Carbon dioxide production of wild type and PDC1 mutant *Saccharomyces cerevisiae* in D-glucose

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Abstract

To study the differences in respiration between wild type and PDC1 mutant *Saccharomyces cerevisiae*, carbon dioxide (CO_2) production was observed in growth media of concentrations 0.10 M, 0.60M and 1.2 M dextrose. Respirometer measurements of the CO_2 produced were taken every five minutes for 30 minutes. The average CO_2 produced per wild type cell in 0.10 M, 0.60 M and 1.2 M was found to be 1.03 x 10^{-9} mL CO_2/cell, 7.18 x 10^{-10} mL CO_2/cell and 5.84 x 10^{-10} mL CO_2/cell respectively, while the values of 6.59 x10^{-10}mL CO_2/cell, 1.27 x 10^{-9} mL CO_2/cell and 9.72 x 10^{-10} mL CO_2/cell were found in the mutant strain. We observed decreasing production of CO_2 in wild type and mutant cells as dextrose concentration increased from 0.6 M to 1.2 M due to the Crabtree effect. The Crabtree effect is characterized by an increase in fermentation in aerobic conditions and a decrease in respiration under excess glucose conditions. Another reason for the drop in CO_2 production from 0.6 M to 1.2 M in mutants may be due to decreased ability to breakdown pyruvate as fast as glycolysis can produce it due to the mutated *pdc1* gene, which results in a pyruvate decarboxylase with impaired function. The results from our study suggest that wild-type strains may preferentially carry out fermentation in higher concentrations of glucose. Mutants also display decreased CO_2 production; however, mutants had higher CO_2 production per cell than wild-type cells suggesting unequal repression of respiration in the two strains.

Introduction

Saccharomyces cerevisiae, baker's yeast, is a single-celled eukaryote capable of growing on a wide array of simple sugars aerobically or anaerobically. In experiments, yeast is generally grown on yeast extract peptone dextrose (YPD), containing dextrose as the carbon source, at 30°C (Sherman 2002). Under anaerobic conditions, *S. cerevisiae* can undergo fermentation where pyruvate produced from glycolysis of sugar is further broken down into ethanol and carbon dioxide (Pronk *et al.* 1996). *S. cerevisiae* are also capable of oxidative phosphorylation of pyruvate in aerobic conditions which can produce more energy than fermentation (Pronk *et al.* 1996). However, under excess glucose conditions aerobic respiration can be repressed and fermentation in aerobic conditions (referred to as fermentation through this rest of this paper) then becomes a main pathway of glucose degradation (Deken 1965). This increase in fermentation under excess glucose conditions is called the Crabtree effect (Deken 1965). The Crabtree effect may have evolved as a mechanism to out-compete other microbes growing on ripening fruit due to the production of ethanol, a substance toxic to other microbes, at the expense of more energy from oxidative phosphorylation (Hagman *et al.* 2013). Also, *S. cerevisiae* have the ability to breakdown

ethanol thereby adopting a “make-accumulate-consume” technique of ethanol metabolism (Hagman *et. al* 2013).

An important step in fermentation is the removal of a carboxyl group from pyruvate to form acetaldehyde, which is carried out by pyruvate decarboxylase isoenzymes (enzymes that differ in amino acid sequence but have the same function) PDC1, PDC5, and PDC6 (Hohmann 1991, Pronk *et. al* 1996). PDC1 is expressed 6 times more than PDC5*;* in addition, PDC6 expression is weak and has little effect on fermentation (Hohmann 1991). Therefore, PDC1 is the pyruvate decarboxylase enzyme that catalyzes the bulk of the pyruvate decarboxylase reactions. Mutant *S. cerevisiae* for all three PDC isoenzymes are therefore compromised in their ability to perform fermentation which results in a reduction of growth of the mutant strain on glucose three-fold when compared to wild type (Flikweert *et al.* 1996).

