Effect of caffeine on the growth rate of wild-type and PDC1 mutant *Saccharomyces cerevisiae*

Geoff J. McKendry, Angelica G. Reyes, Jastej K. Sidhu, Ryan P. Song, Daphne S. Xuan

Abstract

Caffeine is the most consumed drug by humans. In order to understand its effects we must look at the enzymes and pathways it affects to understand what consequences it may have on our own bodies. In this experiment, wild-type *Saccharomyces cerevisiae* and PDC1 mutant *S. cerevisiae* growth rates were compared for both control and caffeine-containing samples. As well, the proportions of wild-type *S. cerevisiae* and mutant *S. cerevisiae* growth rates in caffeine to their respective growth rates in caffeine-free media were compared. Using a χ^2 goodness of fit test we were able to show that there was a significant decrease in the growth rate of wild-type with caffeine compared to wild-type control ($p<0.05$) as well as a significant decrease in the growth rate of mutant with caffeine compared to mutant control ($p<0.05$). A χ^2 goodness of fit test was also used to show that there was a significant decrease in the proportion of wild-type with caffeine to wild-type control compared to the respective mutant proportions ($p<0.05$). This indicates that PDC1 mutant *S. cerevisiae* is more resistant to caffeine than wild-type *S. cerevisiae* and that pyruvate decarboxylase may be affected by this drug.

Introduction

Saccharomyces cerevisiae is one of the most widely used microorganisms in biological research (Ostergaard *et al.* 2000). One reason that *S. cerevisiae* is desirable is for its use in testing drugs which, due to ethical issues, may not be tested on higher-order organisms such as humans (Karathia *et al.* 2011).

One drug that is often of interest is caffeine. Compared to other psychoactive drugs, such as nicotine, alcohol, and illicit drugs, caffeine is the most widely consumed by human beings (James 2011). This raises concerns about how caffeine actually affects human physiology. However, in order to understand how this drug works in humans, we must first understand how it interacts with basic eukaryotic cells.

What is known about the effects of caffeine on *S. cerevisiae* is that it is a kinase inhibitor (Kapitzky *et al.* 2010). More specifically, the drug has an affinity for the TOR1 kinase, which is a subunit of the TORC1 complex (see Figure 1) (Homann *et al.* 2009, *Saccharomyces* Genome Database). This complex is involved in controlling cell growth and division in response to nutrient levels and inhibition of the TOR1 kinase leads to false signaling that the cell is starving (Homann *et al.* 2009). It is also thought that caffeine inhibits cyclic adenosine monophosphate (cAMP), which subsequently affects protein kinase A (PKA), an important enzyme that can control the available energy in the cell (see Figure 1) (Taylor *et al.* 2003, Kuranda *et al.* 2006).

Another pathway to consider is respiration, a process by which energy for the cell is produced (Pronk *et al.* 1996). *Saccharomyces cerevisiae* can engage in two respiratory pathways: anaerobic and aerobic (Pronk *et al.* 1996). In the anaerobic pathway pyruvate is converted to acetaldehyde and carbon dioxide by the enzyme pyruvate decarboxylase (see Figure 1) (Pronk *et al.* 1996). What is of interest is that pyruvate decarboxylase is activated by phosphorylation, which could potentially be affected by the cell's energy levels.

