The effect of temperature on the growth of wild-type *Saccharomyces cerevisiae* and the mutant strain YLR044C.

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Abstract

Saccharomyces cerevisiae is a unicellular organism that obtains energy primarily through alcohol fermentation, where it breaks down glucose into ethanol. We investigated the effect different temperature conditions on the growth rate of *S. cerevisiae* and the YLR044C mutant, a *PDC1* deletion mutant. This mutant has a deletion in the pyruvate decarboxylase gene *PDC1*, consequently reducing the rate of alcohol fermentation. Both mutant and wild-type *S. cerevisiae* were placed in 22°C, 30°C, and 40°C water baths for a set amount of time and then cell density was determined. Temperature was found to affect the growth rate of *S. cerevisiae*. The presence of the mutation was also found to have an effect on growth rate. The exponential growth rates of wild-type and mutant *S. cerevisiae* are significantly different, and temperature has opposing effects.

Introduction

Saccharomyces cerevisiae is a unicellular eukaryotic organism that is commonly used in baking and for brewing alcoholic beverages. It can use either aerobic or anaerobic respiration to break down sugars and obtain the energy required for survival and reproduction. *S. cerevisiae* preferentially respires anaerobically even in the presence of oxygen (Dickinson and Schweizer 2004), resulting in the production of ethanol through alcohol fermentation. As depicted in Figure 1, glucose is converted to ethanol through a series of metabolic steps that involve various enzymes (Nelson and Cox 2005). This metabolic process results in a net production of adenosine triphosphate (ATP), which is used for the maintenance of cellular and metabolic processes within *S. cerevisiae*, and for its growth and reproduction (Nelson and Cox 2005). A change in the fermentation pathway could affect ATP production and consequently the survival, and growth of the organism.

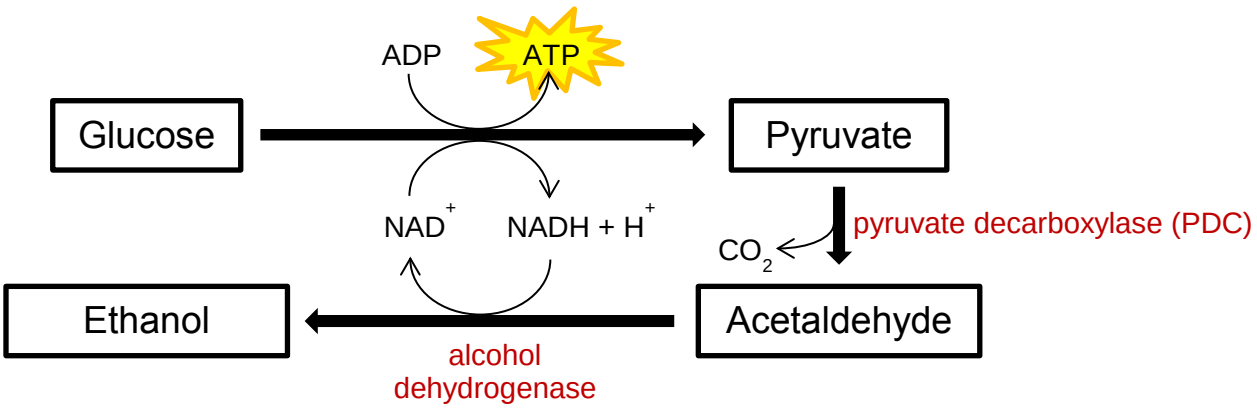

Figure 1. Overview of glucose metabolism by ethanol fermentation based on Nelson and Cox (2005). Glucose is metabolized into pyruvate through multiple steps involving numerous enzymes, yielding a net gain of ATP and net conversion of NAD^+ to NADH + H^+. Pyruvate is decarboxylated to acetaldehyde and CO_2 by the enzyme pyruvate decarboxylase (PDC). Acetaldehyde is finally reduced to ethanol by the enzyme alcohol dehydrogenase, which also regenerates NAD^+ from NADH + H^+. NADH produced during the production of pyruvate from glucose can be used for aerobic respiration. Under anaerobic respiration NAD^+ would be depleted without its regeneration by the reduction of acetaldehyde by alcohol dehydrogenase. This would halt the production of pyruvate from glucose.

