Changes in growth rate of wild-type and *PDC1* mutant *Saccharomyces cerevisiae* in response to caffeine

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Abstract

Saccharomyces cerevisiae is one of the most studied organisms and can provide vital insight into human physiology. With caffeine the most common drug used by humans, the objective of this experiment was to study how varying caffeine concentrations affected the cell counts of wild-type and mutant strains of *S. cerevisiae* over time. We prepared three treatments of caffeine concentrations: 100 mM, 150 mM, and 0 mM with three replicates for each treatment. The response of average cell growth rate was measured from 0 to 6 hours of incubation by counting the number of cells using a haemocytometer. We found a significant difference in growth rate between the wild-type and mutant strains of *S. cerevisiae* using a two-way ANOVA test ($p=0.030$). This is attributed to the deletion of the PDC1 gene in the mutant, resulting in decreased cell growth due to impaired glucose fermentation. Furthermore, there was a significant difference in the growth rate of *S. cerevisiae* at different caffeine concentrations ($p=2.5\times10^{-6}$). There was also a significant difference between the caffeine effect on wild-type compared to its effect on mutant *S. cerevisiae* ($p=0.0099$). We interpret this to be a result of the mutant already functioning with less pyruvate decarboxylase enzyme, so it will be less affected by the presence of caffeine. From our data, we can therefore conclude that mutant *S. cerevisiae* has greater resistance to caffeine than wild-type *S. cerevisiae*.

Introduction

Since being the first eukaryote to have its entire genome sequenced, *S. cerevisiae* has remained at the forefront of genetics research (Ostergaard *et al.* 2000). The species of yeast used in this experiment is *Saccharomyces cerevisia*e. In both our experiment and in the literature, caffeine was found to have an interesting effect on various processes within the yeast.

The mutant used in this experiment is YLR044C, a PDC1 mutant. The PDC1 gene codes for indolepyruvate decarboxylase, which catalyzes alcoholic fermentation. Phenotypic defects caused by its deletion include decreased rate of fermentation as well as increased resistance to caffeine (Schaaff *et al*. 1989).

The objective of this experiment was to test how varying concentrations of caffeine affect abundance of wild-type and mutant strains of *S. cerevisiae* over time. This investigation is important

because testing caffeine as a factor of environmental stress on the *S. cerevisiae* may offer insight to the effects of other stresses such as oxidative stress, heat sensitivity, and acid pH resistance that follow similar pathways (Calvo *et al.* 2009). In this way, the findings can possibly be extrapolated as a model for caffeine effect on the function of human cells. It also provides information regarding the mutant PDC1 gene and its differences from wild-type *S. cerevisiae*. The differences in fermentative growth rate and caffeine resistance have led to the growth rate patterns observed in this experiment.

