Comparison of fermentation rates of a wild-type and YRL044C mutant strain of *Saccharomyces cerevisiae* by ethanol production quantification

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Abstract

Saccharomyces cerevisiae (also known as brewer's yeast) undergoes alcoholic fermentation as a form of metabolism, which is facilitated by pyruvate decarboxylase. The *PDC1* gene is one of three genes that can encode this isoenzyme, and in this experiment, we investigated the difference in fermentation rates of wild-type BY4741A *S. cerevisiae* versus a *PDC1*-deficient YRL044C variant. To calculate these rates, we used a by-product, ethanol, as an indicator of fermentation. Ethanol concentration was calculated from the specific gravity measurement. Equal initial numbers of wild-type and mutant cells were cultivated in a YPD broth for growth and incubated at 32°C in anaerobic conditions to promote growth and fermentation. Over a span of 10 days, we observed a greater slope in ethanol concentration per yeast cell in wild-type *S. cerevisiae.* The slope represents the increase of ethanol concentration of each cell per day, and it was calculated to be $4x10^{-6}$ for the wild-type and $2x10^{-6}$ for the mutant (% per cell per day). Through a two-way ANOVA, our results showed a significant difference between the ethanol production by the two strains. However, a significant difference between the two strains was not found in the pattern of ethanol production over time.

Introduction

Saccharomyces cerevisiae, also known as brewer's yeast, is a strain of yeast that is often utilized as a model organism in the biological sciences. In this study, two strains of *S. cerevisiae,* a wild-type strain (BY4741A) and a YRL044C variant carrying a mutation in the *PDC1* gene were examined. *PDC1*, *PDC5* and *PDC6* are genes that encode an isoenzyme, pyruvate decaryboxylase (Hohmann & Cederberg 1990). *PDC1*, the gene examined in this study, is the most active among all three in encoding pyruvate decarboxylase (Schaaff *et al.* 1989). The role of pyruvate decarboxylase is essential in the physiology of *S. cerevisiae* because it is necessary in order for the organism to carry out a form of pyruvate metabolism, alcoholic fermentation (Pronk, Steensma & Van Dijken 1996). Pyruvate decarboxylase is required for the catalysis of pyruvate into acetaldehyde, which is catalyzed into ethanol later in the fermentation process, as illustrated by Figure 1.

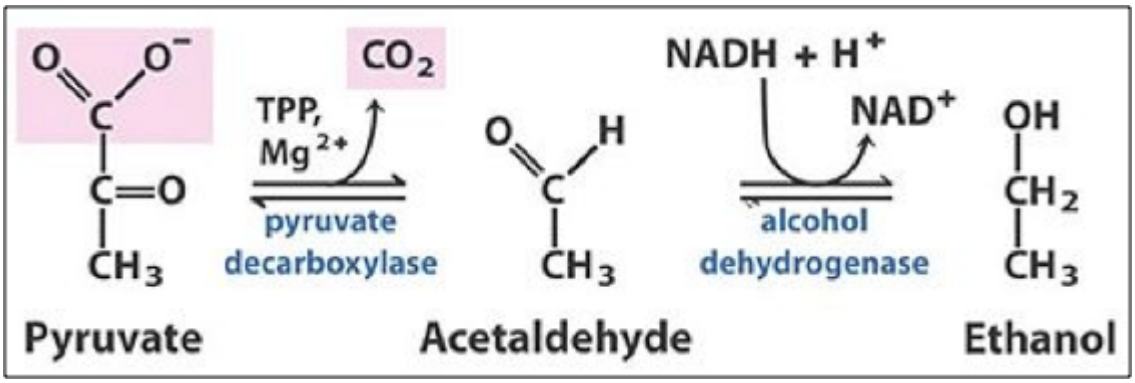

Figure 1. The anaerobic pathway used in alcohol fermentation in *Saccharomyces cerevisiae*, highlighting the role of pyruvate decarboxylase (Protopedia 2016).

Ethanol can therefore be employed as an indicator of pyruvate decarboxylase activity and overall anaerobic fermentation reaction. Schaaff *et al.* (1989) observed that a null mutation in the *PDC1* gene is responsible for a decreased rate of fermentation in the organism, as well as leading to decreased vegetative growth.

