

The effects of glucose concentration on the growth rates of wild type and PDC1 mutant *Saccharomyces cerevisiae*

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Abstract

The PDC1 gene in *Saccharomyces cerevisiae* accounts for a portion of pyruvate decarboxylase enzyme activity during fermentation (Flikweert *et al.* 1996). In our experiment we wanted to determine whether the presence of a mutation in the PDC1 gene influences the effects of increased glucose concentration on the growth rate of *S. cerevisiae*. To do this, we incubated wild-type and mutant strains of *S. cerevisiae* for 24 hours in a 30°C water bath in two different growth media: control (standard, 1X glucose YPD medium) and treatment (2X glucose YPD medium). Then we calculated the growth rates of each cell type in each growth culture using the cell counts taken at scheduled time intervals. We used the two-factor ANOVA to test the statistical significance our results. We found that the growth rate of the wild type was greater in both treatments with an average growth rate of 9.5×10^{-3} cells/min compared to 4.9×10^{-3} cells/min of the mutant (p -value= 9.2×10^{-7}). There was no statistically significant difference in the growth rates of *S. cerevisiae* in different glucose concentrations (p -value=0.771548). Further, we did not see any statistically significant interaction between effects of the mutation and the increased glucose concentration (p -value=0.69927). The PDC1 mutation decreased the growth rate of *S. cerevisiae*; however, increasing glucose concentration of standard YPD medium did not affect the growth rate of wild type and mutant strains of *S. cerevisiae*.

Introduction

Naturally occurring *Saccharomyces cerevisiae* have been found on the surfaces of plants, insects, and gastrointestinal tracts of warm-blooded animals as well as in soils from around the world (Landry *et al.* 2006). As the first eukaryote with a completely sequenced genome and the unprecedented ease at which it can be manipulated, *S. cerevisiae* is an important model organism in biological and biomedical research for cell regulation and genetics studies (Sherman 2002).

Saccharomyces cerevisiae grow in anaerobic and aerobic environments in the presence of simple sugars, using them as a source of energy (Sherman 2002). In anaerobic conditions, *S. cerevisiae* undergo fermentation, a process that is used in beer

brewing to make the beverage alcoholic. In our study, we wanted to investigate the factors that affect this process.

Fermentation is a process that converts pyruvate, a product of glycolysis of sugars, into carbon dioxide and ethanol (Figure 1). It involves a crucial step where a group of pyruvate decarboxylase (PDC) isoenzymes encoded by *PDC1*, *PDC5*, and *PDC6* genes convert pyruvate into acetaldehyde and carbon dioxide (Kellerman *et al.* 1986). The objective of our study was to determine the significance of PDC activity on the growth rate of *S. cerevisiae* exposed to high glucose concentration.

