The Transcriptional Response of Diverse *Saccharomyces cerevisiae* Strains to Simulated Microgravity

LILY S. NEFF¹,², SAMANTHA T. FLEURY³,⁴, JONATHAN M. GALAZKA⁵

¹Space Life Sciences Training Program, Wyle Labs, NASA Ames Research Center; ²Department of Biological Chemistry, Wesley College, Dover, DE; ³Universities Space Research Association, NASA Ames Research Center, Moffett Field, CA; ⁴Department of Biology, University of Virginia, Charlottesville, VA; ⁵Space Biosciences Division, NASA Ames Research Center
Stresses of Spaceflight

Isolation

Closed Environment

Distance from Earth

Space Radiation

Gravity Fields

Credit: NASA
Microgravity

- Space exploration missions place stresses on the space crew and their supporting microbial commensals.
- Reveal a conserved response to the stress of microgravity, measure physiological response.
Why yeast? Why *S. cerevisiae*?

**Yeast**
- Powerful microbial model
- Easy to grow and allows for transcriptomes to be recorded cheaply
- Part of human microbiota

**S. cerevisiae**
- Human colonizer
- Opportunistic pathogen
- Diverse set of strains are readily available
Environmental stress of SMG causes an increased growth of *Candida albicans* in filamentous forms\(^1\)
- Alteration in two genes associated with this hyphal transition
- Evidence of enhanced pathogenicity, fungal pathogen becomes more virulent

\(~300\) million year divergence from *S. cerevisiae*\(^2\)

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Studies conducted show that cells perceive and respond to variations in mechanical forces, i.e. gravity\(^1\).

S. cerevisiae, under SMG, demonstrates random budding than typical bipolar budding pattern\(^1\).

\(^1\)(Sheehan et al. 2007 BMC Genomics. Yeast genomic expression patterns in response to low-shear modeled microgravity)
Hierarchical clustering based on 600 growth rate variables

- clustering represents patterns
- shows diversity in phenotype and physiology

Saccharomyces sensu stricto isolates

Hierarchical clustered based on proliferation rates for 600 traits

Studies on lab strain, S288c

Species are indicated by line color, population by symbol color

Yeast are Diverse!
Central Objective: Reveal a conserved response across all strains or unique to lab strain, $S_{288c}$

How to accomplish this:

1. Screen yeast strains
2. Inoculate HARV vessels
3. RNA extraction
4. Construct libraries
5. Analyze Data
Screening Procedure: YPD Plate

- Create YPD plates (1% yeast extract, 2% peptone, 2% glucose) and YPD liquid culture
- Inoculate YPD plates with strains from cryogenic stock
- Observe for unusual growth (different morphologies):
  - YJM981 (flattened)
  - YJM1401 (wrinkled)
  - YJM996 (normal)
Screening Procedure: Liquid Culture

- Inoculate 5mL YPD broth from overnight “normal” cultures on YPD plates, incubate overnight
- Dilution 10µL:100µL to test OD$_{600}$ using NANODROP 2000 Spectrometer
- 24 hour incubation for microscopy check
- 48 hour incubation for HARV Vessels
Screening Procedure: Microscopy

Top Row (left to right):
- YJM1439 (West African, Clinical)
- YJM1388 (Sake, Non-clinical)

Bottom Row (left to right):
- YJM1248 (West African, Non-clinical)
- YJM627 (West African, Non-clinical)
## Results to Date

### Color Code Key:
- **Gray**: cryogenic stock DNE
- **Red**: Did not have normal phenotype, cannot use
- **Light Green**: Normal Phenotype so far; TBD
- **Dark Green**: All normal, including Microscopy Phenotype; can use in HARV vessel

<table>
<thead>
<tr>
<th>Strain</th>
<th>Clade</th>
<th>Plate</th>
<th>Flask</th>
<th>Microscope</th>
<th>Cleared for HARV</th>
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HARV Vessel Screening

Not cleared for HARV: Screening Stages

- No cryogenic stock: 9.60%
- Plate inspection: 15.40%
- Flask inspection: 11.50%
- Microscopy inspection: 63.50%

Not cleared for HARV: 46.40%
Cleared for HARV: 53.60%
Phylogeny of 94 S. cerevisiae strains based on similarities and differences in genetics.
Selected 34 *S. cerevisiae* strains:

- isolated from clinical and environmental settings
- multiple locations around the world to encompass evolutionary divergence

Credit: Strope et al. 2017 Cold Spring Harbor Laboratory Press. The 100-genomes strains...
High Aspect Ratio Vessel (HARV)

- Simulates microgravity conditions by rotating on vertical plane
- "functional weightlessness"*
  - randomizes the gravitational effect
  - minimizes turbulence (fluid undergoes irregular fluctuations) over surface of cell
- Remain suspended in liquid culture

Purity (degree of contamination) and quality (intactness/integrity) of RNA are essential for examining gene expression.

Degraded samples lead to misrepresentative data and inconsistency in reproducibility.
- Allow for generation of transcriptome information cheaply
- Allows for the investigation of known transcripts and new ones (important for the comparisons)
- Analyze physiology and phenotype
- Allows for the identification of conservation with gene expression profiles
Future Plans of Progression

- Complete simulated microgravity runs for the 32 strains (along with control experiments)
- Complete RNA Extraction and Illumina sequencing of samples using the KAPA mRNA HyperPrep Kit
- Send samples to be sequenced at UCSF and analyze data
Significance

- Systemic understanding of how microbes respond to simulated space flight environment
- Serve as a platform for future flight experiments
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