The objective of this study is to compare the respiration, via carbon dioxide (CO_2) production, of mutant and wild type *S. cerevisiae* in different concentrations of dextrose. We also want to investigate the Crabtree effect in wild-type yeast, well documented in the literature, and make comparisons with observations of the Crabtree effect in yeast lacking PDC1 function which is not as well documented in the literature. We also hope to gain insight into the mechanism responsible for repression of oxidative phosphorylation during the Crabtree effect via observing PDC1 mutant yeast.

Our first alternate hypothesis states that higher dextrose concentration will result in lower CO_2 production in both mutant and wild type *Saccharomyces cerevisiae*. We expect this as there is a repression on respiration (Crabtree effect) under excess dextrose conditions (Deken 1965). Our second alternate hypothesis is that higher dextrose concentration will result in lower CO_2 production in mutant *Saccharomyces cerevisiae* when compared to wild type. Due to the PDC1 mutation, the mutant yeast should display decreased ability to ferment, which will result in lower CO_2 production compared to wild type yeast if the repression on aerobic respiration is similar in both strains (Deken 1965, Flikweert *et al.*1996).

Our first null hypothesis states that higher concentrations of dextrose in growing media will result in higher CO_2 production or have no effect on CO_2 production in both wild type and mutant strains of

Saccharomyces cerevisiae; whereas, our second null hypothesis states that higher concentration of dextrose will result in higher CO_2 production or have no effect on CO_2 production in mutant *Saccharomyces cerevisiae* when compared to wild type.

Methods

The wild type and *pdc1* mutant *Saccharomyces cerevisiae* cells were kept at room temperature in two different 1 L flasks. Each flask had aluminum foil covering the opening of the flask to prevent airborne contamination. Yeast Extract Peptone Dextrose (YPD) broth was provided to us with 0.10 M dextrose concentration. We prepared three different dextrose concentrations as treatments for wild type and mutant yeast cells. There was also a negative control group containing media that had 0.10 M dextrose and no yeast cells. Three replicates were used for each treatment for each strain.

The yeast cells were centrifuged because the yeast culture provided had a concentration of 10^7 cells/mL, and sufficient measurements of CO_2 could only be made with cell counts around 10^8 - 10^9 cells/mL (M. Chow, Lab Technician, personal communication). We performed a cell count using a haemocytometer. 100 μL of cells were fixed with 10 μL of fixative, glutaraldehyde. Wild type and mutant stocks were centrifuged at speed 7 for five minutes (how many rpm)? After each centrifugation, the supernatant was poured into a liquid waste container while the pellet remained in place (See **Figure 2**). More yeast cells were poured into the centrifuge tubes and this process was repeated six times for each strain until all wild type and mutant cells were centrifuged.

Figure 2: Yeast cell pellets on the bottom of 50 mL centrifuge tubes with supernatant

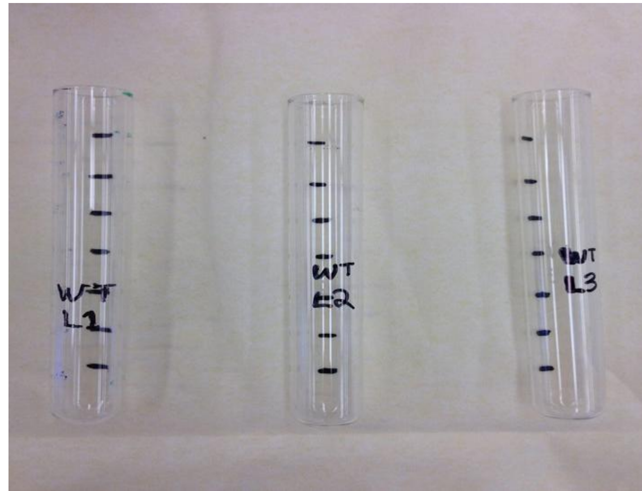

Figure 3: 0.5 mL increment markings on 4 mL respirometer test tubes

The wild type pellets were resuspended in 180 mL YPD broth and the mutant pellets in 100 mL YPD broth, which we calculated would give us roughly equal concentrations of the mutant and wild type solutions. Both wild type and mutant yeast pellets were mixed and vortexed with the 0.10 M YPD broth and then added to a 250 mL beaker. The cells were counted using a haemocytometer and fixative to ensure a cell count of roughly 10^8 cells was achieved.

