The effect of salinity stress on cell count of *Saccharomyces cerevisiae*

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Abstract

Saccharomyces cerevisiae can live in a variety of stressful environments, including varying salinities, making it an ideal model organism to test effects of NaCl concentrations on growth rate. Our experiment was carried out over a nine-hour period and was started by diluting the prepared sample of 400μL of 4M NaCl in YPD into three group concentrations, with four test tubes in each group: 0M NaCl, 1M NaCl and 2M NaCl, with a total of 12 test tubes incubated at 35°C water bath. After increments of 90 minutes, 100μL from each test tube was pipetted into a micro centrifuge tube, in addition to 10μL fixative for further analysis under the microscope. The initial cell count was 3.34×10^5 cells, 3.12×10^5 cells, and 2.81×10^5 for 0M, 1M and 2M NaCl, respectively. The control group experienced a rapid cell proliferation with the final cell count as 9.80×10^6 cells. In contrast, 1M and 2M did not experience similar cell division with the final count as 9.46×10^5 cells and 3.10×10^5 cells respectively. The results suggest that exposure to 1M and 2M NaCl concentrations impair cellular functions and higher salinity has a negative impact on cell count.

Introduction

Saccharomyces cerevisiae is a unique single celled eukaryotic fungus that can tolerate and reproduce in environments of varying pH levels and salinity (Logothetis 2007). *S. cerevisiae's* ability to tolerate a range of sodium chloride (NaCl) concentrations can aid in the determination of the optimal NaCl concentration for maximum cell growth (Schuller 1994). In recent studies, researchers used marine yeast strains for industrial applications (Zaky et al. 2014). The Earth is 70% saltwater; understanding how salinity effects cell growth could aid in better utilization of natural resources (Plummer et al. 2012). Based on this information we proposed the following hypotheses:

H_O - Salinity concentrations will not affect the cell count of *Saccharomyces cerevisiae* over time.

H_A - Salinity concentrations will affect the cell count of *Saccharomyces cerevisiae* over time.

We predict that salinity will affect cell count of *S. cerevisiae* over time because we suspect the placement of *S. cerevisiae* into NaCl solution subjects the organism to osmotic stress (Logothetis 2007). Dehydration caused by NaCl stress leads to improper assembling of actin patches which are critical for cell division (Smith 2001). Figure 1 shows proper cell division with correct assembling of actin patches. Their depolarization can lead to unequal cell division between daughter cells, further resulting in decreased cell division and potential hindering of cell proliferation.

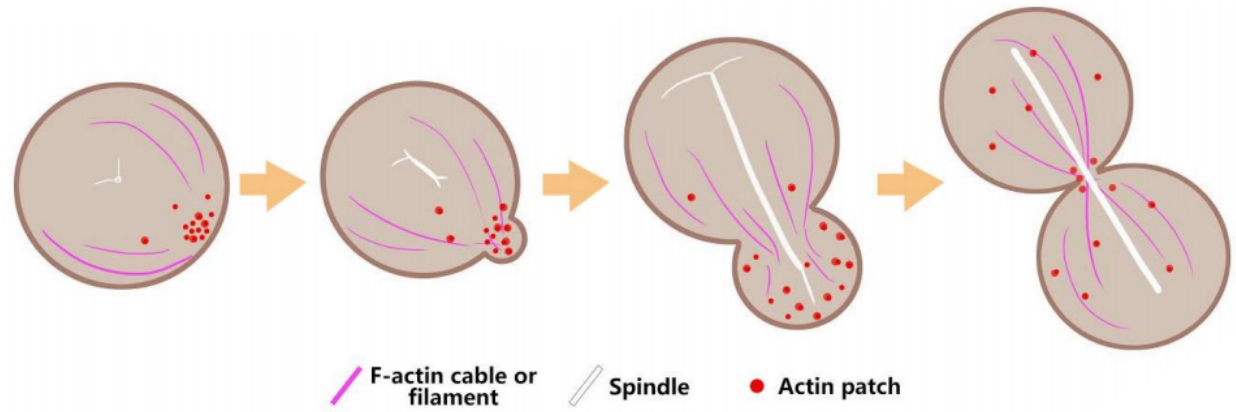

Figure 1. Cell Division: formation of actin patches for proper cell division, resulting in two daughter cells.