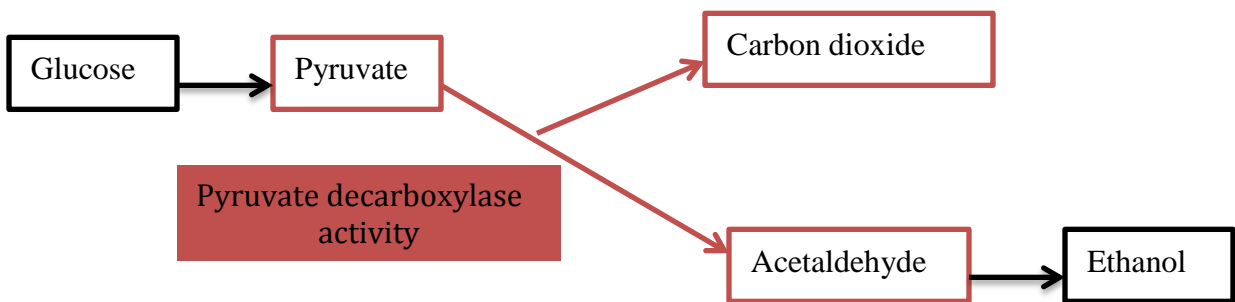


Figure 1. Fermentation in *Saccharomyces cerevisiae*

In our study, we exposed a wild-type strain of *Saccharomyces cerevisiae*, BY4741a, and a mutant strain, YLR044c, to two different glucose concentrations and compared their growth rates. The mutant strain has a deletion of the *PDC1* gene, resulting in decreased PDC activity (Flikweert 1996). We grew the two strains of *S. cerevisiae* in 1X glucose YPD medium and 2X glucose YPD medium. YPD medium is a culture medium for *S. cerevisiae* that contains the necessary nutrients for optimal growth

(Sherman 2002). We used the standard YPD medium for our 1X glucose YPD medium and doubled the glucose concentration for our 2X glucose YPD medium.

Schaaf *et al.* (1989) showed that *PDC1* deficiency in *Saccharomyces cerevisiae* resulted in a 55% decrease in PDC activity compared to that of a wild-type control. Thus, our first alternate hypothesis is the presence of the mutation lowers the growth rate of *Saccharomyces cerevisiae*. Our first null hypothesis is the presence of the mutation increases or has no effect on the growth rate of *Saccharomyces cerevisiae*.

Flikweert *et al.* (1997) showed that excess glucose concentration increased the rate of glycolysis in *Saccharomyces cerevisiae*. Higher concentrations of pyruvate may increase the rate of fermentation. An increase in fermentation leads to an increased energy yield for growth. Thus, our second alternate hypothesis is, *Saccharomyces cerevisiae* grown in 2X glucose YPD medium will have a greater growth rate than those grown in 1X glucose YPD medium. Our second null hypothesis is, *Saccharomyces cerevisiae* grown in 2X glucose YPD medium will have a lower or the same growth rate as those grown in 1X glucose YPD medium.

Under aerobic conditions and in high concentrations of glucose, *Saccharomyces cerevisiae* undergo fermentation and not oxidative phosphorylation, a phenomenon described as the “Crabtree effect” by De Deken (1966). Since the *PDC1* mutation lowers PDC activity, we can expect the mutation to cause *S. cerevisiae* to react differently to the increased glucose concentration than the wild type with normal PDC activity. Thus, our third alternate hypothesis is, there will be an interaction between the effects of increased glucose concentration and of the presence of *PDC1* mutation on the growth rates of *Saccharomyces cerevisiae*. Our null hypothesis is there will be no interaction between the

effects of increased glucose concentration and of the presence of the *PDC1* mutation on the growth rates of *Saccharomyces cerevisiae*.

Methods

We calculated the original concentrations of the wild-type and mutant stock solutions to be 7.92×10^{-7} cells/mL and 5.75×10^{-7} cells/mL, respectively. We prepared three replicates of 10 mL of 2.5×10^5 cells/mL for each cell type in 1X glucose YPD (control) medium and 2X glucose YPD medium (treatment). This procedure is outlined in Figure 2.

For the wild-type control, labeled WTC, we pipetted 10 mL of 1X glucose YPD medium into three 15-mL test tubes and using a micropipette, replaced 31.6 μ L with the wild-type stock solution. Similarly, for the mutant control, labeled MC, we pipetted 10mL of 1X glucose YPD medium into three 15-mL test tubes and replaced 43.5 μ L of it with the *PDC1* mutant stock solution.

For wild type treatment, labeled WTT, we pipetted 5 mL of 1X glucose YPD medium into three 15-mL test tubes and replaced 31.6 μ L of it with the wild-type stock solution. Then we added 5 mL of 3X glucose YPD medium so the glucose concentration in the treatment medium would be double that of the control. Similarly for the mutant treatment, labeled MT, we placed 5 mL of 1X glucose medium, replaced 43.5 μ L of it with the mutant stock solution and added 5mL of 3X glucose YPD medium.