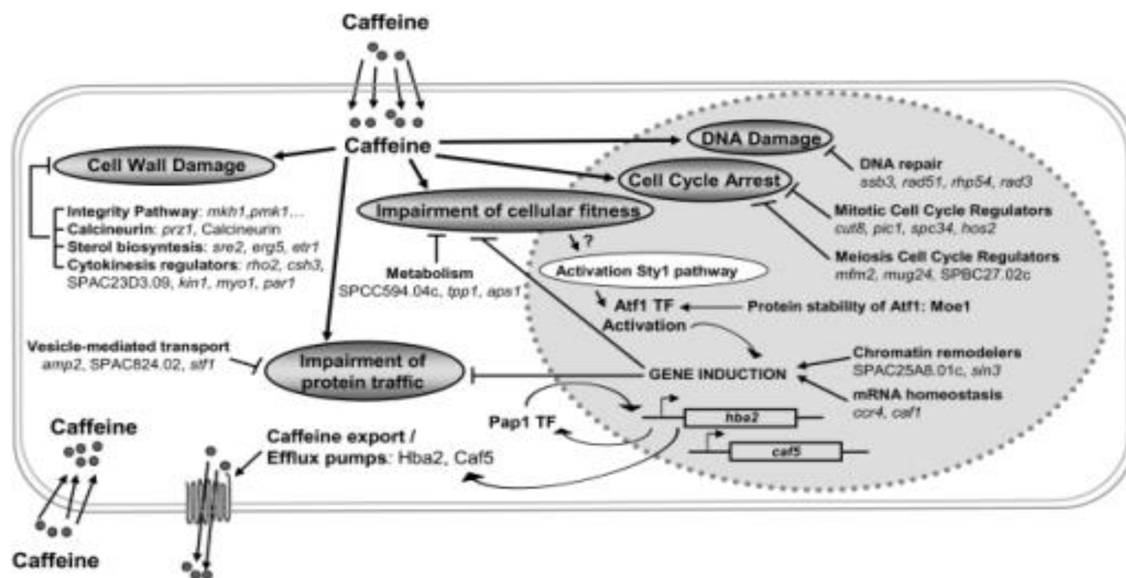

Figure 1: Effect of caffeine on yeast and its defense response. (Image: Calvo *et al.* 2009)

As shown in Figure 1, the effect of caffeine on *S. cerevisiae* is inhibition of cellular growth in addition to impairment of cellular fitness, cell cycle arrest, DNA damage, and most prominently, alteration of the cell wall architecture. The cell wall is affected via the yeast cell wall integrity mitogen-activated protein kinase (CWI-MAPK) pathway, which regulates responses to adapt to cell wall stress (Kuranda e*t al.* 2006). The Tor1 kinase in yeast is a target of caffeine. Once this kinase is inhibited, it activates the Pkc1p-Mpk1p kinase cascade, which is a mechanism that is activated when the cell wall integrity is threatened (Kuranda e*t al.* 2006). These are the mechanisms within the cell wall integrity pathway that consequently lead to dysfunctions in the cell wall (Figure 1).

The cell wall is critical for cell expansion during growth and morphogenesis, with growth being the rate-limiting factor for cell proliferation (Johnston *et al.* 1977). This means the cell needs to grow to a sufficient size before it undergoes division; if cells stop dividing there will not be an observed increase in abundance. The cell wall also functions to maintain cell shape, which is essential in the formation of a bud and hence cell division (Cid *et al.* 1995). These are all ways in which the response we are measuring, growth rate via cell count, is affected by the presence of caffeine.

Our hypotheses are:

H_{01}: Presence of the PDC1 mutation has no effect or increases growth rate of *Saccharomyces cerevisiae*.

H_{A1}: Presence of the PDC1 mutation decreases growth rate of *Saccharomyces cerevisiae*.

H_{02}: Caffeine concentration has no effect or increases growth rate of *Saccharomyces cerevisiae*.

H_{A2}: Increase in caffeine concentration decreases growth rate of *Saccharomyces cerevisiae*.

H_{03}: The effect of caffeine concentration on the change in growth rate of *Saccharomyces cerevisiae* is the same in wild type and mutant.

H_{A3}: The effect of caffeine concentration on the change in growth rate of *Saccharomyces cerevisiae* is not the same in wild type and mutant.

Methods

We began the experiment with a sample of each of the wild-type and mutant *S. cerevisiae.* To ensure a concentration of $7x10^7$ cells/mL, we went through the process of counting both samples; for each of our samples, we vortexed the tube for 15 seconds to ensure even distribution of cells. We then transferred 100 µL of each sample into two new 500 µL plastic tubes for counting. After fixing the cells by adding and thoroughly mixing 10 µL of Prefer fixative to each tube, we finger

vortexed each tube before loading 10 μL of the sample into the haemocytometer for counting. Our procedure is outlined in Figure 2.

