The fermentation rate of *PDC1* mutant and wild-type *Saccharomyces cerevisiae* as measured by carbon dioxide production at 30ºC

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Abstract

Saccharomyces cerevisiae is a unique organism because of its ability to perform both aerobic and anaerobic fermentation. The objective of our experiment was to compare and analyze the fermentation rates of the wild type and the *PDC1* mutant of *S. cerevisiae* by measuring the level of CO_2 produced per cell at 30ºC. Both the mutant and wild-type forms were grown in YPD media and incubated at 30ºC; the rate of fermentation was measured at regular intervals. 95% confidence intervals of the mean level of CO_2 production per cell for mutant and wild-type yeast as well as the t-test analysis provided us with sufficient evidence to statistically reject our null hypothesis and provided us with support for the alternate hypothesis: the CO_2 production level of wild-type *S. cerevisiae* is greater than that of the *PDC1* mutant at this temperature. Thus, it can be concluded that the wild type is more efficient in fermentation as it has a higher rate of CO_2 production.

Introduction

Saccharomyces cerevisiae is a unique eukaryotic microorganism, also called yeast, which can thrive under aerobic and anaerobic conditions (Ter Linde *et al.* 1999). The objective of our experiment was to compare the fermentation rate of wild-type and mutant *S. cerevisiae* in order to ascertain the effect of mutation on the *PDC1* gene of yeast. The experiment will be done by measuring levels of CO_2 production under the optimal temperature of 30°C in 75 minutes

S. cerevisiae is useful in various food and fuel industries because of its ability to ferment ethanol (Berlowska *et al.* 2009, Figure 1). While it is able to ferment at 15°C to 35°C, its optimal temperature range ranges from 25°C to 30°C (Torija *et al.* 2003).

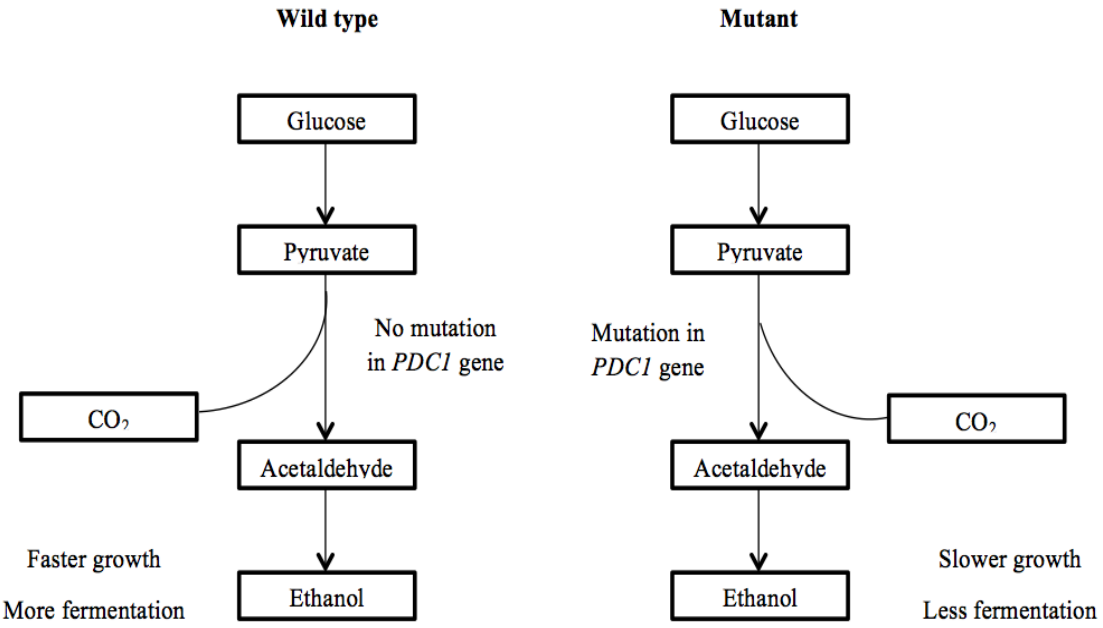

Figure 1**.** Simplified version of ethanol fermentation pathway for wild-type and mutant yeast (Morton 1995).