However, even though the cell may be subjected to the stress, we still expect an increase in overall cell count; this is due to the production of trehalose, a sugar made of two glucose units. Trehalose aids in stress regulation by accumulating underneath the cell membrane and protecting the biomolecules by maintaining cell membrane integrity as shown in Figure 2 (Iturriaga 2009). This protects the cell from shrinkage and allows the cell to survive under high stress environments.

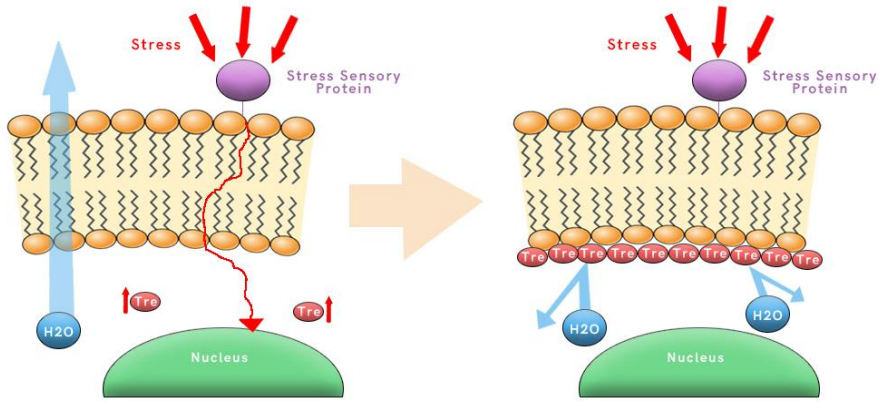

Figure 2. Trehalose model: detection of stress signals causes the nucleus to actively transcript for trehalose. Newly made molecules have signal peptides to be translocated under/within the bilayer. Causing: increased membrane strength, decreased permeability, decreased H_2O diffusion outwards, and decreased chance of shrinkage for the cell.

We expect an increase in overall cell count for all our conditions, however cells exposed to NaCl will proliferate at a slower rate, resulting in less of an increase in cell count.

Methods

Yeast culture

We were provided with 30,000µL of *S. cerevisiae*, 10,000µL of 4.0 M NaCl yeast extract peptone dextrose (YPD) and 30,000µL neutral YPD. The YPD medium contained 20g Tryptone (pancreatic digest of casein), 10g of yeast extract, and 20g of Dextrose.

Procedure

The initial yeast solution was diluted to reach the minimum cell count of $2x10^5$ cells per mL to be viewed in the hemocytometer.

We then proceeded to make the different concentrations required for the experiment. For the 0M control group, we added 1000µL of stock solution of *S. cerevisiae* and 1000µL YPD. To make the 1M experimental group, we added 500µL 4.0M NaCl, 500µL neutral YPD, and

1000µL stock solution of *S. cerevisiae*. Lastly, the 2M group was made by adding 1000µL stock solution of *S. cerevisiae* and 1000µL 4.0 NaCl. The above steps were repeated four times for each of the control and the two experimental groups (Figure 3).

For the next nine hours, we placed the 12 test tubes in a rack for a water bath incubated at 35°C. At 90-minute intervals, we took out the test tubes, vortexed, and prepared the micro centrifuge tubes with 100µL sample, and 10µL fixative. We extracted three pseudoreplicates from each of the four replicates for the three different concentrations, as depicted in Figure 3.

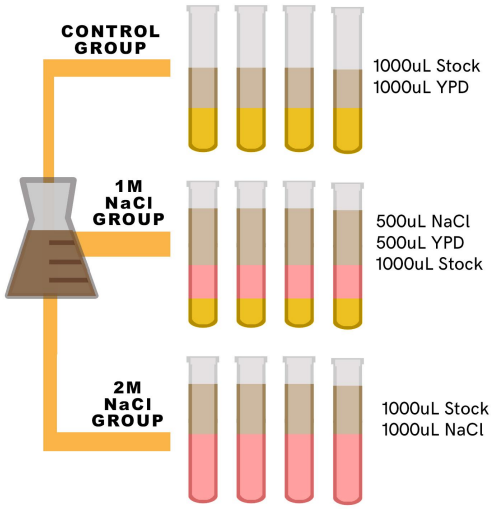

Figure 3. Experimental setup diagram. Diagram of the experimental setup: three experimental groups, four replicates of each concentration.