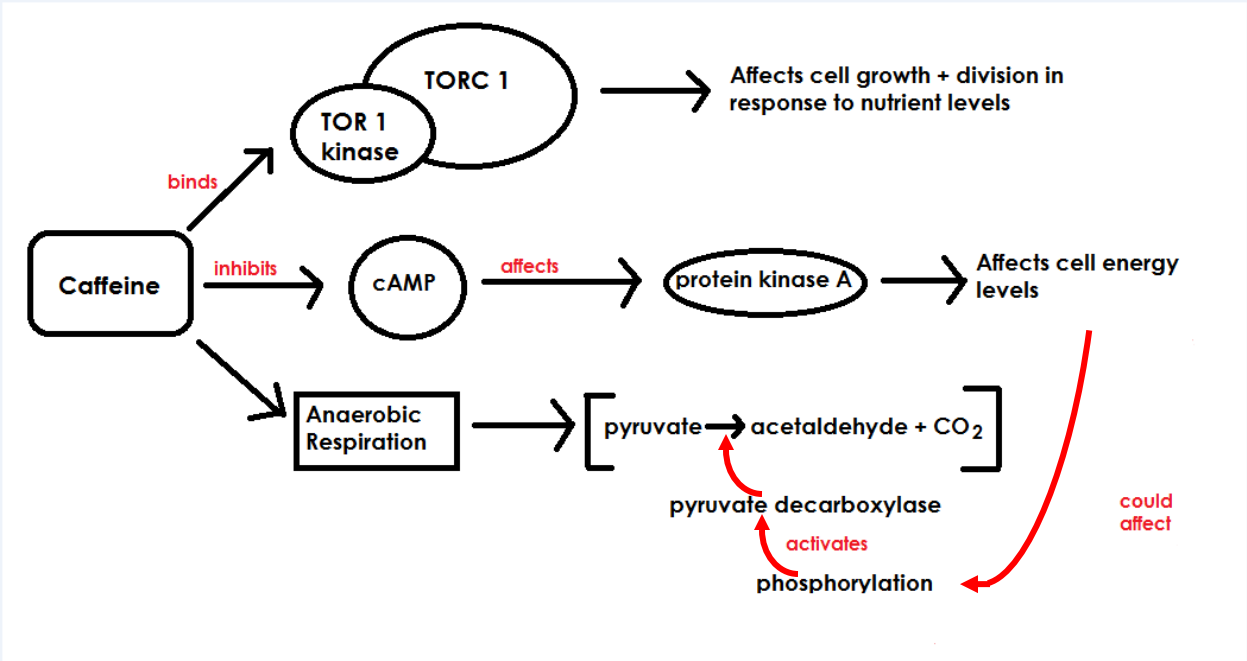

Figure 1. The effect of caffeine on TOR1 and cAMP in *S. cerevisiae* cells and the potential association with pyruvate decarboxylase.

In order to test whether caffeine affects the pyruvate decarboxylase enzyme specifically, wild-type *S. cerevisiae* would have to be compared to a mutant lacking this enzyme. To do this, mutant *S. cerevisiae* cells with a deletion in the PDC1 gene, which codes for one of three isozymes in pyruvate decarboxylase, were used in this experiment (*Saccharomyces* Genome Database).

There are three hypotheses that need to be tested to show whether or not caffeine affects the pyruvate decarboxylase enzyme. They are as follows:

H_{A1}: In the presence of caffeine there will be a decrease in the growth rate of wild-type *Saccharomyces cerevisiae.*

H_{O1}: In the presence of caffeine there will be an increase or no change in growth rate of wild-type *Saccharomyces cerevisiae*.

H_{A2}: In the presence of caffeine there will be a decrease in the growth rate of mutant *Saccharomyces cerevisiae*.

H_{O2}: In the presence of caffeine there will be an increase or no change in growth rate of mutant *Saccharomyces cerevisiae*.

H_{A3}: The growth rate of wild-type *Saccharomyces cerevisiae* in the presence of caffeine is decreased compared to the growth rate of mutant *Saccharomyces cerevisiae* in the presence of caffeine.

H_{O3}: The growth rate of wild-type *Saccharomyces cerevisiae* in the presence of caffeine is unchanged or increases compared to the growth rate of mutant *Saccharomyces cerevisiae* in the presence of caffeine.

The first two alternative hypotheses were formulated from literature indicating that the TOR1 kinase and cAMP would be inhibited in the presence of caffeine, leading to decreased growth rate (Kuranda *et al.* 2006, Homann *et al.* 2009). Based on this information we believe that if the pyruvate decarboxylase enzyme is associated with either of these two processes, then cells containing a mutated PDC1 gene will be less affected as they already have a decrease of function for that enzyme.