Pyruvate decarboxylase (PDC) is one of the key enzymes used in ethanol fermentation, and it allows for decarboxylation of pyruvate, which produces CO_2 and acetaldehyde (Berlowska *et al.* 2009). Seeboth *et al.* (1990) stated that if the *PDC1* gene that codes for PDC was affected by a deletion mutation, there would be a decrease in translation of PDC to around 60% to 70% of wild type's activity. They also declared that this activity of the mutant is due to the presence of a *PDC5* gene. The *PDC5* gene codes for the isoenzyme that can replace the *PDC1* gene product as a catalyst. However, when compared to PDC, it is 20% less effective (Hohmann and Cederberg 1990). This mutated strain is called *PDC1* mutant (Seeboth *et al.* 1990).

Based on the discussion of Seeboth *et al.* (1990) and Hohmann and Cederberg (1990) on the effect of mutation on *S. cerevisiae*, we decided on our hypotheses: our null hypothesis states that the CO_2 production of the wild type will be less than or equal to the

CO_2 production of the mutant and our alternate hypothesis states that the CO_2 production of wild type will be greater than CO_2 production of the mutant.

Methods

The mutant and wild-type yeast cells were kept in a large flask on a shaker at 30ºC. We were also provided with yeast-extract peptone-dextrose, also known as YPD, which is a medium for yeast growth. In this experiment, we tested the CO_2 production of the wild-type and *PDC1* mutant yeast cells at 30ºC. We had one control treatment (no yeast cells present) for the wild type and another one for the mutant. We prepared four replicates for each yeast strain.

We measured the initial cell concentration of the yeast culture with a haemocytometer and found that it had an initial stock concentration of 10^6 – 10^7 cells/mL. In order to have a measurable amount of CO_2 produced, we had to concentrate the cell count to 10^9 cells/mL (Sherman 2002). To reach this target magnitude, the initial yeast culture had to be concentrated by about 100x. We did this by centrifuging the yeast strains for 5 minutes. The supernatant was discarded after centrifuging and the white pellet was collected. Both yeast strains were then suspended in 10mL of YPD medium and mixed until the pellet was gone. The final cell count indicated the concentration was approximately $5x10^9$ cells/mL, which was sufficient to be used in our experiment.

After the yeast culture was prepared, the solutions were added to the respirometer tubes to measure CO_2 production. The respirometer setup consisted of a small inverted test tube containing the yeast culture inside a large 20 mL test tube also containing some excess yeast culture to provide pressure and ensure consistency of cell numbers inside the smaller test tube. We also prepared a respirometer for the control group that only

contained the YPD growth medium (Figure 2). The respirometers were then placed inside a water bath at a temperature of 30ºC (Figure 3)**.**