We investigated the effect of a particular deficiency in the fermentation pathway on the growth of *S. cerevisiae* at various temperatures. *PDC1* encodes for a PDC isozyme that is shown in Figure 1. Our mutant has a loss of function of *PDC1*. Seeboth *et al.* (1990) found that the absence of the PDC1 isozyme induces increased expression of PDC5, another isozyme of PDC.

A number of studies have been performed on the effect of temperature on the growth rate of various yeast species including *S. cerevisiae* (Walsh and Martin 1997, Merrit 1965). From these studies we decided to analyse the exponential growth rate of *S. cerevisiae* at the following three temperatures: 22°C, a control temperature at which growth is slow; 30°C, the optimal growth temperature, and 40°C, a temperature which is nearly fatal.

Our hypotheses are:

H_{01}: Temperature has no effect on the exponential growth rate of *S. cerevisiae*.

H_{A1}: Temperature has an effect on the exponential growth rate of *S. cerevisiae*.

H_{02}: The presence of the *PDC1* deletion mutation has no effect on the exponential growth rate of *S. cerevisiae*.

H_{A2}: The presence of the *PDC1* deletion mutation has an effect on the exponential growth rate of *S. cerevisiae*.

H_{03}: Temperature has no differential effect on the exponential growth rate of wild-type *S. cerevisiae* and the *PDC1* deletion mutant *S. cerevisiae*.

H_{A3}: Temperature has a differential effect on the exponential growth rate of wild-type *S. cerevisiae* and the *PDC1* deletion mutant *S. cerevisiae*.

Prediction for H_1: The exponential growth rate of *S. cerevisiae* will be higher at 30°C than at 22°C. This is based on previous studies which show that the growth rate is higher at temperatures near 30°C than at 22°C (Walsh and Martin 1997, Merrit 1965). We also predict that cells at 40°C will have the lowest growth rate as that is near cell death of 43°C (Mensonides *et al.* 2002).

Prediction for H_2: The growth rate of our *PDC1* deletion mutant will be slower than the wild type at all temperatures since there is reduced PDC catalytic activity in the mutant (Seeboth *et al.* 1990) and therefore a lower rate of ATP production leading to a lower growth rate.

Prediction for H_3: Temperature will not have a differential effect on the growth rate of wild-type and mutant *S. cerevisiae*. Since overall metabolic rate is higher at higher temperatures (Nelson and Cox 2005) we expect growth to be higher at higher temperatures in both wild-type and mutant *S. cerevisiae*.

Methods

Wild-type *S. cerevisiae* were obtained at a starting concentration of 5.3×10^7 cells/mL; the *PDC1* deletion mutant had an original concentration of 7.8×10^7 cells/mL. These concentrations were determined using a haemocytometer, which is described in detail below. The starting concentration of wild-type and mutant yeast had to be diluted in order to prevent the yeast

population from reaching a plateau and to improve the ease of cell counting. The lag phase is the initial period of a growth curve where not much growth is observed. We then enter the exponential growth phase in which the cells double at an exponential rate. After this the cells begin to level off due to factors such as lack of nutrients or space (Jasnos *et al.* 2005), this is the plateau. We diluted by a factor of 100 resulting in the new starting concentration of wild type to be 5.3×10^6 cells/mL and mutant to be 7.8×10^7 cells/mL at time zero. From the total volume, four replicates of wild type and four of mutant were placed in a water bath at each temperature (22°C, 30°C and 40°C).

Every hour, 50 µL of wild-type or mutant cells in media were removed from each tube and fixed with Prefer for a total of nine time point samples. The fixed cells were later used for counting under the haemocytometer using an Axio compound microscope. This process was carried out for all four replicates at each temperature.