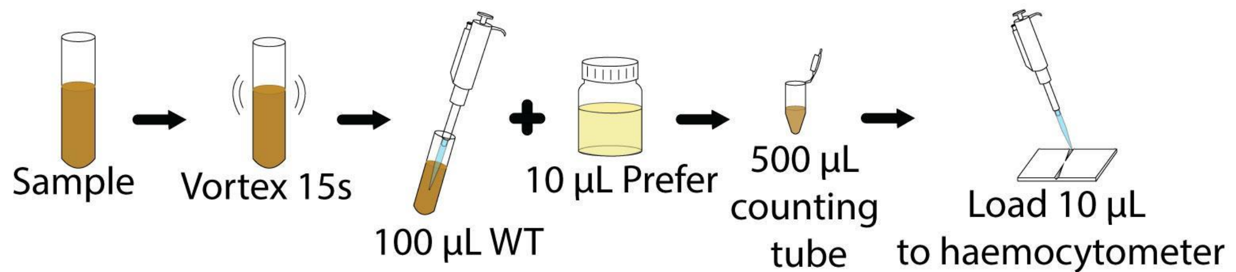

Figure 2: The procedure used to count cells of *S. cerevisiae* to determine concentration of the sample.

We found initial concentrations of 7.166×10^{7} cells/mL for wild-type and 7.553×10^{7} cells/mL for mutant *S. cerevisiae.* We diluted both with yeast growth medium (YPD) to bring each to a concentration of 1.2×10^{6} cells/mL. We then prepared the treatments, depicted in Figure 3, combining 2.5 mL of sample with 2.5 mL of either the 200mM or 300mM of caffeine treatment into a 6 mL test tube to reach 5 mL of sample, with a concentration of 6.0×10^{5} cells/mL. Our treatments were 0 mM caffeine for wild-type *S. cerevisiae,* which we labelled WTC; 100 mM of caffeine for wild-type *S. cerevisiae,* which we labelled WTA; 150 mM of caffeine for wild-type *S. cerevisiae* (WTB); 0 mM of caffeine for mutant *S. cerevisiae* (MUTC); 100 mM of caffeine for mutant *S. cerevisiae* (MUTA); and 150 mM of caffeine for mutant *S. cerevisiae* (MUTB). We had 3 replicates for each treatment.