Figure 2. The respirometer set-up

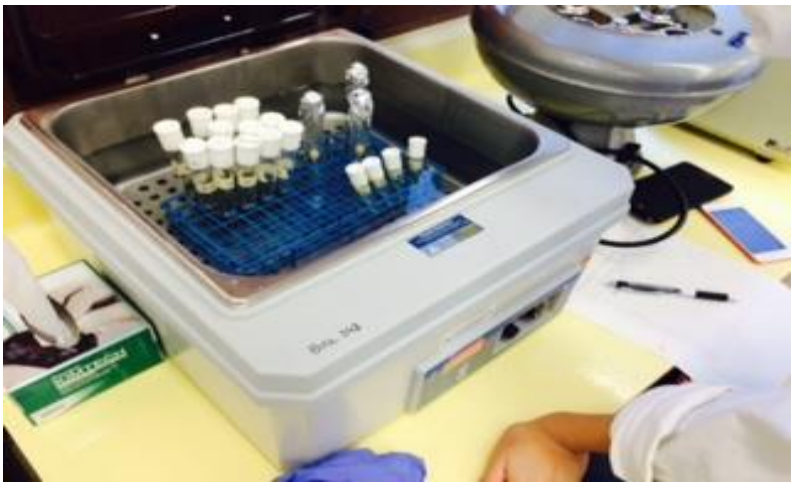

Figure 3. The setup of the respirometers inside the water bath at 30ºC

We could observe CO_2 being produced inside the smaller test tubes because of the air bubble forming at the top. The test tubes were monitored for 75 minutes and measurements were taken every 5 minutes. A caliper was used to measure the height of

bubble formation in millimeters inside the smaller test tube (Figure 4). We multiplied every interval by a factor of 0.694 to convert the micrometer ruler readings of CO_2 production in respirometer sets from millimeters into milliliters. The volume for each strain was then divided by the total number of cells counted for that strain earlier to determine the volume of CO_2 produced per cell.

Figure 4**.** Measuring the height of bubble formation using a caliper

After plotting the data for each wild-type and mutant yeast culture, the amount of CO_2 produced per cell during the period of exponential growth was averaged over the four replicates to come up with a mean value for each of the wild-type and mutant strains. We calculated the 95% confidence interval for the mean value for both the wild-type and mutant strains to see if there was a significant difference between the two. Lastly, a t-test was performed to determine any significant difference between the mean values of CO_2 production per cell for wild-type versus mutant yeast.

Results

At 30°C, the mean level of CO_2 per cell produced by the wild-type yeast was 3.7 times larger than that of the mutant yeast during the exponential phase. Additionally, the

95% confidence intervals of the means were notably small and did not overlap at all (Figure 5). Overall, all wild-type replicates produced about the same mean level of CO_2 per cell over the exponential phase. In addition, all except one of the mutant replicates produced about the same amount of CO_2 per cell during the exponential phase. The distinct mutant replicate produced approximately 1.7 times less CO_2 per cell than the other mutant replicates; however, it did not pose a noticeable impact on our final results and analysis.