We placed the fixed samples under an Axiostar microscope and counted the cells with a haemocytometer. For a more accurate population count, we counted a minimum of 100 cells in their respective grids. We averaged the three pseudo replicates for each replicate. The mean of the replicates was then used to represent the overall concentration of the group. Figure 4 and Figure 5 are graphical representation of the total cell count; the number and size of the grids

were recorded and photos were taken of the cell culture with a DinoXcope (Figure 6). A one-way ANOVA test was conducted on the data.

Results

Average initial cell counts were 3.34 x 10^5 cells, 3.12 x 10^5 cells, and 2.81 x 10^5 cells for 0M, 1M, and 2M NaCl respectively. While the three treatments initially had comparable cell counts, by 180 minutes differences were present. The cell culture grown in the control treatment (0M NaCl) had the largest increase in cell numbers. By the end of the experiment, the average values of the cell counts were 9.80 x 10^6 cells, 9.46 x 10^5 cells and 3.10 x 10^5 cells for the 0M, 1M, and 2M NaCl respectively.

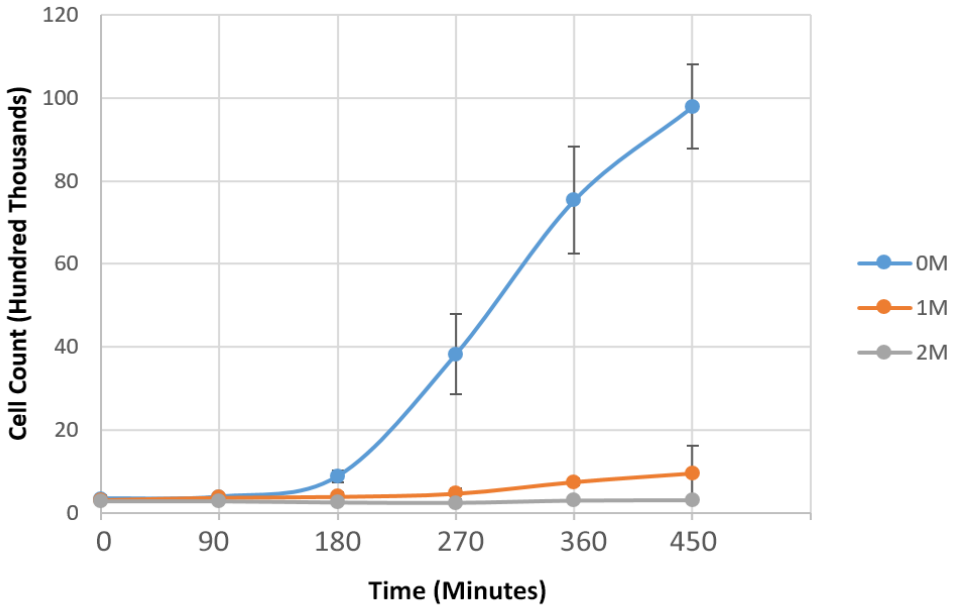

Figure 4. The cell count of *S. cerevisiae* in varying salt concentrations of 0M NaCl, 1M NaCl, and 2M NaCl over 450 minutes. N=4 for each treatment. p-value = 0.036 between groups.

On closer examination of the results of 1M NaCl and 2M NaCl treatments, the 1M NaCl treatment underwent a similar pattern of cell growth that 0M NaCl had – albeit on a smaller scale. The 1M NaCl treatment's cells appeared to exit lag phase and enter log phase by 270

minutes. The 2M NaCl cells in contrast, hover in equilibrium around the 3.00 x 10^5 cells, seemingly never leaving the lag phase of their growth cycle seen in Figure 5, which is a magnification of Figure 4. As a stark contrast to the other two treatments, that there was an initial decrease in cell count for the 2M culture until 350 minutes.