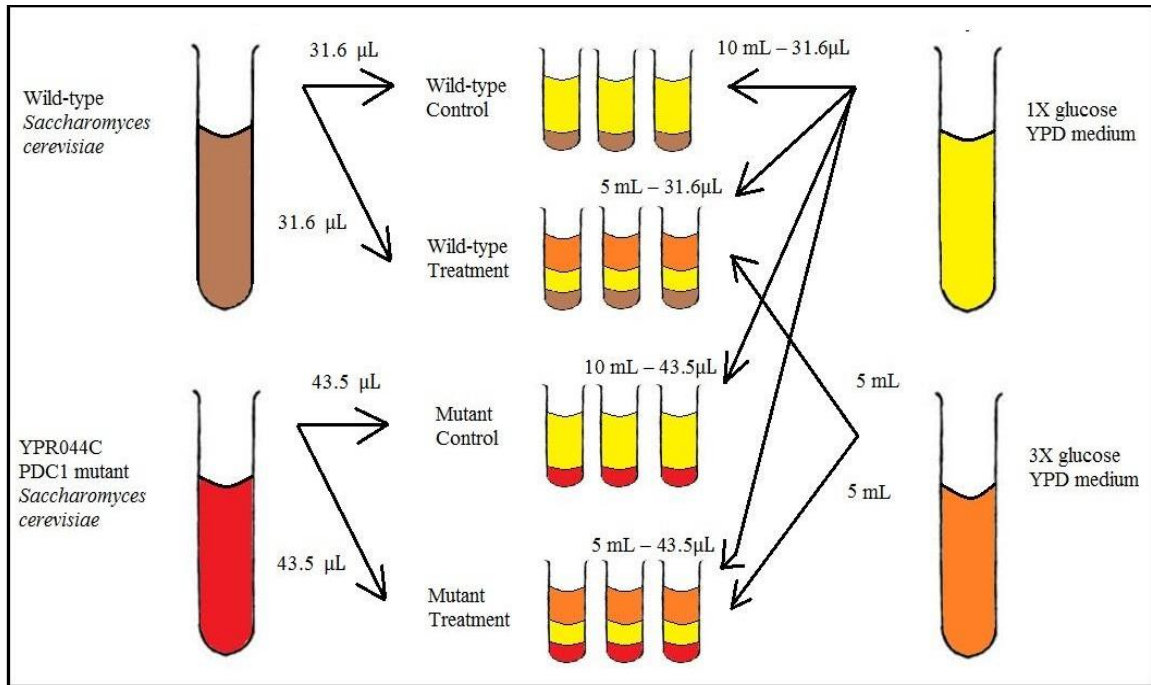


Figure 2. Description of the preparation of cell culture replicates for wild type control, wild type treatment, mutant control and mutant treatment.

To grow our cultures, we incubated the 12 replicates of wild-type and mutant *Saccharomyces cerevisiae* in a 30°C water bath for 24 hours and counted the number of cells present at time(t) = 0, 30, 90, 210, 450 and 1,440 minutes. At each time, we removed our samples from the water bath and vortexed each test tube to ensure that the cells were evenly distributed in the media. We then pipetted out 100 µL of our samples into separate 1.5 mL microcentrifuge tubes and added 10 µL of Prefer to fix the cell number. We pipetted 10 µL of the content in each microcentrifuge tube onto a haemocytometer and counted the cells present using an Axio Star microscope and a Dino-Lite USB Digital Microscope (see Figure 3). We counted at least 30 cells.