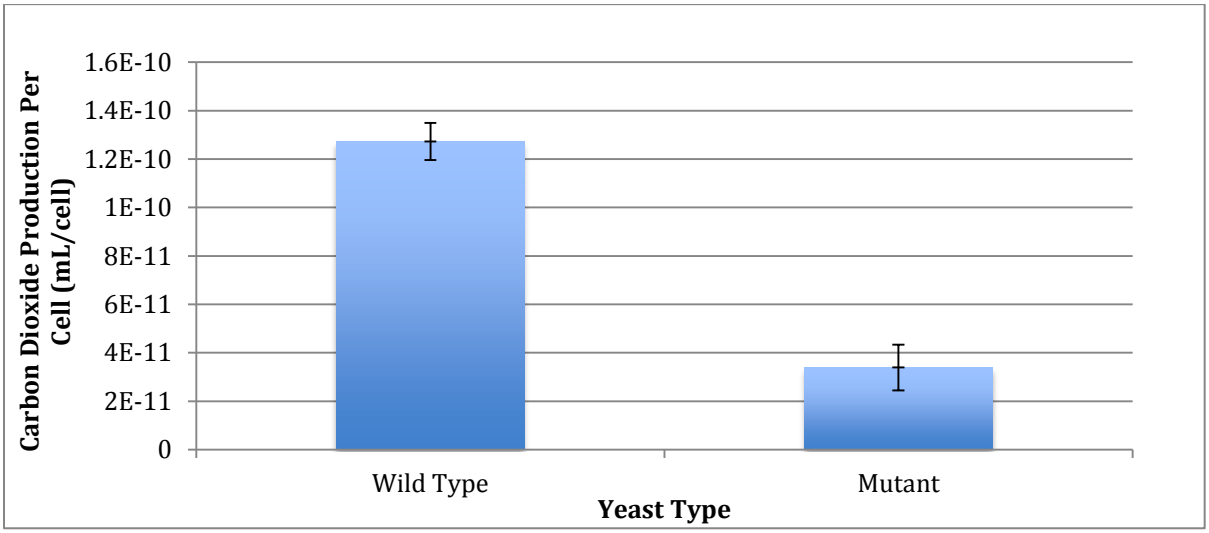

Figure 5. Mean level of CO_2 production per cell during the exponential phase for wild-type and mutant *S. cerevisiae* at 30°C. Error bars represent 95% confidence intervals.

The shape of the graph of CO_2 production per cell against time as depicted by the moving average regression line for wild-type replicates (Figure 6), which is a better fit better to an S-shaped curve than is that of the mutant replicates (Figure 7).

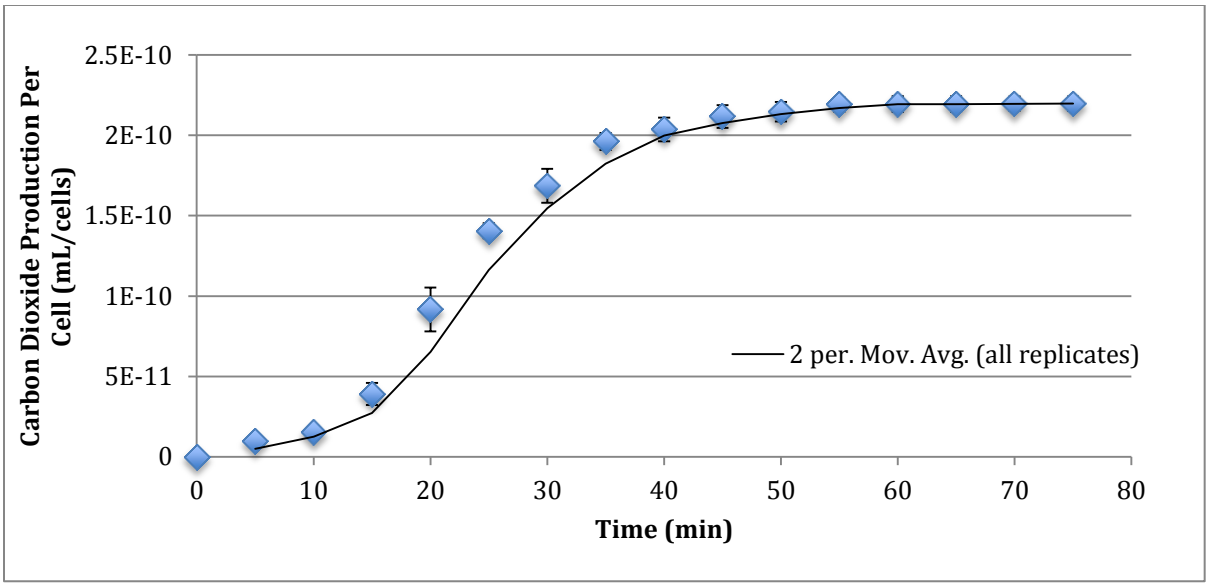

Figure 6. The mean level of CO_2 production per cell for all wild-type replicates during the full 75 minutes of experiment at 30°C. The moving average regression line (period=2) represents an average for CO_2 production per cell for all replicates combined. Error bars represent 95% confidence intervals.