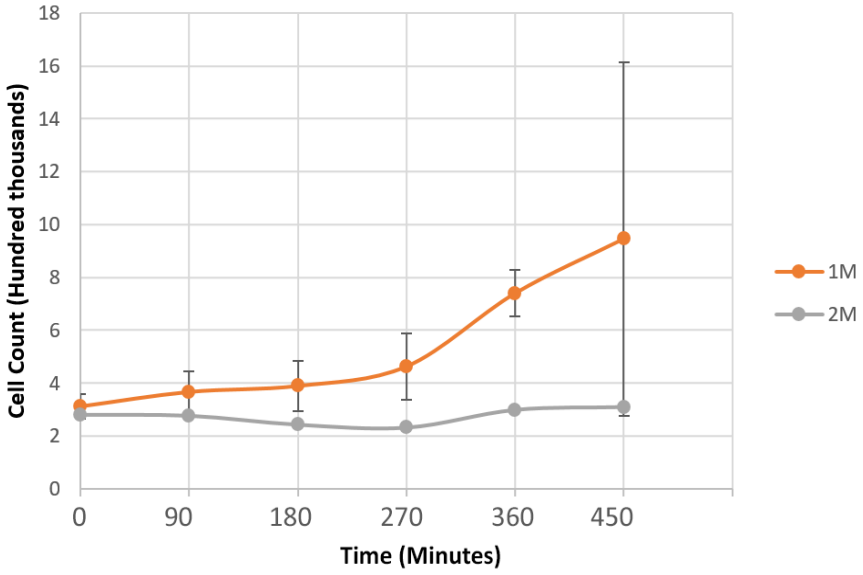

Figure 5. The cell count of *S. cerevisiae* in varying salt concentrations of 1M NaCl and 2M NaCl over 450 minutes. This is a magnification of Figure 4 for clarification.

During the experiment, we noted that the yeast cells from zero to 180 minutes were well distributed. However, after time 270 minutes, many cells were grouped in pairs (Figure 6).

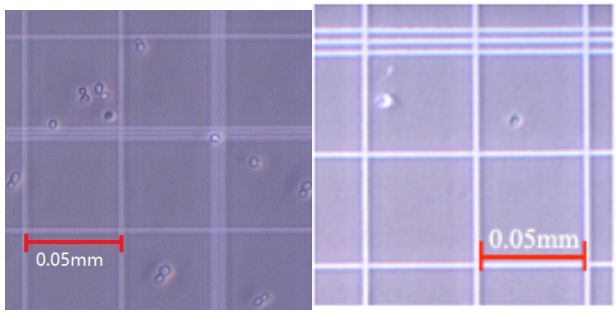

Figure 6. Cell images under DinoXcope at 270 minutes (100x magnification). (Left) doubled cells observed at 9-hour mark for control culture, suggesting cell division; (right) singled cells observed at 9-hour mark for 2M NaCl culture. Image taken by Serena Wang.

The one-way ANOVA test yielded a p-value of 0.036. This is a significant value as it is less than the required 0.05.

Discussion

Overall

Our results suggest a negative correlation between salinity and cell growth. The one-way ANOVA test yielded a p-value of 0.036 and since it is less than 0.05, we can verify that it is statistically significant. This conclusion allows us to reject the null hypothesis and support the alternative hypothesis that the concentration of NaCl does affect the cell count of *S. cerevisiae* over time. The control *S. cerevisiae* culture had the highest cell count of 9.80×10^6 cells, and a doubling period of 77.39 minutes, whereas a decrease in overall cell count was observed in 1M and 2M experimental groups.

In an experiment conducted by Sherman (2002), a doubling time of 90 minutes was reported when a wild-type culture was placed in YPD medium at 30°C. However, since our culture was incubated at 35°C, the lag phase was shortened. This could result in a faster doubling time for our control group, given the optimal growth temperature for *S. cerevisiae* is between 32.3°C - 35°C (Salvadó et al. 2011).

Figure 4 illustrates a more gradual growth curve and lower cell count in the 1M and 2M experimental groups compared with the 0M control culture. The total cell count for the 2M group 2 was 2.81×10^5 cells, suggesting this treatment may not have entered the log phase.

Our results are consistent with previously published literature, Andre et al. (1991) and Abdel et al. (2010), both suggest that higher salinity would have a negative influence over the cell growth of *S. cerevisiae*.