Each resuspended yeast strain was divided into three labeled flasks according to the different dextrose concentrations. A 10 mL glass pipette was used to divide the suspended mixtures into thirds. For wild type, 4.96 grams of dextrose was added for 0.6 M and 10.9 grams was added for 1.2 M. For mutant, 2.97 grams was added for 0.6 M and 6.54 grams were added for 1.2 M. No extra dextrose was added to 0.1 M as our YPD broth had 0.1 M dextrose already.

Respirometer sets, which consisted of one 4 mL and one 15 mL test tube, were used to calculate the CO_2 production. The 4 mL test tube was marked with 0.5 mL increments, which were measured using a micropipette as shown in **Figure 3**. Once the respirometers were set up, they were placed in a test tube rack in a 30°C water bath. The times that each respirometer was placed in the water bath were recorded. The CO_2 production rate was observed every five minutes for 30 minutes. The CO_2 produced displaced

the yeast mixture; therefore we used a plastic pipette to remove excess yeast mixture from the 15 mL test tube.

At the end of the 30 minute observations, the amount of CO_2 produced per cell was averaged for each dextrose concentration for each strain. The volume of CO_2 produced was divided by the number of cells to yield CO_2 produced per cell. 95% confidence intervals were calculated for each yeast strain to determine if there were significant differences among the different dextrose concentrations. If the intervals overlapped, the results were not significantly different but if they did not overlap, the results were significantly different. A t-test was used to determine significant differences between the wild type and mutant strains.

Results

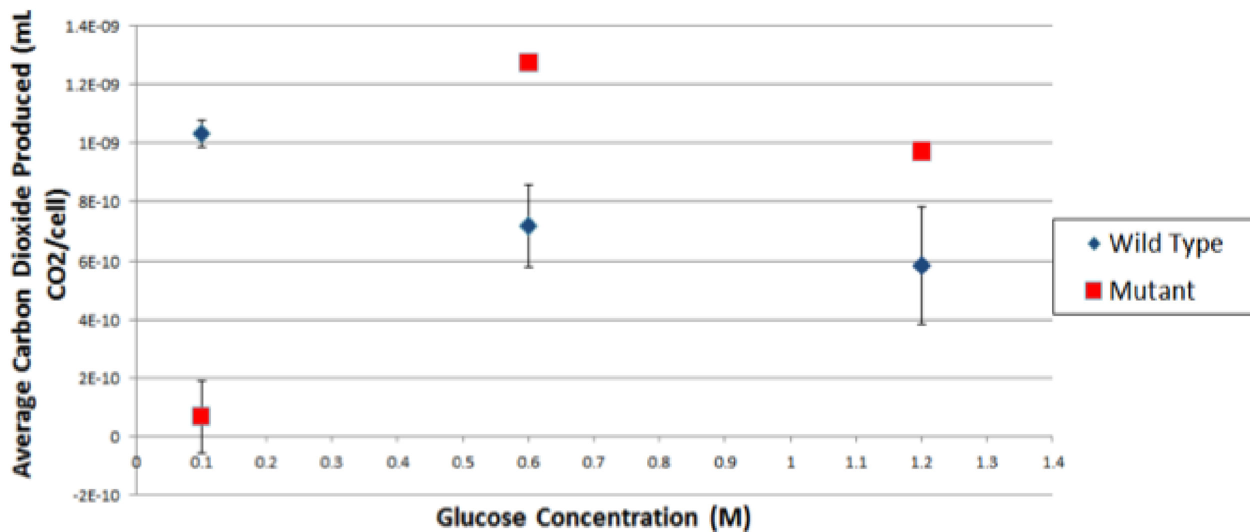

Figure 4: Average mL of CO_2 produced per wild type and PDC1 mutant cell at 0.10 M, 0.6 M and 1.2 M dextrose concentrations after 30 minutes. Note: Error bars represent 95% confidence intervals of the average carbon dioxide produced. Error bars are present for the PDC1 mutant in the 0.6M dextrose concentration, but are not visible at this scale of the graph. No error bars are present for the PDC1 mutant in the 1.2M concentration of dextrose n=2; n=3 for all other treatments).