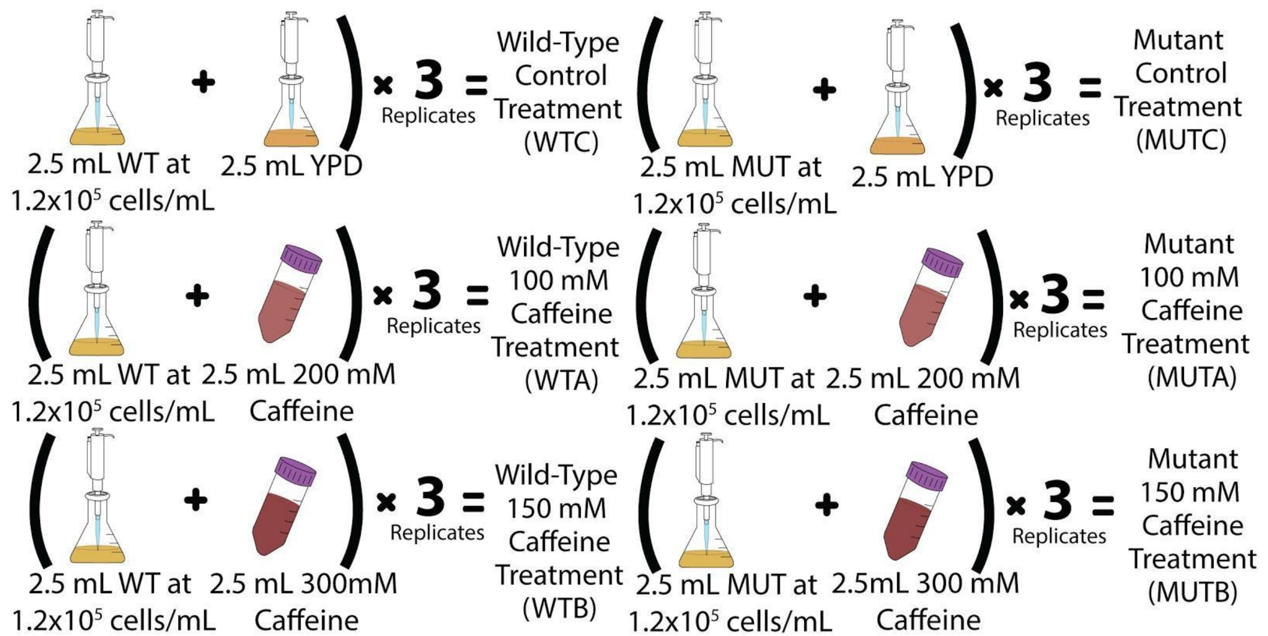

Figure 3: The components used to create all 9 treatments of our experiment: WTC, WTA, WTB, MUTC, MUTA, MUTB.

After preparing the samples, we counted the cells again to find cell counts for time t=0 hours. We used the procedure previously outlined, repeating this method of preparation for counting each replicate in each sample. We then placed our samples into the incubator on a shaker at 30°C, and repeated the counting process at times t=2 h, t=4 h, t=6 h, t=19 h, and t=21 h.

At t=19 h and t=21 h, our control treatments for both wild-type and mutant *S. cerevisiae* were too densely populated for accurate counting. For easier counting, we diluted the sample for counting by a factor of 10 by combining 10 µL of the sample with 90 µL of YPD, adding the usual 10 µL of Prefer, then counting as usual.

We calculated the growth rates of each treatment, grouping the 3 replicates for each, then graphed the average growth rates with 95% confidence intervals. We used a two-way ANOVA test for our three hypotheses, determining the effect of cell type and caffeine concentration, each individually and combined, on growth rate, averaged between t=0 h and t=6 h. This period of time during incubation was selected as it represents the exponential phase of the cell growth curve for

all treatments. The two factors analyzed were caffeine concentration, with three levels (0, 100, and 150 mM), and cell type, with two levels (wild-type and mutant *S. cerevisiae*).

Results

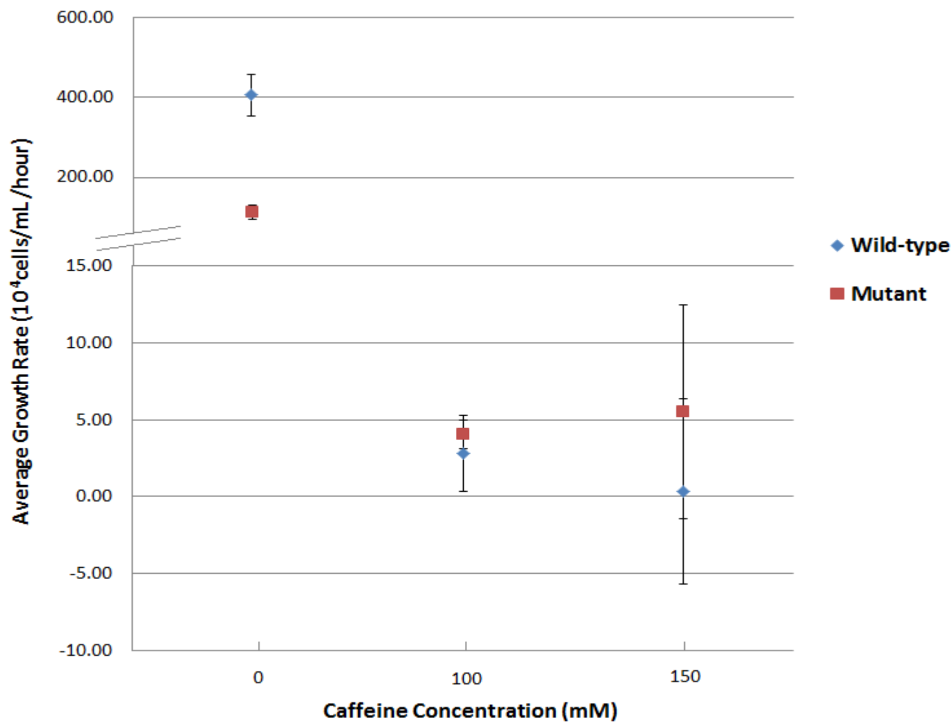

Figure 4: The mean growth rate of wild-type and mutant *S. cerevisiae* replicates between 0 to 6 hours at 0, 100, and 150 mM caffeine concentrations are represented with 95% confidence intervals.