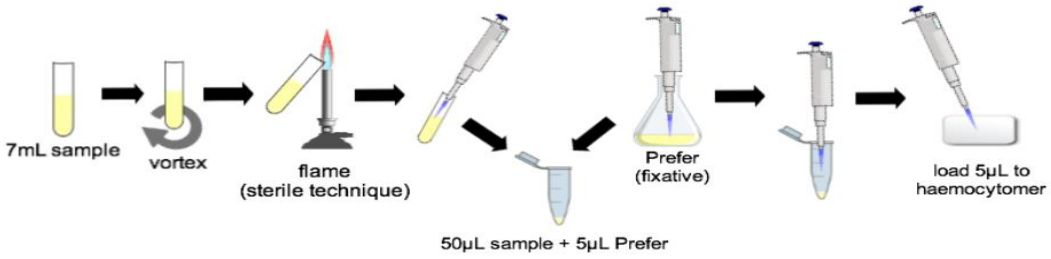

Figure 2. Overview of the procedure used to count cells of *S. cerevisiae* (wild type and mutant) used to calculate cell densities for varying temperatures (22°C, 30°C and 40°C) at one-hour time intervals.

It is important to note that the 40°C water bath had alterations to the set temperature, which drastically raised the temperature of the water to roughly 80°C killing the cells before any growth could occur (Mensonides *et al.* 2002). In order to replace the lost temperature point, a new cell culture was set up. Due to time constraints only four samples of each mutant and wild type were made and fewer cell counts were obtained at 40°C.

A two-way analysis of variance (ANOVA) was carried out on the growth rate for the first two temperatures of 22°C and 30°C. A two-way ANOVA determines if there is a difference between groups of each independent variable as well as if there are any interactions between them. If the calculated *p*-value is lower than the selected level of significance (*p*-value of 0.05) then the results are statistically significant. Because data for the 40°C treatment could only be collected for 4 hours instead of 8 hours, we did not include the 40°C treatment in the analysis.

Results

From Figure 3, we see the differences in lag time and the plateaus between the mutant and wild-type. The duration of the experiment was too short to see the plateau for all temperatures except for the mutant in Figure 3a. The *PDC1* deletion mutant is referred to as "mutant" in the graphs and legends.

From the growth curves below (Figure 3), we are able to determine where each temperature had exponential growth. This was found over the points which contained the most positive slope. We then determined the average exponential growth by averaging each value for all replicates during the same time period. As shown in Figure 3b, the exponential growth occurs between hours 3-4 for the mutant at 22°C, 5-8 hours for the wild-type at 22°C and 3-7 hours for both the mutant and wild type at 30°C.