As shown in Figure 2, the distinguishing phenotypes of each strain are shown: the wild-type cells tended to be in clusters while the mutant cells tended to be grouped in pairs.

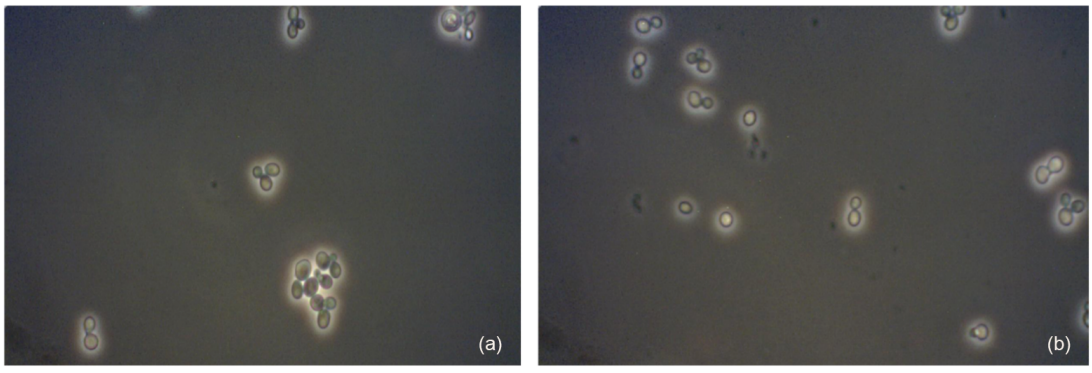

Figure 2. ***(a)*** Wild type specimen of *S. cerevisiae* viewed at 400x magnification. ***(b)*** YRL044C mutant specimen of *S. cerevisiae* viewed at 400x magnification.

Based on these findings, it is believed that the wild-type strain of *S. cerevisiae* will produce a greater amount of ethanol than the YRL044C strain. Examining the role of *PDC1* in ethanol production is important because, due to its wide variety of commercial and industrial applications, the amount of ethanol yielded by different strains of *S. cerevisiae* can be calculated and exploited. For example, the results of this study could be used to control the ethanol contents of consumable alcohol.

Three sets of hypotheses were generated to guide our study. Our first set of hypotheses looks at the presence of the *PDC1* mutation and its effect on ethanol production, with the null hypothesis stating that the presence of this mutation has no effect on ethanol production and the alternate hypothesis stating the presence of the *PDC1* mutation will have an effect on ethanol production. We predicted that the deletion mutation at the *PDC1* gene would hinder ethanol production: Schaaff *et al.* (1989) correlate *PDC1* with pyruvate decarboxylase activity; therefore, with a lack of *PDC1*, less pyruvate will be catalyzed by pyruvate decarboxylase into acetaldehyde, which is the precursor of ethanol (Figure 1). The presence of *PDC5* and *PDC6* means that the mutant would still be able to produce ethanol, however, to a lesser degree compared to the wild type (Yoshimoto *et al.* 2001). The second set of hypotheses addressed the effect of time on ethanol production, the null hypothesis stating time has no effect on ethanol production and the alternate hypothesis stating that time would have an effect on ethanol production. In this instance, our prediction was that with the passage of time one would see an increase in total ethanol produced, as it would accumulate over time within its sealed container. Lastly, the third null hypothesis stated that the pattern of ethanol production over time would not differ between the wild-type and YLR044C strain of *S. cerevisiae* and our alternate hypothesis

stated that the pattern of ethanol production over time would differ between the two yeast strains. Our prediction was that the pattern of ethanol produced between the wild-type and mutant strains over time would not differ in their increase, as their *PDC1* expression (or lack thereof) should remain consistent over the course of the experiment.

Methods

The UBC Biology Program provided wild-type *S. cerevisiae* suspended in yeast peptone dextrose (YPD) broth, mutant *S. cerevisiae* strain (YRL044C) in YPD broth, and YPD broth (control - absence of live yeast). We determined the concentrations of the wild-type and mutant cells in their respective media. After determining cell concentrations, we diluted the wild-type sample to match the concentration of the mutant sample. As beer is commonly produced with a yeast concentration of 15 $x10^{-6}$ to $20x10^{-6}$ cells/mL, our concentrations were one quarter of this value to prevent ethanol overproduction, as an excessive concentration of ethanol can be lethal for yeast (Pires & Brányik 2015; Ohta *et al.* 2015).