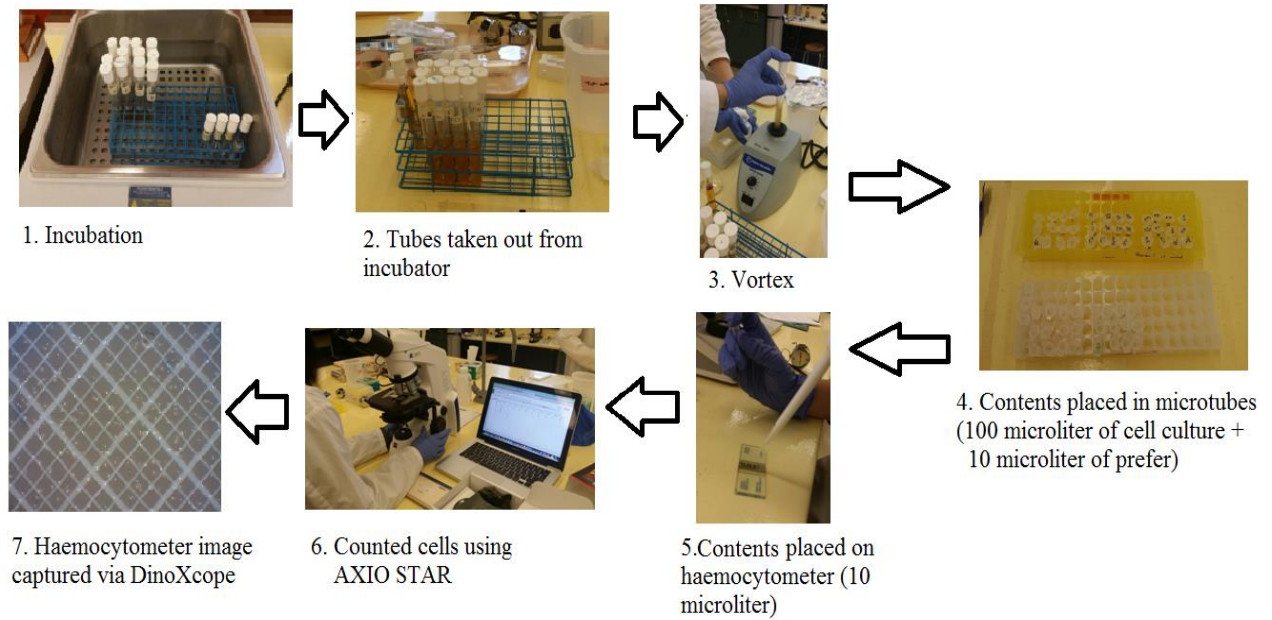


Figure 3. Sample collection and cell counting method.

To analyze our data, we separately plotted the cell counts of each of the 12 replicates of wild-type and mutant *Saccharomyces cerevisiae* on a semi-log graph. Using Excel, we fit exponential best-fit lines for each cell type grown in the different concentrations of glucose. We obtained the growth rates of each replicate from the equation of the best-fit line, given in the general form: $N(t) = N_0 e^{kx}$, where k is the growth rate in cells/min. We then used the two-factor ANOVA with replication on Excel to determine statistical significance in the differences in growth rates between cell types, glucose concentrations and the interaction of the two factors.

Results

We can see in Figure 4 that there is an exponential increase in both wild-type and mutant *Saccharomyces cerevisiae* in both 1X and 2X glucose YPD media. It is also clear

from Figure 5 that the growth rates of the wild type in both concentrations of glucose are greater than that of the mutant. The growth rates for wild-type control, mutant control, wild-type treatment and mutant treatment were 9.6×10^{-3} cells/min, 5.0×10^{-3} cells/min, 9.3×10^{-3} cells/min and 5.0×10^{-3} cells/min, respectively. We excluded the cell counts at $t=1,440$ minutes because the time between $t=450$ and $t=1,440$ minutes was beyond the log phase of growth.

The error bars for both graphs show the 95% confidence intervals. In both figures, we see that the error bars of wild-type *S. cerevisiae* for treatment and control overlap with each other and those of the mutant *S. cerevisiae* treatment and control overlap with each other. In both cases, the spread between the replicates do not seem very large.

