The Transcriptional Response of Diverse Saccharomyces cerevisiae Strains to Simulated Microgravity

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Background

Spaceflight imposes multiple stresses on biological systems resulting in genome-scale adaptations.

- Must understand in order to clarify and reduce the risks associated with spaceflight

Risk of infection by microbes present in spacecraft and microbial commensals

Previous studies of simulated microgravity have shown:

- Increased growth of *Candida albicans* in filamentous forms; with enhanced pathogenicity and increased virulence[4]
- *S. cerevisiae* does not demonstrate typical bipolar budding pattern, instead random[2]

Hierarchical clustering of *Saccharomyces sensu stricto* isolates demonstrates the lab strain, S288c, responds to 600 traits in an atypical manner[3]

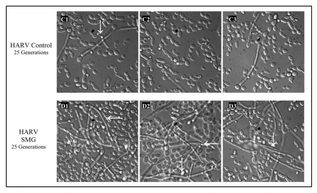


Figure 1 displays increased filamentous form of *Candida albicans*.

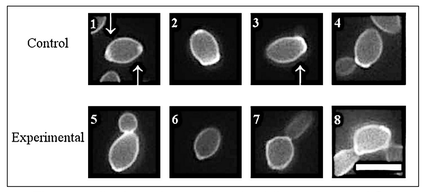


Figure 2 displays the random budding pattern of S. cerevisiae in simulated microgravity.

Objective

- **Determine if diverse *Saccharomyces cerevisiae* strains exhibit a conserved response to simulate microgravity.**

Method of Study

Simulated microgravity conditions using a High Aspect Ratio Vessel (HARV):

- Randomizes gravitational vector
- Cells experience a "functional weightlessness"
- Remain suspended in liquid culture

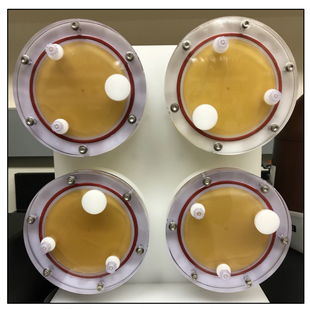


Figure 3 displays the HARV Vessel inoculated with S. cerevisiae strains in YPD Broth.

Transcriptional response will be documented using RNA-sequencing:

- Analyze physiology and phenotype indirectly
- Identification of conservation with gene expression levels
- Generate data quickly and cheaply to investigate known and new transcripts

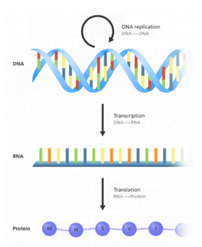


Figure 4 demonstrates the Central Dogma of Molecular Biology.

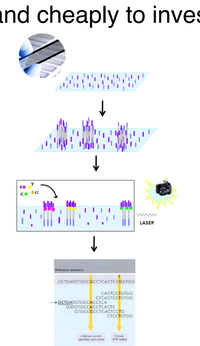


Figure 5 demonstrates an overview of the Illumina Sequencing System.

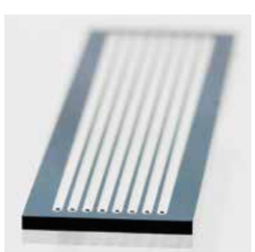


Figure 6 displays the eight lane flow cell which allows for simultaneous analysis.

Screening Procedure

YPD (1% yeast extract, 2% peptone, 2% glucose) **Plates:**

- Inoculate using cryogenic stock
- Observe for different morphologies

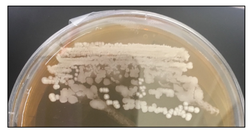


Figure 7 displays the flattened colony phenotype of YJM981.

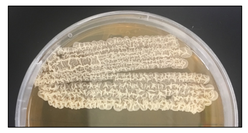


Figure 8 displays the wrinkled colony phenotype of YJM1401.

Figure 9 displays the normal colony phenotype of YJM996.

Liquid Cultures:

- Inoculate 5mL culture test tube overnight samples using "normal" cultures from the YPD plates
- Inoculate from culture test tubes to 250mL flasks to observe for aggregation; 24 hour incubation for microscopy check

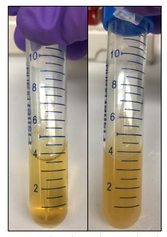


Figure 10 displays how the liquid cultures were inoculated and grown.

Microscopy:

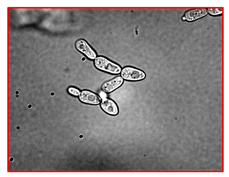


Figure 11 displays the microscopy result for YJM1439.

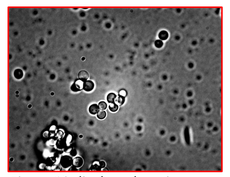


Figure 12 displays the microscopy result for YJM1388.

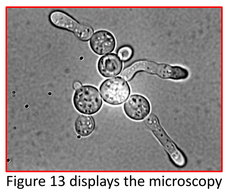


Figure 13 displays the microscopy result for YJM1439.