Methods

We started with two large test tubes containing 20 mL of approximately 1 x 10^7 cells/mL of *S. cerevisiae* in each: one tube contained cells of the BY4741A wild-type strain and the other contained cells of the YLR044C mutant strain. We also had a large flask of YPD (Yeast Extract Peptone Dextrose) growth medium and 20 mL of 100 mM caffeine. First, we measured the concentration of the cells in the test tubes. We ensured that the cells were evenly distributed in their medium by vortexing the tubes before we pipetted out 100 µL of both mutant and wild-type cells into microcentrifuge tubes for counting. We then added 10 µL of Prefer™ fixative and diluted the samples by 10 times

to make counting easier. To count the cells, we pipetted 10 µL of the samples into a haemocytometer and counted using the haemocytometer grid with a volume of 10^{-4} mL.

Using our cell counts, we calculated the volumes we needed to take from the mutant and the wild-type test tubes to mix with YPD in order to obtain 75 mL at 2 x 10^4 cells/mL of both mutant and wild-type cells. We then used these to produce our desired 1 x 10^4 cells/mL replicates for caffeine treatments (50 mM caffeine) and controls by diluting with either YPD or 100 mM caffeine (see Figure 2). We added 1.5 mL of our mutant stock to six test tubes and 1.5 mL of wild-type stock to the other six test tubes. Then, we added 1.5 mL of YPD to each of the three mutant-containing tubes and the three wild-type-containing tubes. For the remaining six tubes, we added 1.5 mL of 100 mM caffeine to each. In total, we had twelve 6 mL test tubes, with three replicates for each of the mutant and wild-type controls, and mutant and wild-type treatments. We chose to test 50 mM caffeine because it is an intermediate concentration within the range tested in a previous study (Bard *et al.* 1980).