As shown in Figure 3, we filled 50mL Falcon tubes with the diluted wild-type solution, mutant solution, and the YPD broth (control). We set up 36 50-mL Falcon tubes to use on four days dedicated to data collection (over a nine day period following the setup) (Figure 3). Three wild-type samples, three mutant samples and three controls were prepared for each of the four days. We transferred our YPD broth samples to the Falcon tubes and filled each one to the brim, approximately 60mL, to ensure that the yeast grew in as anaerobic an environment as possible. This promoted the cells' execution of the fermentation reaction. We stored the Falcon tubes in a 32°C incubator to imitate the methodology employed by Flikweert *et al.* (1999), who incubated their *PDC*-negative samples at 30°C.

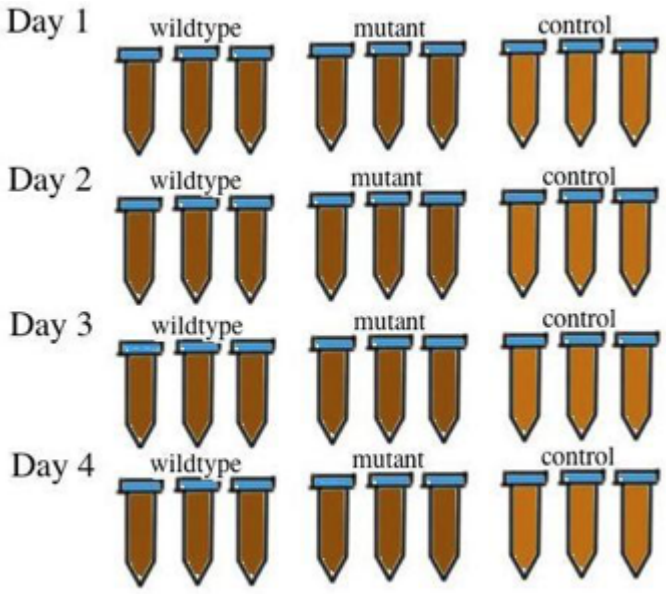

Figure 3. Diagram of experimental setup.

On day 0, we took baseline measurements to calibrate the hydrometer to the YPD media. On each of the subsequent four days, we took measurements of three wild-type, three mutant and three control samples, obtained from the incubator. We measured and checked the incubator temperature to ensure consistent conditions. One at a time, we poured 50mL from each Falcon tube into a 50-mL graduated cylinder without disturbing the aggregation of cells at the bottom of the tube. We took the temperature of the first sample and worked efficiently to ensure as little variation in temperature throughout the procedure as possible. Specific gravity was then measured using a hydrometer. Following each hydrometer measurement, we rinsed the apparatus with 70% ethanol, followed by distilled water, and dried the hydrometer before taking the next reading. This was done to prevent the mixing of samples and to avoid any contamination. We poured the contents of the graduated cylinder back into the respective Falcon tube and

determined cell concentrations of the samples. The control sample was measured for specific gravity only. This was done on each day for a total of four days over a period of ten days.

To analyze the data, we performed a two-way ANOVA test. This showed the effect on ethanol production, by wild-type and mutant yeast strains and time (measurement days). We compared our results to a *p*-value of 0.05.

Results

The fermentation rate of each strain of *S. cerevisiae* over the course of the experiment is shown in Figure 4.