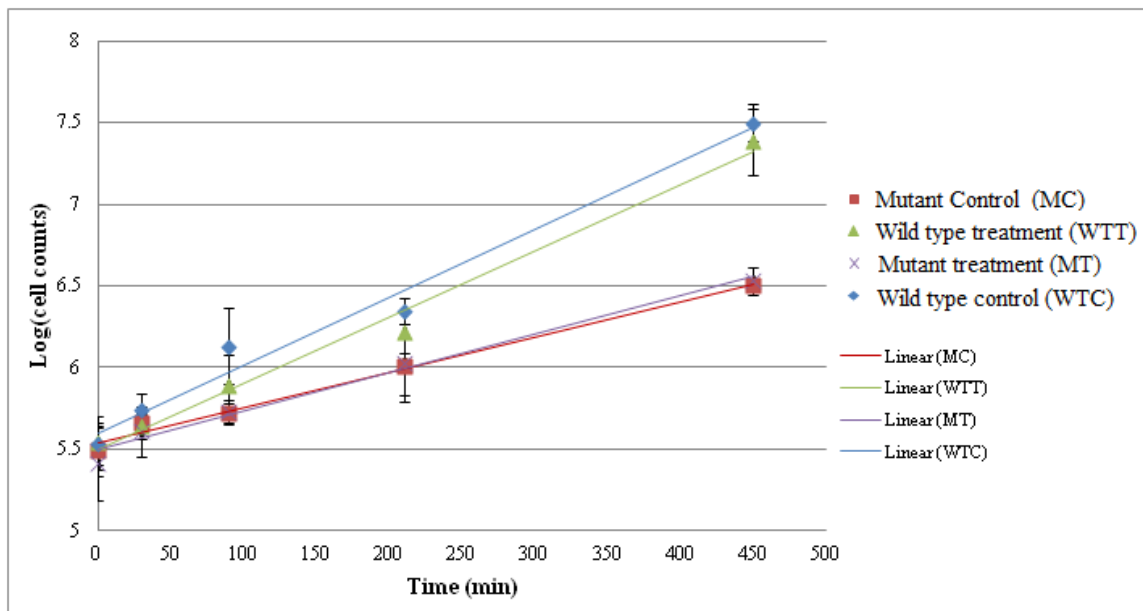


Figure 4. The logarithm of average cell counts of wild-type and mutant *Saccharomyces cerevisiae* in standard (control) and 2X glucose (treatment) YPD media from $t=0$ to $t=450$ minutes. Error bars are the 95% confidence intervals. (MC: mutant control, WTT: wild-type treatment, MT: mutant treatment, WTC: wild-type control)