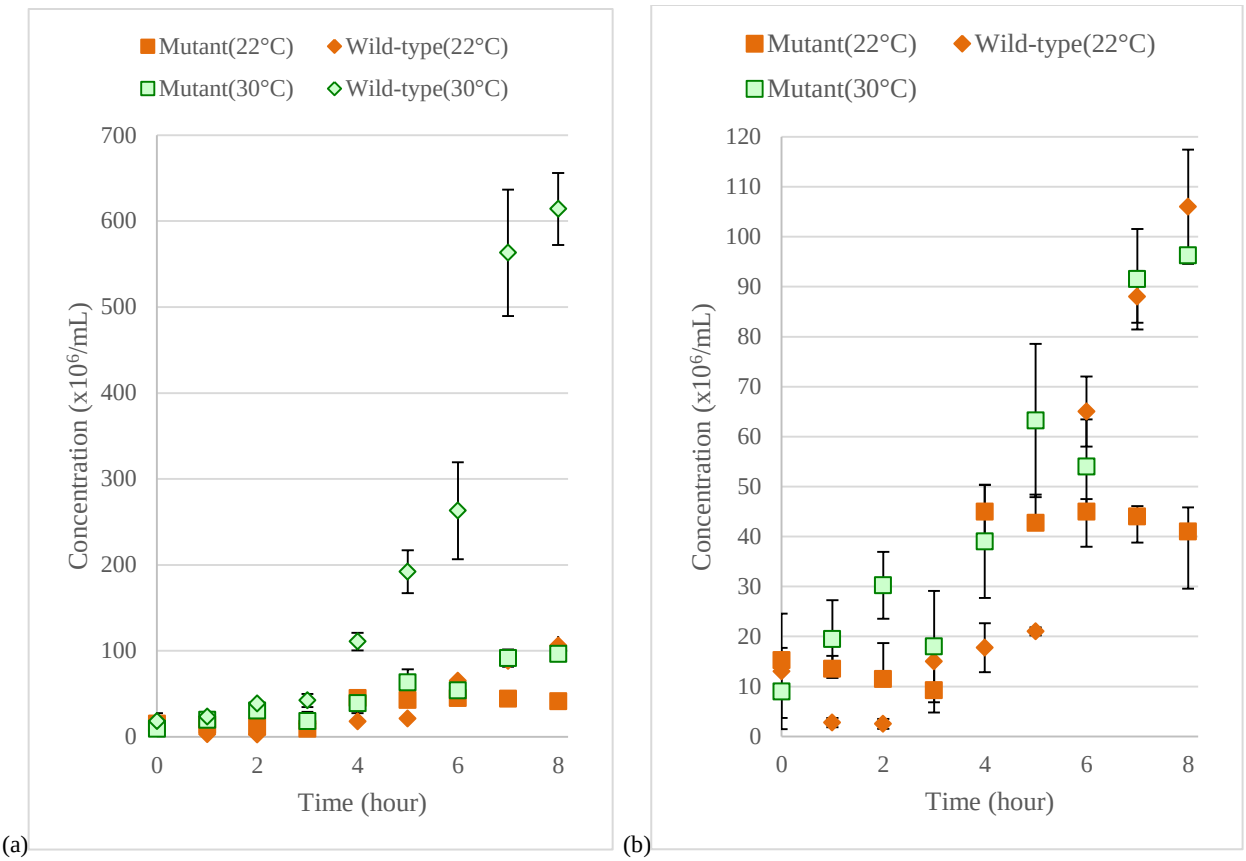

Figure 3. (a) Growth curve of mutant and wild-type *S. cerevisiae* at 22°C and 30°C. Error bars show 95% confidence intervals. (b) Growth curve of mutant and wild-type *S. cerevisiae* excluding wild-type at 30°C. Error bars show 95% confidence intervals.

The exponential growth rates of the wild-type and mutant are shown in Figure 4. We see that temperature does have an effect on growth rate, p-value = $4\text{x}10^{-6}$ (H_1). This graph also shows that there are differences between the mutant and wild-type growth rates with a p-value of $4\text{x}10^{-7}$ (H_2). We can see that at a temperature of 22°C the rates are very similar for mutant (35.8±5.0) and wild type (28.3±4.0). However, there is a significant difference (p-value = $1\text{x}10^{-7}$) between the two strains at 30°C (H_3), the mutant was 18.4±3.9 and wild-type was 130.2±19.4.