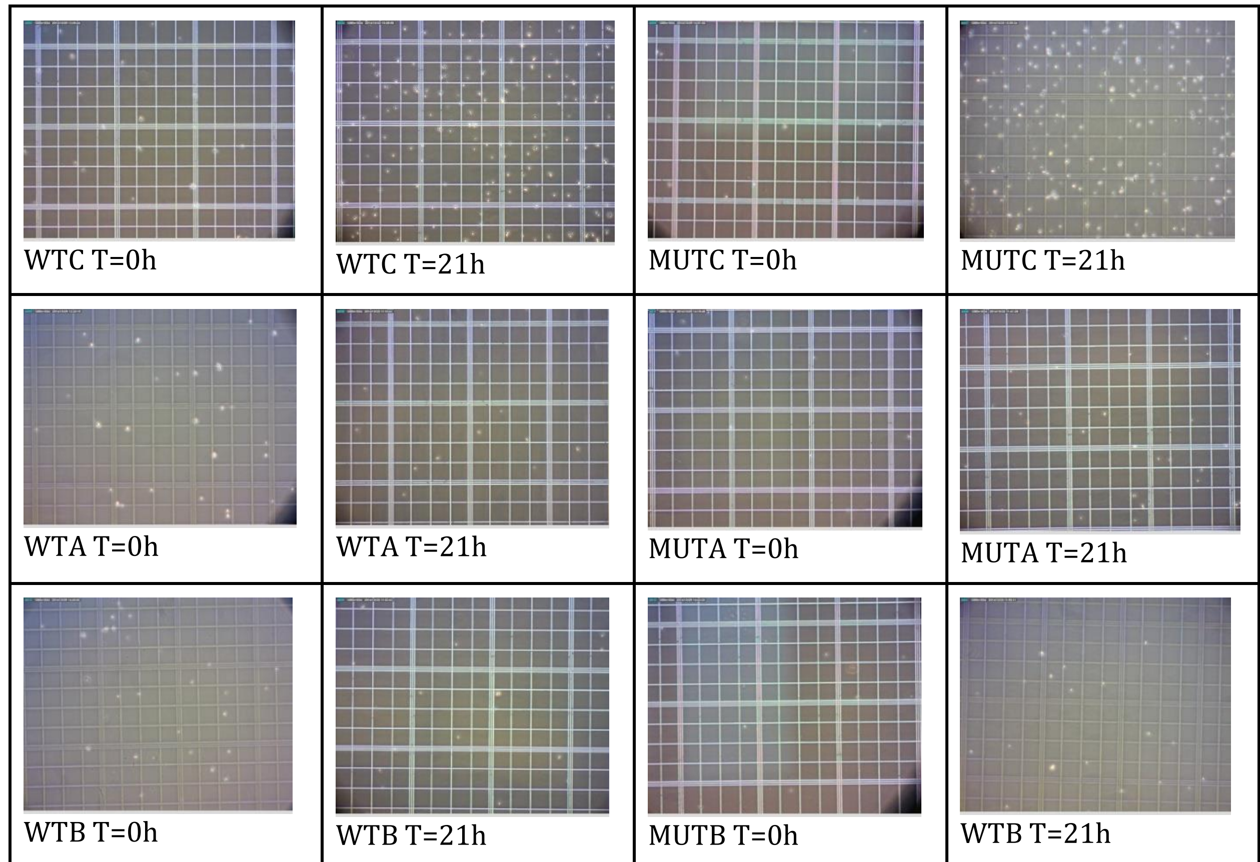

Figure 5: DinoXcope photographs at 100x magnification on an Axio compound microscope taken for WTC replicate 1 at 0 and 21 hours (top row, left); WTA replicate 1 at 0 and 21 hours; WTB replicate 1 at 0 and 21 hours (bottom row, left); MUTC replicate 1 at 0 and 21 hours (top row, right); MUTA replicate 1 at 0 and 21 hours; MUTB replicate 1 at 0 and 21 hours (bottom row, right).