For each dextrose concentration we calculated the CO_2 produced per cell after 30 minutes by taking the average of the three replicates, and then calculated the 95% confidence intervals (see **Figure 4).** The wild type strain of *Saccharomyces cerevisiae* showed a significant difference in the amount of carbon dioxide produced per cell when comparing the low (0.1 M) and medium (0.6 M) dextrose

concentrations and when comparing the low (0.1 M) and high (1.2 M) concentrations. Since the confidence intervals for the averages of CO_2 produced at 0.6 M and 1.2 M overlap, they are not significantly different. The wild type strain showed a decrease in average carbon dioxide produced per cell as the dextrose concentration increased from 0.1M to 0.6M to 1.2M, producing $1.03 \times 10^{-9} \pm 4.5 \times 10^{-11}$, $7.18 \times 10^{-10} \pm 1.40 \times 10^{-10}$, and $5.84 \times 10^{-10} \pm 1.97 \times 10^{-10}$ mL/cell respectively. The overall trend for the wild type strain show that increases in dextrose concentration decreases the amount of carbon dioxide produced (**Figure 4**).

The mutant strain of *Saccharomyces cerevisiae* showed significant differences when comparing each of the three dextrose concentrations. The mutant strain increased its carbon dioxide production from $6.59 \times 10^{-11} \pm 1.23 \times 10^{-10}$ mL/cell to $1.27 \times 10^{-9} \pm 1.13 \times 10^{-11}$ mL/cell when going from a 0.10 M to 0.60 M dextrose medium. However, in the 1.2M dextrose solution, the mutant strain decreased the amount of carbon dioxide produced to $9.72 \times 10^{-10} \pm 0$ mL/cell. Since values of CO_2 produced by the two mutant replicates in high concentrations of dextrose were the same, there is no deviation from the mean value, and therefore no range for a confidence interval. There does not appear to be a trend in the data for the mutant strain (**Figure 4**), as the amount of carbon dioxide produced first increases with increasing dextrose concentration, and then decreases with a further increase in dextrose concentration.

Also, measurements of CO_2 production were made on a negative control containing only YPD growth media and no yeast cells. No CO_2 was produced in the respirometer of the negative control, indicating that all CO_2 produced and measured was a result of yeast-sugar biochemistry.

Sample Calculations

Concentrating amount:

Wild type initial concentration: 6.2×10^{7} cells/mL

Mutant initial concentration: 3.3×10^{7} cells/mL

Wild type in 180 mL: $(6.2 \times 10^{7}$ cells/mL x 1000mL) x (1L /180mL) = 3.4×10^{8} cells/mL

Mutant in 100mL: $(3.3 \times 10^{7}$ cells/mL x 1000mL) x (1L/100mL) = 3.3×10^{8} cells/mL

Amount of dextrose to add to achieve desired molarity:

Wild type: Have 180mL, able to make 3 replicates of 50mL each at every concentration.

Start with 0.1M YPD broth want 0.6M dextrose and 1.2M dextrose solutions.

Number of moles of dextrose in 50 mL of a 0.1M solution = (Conc.) x (Volume) = 0.1M x 0.05L= 0.005 moles

0.6M:

Need 50 mL of a 0.6M solution: (0.05L) x (0.6M) = 0.03 moles

Therefore, need to add 0.03-0.005 = 0.025 moles of dextrose to 0.1M solution

0.025 moles x (198.2g/mol) = **4.96 g dextrose**

Number of Cells in each wild-type respirometer (4 mL):

Number of cells = (4 mL) x (6.5×10^8 cells/mL) = 2.6×10^9 cells

Amount of CO_2 produced per wild type cell of replicate 1 in 0.10 M of dextrose:

Amount of CO_2 per cell $= \frac{2.8 \text{ mL CO2}}{2.6 \times 10^9 \text{cells}} = 1.08 \times 10^{-9}$ mL CO_2/cell