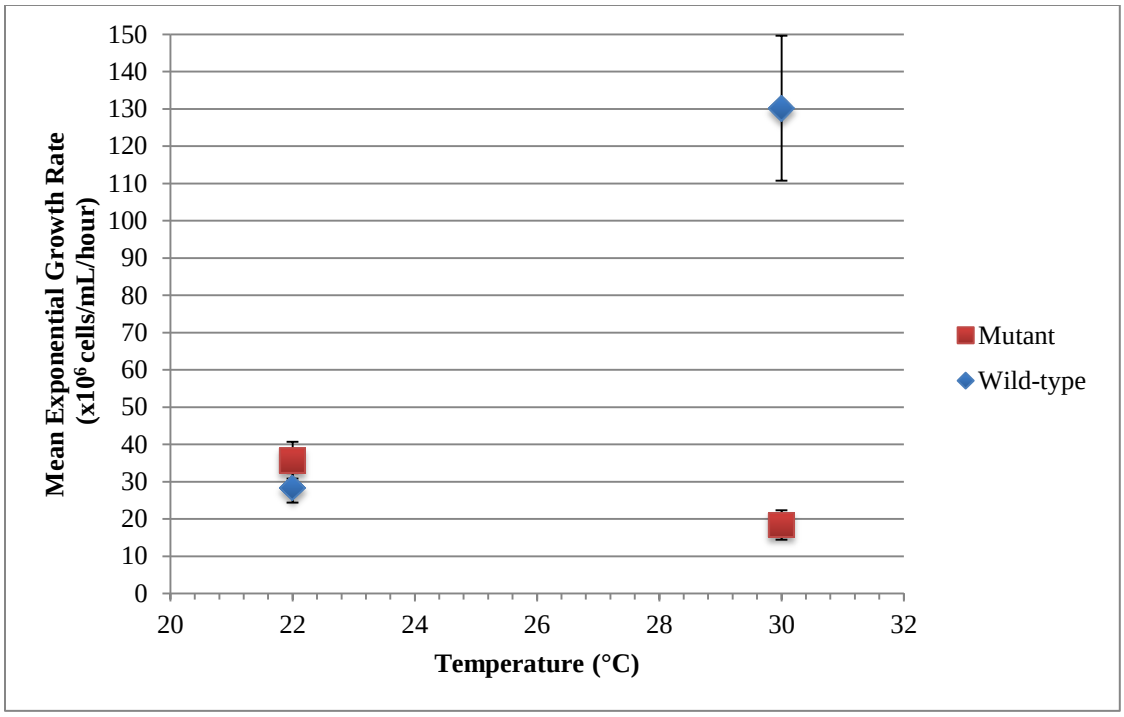

Figure 4. Exponential growth rate of *S. cerevisiae* for temperatures of 22°C and 30°C. Error bars represent the 95% confidence intervals.

Discussion

We analyzed the data from the 30°C and 22°C temperatures for both wild-type and mutant strains of *S. cerevisiae*. This provided enough data for each temperature to see the lag and exponential portions in our growth curves (Figure 4).

We reject H_{01} - Temperature has no effect on the exponential growth rate of *S. cerevisiae* - and provide support for H_{A1} - Temperature has an effect on the exponential growth rate of *S. cerevisiae* ($p<0.05$). This agrees with our predictions based on the literature which shows that the growth rate of *S. cerevisiae* increases with increasing temperature, until a lethal temperature of about 43°C (Walsh and Martin 1997, Jones and Hough 1970, Merrit 1965, Arroyo-López *et al.* 2009). The higher cell concentration of the wild type at 30°C (~6 x 10^8 cells/mL) than at 22°C (~1 x 10^8 cells/mL) confirms that a temperature of 31-35°C is optimal for *S. cerevisiae* growth, as shown by Walsh and Martin (1997). The reason we see this phenomenon is because

temperature has an effect on enzyme kinetics, and in turn the overall metabolism of the organism (Nelson and Cox 2005). Catalytic rate of an enzyme increases with increasing temperatures until its denaturation temperature, which is approximately 40°C for most enzymes (Nelson and Cox 2005). A higher rate of metabolism in the organism results in a higher rate of ATP production and a higher rate at which ATP is utilized for growth – ultimately increasing growth rate.

We also reject H_{02} - The presence of the *PDC1* deletion mutation has no effect on the exponential growth rate of *S. cerevisiae* - and provide support for H_{A2} - The presence of the *PDC1* deletion mutation has an effect on the exponential growth rate of *S. cerevisiae* since $p<0.05$. This agrees with our prediction. Previous studies show decreased CO_2 production in the *PDC1* mutant (Kabolizadeh *et al*. 2013, Hosseini *et al*. 2015, Zimmermann and Entian 1997), which is an indication of, reduced PDC activity (Figure 1). This in turn reduces the rate of ATP production. As ATP is essential for the growth of *S. cerevisiae*, reduced ATP levels stunt growth (Nelson and Cox 2005). Also, Seeboth *et al.* (1990) have shown that PDC's catalytic activity is much lower in the *PDC1* mutant than in the wild type. PDC5, the isozyme expressed in the absence of PDC1, has lower catalytic activity than PDC1 (Hohmann and Cederberg 1990, Seeboth *et al.*1990), which explains why the wild type has a higher exponential growth rate than the mutant.