The growth rates for each sample were determined from the slope of linear regression lines from 0 to 6 hours of incubation. This period of time represents the exponential growth phase of *S. cerevisiae* during the cell growth cycle. The mean growth rates were used for the two-way ANOVA test at 0.05 significance level, where testing H_{01} for effect of cell type yielded the p-value of 0.030, testing H_{02} for effect of caffeine concentration yielded the p-value of 2.5×10^{-6}, and testing H_{03} for effect of caffeine concentration on change in growth rate in the different cell types yielded the p-value of 0.0099. All of these p-values are less than 0.05, thus indicating a significant difference in the response of mean growth rates for the factors tested.

In Figures 4 and 5, differences in mean growth rate and abundance between wild-type and mutant are observed. There is a trend of mutant samples having a higher mean growth rate than wild-type when exposed to caffeine, while the opposite is true for when there is no caffeine present. Wild-type control samples have a higher mean growth rate of 4.1×10^6 cells/mL/hour, compared to mutant control samples, which have a mean growth rate of 1.1×10^6 cells/mL/hour. Mean growth rates at 100 mM caffeine are 2.8×10^4 cells/mL/hour for wild-type samples and 4.1×10^4 cells/mL/hour for mutant samples, with a mean growth only slightly higher than that of wild-type as seen in Figure 4. Finally, mean growth rates at 150 mM caffeine are 3.4×10^3 cells/mL/hour for wild-type samples and 5.6×10^4 cells/mL/hour for mutant samples. Although the error bars representing confidence intervals for the caffeine samples overlap with each other, a trend of higher mean growth rate is seen for mutant samples growing in caffeine. The wild-type control has a mean growth rate much larger than that of the mutant control and the error bars do not overlap for these data points.

The DinoXcope images in Figure 5 were captured with an Axio compound microscope at 100x magnification and show both wild-type and mutant *S. cerevisiae* cells became smaller in size during growth in caffeine. There is also a visibly lower abundance of cells in caffeine samples compared to the control samples.

Discussion

The presence of the mutation does have a significant effect on the growth rate of *S. cerevisiae*, since $p<0.05$, we reject our first null hypothesis and provide support for our first alternate hypothesis. This finding is supported by van Maris *et al.* (2004) and Schaaff *et al.* (1989), where it was found that the deletion of the PDC1 gene results in decreased production of pyruvate decarboxylase, an enzyme necessary for glucose fermentation. This impairment in ability to ferment glucose causes PDC1 mutant strains of *S. cerevisiae* to produce less energy, and as energy is

essential for cell proliferation (Johnston et al. 1977); this results in decreased rate of cell growth when compared to wild-type *S. cerevisiae.* This is supported by the data shown in Figure 4 where the difference in mean growth rate between the wild-type and mutant controls is observed and wild-type *S. cerevisiae* has greater rate. Figure 5 also shows a difference in the abundance of cells in wild-type and mutant samples viewed, with caffeine exposed samples having fewer cells in the field of view.

Since $p<0.05$, we reject our second null hypothesis and provide support for our second alternate hypothesis, indicating that the concentration level of caffeine has an effect on the growth rate of *S. cerevisiae*. This finding is supported by Kuranda *et al.* (2006), who found that caffeine causes a drop in intracellular levels of cyclic adenosine monophosphate (cAMP), which then triggers a reaction in protein kinase A (PKA), an important enzyme that can control the available energy in the cell (Taylor *et al*. 2003, Kuranda *et al*. 2006). The resultant change in available energy in the cell may be the cause of the decreased growth rate of *S. cerevisiae,* as energy is needed to bring cells to a sufficient size before division is possible (Johnston *et al.* 1977). The result of this mechanism is exemplified in our findings where we found that the presence of caffeine decreases growth rate in both wild-type and mutant *S. cerevisiae* (Figure 4). The effect of increasing caffeine concentration is shown when looking at the decreased growth rates when caffeine concentration is increased to 150 mM for both wild-type and mutant samples compared to caffeine concentration of 100 mM.