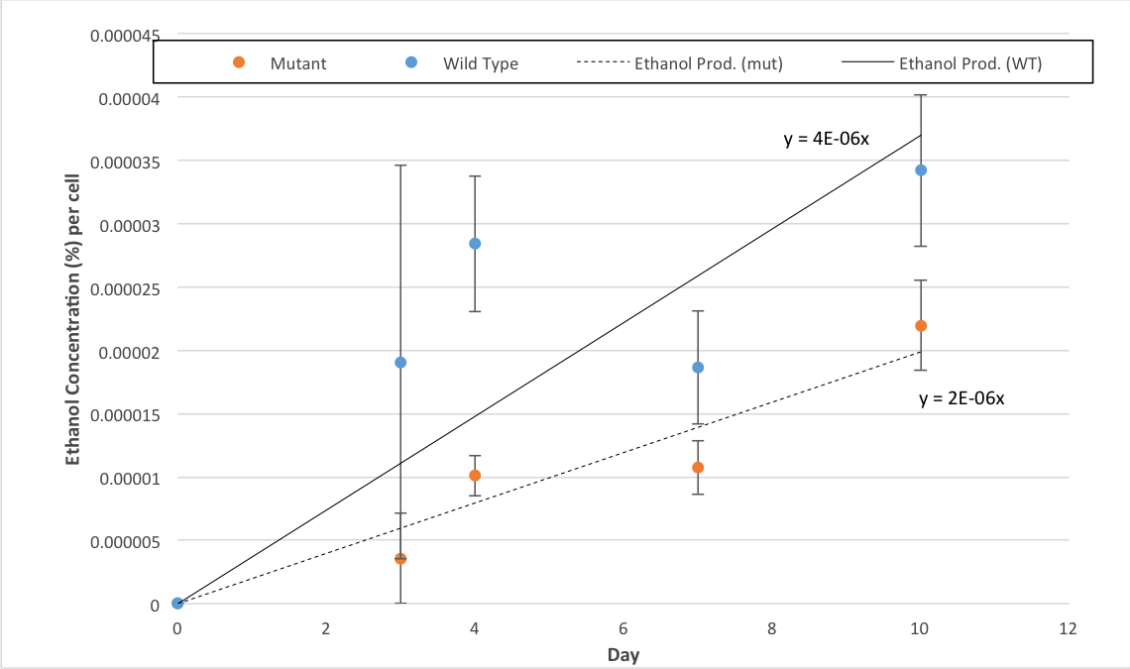

Figure 4. Mean ethanol concentrations per cell; 95% confidence intervals indicated by vertical error bars. Means calculated from three measurements obtained for each wild type and mutant each day, specific gravity measurements obtained by a hydrometer were converted to ethanol concentration. (*n*=3 for each data point).

The line of best fit, through each of the calculated means of wild-type and mutant samples on each day, show a linear rate of alcohol production (Figure 4). The slope represents rate of ethanol production over the four days that data were taken; the calculated slope of the wild-type and mutant *S. cerevisiae* were $4x10^{-6}$ and $2x10^{-6}$ (% per cell per day), respectively. A two-fold difference was shown between the wild-type and mutant fermentation rates. The 95% confidence intervals, shown for each daily mean for the wild-type and mutant strain, display greater variation within the wild-type samples when compared to the mutant. These confidence intervals also show that the final data point for the mutant strain is significantly higher than the preceding points of the mutant strain; this was not seen in the wild-type. Based on the data points shown, the general trend of ethanol production per cell is increasing. Figure 4 also shows that, with the progression of time, the wild-type strain produced a greater amount of ethanol per cell than the mutant strain.

The results of a two-way ANOVA yielded the following *p*-values: For the first set of hypotheses, a *p*-value of 0.0003 was obtained. For the second set of hypotheses, a *p*-value of 0.0060 was obtained. For the third set of hypotheses, a *p*-value of 0.6413 was obtained.

Discussion

The two-way ANOVA test was run on the two independent variables, strain (wild-type and mutant) and time, and the measured dependent variable was ethanol production. Statistical analysis yielded significant results when the independent variables were taken into account separately; however, the test did not yield significance when the two independent variables were analyzed together.

The first null hypothesis was rejected, supporting the alternate hypothesis that presence of the YRL044C mutation has an effect on ethanol production. A *p*-value of 0.0003 was reported at the significance level of 0.05 (p-value $\leq$ 0.05). From our results, we observed that the rate of fermentation between the two strains of *S. cerevisiae* is different. Based on the phenotypic effect of the YRL044C mutant, which is reduced pyruvate decarboxylase activity (Schaaff *et al.* 1989), we predicted that the mutant strain would have a lower rate of ethanol production compared to the wild-type. This prediction was supported by our results; Figure 4 shows that the rate of fermentation in the wild type is twice that of the mutant strain. This was determined by calculating and comparing the slopes of each line of best fit.

