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Acta Astronautica 60 (2007) 460–471
ACTA ASTRONAUTICA
www.elsevier.com/locate/actaastro

Haploid deletion strains of *Saccharomyces cerevisiae* that determine survival during space flight

Kelly Johanson[a], Patricia L. Allen[a], Romer A. Gonzalez-Villalobos[a, b], Jacqueline Nesbit[c], Cheryl A. Nickerson[d], Kerstin Höner zu Bentrup[e], James W. Wilson[d], Rajee Ramamurthy[d], Riccardo D'Elia[a], Kenneth E. Muse[f], Jeffrey Hammond[a], Jake Freeman[g], Louis S. Stodieck[g], Timothy G. Hammond[a, b, h, *]

[a] *Department of Medicine/Section of Nephrology, Tulane University School of Medicine, Tulane Environmental Astrobiology Center, USA*
[b] *Tulane Hypertension and Renal Center of Excellence and Department of Physiology, USA*
[c] *Tulane Department of Biochemistry, USA*
[d] *Center for Infectious Diseases and Vaccinology, The Biodesign Institute, Tempe, AZ 85287, USA*
[e] *Program in Molecular Pathogenesis and Immunity, Department of Microbiology and Immunology, Tulane University School of Medicine, New Orleans, LA 70112, USA*
[f] *Coordinated Instrumentation Facility, Tulane University, New Orleans, LA 70118, USA*
[g] *BioServe Space Technologies, University of Colorado, Boulder, CO 80309, USA*
[h] *Southeast Louisiana Veterans Healthcare Network, New Orleans, LA 70112, USA*

Available online 13 October 2006

Abstract

This study identifies genes that determine survival during a space flight, using the model eukaryotic organism, *Saccharomyces cerevisiae*. Select strains of a haploid yeast deletion series grew during storage in distilled water in space, but not in ground based static or clinorotation controls. The survival advantages in space in distilled water include a 133-fold advantage for the deletion of PEX19, a chaperone and import receptor for newly- synthesized class I peroxisomal membrane proteins, to 77–40 fold for deletion strains lacking elements of aerobic respiration, isocitrate metabolism, and mitochondrial electron transport. Following automated addition of rich growth media, the space flight was associated with a marked survival advantage of strains with deletions in catalytically active genes including hydrolases, oxidoreductases and transferases. When compared to static controls, space flight was associated with a marked survival disadvantage of deletion strains lacking transporter, antioxidant and catalytic activity. This study identifies yeast deletion strains with a survival advantage during storage in distilled water and space flight, and amplifies our understanding of the genes critical for survival in space.
Published by Elsevier Ltd.

Keywords: Yeast; Deletion series; Suspension culture; Space flight

1. Introduction

The cellular response to environmental stimuli is mediated by phenotypic changes ultimately driven by alterations in gene expression [1–3]. We examine the phenotypic and genotypic changes that are dictated by the unique conditions that exist in space, conducting a

* Corresponding author. Department of Medicine/Section of Nephrology SL-45, Tulane University School of Medicine, 1430 Tulane Avenue, New Orleans, LA 70112, USA. Tel.: +1 504 556 7225; fax: +1 504 988 1909.
E-mail address: thammond@tulane.edu (T.G. Hammond).

0094-5765/$ - see front matter Published by Elsevier Ltd.
doi:10.1016/j.actaastro.2006.09.011

genomic study using the yeast, *Saccharomyces cerevisiae*, as a model system. Three major factors make this the optimal reagent for analysis of the genes which determine survival in space: *S. cerevisiae* has grown well in flight experiments [4,5], novel reagents are available to answer such questions in this organism [6–9], and the regulatory mechanisms that are operative in yeast cells are largely conserved throughout evolution [1,10].

As part of a large-scale effort to examine the function of all yeast genes, a consortium of investigators created a series of heterozygous and homozygous diploid strains and haploid strains of both mating types, each containing a deletion of a single gene allele [7–9]. A selectable marker that carries adjacent gene specific oligonucleotide sequences replaces this gene. These sequences serve as an identifier of the locus essentially acting as a barcode. The individual strains can then be pooled so that all are represented in equimolar amounts, and grown en mass [7–9]. The result of this genomic approach to a selective growth condition has been coined "fitness profiling" and has advantages over conventional genetic screens that frequently have unavoidable biases [7–9]. Additionally, as almost all of the known open reading frames (ORFs) are represented in the pool, regardless of having a known function, (96% of known ORFs) this methodology presents, essentially, a saturation genetics approach.

Aliquots of haploid yeast deletion series stored quiescent in distilled water were flown in space on space shuttle Atlantis mission STS-112 in October 2002, inoculated into rich growth media by the computer driven automated hardware, and the growth stopped by automated addition of a fixative. Ground controls included clinorotation and static replicates in hardware identical to the flight equipment. This article reports the genetic and structural analysis of these samples.

2. Materials and methods

2.1. Strains, media and chemicals

The *S. cerevisiae* haploid deletion series (MAT a his3D1, leu2D, met15D ura3D) was from Invitrogen (Carlsbard, CA). Culture and media components were obtained from Difco (Detroit, MI). All oligonucleotides were synthesized by Qiagen (Valencia, CA). All other reagents were from Sigma (St. Louis, MO) unless noted.

2.2. Haploid deletion pool

Ninety six well plates containing the haploid deletion series were thawed and 10 μL transferred from each well into 990 μL of fresh YPD. The cultures were grown at 25 °C with mild agitation for 4–5 days. OD_{600} was measured at the end of the growth period and used to calculate the cell concentration. Aliquots equivalent to 3×10^7 cells were removed from each tube and combined together in a 500 mL sterile flask. The pool was gently swirled to mix, then aliquoted into a series of 50 mL conical tubes and stored at 4 °C.

2.3. Cell preparation

Aliquots of 3×10^6 cells were removed from the haploid pool and pelleted by centrifugation at 3000 rpm for 5 min in 1.5 mL microcentrifuge tubes. The cell pellets were washed with sterile dH_20 and each was resuspended in 1 mL sterile dH_20. Cells were loaded immediately into flight tubes and stored at 4 °C.

2.4. Fluid processing apparatus (FPA) loading

The flight tubes known as FPAs are glass barrels surrounded by a Lexan sheath. Each glass barrel can be divided into three chambers using stoppers, and fluid can be delivered with high volumetric accuracy via pass throughs between chambers using a computer driven motor. Before autoclaving, each glass barrel, rubber stoppers, and mixers were lightly coated with silicone lubricant (Sigmacote, Sigma, St. Louis, MO). A rubber stopper was placed about 1 inch from the bottom of the glass barrel, before the hardware was wrapped in foil and autoclaved at 121 °C for 20 min.

FPA assembly was performed in a laminar flow hood. First, 3 mL of 1.1X YPD media was added to the glass barrel. The mixer was then inserted and placed to remove all air in the media chamber. Next, the top and middle chambers were loaded with 1 mL of RNA Later II and the cell suspension respectively. Finally, each glass barrel was loaded into a Lexan sheath to form a FPA, and was pressure tested and loaded into a container known as a group activation pack or GAP, which holds eight FPAs to facilitate simultaneous activation. Each GAP was stored at 4 °C until transfer to the shuttle or the incubator for ground controls. A motor in the center of the GAP between the FPAs was computer controlled to activate and stop the experiment by screwing a plate down to push the syringes of the FPAs, moving reagents between chambers via pass through channels. The entire volume was temperature controlled initially at 37 °C to facilitate other experiments in the hardware volume, then at 24 °C for the yeast experiments. Clinorotation [11,12] and static ground control were