The Transcriptional Response of Diverse Saccharomyces cerevisiae Strains to Simulated Microgravity

Lily S. Neff, Samantha T. Fleury, Jonathan M. Galazka

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.
- S. cerevisiae does not demonstrate typical bipolar budding pattern, instead random.

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

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.

Transcriptional response will be documented using RNA-seq:
- Analyze physiology and phenotype indirectly.
- Identification of conservation with gene expression levels.
- Generate data quickly and cheaply to investigate known and new transcripts.

Screening Procedure

YPD (1% yeast extract, 2% peptone, 2% glucose) Plates:
- Inoculate using cryogenic stock
- Observe for different morphologies

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.

Microscopy:

Organizing the Data:

Strain: Cline: Plate: Flask: Microscope: Clear for HARV
YRM248: European: Clinical: Green: Green: Green
YRM978: European: Clinical: Green: Green: Green
YRM993: European: Clinical: Green: Red: Red
YRM996: European: Clinical: Green: Red: Red
YRM990: European: Clinical: Green: Red: Red
YRM975: European: Clinical: Green: Red: Red
YRM981: European: Clinical: Green: Red: Red
YRM1447: Malaysian: Non-clinical: Green: Red: Red
YRM1190: Mosaic: Clinical: Green: Green: Green
YRM555: Mosaic: Clinical: Green: Green: Green
YRM430: Mosaic: Clinical: Green: Red: Red

Gray: no cryogenic stock, Red: did not pass as normal phenotype, Green: normal phenotype so far.

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

Strain Data

Diversity of Selection based on Population

- European (13 out of 14)
- West African (3 out of 14)

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

Techniques

RNA Nano LabChip Bioanalysis
- Analyzes purity (degree of degradation) 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.

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.

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.

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)

Acknowledgements

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

References

1. Stope et al. 2015 Genome Res. The 100-genomes strain, an S. cerevisiae resource that illuminates its natural phenotypic and genotypic variation and emergence as an opportunistic pathogen.