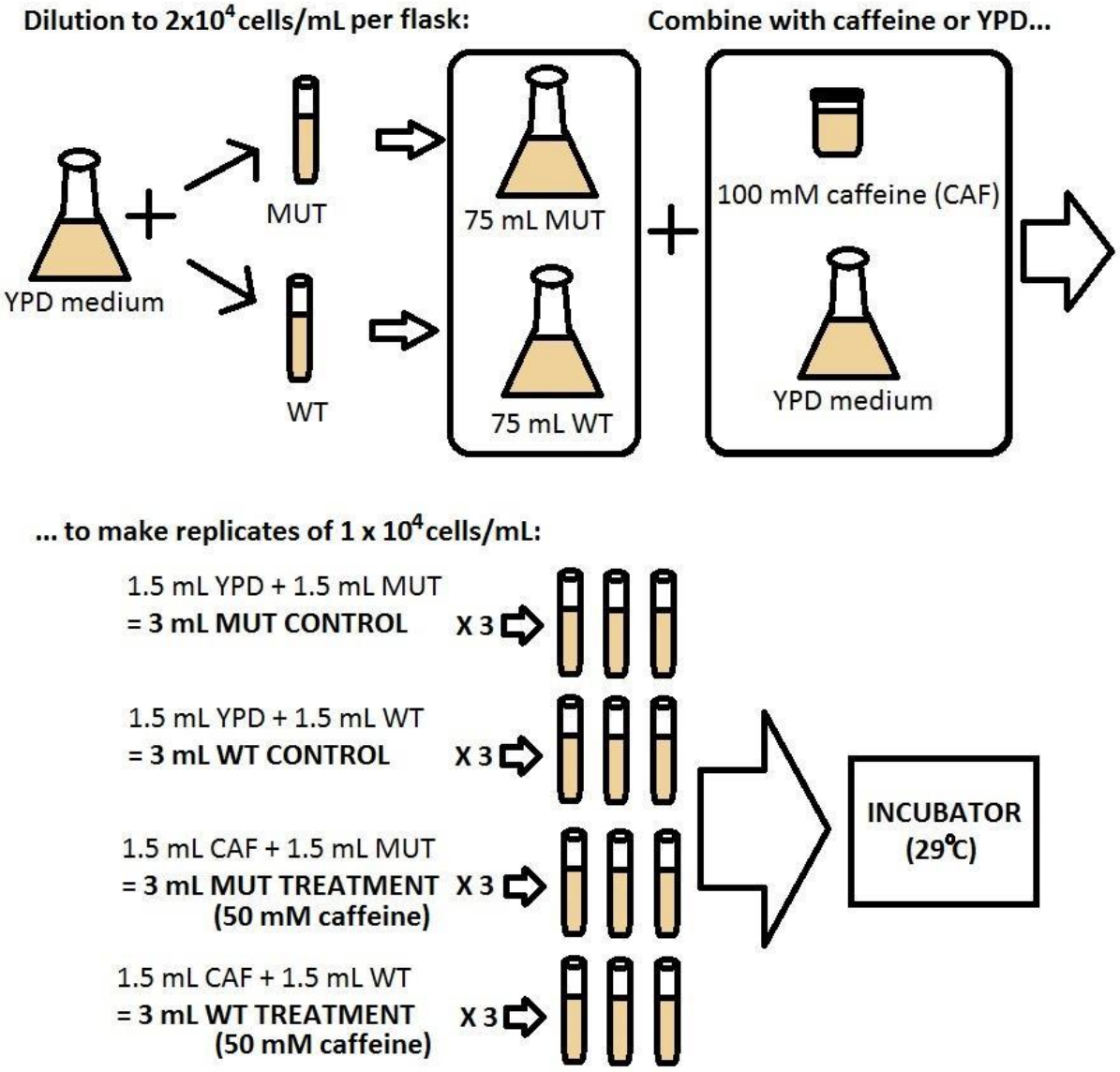

Figure 2. Experimental set-up procedure. These are the steps taken before beginning the *S. cerevisiae* experiment.

To begin the experiment, we obtained our first samples of the 12 replicates for t=0 hours by pipetting out 50 µL samples into a microcentrifuge tube and adding 5 µL of fixative. Then, we placed the 12 test tubes into the incubator set at 29^{o}C, similar to that of the recommended 30^{o}C (Tortora *et al.* 1982). Every two hours until the tenth hour (t=2, 4, 6, 8, 10 hours), we took out samples and fixed them using the same method as

described above. We obtained our final samples at t=23 hours. To count the cell population sizes of all of our samples, we first ensured the cells were distributed evenly by pipetting the sample up and down in its tube, then we pipetted 10 μL of each sample into a haemocytometer, and counted using the 10^{-4} mL grid. We concentrated the samples by two times at t=0 and t=2 hours for easier counting. We did this by centrifuging the tubes, decanting them, and resuspending the pellets in 27.5 μL of YPD. We counted all budding cells as two cells.

For the analysis of our data, our first step was to average the cell population sizes over the three replicates for each treatment or control at each sampling time. To analyze the data for hypothesis three, we also calculated the proportions of the wild-type treatment cell population sizes divided by the wild-type control cell population sizes as well as the proportions of the mutant treatment cell population sizes divided by the mutant control cell population sizes, and graphed these proportions over time. We analyzed using proportions because our starting cell concentrations measured at t=0 were not equal. Finally, we tested the significance of the difference in growth rates for each of the hypotheses, by using the χ^2 goodness of fit test with two categories. We calculated the χ^2 value for each hypothesis, compared them to the critical χ^2 value at p=0.05.

Results

Table 1. Table of cell counts at time t in cells/mL for *S. cerevisiae* wild-type, wild-type with caffeine, mutant, and mutant with caffeine.