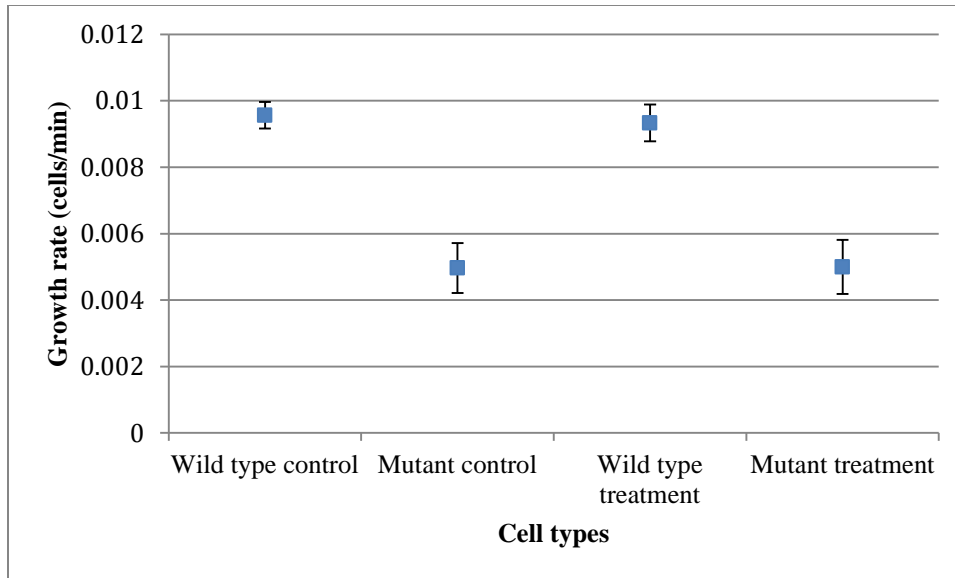


Figure 5. The average growth rate of wild-type and mutant *Saccharomyces cerevisiae* in 1X (control) and 2X glucose (treatment) YPD media from t=0 to t=450 minutes. Error bars are the 95% confidence intervals.

Using the two factor ANOVA with replication, we obtained p -values of 9.2×10^{-7} , 0.771548 and 0.69927 for hypothesis one, two and three respectively. The p -value for hypothesis one, between cell types, is less than $p=0.05$ while the p -values for hypotheses two and three, between treatments and interaction respectively, are greater than $p=0.05$.

The cell cultures were initially brown with pellets at the bottom of the test tubes. Once we diluted the cell cultures, the color changed from brown to a lighter brown with a yellow tint. We saw no visible changes in the cultures until t=1,440 minutes. Pellets reappeared at the bottom of the test tubes, as can be seen in Figure 6a. There was an increase in turbidity observed after vortexing the cell cultures, demonstrated by the cloudiness of the culture in Figure 6b.

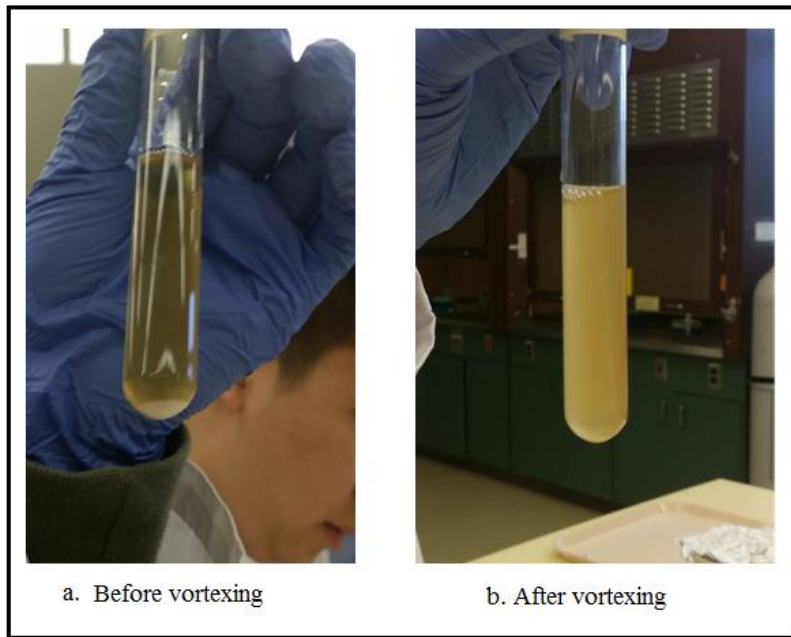


Figure 6. a) Pellet visible at the bottom of test tube of WTC replicate 1, at t=1,440 minutes. b) Turbidity visible in test tube of WTC replicate 1, at t=1,440 minutes.