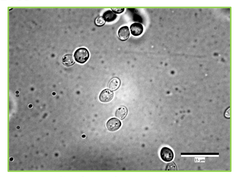


Figure 14 displays the microscopy result for YJM1248. It was cleared for HARV use based on the normal phenotype.

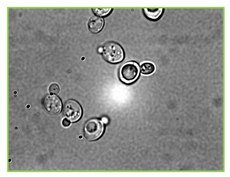


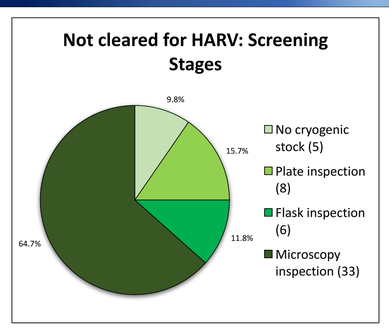
Figure 15 displays the microscopy result for YJM244. It was cleared for HARV use based on the normal phenotype.

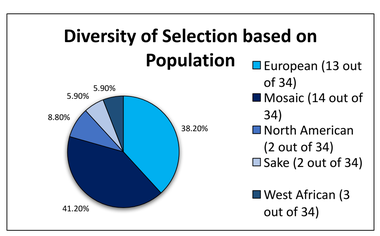
Organizing the Data:

Strain	Clade	Plate	Flask	Microscope	Clear for HARV
YJM248	European, Clinical				
YJM978	European, Clinical				
YJM993	European, Clinical				
YJM996	European, Clinical				
YJM990	European, Clinical				
YJM975	European, Clinical				
YJM981	European, Clinical				
YJM1447	Malaysian, Non-clinical				
YJM1190	Mosaic, Clinical				
YJM555	Mosaic, Clinical				
YJM470	Mosaic, Clinical				

Gray: no cryogenic stock, **Red:** did not pass as normal phenotype, **Green:** normal phenotype so far, **Dark Green:** all normal phenotype; clear to use for HARV Vessels

Strain Data





Forty-four strains cleared for HARV use
Selected *S. cerevisiae* strains:

- Isolated from clinical and environmental settings
- Multiple locations around the world to encompass evolutionary divergence

Controls:

- Salt Osmotic Stress Test
- HARV Vessels at 1*g* (horizontal orientation)

Techniques

RNA Nano LabChip Bioanalysis

- Analyzes purity (degree of contamination) and quality (intactness/integrity) of RNA
- Essential for examining gene expression
- Contamination leads to degradation of RNA samples and inhibition of enzymes
- RNA integrity is important for all mRNA species are represented in cDNA sample

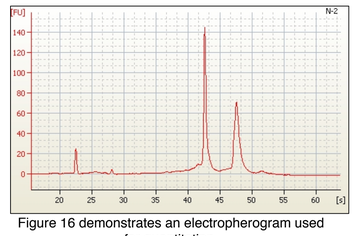


Figure 16 demonstrates an electropherogram used for quantitation.

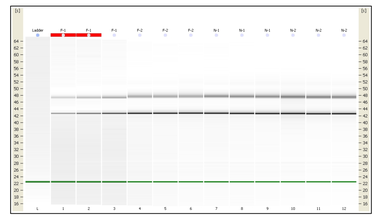


Figure 17 displays the gel used for visual inspection of RNA quality.

Protocols:

RNA Isolation

The Direct-zol RNA MiniPrep Kit instructions were completed with the following revisions:

- Mechanical lysis: 2 repetitions of 60 seconds and set at 4,200 oscillations/minute (60 second rest on ice between repetitions)
- 500 μL of 95% ethanol added to the homogenate
- Centrifugation was at 10,000 x *g*

Figure 18 displays the Direct-zol RNA MiniPrep Kit.

Quantification of RNA Samples

The Qubit RNA BR Assay Kit was used to provide an accurate method to quantify the twenty-four RNA samples from the salt osmotic stress test.

Figure 19 displays the Qubit RNA BR Assay Kit.

Library RNA-Seq Construction

The KAPA mRNA HyperPrep Kit was used for Illumina sequencing by constructing stranded mRNA-Seq libraries from 500ng of intact total RNA.

- Revisions will be made to the PCR amplification step (only twelve cycles were completed but more are necessary)

Figure 20 displays KAPA mRNA HyperPrep Kit.

Acknowledgements

- Financial support was provided through KBRWyle. I would like to thank the SLSTP program and the scientists of the lab I worked in.

References

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2. Sheehan et al. 2007 BMC Genomics. Yeast genomic expression patterns in response to low-shear modeled microgravity.
3. Warringer J, Zo¨rgo¨ E, Cubillos FA, Zia A, Gjuvsland A, et al. (2011) Trait Variation in Yeast Is Defined by Population History. PLoS Genet
4. Altenburg et al. 2008 Geno. Prot. Bioinfo. Increased Filamentous Growth of *Candida albicans* in Simulated Microgravity