Time (hr)	0	2	4	6	8	10	23
WT Control	5.17×10^4	3.80×10^4	4.00×10^5	8.10×10^5	1.65×10^6	3.14×10^6	1.21×10^8
WT Caffeine	6.33×10^4	4.07×10^4	3.30×10^4	5.13×10^4	7.70×10^4	7.70×10^4	1.10×10^4
M Control	9.00×10^4	1.83×10^4	1.43×10^5	2.51×10^5	4.36×10^5	4.36×10^5	7.73×10^7
M Caffeine	6.73×10^4	3.10×10^4	6.97×10^4	6.97×10^4	7.33×10^4	7.33×10^4	7.33×10^4

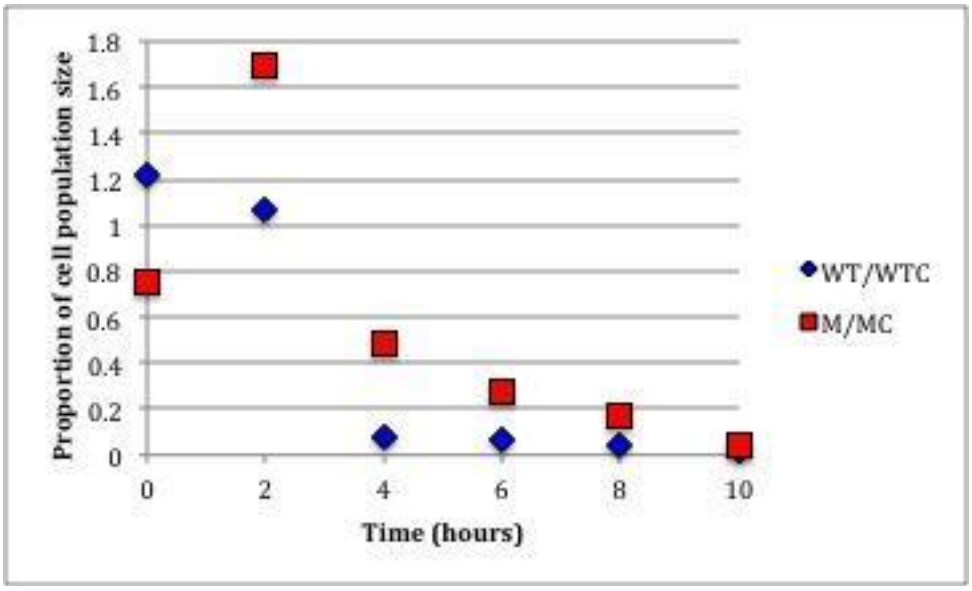

Figure 3. Proportion of cell population size from t=0 to t=10 hours for *S. cerevisiae* wild-type with caffeine:wild-type control and mutant with caffeine:mutant control.

In Figure 3 we can see a decrease in the proportion of cell population size in *S. cerevisiae* for both the mutant and the wild-type. Looking at Table 1 it can be seen that the wild-type control and the mutant control both have cell densities that are increasing at a greater rate than their mutant caffeine-treated counterparts. T=23 hours is not included in Figure 3 in order to see the trend more clearly and not skew the scale, but if it is plotted it continues in the same trend as the graph, WT:WTC at t=23 hours is 9.09×10^{-5} and M:MC at t=23 hours is 9.48×10^{-4}.

Using the χ^2 goodness of fit test with two categories we calculated a χ^2 value of 1.87×10^8 for hypothesis one, a χ^2 value of 5.49×10^7 for hypothesis two, and a χ^2 value of 5.54×10^5 for hypothesis three. Comparing these values to a critical χ^2 value of 3.84 at $\alpha=0.05$ with one degree of freedom, we can say that all three of these χ^2 values result in a p value of less than 0.05.