The 95% confidence intervals of the average carbon dioxide production for the wild type strain at 0.10 M dextrose:

Average carbon dioxide produced per wild-type cell after 30 minutes = $(1.08 \times 10^{-9} + 1.02 \times 10^{-9} + 1.00 \times 10^{-9})/3 = 1.03 \times 10^{-9}$ mL CO_2/cell in 0.10 M dextrose

Variance = $[(1.077 \times 10^{-9} - 1.032 \times 10^{-9})^2 + (1.019 \times 10^{-9} - 1.032 \times 10^{-9})^2 + (1.00 \times 10^{-9} - 1.032 \times 10^{-9})^2] / (3-1) = 1.609 \times 10^{-21}$

Standard Error = $\frac{\sqrt{1.609 \times 10^{-21}}}{\sqrt{3}} = 2.316 \times 10^{-11}$

95% confidence intervals = $\pm$ 1.96 x (standard error) = 1.96 x (2.316×10^{-11}) = 4.5×10^{-11}

Lower Limit = Average CO_2 produced - Standard Error

= 1.03×10^{-9} mL CO_2/cell - 4.5×10^{-11}

= 9.87×10^{-10} CO_2/cell

Upper Limit = Average CO_2 produced + Standard Error

= 1.03×10^{-9} mL CO_2/cell + 4.5×10^{-11}

$= 1.08 \times 10^{-9}$ CO_2/cell

Sample T-test for mutant and wild type strains at 0.1M dextrose

$t = (x_1 - x_2)/s\sqrt{\frac{1}{n_1} + \frac{1}{n_2}}$

Where x_1 is the mean of our wild type and x_2 is the mean of our mutant, n_1 and n_2 are the number in each sample (both are 3) and s is the combined standard deviation of the two samples.

$$s^2 = \frac{\Sigma(x - x_1)^2 + \Sigma(x - x_2)^2}{n_1 + n_2 - 2}$$

$\Sigma(x - x_1)^2 = 3.5 \times 10^{-20}$

$\Sigma(x - x_2)^2 = 2.325 \times 10^{-20}$

$$s^2 = 3.5 \times 10^{-20} + 2.325 * 10^{-20}/(3 + 3 - 2) = 6.689 \times 10^{-21}$$

$$s = 8.178 \times 10^{-11}$$

$t = (1.03 \times 10^{-9} - 6.659 \times 10^{-11})/(8.178 \times 10^{-11}\sqrt{\frac{1}{3} + \frac{1}{3}})$

t=14.428

Since our t value of 14.428 is greater than the p value of 2.776 at 4 degrees of freedom, we are sure that our two samples are significantly different 95% of the time.

Discussion

Based on 95% confidence intervals we reject our first null hypothesis and support our first alternate (higher dextrose will result in lower CO_2) for the wild type *S. cerevisiae*; however, we fail to reject our first null hypothesis for the mutant *S. cerevisiae*.

The observed trend in the wild type strains is that as dextrose concentrations increase, the CO_2 produced per cell decreases. There is a statistical difference between the 0.1 M and 0.6 M concentrations, as well as a statistical difference the 0.1 M and 1.2 M concentrations, because the 95% confidence intervals of these two pairs of data do not overlap. We cannot say that there is a statistical difference between the 0.6 M and 1.2 M concentrations, because the confidence intervals overlap. However, one can still notice a trend in which the CO_2 produced decreases with increasing dextrose concentration. In an environment with high levels of glucose, *S. cerevisiae* break down the glucose *via*

fermentation, not through aerobic respiration (Deken 1965). This is called the “Crabtree effect”. Since fermentation produces less CO_2 than aerobic respiration, the amount of CO_2 produced decreases with increasing dextrose concentrations. A possible cause of the Crabtree effect could be that in a high glucose medium, the yeast cells are able to obtain enough ATP through substrate-level phosphorylation, making it unnecessary to obtain ATP from oxidative phosphorylation (Thomson *et. al* 2005). The cells can perform fermentation which allows them to outcompete ethanol sensitive organisms (Hagman *et. al* 2013). When the cell represses oxidative phosphorylation, the amount of CO_2 it produces decreases, which could be a possible explanation for the observed trend in the wild type strain in **Figure 3**.

