression formula shown within the figure was used to calculate intracellular trehalose concentrations within a test group of wild-type cells as shown in Table 1 (below).

Table 1. Intracellular trehalose content. Using the linear regression formula from Figure 5, intracellular trehalose concentrations were successfully calculated for the wild-type test cells.

Strain	Trehalose concentration (ug/mL)
wt-1 - 1x107	0.247
wt-1 - 5x107	1.158
wt-1 - 1x108	2.413
wt-1 - 2x108	4.161
wt-2 - 1x107	0.202
wt-2 - 5x107	1.218
wt-2 - 1x108	2.293
wt-2 - 2x108	4.183
wt-3 - 1x107	0.209
wt-3 - 5x107	1.315
wt-3 - 1x108	2.518
wt-3 - 2x108	4.437

Conclusions/Future Work

Testing the desiccation tolerance of twenty *rad51* re-isolates revealed that re-isolates L2, L5 and L14 are potential candidates for whole-genome sequencing. This study will continue for the next few months and other potential candidates might be indicative of desiccation tolerance. Furthermore, with the intracellular trehalose protocol that we have optimized, we will run a full study on determining how intracellular trehalose levels change during prolonged desiccation and determine which genes are responsible for enabling desiccation tolerance in *S. cerevisiae*.

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