We also reject H_{03} - Temperature has no differential effect on the exponential growth rate of wild-type *S. cerevisiae* and the *PDC1* deletion mutant *S. cerevisiae* - and instead provide support for H_{A3} - Temperature has a differential effect on the exponential growth rate of wild-type *S. cerevisiae* and the *PDC1* deletion mutant *S. cerevisiae* - since $p < 0.05$. We had predicted that temperature would not have a differential effect on the exponential growth rate of the mutant. We predicted that the exponential growth rate would be higher at higher temperatures in

both the mutant and wild-type. The effect of temperature on exponential growth of the mutant is opposite to that of wild-type *S. cerevisiae* which has a much higher exponential growth rate at 30°C than at 22°C (Figure 4). As mentioned previously the decrease in function of *PDC1* increases the expression of the PDC5 isozyme (Seeboth *et al.* 1990) and it is the main enzyme responsible for pyruvate decarboxylation in our mutant (Hohmann and Cederberg 1990). Therefore our results could suggest that the catalytic rate of PDC5 is higher at 22°C than at 30°C. However it should be noted that our experimental design is not sufficient to show that the catalytic rate of PDC5 is higher at 22°C than at 30°C. The PDC5 enzyme would have to be isolated and its kinetics determined *in-vitro* at different temperatures to provide support for this theory.

There are many other pathways, besides alcohol fermentation, which could have been affected by knockout of *PDC1* and here is one way we propose to test this. If we add NAD^+ to the growth media we can bypass the need for PDC5 and alcohol dehydrogenase (ADH) in the fermentation pathway of the *PDC1* deletion mutant (Figure 1). If we then rescued the wild-type exponential growth in the *PDC1* deletion mutant at both 22°C and 30°C it could suggest that either PDC5 or alcohol dehydrogenase (ADH) are solely responsible for the reduced growth rate seen in the mutant at 30°C (Figure 4). If the addition of NAD^+ does not recover the wild-type exponential growth rate it could suggest that deletion of *PDC1* has other effects beyond the fermentation pathway.

Sources of error could have been due to lack of thorough mixing, the process of sampling, or human error. Insufficient mixing of our sample could have led us to a lower and inaccurate overall average cell count. In order to reduce this source of error we made sure that all group members vortexed each sample for three seconds at the highest speed before moving on to

the next step. Another source of error could have come from time differences in our sampling process. Cells that were sampled and fixed first may have a slightly lower count than cells sampled and fixed a few minutes later. However, to reduce the effect of this error on our experiment we extracted the samples in the same order each hour. This allowed for the growth of the replicates to be around the same at each time interval. Lastly, because our experiment largely depended on cell counts there could have been human error and variability when performing haemocytometer readings. This could have drastically affected the variation in our results because one person may have counted differently compared to another group member. We minimized this by establishing criteria for what cells are, and each person counted a mixture of wild-type and mutant cells. This eliminated confounding variables.

Conclusion

Our statistical analysis enabled us to reject all our null hypotheses and provide support for all our alternative hypotheses. Temperature has an effect on the exponential growth rate of *S. cerevisiae*, and as we predicted the rate is much higher at the optimal temperature of 30°C than at 22°C. The presence of the *PDC1* mutation has an effect on the exponential growth rate of *S. cerevisiae*, in agreement with our prediction based on the PDC activity and CO_2 production in the mutant, which were determined in previous studies. Contrary to our predictions temperature has a differential effect on the exponential growth rate of wild-type *S. cerevisiae* and the *PDC1* deletion mutant *S. cerevisiae*. Temperature has different effects on growth in the wild type and mutant and further experiments could be conducted to determine the precise mechanism for this phenomenon.

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