As the concentration of dextrose increases in the treatments for the PDC1 mutant strain, we would expect to see a trend similar to the one observed in the wild type strain; where increases in dextrose concentration result in a decrease of CO_2 produced. We see a significant decrease in CO_2 from 0.60 M to 1.2 M; however, we see a significant increase in CO_2 production from 0.10 M to 0.60 M of dextrose. Research done by Pronk *et al.* (1996) suggests that pyruvate decarboxylase activity may still ensue even after the disruption of the *pdc1* gene, as *pc5* and *pdc6* genes also encode for pyruvate decarboxylase. They found that in strains where the *pdc1* and *pdc5* genes or all 3 *pdc* genes have been disrupted, the mutants displayed significantly decreased pyruvate decarboxylase activity and therefore decreased growth and decreased production of CO_2. However, even if the *pdc1* gene is disrupted, there is still functional *pdc5* and *pdc6* genes present, which may be enough for sufficient pyruvate decarboxylase activity, and could explain our results in increasing CO_2 production from 0.1M to 0.60M. As our mutant is reported to have defective *pdc1* genes only, the *pdc5* and *pdc6* genes present may produce functional pyruvate carboxylases, thus allowing the continuous production of CO_2. Our observed results were unexpected in comparison to our hypothesis as we did not account for the activity of other genes that could potentially affect CO_2 production in the mutant strain of *S. cerevisiae*.

Based on our statistical analysis with a two-sided t-test ($\alpha = 0.05$), we fail to support our second alternate hypothesis that states that higher dextrose concentrations will result in lower CO_2 production in mutant yeast compared to wild type yeast, *Saccharomyces cerevisiae*. Therefore, we fail to reject our

second null hypothesis which states that at higher dextrose concentrations, CO_2 production will increase or remain the same in mutant yeast compared to wild type.

We were able to conclude that our mutant and wild type strains were significantly different from each other in the low (0.1M) and medium (0.6M) dextrose concentrations, but not in the 1.2 M dextrose medium. The mutant *Saccharomyces cerevisiae* had statistically higher CO_2 production (1.27×10^{-9}mL/cell) at 0.6 M concentration of glucose than the wild type cells (7.18×10^{-10}). This is surprising because without PDC1 activity the ability of the mutant yeast to do fermentation is greatly reduced (Flikweert *et. al* 1996). One explanation for the increased CO_2 levels for the mutant yeast may be an increased activity of PDC5 which could be due to an increased level of expression of the *pdc5* gene in the mutant to compensate PDC1 inactivity (Hohmann 1991). Another possibility that could result in increase of CO_2 production could be an increase in the rate of oxidative phosphorylation in the mutant. This would suggest that the mechanism of respiration inhibition shown in the Crabtree effect may not be due solely to glucose concentration of the medium as the two strains may show different levels of oxidative phosphorylation at 0.6 M glucose concentration. Instead, the repression of respiration during the Crabtree effect may be due to a decreased demand for energy when glucose is abundant allowing the cell to sacrifice energy from oxidative phosphorylation and instead use fermentation. This would result in a lower yield of energy; however, fermentation has the beneficial effect of ethanol production, which would eliminate ethanol-sensitive competition growing on the same carbon source (Hagman *et. al* 2013). Our mutant yeast would have a decreased ability to perform fermentation and the decrease in energy production would not allow for the sacrificing of energy for ethanol. Therefore, oxidative phosphorylation is not repressed and more CO_2 production is observed: the species responsible for the repression of respiration may not directly be the glucose in the media (Deken 1965).