Cellular Physiology

Sodium is essential for cell growth and takes part in many fundamental cellular mechanisms. The concentration of Na^+ cations across the membrane establishes an osmotic gradient, and generates membrane potential for other metabolic pathways to function. By introducing salinity shock, the gradient was disturbed. To counterbalance the stress, water diffuses out of the *S. cerevisiae*'s cytoplasm, causing osmotic shrinkage and dehydration. In addition to the disrupted homeostasis of membrane ion microenvironment, cell division was also expected to be hindered by osmotic dehydration. Smith (2001) proposed that osmotic dehydration causes depolarization of actin patches. Young et al. (2004) showed that during the budding of *S. cerevisiae*, the actin patches are required to assemble and disassemble rapidly. Therefore, impaired actin assembly would hinder the cell from accomplishing cell division, resulting in only singlet cells. No budding cells were observed in Figure 6 (right), which in turn provides a possible explanation for the much lower overall cell count in 1M and 2M NaCl groups in Figure 4.

In the preliminary growth stage for the 2M NaCl treatment, we observed a decrease in total cell count - indicating that the cells were dying between time 0 and time 2. Huh et al. (2002) reported that programmed cell death (PCD) occurred after the yeast culture was exposed to 1.5M NaCl medium for 1 hour. Huh et al. (2002)'s study further suggests that clonogenic survival rate decreases drastically due to DNA fragmentation caused by salt shock. Since our 2M

experimental group had an even higher salt concentration than used in the study by Huh et al. (2002), we expect similar PCD trends. Results suggest a decrease in total cell count was caused both by PCD and osmotic shrinkage. However, integrity of the cells is maintained by self-rescue mechanisms and the upregulation of osmo-instigated gene expression of certain molecules.

Trehalose and Osmotic Stress Protection

Trehalose is a sugar often found in plants, animals, and microorganisms such as *S. cerevisiae*. Its main function is to store carbohydrates as energy source and release glucose when the cell is in need (Higashiyama, 2002). However, in *S. cerevisiae*, trehalose also functions as a stress protection molecule, which accumulates during stress-induced adaptation (Meikle et.al, 1991). Trehalose accumulation aids the cell membrane in maintaining its integrity, preventing osmotic shrinkage, and stabilizing membrane bound proteins (Iturriaga 2009). The transcription of trehalose is stress regulated. Upon NaCl shock, the ionic balance across the membrane is disturbed and consequently a stress signal is sensed by the transcription factor, causing upregulation of trehalose shown in Figure 2 (Ren et.al, 2011). This may explain why our control group appeared to have entered log phase by 120 minutes, whereas in 1M NaCl culture, log phase started by 180 minutes – the ongoing processes of transcriptional modification takes time.

Figure 5 shows that our 2M NaCl culture did not follow the same trend as the 1M sample, and seemingly had a prolonged lag phase. According to Reina-Bueno et.al (2012), the total trehalose concentration increased by 2.8-fold in 2.5M NaCl condition, however the overall accumulation of the molecules remained too low to counterbalance the osmotic pressure. It is possible that the *S. cerevisiae* strain provided could not tolerate salinity as well; hence at 2M

NaCl the accumulation of trehalose was unable to counterbalance the salinity stress, therefore inhibiting readjustment and rapid proliferation.

This finding provides insight into possible future studies, an inspection of the genetic level should be conducted, focusing on osmoregulated transcription factor at 1M and 2M NaCl concentrations.

Relevant errors

Although our experiment showed promising results and had similar outcomes to previously published literature, there were still potential sources of error. Initially, when we were making the stock solution, we had difficulties deciding on the final volume of *S. cerevisiae* to be added into the YPD. Our ideal initial cell count should be approximately $2x10^5$ cells, however after the dilution; we counted 3.34×10^5 cells. This suggests possible pipetting errors and inaccurate calculations. The sample count could have been biased, despite our efforts to try to randomize the grids on the haemocytometer. Lastly, we encountered sample shortages for recounting; as a result, we were unable to verify some inconsistencies in our raw data.

Conclusion

The one-way ANOVA test yielded a $p\text{-value} < 0.05$, which allowed us to reject our null hypothesis and support the alternative hypothesis. As predicted, the salinity did affect the cell count of *S. cerevisiae* over time. Experimental data suggests that *S. cerevisiae* proliferates most efficiently under minimum salinity, maximizing cell count and further enhancing our knowledge on the *S. cerevisiae* in the maricultural and biofuel industry.

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