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Osmotic Stress Survival and Thermal Tolerance in *Saccharomyces cerevisiae*

Tiffany Fraser

ABSTRACT

Saccharomyces cerevisiae (yeast) is a single-celled eukaryotic model organism, often used to study more complex eukaryotic systems. When exposed to a stressor, yeast cells can respond in a variety of ways including the expression of various Heat Shock Proteins (HSPs) and the initiation of the MAP and HOG pathways. This study tested whether selection by one environmental stress improves the ability of yeast to thrive in subsequent stressful environments. Stressors included hyperosmotic and hyperthermic conditions. The yeast's responses were monitored by comparing the absorbance values that were measured in the Spectronic Genesys 2 spectrophotometer. Results revealed that there was an increased absorption difference (ie: growth rate) in the cells that experienced the 1.8 M osmotic stressor followed by the temperature stressors at 27°C and 35°C. This shows that yeast cells have similar response mechanisms for different environmental stressors, and that, therefore, selection for resistance to one stressor may inadvertently select for resistance to another.

Keywords: *multiple-stress resistance, Saccharomyces cerevisiae, osmotic stress, thermal tolerance*

INTRODUCTION

Appropriate cellular environments are essential for cell growth. When a cell's environment is not considered to be optimal, a cellular response occurs within the cell in order to avoid cell death (Gasch, 2014). These responses have been studied extensively across many forms of life, and "are essential for survival" (Shor, et al., 2013).

Saccharomyces cerevisiae are single-celled eukaryotic model organisms, commonly known as yeast. These cells are small in size, with a short generation time, yet still manage to have the complex internal cell structures that are found in all eukaryotes (Duina, et al., 2014). Yeast cells have been used to study many different fields and subfields ranging from synthesis of complex biofuels to fundamental biological questions (Gasch, 2014). Yeast has been used recurrently in the science community, because they are safe to work with, have been well characterized, and are customizable, in the sense that a scientist can choose which specific strain or genotype to use (Gasch, 2014).

There are many different types of stress that have been induced on yeast cells, including temperature change by heat shock or low temperature environments, nutrient change by starvation or overexposure to an amino acid, glucose, etc, exposure to chemicals such as oxidizing agents, and osmotic stress (Gasch and Werner-Washburne, 2002). When exposed to increased osmolarity, *S. cerevisiae* react to the stress by evoking environmental stress responses, ranging from loss of turgor, cell shrinkage, and eventually growth arrest (Gasch, 2014). The study of stress response in yeast is important today, because the "implications of cellular stress responses to human physiology and diseases are manifold" (Fulda, et.al, 2010). In recent years, scientists have been conducting experiments to select for a multiple-stress resistant phenotype of *Saccharomyces cerevisiae* (Botstein and Gerald, 2011).

There is interest in these advancements for many reasons. One example is in industrial yeast, where multiple stress resistance is a preferable trait "because practically all biotechnological processes and traditional food-manufacturing processes expose cells to simultaneous or sequential combinations" of various environmental stressors. In these contexts stress is seen in the production of baker's yeast, baking, brewing, distiller's fermentations, and wine making (Çakar, et al., 2005).

As model organisms, yeast cells have been found to respond to stressful environments much like other larger eukaryotic organisms (Yang et al., 2006; Mager and Siderius, 2002). By focusing on the molecular mechanisms associated with stress response and cell death, a correlation could be found in other organisms or previous studies done on yeast cells by scientists around the world (Botstein and Gerald, 2011; Duina, et al., 2014). In recent years, studies have correlated mechanisms in yeast cells to mammalian cells; these advancements have led to other studies in cancer, DNA, and drug research (Botstein and Gerald, 2011; Yang, et al., 2006).

This study uses yeast cells as model organisms to see if selection for hyperosmotic stress survival yields increased hyperthermic tolerance in *Saccharomyces cerevisiae*. Various NaCl concentrations are used as the osmotic stressor and four different temperature environments are used as the thermal stressor. With these stressors a two part

process is conducted to see if a resistance phenotype occurs.

MATERIALS AND METHODS

This experiment was conducted in the Biology Research lab in the Melhorn Science Hall of McPherson College. A pilot study was first carried out to identify the most stressful osmolarity environment. The yeast growth broth for the pilot study was prepared by adding 3.00g of Yeast Extract and 5.00g of Bacto Peptone in 1 L of DI water, stirred until completely dissolved. The broth was then distributed by 100 mL increments into ten 250 mL Erlenmeyer Flasks along with the designated amount of NaCl to create the various osmotic environments (control, 0.2 M, 0.4 M, 0.6 M, 0.8 M, 1.0 M, 1.2 M, 1.4 M, 1.6 M, and 1.8 M). The solutions were then autoclaved and .010 g of Fleischmann's Bread Machine Yeast was added to each Erlenmeyer flask. The yeast were grown on the New Brunswick Scientific C24 Incubator Shaker at 100r.p.m. and 27.0°C for 48 hours. The absorbance of each flask was measured at 600nm every hour until samples reached 1.00 A.

The yeast broth was then prepared as previously discussed along with the appropriate amount of NaCl to create the 1.8 M environment. The molarity was verified using the Vapro Osmometer. The broth measured out to be 1.7 M. The broth was then distributed in 10 mL increments to 40 test tubes, the test tubes were autoclaved and .010 g of yeast were added to each test tube. The yeast were cultivated on the New Brunswick Scientific C24 Incubator Shaker at 100r.p.m. and 27.0°C for 24 hours. Each tube's absorbance at 600nm was measured at 0 hours and 24 hours using the Spectronic Genesys 2 spectrophotometer.