Sample calculation of mean for WTC at t=0:

$$\frac{(1.00 \times 10^4) + (5.00 \times 10^4) + (9.50 \times 10^4)}{3}$$

$$= 5.17 \times 10^4$$

Sample calculation for χ^2 value for hypothesis one at t=2:

$$X^2 = \sum \frac{(\text{observed - expected})^2}{\text{expected}}$$

Observed: WT with caffeine at t=2 (4.07×10^4)

Expected: WT at t=2 *percent change between t=2 and t=0 ((1 + (-0.265)) * (6.33×10^4))

$$\chi^2 = \frac{(4.07 \times 10^4) - (1 + (-0.265)) * (6.33 \times 10^4)^2}{(1 + (-0.265)) * (6.33 \times 10^4)}$$

$$= 7.40 \times 10^2$$

Sample calculation of proportion (*p*) of cell density for WT/WTC at t=4:

$$p = \frac{WT}{WTC}$$

$$= \frac{3.30 \times 10^4}{4.00 \times 10^5}$$

$$= 8.26 \times 10^{-2}$$

$$= 0.0826$$

Discussion

Based on our statistical analysis, we reject H_{O1} and therefore support H_{A1}. The results from our χ^2 goodness of fit test indicates that the growth rate of wild-type *S. cerevisiae* in the presence of caffeine is significantly decreased compared to the growth rate of wild-type control in the absence of caffeine. This difference in growth rate is demonstrated in Table 1 starting at t=4 hours. The cell population size of wild-type *S. cerevisiae* in the absence of caffeine is an order of magnitude greater compared to the cell population size of wild-type *S. cerevisiae* in the presence of caffeine. This difference in growth rate is much more evident at t=23 hours, where the cell population size of wild-type *S. cerevisiae* in the absence of caffeine is four orders of magnitude greater.

Based on our statistical analysis, we also reject H_{O2} and therefore support H_{A2}. The results from our χ^2 goodness of fit test indicates that the growth rate of mutant *S. cerevisiae* in the presence of caffeine is significantly decreased compared to the growth rate of mutant control in the absence of caffeine. This difference in growth rate is demonstrated in Table 1 starting at t=4 hours. The cell population size of mutant *S. cerevisiae* in the absence of caffeine is 1.43×10^5 cells/mL compared to 6.97×10^4 cells/mL for mutant *S. cerevisiae* in the presence of caffeine. Our results also show a visibly larger difference at t=23 hours, where the cell population of mutant *S. cerevisiae* in the absence of caffeine is three orders of magnitude greater.

Therefore, from our results we can see that the presence of caffeine affects both wild-type and mutant *S. cerevisiae*. Caffeine is a kinase inhibitor that inhibits the function of the TOR1 kinase and thus interferes with the TOR pathway (Powers and Walter 1999, Kapitzky *et al.* 2010). TOR is a signal transduction pathway that is an

important mechanism in controlling cell growth in eukaryotic organisms (Powers and Walter 1999). This pathway depends heavily on nutrient availability (Reinke *et al.* 2004). Therefore, when there is an abundant supply of nutrients, the TOR pathway promotes cell growth (Homann *et al.* 2009). Consequently, when there is a lack of nutrients, the TOR pathway slows cell growth (Homann *et al.* 2009). When caffeine is in the presence of both our wild-type and mutant, it leads to false signaling that the cell is starving (Homann *et al.* 2009). Because the TOR pathway believes there is a lack of nutrients, it slows cell growth. As a result, we see a decrease in cell population size in both wild-type and mutant *S. cerevisiae*.

In addition, caffeine inhibits cAMP (Taylor *et al.* 2003, Kuranda *et al.* 2006). cAMP is a secondary messenger derived from adenosine triphosphate (ATP), which is used for intracellular signal transduction and is important in many biological processes (Tortora *et al.* 1982). When cAMP is inhibited by caffeine, PKA is affected (Taylor *et al.* 2003, Kuranda *et al.* 2006). This leads to a decrease in energy levels that subsequently leads to a decrease in cell growth for both wild-type and mutant *S. cerevisiae* (Taylor *et al.* 2003, Kuranda *et al.* 2006). From previous studies, caffeine is known to interfere with metabolism of nucleotides and nucleic acids (Tortora *et al.* 1982). More specifically, adenine nucleotides, including cAMP, are disrupted (Tortora *et al.* 1982). This is supported by experimental evidence that caffeine treatment reduced cAMP levels by 50% at t=5 minutes (Tortora *et al.* 1982).