The second null hypothesis, stating that time has no effect on ethanol production, was also rejected, and the alternate hypothesis, that time does have an effect on ethanol production, was supported. A *p*-value of 0.0060 was reported (p-value $\leq$ 0.05). In regards to our second prediction, we suggested time does have an effect on ethanol production, and this is consistent with our results. As seen in Figure 4, ethanol concentration per cell was highest at the last measurement for both wild-type and mutant-strains. Ethanol has been studied and shown to negatively impact cell growth and reproduction (Kubota *et al.* 2004). Therefore, while ethanol continues to build up in the medium, the rate of cell growth will likely be decreased, thus producing the observed results of increased ethanol concentration per cell. For further studies, it is possible to enhance yeast tolerance to ethanol and reduce the rate of cell death (Ohta *et al.* 2016).

However, we failed to reject the third null hypothesis, which states that the pattern of ethanol production over time did not differ between the wild-type and mutant strains. A *p*-value

of 0.6413 was reported (p-value not $\leq$ 0.05). Our prediction, the pattern of ethanol production being the same for both wild-type and mutant strains, is supported by our results. Figure 4 shows the ethanol concentrations per cell of both the wild-type and mutant strains continuing to increase throughout the span of 10 days.

The underlying genetic variation between our two strains of *S. cerevisiae* is made clear through this experiment and our results. As stated in the study conducted by Schaaff *et al.* (1989), the null mutation in the *PDC1* gene that is characteristic of the YRL044C mutant results in two main phenotypic effects: the mutant shows both decreased fermentation rate and decreased vegetative growth. Schaaff *et al.*'s (1989) research states that mutants lacking the *PDC1* gene will show decreased pyruvate decarboxylase activity. Less pyruvate is catalyzed into acetaldehyde, which is later catalyzed into ethanol. In our experiment, the lower rate of fermentation is clearly exhibited and we correlated this with the production of ethanol, which can be seen in Figure 4. However, due to the presence of other pyruvate encoding genes including *PDC5* and *PDC6*, ethanol production is still to be expected in the *PDC1*-negative mutant and we observed this in our results. This is consistent with a past study by Seeboth *et al.* (1990) where *PDC1* negative mutants still maintains 60-70% activity of wild-type pyruvate decarboxylase, and it was reasoned that other genes such as *PDC5* would increase in productivity.

Sources of uncertainty and variation included the initial cell count of wild-type and mutant strains. Our experiment relied on the fact that there was the same number of cells initially in the mixture so that the data collected on the next day compared the ethanol produced from the same initial number of cells. The subsequent data points for ethanol production were compared by a per cell basis. However, if initial number of cells in the two different groups varied, this

would have meant that the mean amount of ethanol produced per cell would not be comparable. We took a haemocytometer count before diluting the wild-type solution, but did not confirm cell count in each sample after the dilution. For actual haemocytometer counts, we were uncertain if the yeast cells that were counted were all alive. Therefore, we cannot be sure the ethanol concentration is on a per cell basis with regards to the ratio of the presence of live to dead cells.

In addition, an effort was made to prevent aerobic respiration from occurring within the Falcon tubes. Carbon dioxide and ethanol are products of anaerobic fermentation, whereas carbon dioxide and water are products of aerobic fermentation (Sridhar & Saucedo 2015). As we were using ethanol production as an indicator of anaerobic fermentation, we did not want to allow aerobic respiration to occur. We addressed this concern by filling the Falcon tubes to the brim and putting the lid on top. However, it is possible that some air may have been trapped in some of the samples, introducing variability and variation within and between days. Furthermore, when resuspending the cells in preparation for hydrometer analysis, it became obvious that Falcon tubes are not reliably airtight: when shaking tubes to resuspend the pellet and creating an increase in internal pressure, leakage tended to occur.

Conclusion

From our study comparing the fermentation rates of wild-type and YRL044C mutant strains of *S. cerevisiae,* we concluded that there was a statistically significant difference in alcohol fermentation between the wild-type and the mutant; (p-value of 0.0003). A greater fermentation rate was seen in the wild-type strain. It was also concluded that the passage of time had an impact on ethanol production; increased time increased the amount of ethanol produced

(*p*-value of 0.0060). There was no significant difference between wild-type and mutant strains in the pattern of ethanol production over time.

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