Because $p<0.05$, we reject our third null hypothesis and provide support for our third alternate hypothesis. From this, we can deduce that the effect caffeine has on the change in growth rate of *S. cerevisiae* differs between wild-type and mutant. In Figure 4, it shows that both controls grow with high mean growth rates, while wild-type and mutant treated with 100 mM of caffeine grew at lower rates, and wild-type and mutant treated with 150 mM caffeine grew at even lower rates. Furthermore, in caffeine treatments of 100 mM and 150 mM, mutant mean growth rates were

higher than wild-type mean growth rates. The significant difference in the effect of caffeine on wild-type *S. cerevisiae* compared to mutant can be attributed to the altered PDC1 gene in the mutant. In previous gel electrophoresis analysis, our mutant *S. cerevisiae* sample, YLR044C, was found to have an insertion at the PDC1 gene, which would change the structure and most likely functionality of the resulting protein. This gene is responsible for producing the protein pyruvate decarboxylase (PDC1), an enzyme which catalyzes alcoholic fermentation (Schaaff *et al.* 1989). The caffeine targets the Tor1 kinase in yeast. Once this kinase is inhibited, cyclic adenosine monophosphate (cAMP) levels decrease which, in turn, decreases phosphorylation and the activity of pyruvate decarboxylase (Schaaff *et al*. 1989). If the mutant with a mutated PDC1 gene is already functioning with decreased pyruvate decarboxylase enzyme, it will be less impacted by the presence of caffeine (Schaaff *et al.* 1989). Therefore, this biological reasoning supports our hypothesis that caffeine affects the growth rate of mutant *S. cerevisiae* differently than the wild-type and shows that, in fact, the mutant has greater resistance to caffeine than the wild type.

A source of error in our experiment could have been lack of thorough mixing. As we had so many replicates to be mixed at six different time intervals, there is a possibility that a sample was not sufficiently mixed before counting. For example, there is some variation in our data as our WTA sample (100 mM caffeine treatment to wild-type) decreased in abundance between 2 and 4 hours, but increased greatly in abundance between 4 and 6 hours. This could be due to improper vortexing or mixing of a replicate, decreasing average cell count and leading to an inaccurate representation of abundance of *S. cerevisiae* measured at this time. Improper mixing may have also occurred for measurement of other data points, though the effects may not have been as extreme as this example. Another source of error could have been a difference the amount of time spent preparing the samples for counting; the process of fixing all the samples for counting required approximately 20 minutes at each time interval, allowing samples prepared last to grow for longer than samples

prepared first. To correct for this, samples were prepared in the same order each time, thus allowing each replicate to grow for approximately the same amount of time.

If these possible errors and inconsistencies were eliminated, precision would improve and the observed 95% confidence interval error bars in Figure 4 would be much smaller and have less overlap with other mean growth rates. Although the confidence intervals were large for certain samples, the mean growth rates are quite different, therefore the effects of cell type and caffeine are significant.

Conclusion

The results of our experiment offer insight into the response of *Saccharomyces cerevisiae* to environmental stress and confirm literature findings regarding the effect of caffeine on wild-type and mutant *S. cerevisiae*. Analysis of our collected data caused us to reject our first null hypothesis and provide support for our first alternate hypothesis, indicating a significant difference in growth rate of wild-type and mutant *S. cerevisiae*, where wild type had increased growth rate when compared to mutant growth rate. We reject our second null hypothesis and provide support for our second null hypothesis, showing a significant decrease in growth rate of *S. cerevisiae* when exposed to different concentrations of caffeine. We also reject our third null hypothesis and provide support for our third alternate hypothesis, showing that growth rate of wild-type *S. cerevisiae* in response to increased caffeine concentration is lower than growth rate of mutant *S. cerevisiae* in response to increased caffeine concentration.

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