Discussion

A p -value of 9.2×10^{-7} between cell types indicates that the difference seen between the growth rates of the wild-type and mutant strains is statistically significant. As can be seen in Figure 5, the average growth rate of wild type was 9.5×10^{-3} cells/min and that of the mutant was 4.9×10^{-3} cells/min. This provides support for our first alternate hypothesis, that the presence of the mutation decreases the growth rate of *S. cerevisiae* and thus we reject our first null hypothesis. The decrease in the growth rate of *S. cerevisiae* mutant was likely due to the decrease in fermentation caused by the decrease in PDC activity (Flikweert *et al.* 1996). This is in agreement with the findings of Schaaf *et al.* (1989) who showed a 55% decrease in pyruvate decarboxylase activity in *PDC1* deficient mutants. Thus, the deletion of *PDC1* gene decreased the growth rate of *S. cerevisiae*.

A *p*-value of 0.771548 between different glucose concentrations suggests that the difference in the growth rates of *S. cerevisiae* between the control and treatment was not statistically significant. The growth rates of the mutant grown in 1X and 2X glucose concentrations were the same— at 5.0×10^{-3} cells/min. The growth rates of the wild type grown in 1X glucose YPD medium was higher at 9.6×10^{-3} cells/min than that of the wild type grown in 2X glucose YPD medium, which had a growth rate of 9.3×10^{-3} cells/min. However, our analysis showed that the probability of this difference being due to the difference in glucose concentration is less than 5%. Thus, we fail to reject our second null hypothesis, that *Saccharomyces cerevisiae* grown in 2X glucose YPD medium will have a lower or the same growth rate as those grown in 1X glucose YPD medium.

There may be a glucose concentration that causes the pyruvate to be saturated, resulting in the growth rate being limited by pyruvate decarboxylase activity. We see a similar saturation of enzymes in oxidative respiration, where excess glucose concentrations resulted in the generation of pyruvate at an amount exceeding the capacity of oxidative breakdown (Fiechter and Gmünder 1989). Since we did not see a difference in the growth rates between *S. cerevisiae* grown in 1X and 2X glucose YPD media, we infer that the concentration of glucose in our 1X glucose YPD medium already saturated pyruvate decarboxylase. Thus, doubling the concentration of standard YPD medium did not result in an increase in the growth rate of *S. cerevisiae*.

With a *p*-value of 0.69927, we failed to reject our third null hypothesis as there was not a significant degree of interaction found between the effect of glucose concentration on growth rates and the presence of the *PDC1* mutation in *Saccharomyces*

cerevisiae. This indicates that the variables of glucose concentration and cell type were independent of one another.

There are sources of uncertainty that could have affected our results. One source could be our decision to double the standard YPD medium for our treatment. This is because YPD medium is designed for the culturing of *S. cerevisiae*, so the glucose concentration in the 1X glucose YPD medium was already high enough that glucose was no longer a limiting factor in growth (Sherman 2002). This high glucose concentration would saturate the pyruvate decarboxylase in the 1X control, so increasing the glucose concentration in the 2X treatment would not have a significant effect on the growth rate.

A potential future experiment could be to determine whether a decrease in the glucose concentration of standard YPD medium would result in a lower growth rate in wild-type and mutant *Saccharomyces cerevisiae*. Doing so would help us determine whether there was actually a saturation of pyruvate decarboxylase in our 1X glucose YPD medium. If *S. cerevisiae* grown in lower glucose concentrations have a lower growth rate than those grown in standard YPD medium, we could provide support for the presence of pyruvate decarboxylase saturation in our study.

Conclusion

We rejected our first null hypothesis, with our findings indicating higher growth rates in wild-type *Saccharomyces cerevisiae* when compared to mutant *Saccharomyces cerevisiae* in both control and treatment, likely due to reduced pyruvate decarboxylase activity in the PDC1 mutant. We failed to reject our second null hypothesis because there was no statistically significant increase in the growth rate of *Saccharomyces cerevisiae*

grown in 2X glucose YPD medium treatment. This was probably caused by growth rate limitations resulting from pyruvate decarboxylase saturation due to high glucose concentrations. We also failed to reject our third null hypothesis as we found that there was no statistically significant interaction between the effects of glucose concentration and the presence of the PDC1 mutation on the growth rate of *S. cerevisiae*.

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