The consecutive stress environment was prepared by designating four temperature-controlled incubators to various temperatures (18°C, 27°C, 35°C, 40°C) and by preparing 0.6 M NaCl yeast broth. The yeast broth was prepared just as the 1.8 M broth was; however, the NaCl amount varied. Ten mL of 0.6 M NaCl yeast broth were transferred into 40 test tubes. Tubes of yeast growth medium with 0.6M NaCl were then inoculated with 0.010 mL samples from the previous 1.8 M culture fractions. Measurements of the cultures' absorbance were again taken at 0 hours and 24 hours.

RESULTS

The data shown in Figure 1 is a visual representation of the data collected from a Two Way Analysis of Variance. The Two Way ANOVA showed that there was a significant interaction in the absorbance values within the temperature stressor (p= < .001) and in the absorbance values following both the osmotic and the temperature stressors (p= < .001). There was not a significant difference in the osmotic absorbance values alone (p= .244).

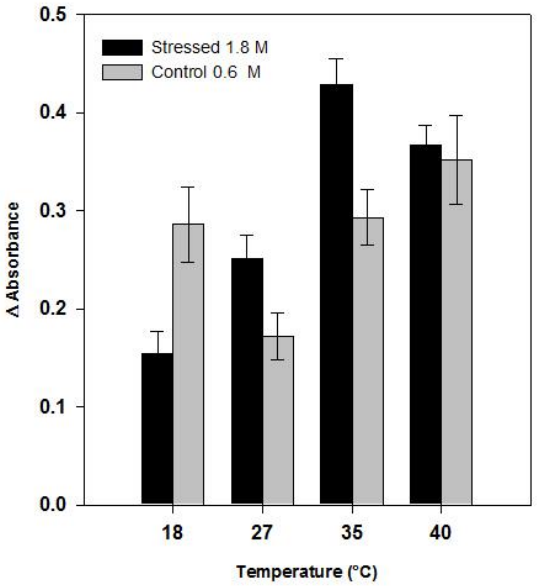

Figure 1. Comparisons between growth rates in stressed and controlled yeast cells after the 1.8 M osmotic and subsequent temperature stressors. Error bars represent the standard error of the least squared means across ten tubes per temperature condition.

Table 1. Two Way ANOVA results ran for stressed and control group yeast cell data after temperature and osmotic stressors.

Source of Variation	DF	SS	MS	F	P
Osm	1	0.0122	0.0122	1.378	0.244
Temp	3	0.4180	0.1390	15.692	<.001
Osm x Temp	3	0.200	0.0667	7.517	<.001
Residual	72	0.6390	8.8700 e-3		
Total	79	1.2690	0.0161		

Figure 1 and Table 1 show that there is an increased absorption difference/ growth rate in the cells that experienced both the 1.8 M osmotic stressor and the temperature stressors at 27°C and 35°C.

DISCUSSION

Cells have shown resistance phenotypes in experiments dealing with multiple stressors including oxidative, freezing–thawing, high-temperature and ethanol stress (Çakar, et al., 2005). The cells that grew throughout the freeze-thawing, also grew throughout the high temperature stressor; however,

the combination of osmotic and thermal stressors is yet to be studied (Çakar, et al., 2005). Thus, the focus of this study was to observe the effect of consecutive osmotic and thermal stressors on *S. cerevisiae*. The data collected shows that the upshift in osmolarity played a role in the cell's ability to survive in two of the four thermal stressors.

The effects of osmotic stress on yeast cells are observed within a mechanism designated for osmotic stress response, "a mitogen-activated protein (MAP) kinase cascade the high-osmolarity glycerol (HOG) pathway which orchestrates part of the transcriptional response" (Hohmann, 2002). These pathways are ubiquitous in eukaryotic cells; the HOG pathway in these cells is "stimulated by an osmotic upshift" (Hohmann, 2002). This initial tolerance to the 1.8 M NaCl stressor and MAP/HOG pathway response could be a mechanism that leads to the thermal tolerance in the subsequent heat stressors in *S. cerevisiae.*

The heat shock response is arguably "one of the most powerful adaptation mechanisms", which is "a highly conserved program of changes in gene expression that result in the repression of the protein biosynthetic capacity and the induction of a battery of cytoprotective genes encoding the heat shock proteins" (Verghese, et. al., 2012). A variety of HSPs work to "protect thermally damaged proteins from aggregation, unfold aggregated proteins, and refold damaged proteins or target them for efficient degradation" (Verghese, et. al., 2012). Heat shock proteins are a mechanism that cells use when stressed by both osmotic and thermal stressors; "HSP90 is an ubiquitous molecular chaperone, which plays a crucial role in maturation and activation of a wide array of client proteins under normal and stress-related conditions" (Raman, et. al, 2015). The expression of HSP90 could be a possible mechanism attributed to the yeast's thermal tolerance in the 27°C and 35°C environments. This mechanism could be tested by using microarrays- testing for heat shock protein, a technique similarly carried out by Yang et al., 2006. Yang and colleagues found that "Hsp90 is shown to be required for proper adaptation to high osmolarity via a novel signal transduction pathway that operates parallel to the HOG pathway and requires Cdc37p;" thus, showing that perhaps after both stressors *S. cerevisiae* could be tested in this way.

The data acquired from this experiment can be applied to further advancements in other eukaryotes undergoing stressful environments, to experiments dealing with yeast stress resistance and it's correlation to HSP90, as well as to the possible inheritability of the multiple-stress resistance phenotype (Botstein and Gerald, 2011). The conclusions made may be linked to the possible cell mechanisms and HSP90 dependence shown in virulence, fungal development, and animal health (O'Meara and Cowen, 2014; Roberts, et al., 2010).

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