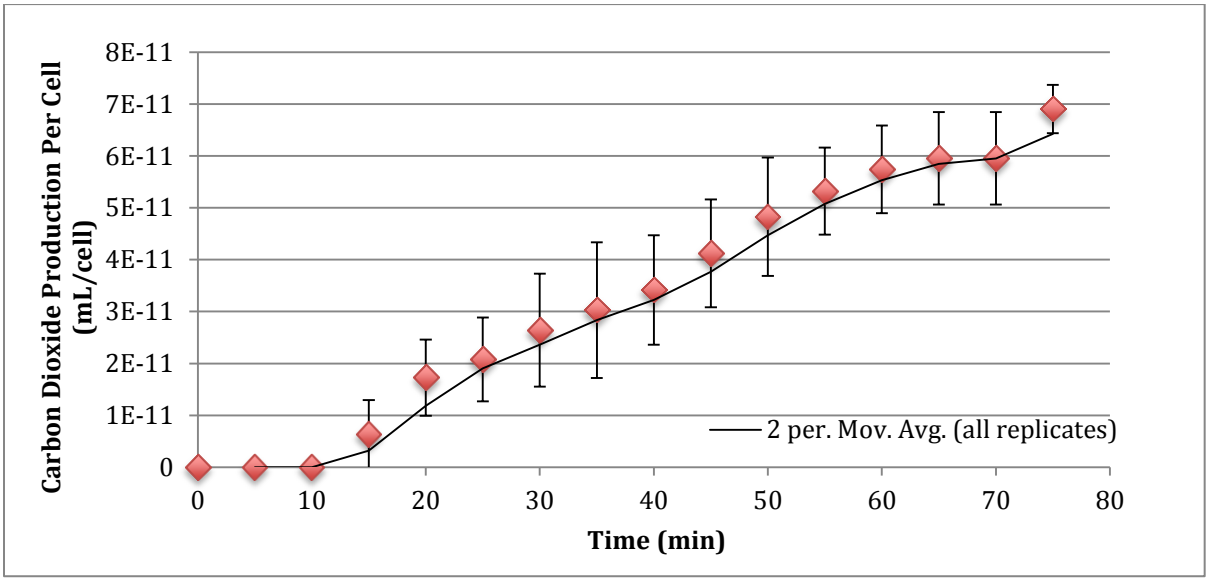

Figure 7. The mean level of CO_2 production per cell for mutant replicates during the full 75 minutes of experiment at 30°C. The moving average regression line represents an average for CO_2 production per cell for all replicates combined. The error bars represent 95% confidence intervals.

The slope of the linear regression line for average CO_2 per cell produced over the exponential phase for the wild-type yeast was 8 times larger than that of the mutant replicates.

The t-test analysis yielded a p-value of $7.8x10^{-6}$. Because this value is smaller than 0.05, there must be a significant difference in the mean level of CO_2 production per cell between the wild-type and mutant yeast cells at 30^oC.

Lastly, no CO_2 was produced in the negative procedural control respirometer that was only filled with YPD extract.

Discussion

The analysis of our results show a p-value for the difference in mean level of CO_2 production per cell for wild-type and mutant *Saccharomyces cerevisiae* that is much smaller than 0.05. In addition, the 95% confidence intervals for the mean levels for wild-type and mutant CO_2 production per cell during the exponential phase do not overlap with one another (Figure 5). These findings allow us to reject our null hypothesis and provide support for the alternate hypothesis that the amount of CO_2 production by wild-type *S. cerevisiae* is greater than that of the mutant strain at 30ºC.

The mean level of CO_2 production per cell at 30ºC by the wild-type yeast cells was significantly greater than mutant yeast cells. This was expected as *PDC1* mutant yeast cells have a diminished fermentative capacity resulting in a lower amount of CO_2 being produced (Hohmann and Cederberg 1990). This is because a cell containing a mutant *PDC1* gene would result in the synthesis of a mutated *PDC1* enzyme, which is expected to produce very little to no CO_2, which could potentially lead to cell death

(Pronk et al. 1996, Dijken et al. 1993). However, our data clearly indicate that some CO_2 production was observed for the mutant strains of *S. cerevisiae* in our experiment.

The production of CO_2 despite having a mutated *PDC1* gene is due to the activation of the *PDC5* gene, which is 20 percent less active than the *PDC1* enzyme in wild-type yeast (Hohmann and Cederberg 1990). Therefore, lower levels of CO_2 production per cell are expected for the mutant yeast. This correlates with our data where the mean level of CO_2 produced per cell by the wild-type yeast is significantly greater than that for the mutant yeast.