We found that at 1.2M concentration of glucose the CO_2 produced by wild type and mutant were not significantly different; however, the CO2 produced in mutants was significantly less than wild type at 0.6M. The drop in CO_2 production in mutants could be due to a weak repression of respiration due to increased glucose metabolites as pyruvate dehydrogenase is not able to metabolize pyruvate as fast as it is produced (Flikweert *et. al* 1996). Without a working pyruvate decarboxylase 1 enzyme, pyruvate

dehydrogenase (enzyme responsible for the pyruvate to acetyl CoA reaction) may become the bottleneck of metabolism as glycolysis increases due to increased glucose and pyruvate starts to accumulate. In wild-type yeast, PDC1 can break down pyruvate into acetaldehyde which can be shunted into the TCA cycle (Flikweert *et. al* 1996). Without this shunt the accumulation of glucose intermediates may be repressing further glycolysis and result in a decrease in the CO_2 produced by the mutant yeast cells.

When observing the CO_2 production in the wild type and mutant for 0.6 M, we notice that the amount of CO_2 produced in mutant is more than wild type. As stated earlier, we expected CO_2 production to be less in increasing dextrose concentrations due to the mutation of pyruvate decarboxylase activity. Our cell count can be an explanation as the mutant cell count was 1.08 times greater than our wild type cell count, resulting in more cells which could then produce more CO_2. A limitation related to this is in our 0.1 M mutant treatment where our mutant yeast was in the 0.1 YPD broth for the longest amount of time before being incubated. Thus the mutant yeast cells could have used up most of the dextrose prior to our measurements, which could explain why the least amount of CO_2 was produced in the 0.1 M mutant strain.

One possible source of error could come from a change in total volume of our solutions when we added the YPD broth and dextrose. We measured our concentrations before adding dextrose and the YPD broth, and the addition of these could have significantly increased the total volume of our samples, causing a slight change in the concentration of cells.

Another source of error could have occurred when we were using the plastic pipette to remove the excess growth medium in the respirometer. As the CO_2 was being produced, it was displacing the growth medium; therefore we removed excess growth media with the plastic pipette to be able to read the CO_2 measurements on the test tube. However, it may have altered the CO_2 displacement because as the pipette was taking in medium, it may have sucked in growth medium from the respirometer as well.

A limitation in our study involves the length of time the wild type and mutant strains were in the flask. We were unable to record CO_2 observations simultaneously for both wild type and mutant because we were not able to add the dextrose, set up the respirometers, place them in the water bath and observe

the CO_2 measurement for 18 replicates. Therefore, we measured wild-type CO_2 production before mutant CO_2 production. A source of error is that the wild-type and mutant strain measurements were not taken at the same time. A consequence that could arise from this situation is that the mutant cells may have increased their cell count while wild type measurements were being taken. According to Sherman (2002), yeast has a doubling time of 90 minutes in YPD broth therefore the mutant strain may have somewhat increased its cell count size to some extent.

Another limitation is that the readings on the respirometer were subjective due to the increment size. Increments of 0.50 mL were used for the ease of reading the measurements as yeast was producing CO_2. Although our team came up with a unanimous observation for each measurement, the observation was still subjective. However, if we made smaller increments such as 0.25 mL or less, the readings on the respirometer would be more precise leading to more accurate measurements. If time was not a limited factor, more precise markings with a finer tip marker would increase accuracy greatly.

Conclusion

When comparing the average CO_2 production rate per cell for wild type and mutant, we observed the Crabtree effect in our wild type strains and a decrease in CO_2 concentration with increasing dextrose concentration in our mutant strain which may be due to glucose metabolite accumulation. Furthermore, our mutant strain had a greater CO_2 production rate per cell for dextrose concentrations at 0.6 M than our wild type yeast at the same concentration possibly due to overexpression of PDC isoenzymes or unequal repression of oxidative phosphorylation in mutant and wild type strains.

Acknowledgements

We would like to thank Dr. Carol Pollock for providing guidance and feedback for planning and analyzing results in our experiment, Mindy Chow for providing us with all the necessary equipment, setting up our yeast cultures and allowing us to enter the lab early to centrifuge our yeast cells, and Hayley Kenyon for helping us with any issues or questions we had during the experiment. We would also like to thank the University of British Columbia for allowing us the opportunity to take Biology 342 and provide us with valuable lab study experience.

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