Looking at the general plot of CO_2 production per cell for each of the wild type and mutant, we can see that they both follow the same pattern. In both Figures 6 and 7, we see the wild-type and the mutant strains initially enter a lag phase, consisting of very little to no CO_2 production. During this lag phase, present in all wild-type and mutant replicates, the yeast cells are adjusting to the YPD medium that they have been immersed in and prepare to use the glucose and nutrients present in the medium for their needs (Rastogi 1997). This lag phase is then followed by an exponential phase, seen in both the wild-type and mutant cells. During this phase, we observe the fastest rate of growth for each of the two strains and there is an exponential increase in the level of CO_2 production at 30ºC by each type of yeast cell. The slope of this exponential phase can also be representative of the fermentative ability of the wild-type and mutant yeast cells, where we see that the wild-type yeast cells grow much faster compared to the mutant cells. This slower rate of growth observed in the mutant yeast cells is due to the *PDC1* mutation present, leading to the activation of the *PDC5* isoenzyme which will be used to convert pyruvate into acetaldehyde and eventually produce CO_2 (Hohmann and Cederberg 1990).

After the exponential phase, we see the wild-type yeast cells entering a plateau phase where the amount of CO_2 produced per cell at 30ºC remains unchanged. We can also see our mutant yeast cells entering this stationary phase at the end of the experiment. An important observation worth noting is that the mutant yeast cells enter a plateau phase about 25 minutes later compared to the wild-type cells, which is once again due to the reduced fermentative capability of the mutant cells (Hohmann and Cederberg 1990). It is possible that had we continued our measurements of CO_2 production, we would have been able to see a more distinct stationary phase for our mutant yeast cells.

One limitation of this study was in the measurement of CO_2 production. The respirometer setup consisted of a small test tube, which was marked by hand at 0.50 mL intervals, as it would have been too time consuming and untidy to mark out smaller intervals such as 0.1 or even 0.25 mL intervals. As a result of this, CO_2 measurements were made using a caliper and were therefore somewhat subjective at times, as a measurement of 1.7 mL, for example, could have been 1.6 mL or 1.8 mL. This can be improved in the future by using more accurate instruments such as incremented test tubes.

An assumption made in the study was that the glucose concentrations remained constant throughout the experiment. However, as yeast metabolized, the glucose concentration would have decreased over time and less glucose would have been present for the remaining cells, thus reducing their amount of CO_2 production over time.

Another important assumption made was regarding the number of yeast cells throughout the measurements. Since it takes 90-140 minutes for yeast cells to grow and divide (Sherman 2002), we assumed that the number of wild-type and mutant yeast cells

remained constant throughout the 75 minutes that measurements took place. However, it is quite possible that during this time some yeast cells may have been at earlier stages of development and thus reproducing new cells. This would cause the actual number of yeast cells present in each replicate to be greater than measured and a lower amount of CO_2 actually being produced per cell.

A possible future experiment could be to compare the level of CO_2 production at different temperatures to see which of the wild type or the mutant has greater CO_2 production at lower temperatures. Also, levels of ethanol production at different starting glucose concentrations could be compared for the wild-type and mutant yeast cells. This would be useful as ethanol is produced only through fermentation, and therefore, findings of such a study could tell us at what glucose concentration yeast cells begin fermentation, and which of wild-type or mutant strains start fermentation at lower glucose levels.

Conclusion

The statistical analysis allows us to reject our null hypothesis and also gives us evidence to support our alternative hypothesis that CO_2 production level of the wild-type *S. cerevisiae* is greater than the CO_2 production level of the *PDC1* mutant at 30°C. Thus we can conclude that the wild-type *S. cerevisiae* are more efficient in fermentation as their rate of CO_2 production is higher compared to the mutant.

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