Based on our statistical analysis, we reject H_{O3} as well, and therefore support H_{A3}. The results from our χ^2 goodness of fit test indicates the growth rate of wild-type *S. cerevisiae* with the addition of caffeine is decreased compared to the growth rate of

mutant *S. cerevisiae* with the addition of caffeine. This suggests that although both wild-type and mutant *S. cerevisiae* decreased in growth rate in the presence of caffeine, the mutant showed greater resistance. From Figure 3, we can see the proportion of cell population size for mutant with caffeine and mutant control is greater than the proportion of cell population size for wild-type with caffeine and wild-type control.

Our mutant *S. cerevisiae* contains a deletion in the *PDC1* gene, which is a structural gene for pyruvate decarboxylase (Schaaff *et al.* 1989). Pyruvate decarboxylase is an important enzyme in alcoholic fermentation and is activated by phosphorylation (Schaaff *et al.* 1989). Inhibition of cAMP pathways by caffeine, however, could decrease phosphorylation and subsequently decrease the activity of pyruvate decarboxylase (Schaaff *et al.* 1989). Therefore, we can conclude that because our mutant *S. cerevisiae* already has a decrease of function in the pyruvate decarboxylase enzyme, it will be less affected by the presence of caffeine. Thus, our mutant *S. cerevisiae* shows greater resistance to caffeine.

There were some errors that we encountered during our experiment. For the counts at t=0 and t=2 hours, the tubes containing the sample cells were not vortexed prior to pipetting. This resulted in cell counts of 0 cells/mL for both wild-type and mutant, which most likely occurred because most of the cells had sunk to the bottom. Therefore, we had to centrifuge and resuspend to retrieve the cells. In addition they were not shaken during the first two hours of incubation. Thus, our results for t=0 and t=2 hours may not be accurate, as the method of cell growth and cell collection were slightly altered from the rest of the time intervals. Furthermore, when collecting the cells, we simply pipetted 50 µL samples into microcentrifuge tubes. An alternative method could

be collecting the samples by centrifugation, which would separate the cells from the medium, wash with a buffer and resuspend (Tortora *et al.* 1982). This method could potentially give a more accurate cell count. At t=0 and t=2 hours, we used a similar method as the one used by Tortora (1982) as we did not initially obtain any cells at these two times. However, this would be impractical due to time constraints if we were to perform this way for all of our replicates. Improper cell counting could also have been an issue. We used haemocytometers viewed under an Axio compound microscope to count the number of cells within a 10^{-4} mL grid. However, which 10^{-4} mL grid to view was not determined among our peers, as some grids contained no cells. Perhaps we could have used another method, such as using measurements of dry weights by filtration and calculating the cell count from photographs (Johnston *et al.* 1977). This could increase the accuracy of our results.

Conclusion

Through our statistical analysis, we reject all three of our null hypotheses and provide support for our alternative hypotheses, which is that in the presence of caffeine, the growth rate of both the wild-type and mutant *S. cerevisiae* is significantly decreased, and the growth rate of wild-type *S. cerevisiae* is significantly decreased compared to the growth rate of mutant *S. cerevisiae.* The latter demonstrates that caffeine could have an effect on the pyruvate decarboxylase enzyme.

Acknowledgements

We would like to thank Dr. Carol Pollock for her guidance in planning and performing this experiment. She was instrumental in helping us formulate structured and

testable hypotheses. She oversaw that the methods of our experiment would produce viable results and was in lab at all times during the experiment to provide her expertise if help was needed. Furthermore, Dr. Pollock refereed our data analysis making sure our statistics and graphs represented our hypotheses well.

We would also like to thank our technician Mindy Chow for helping us determine what materials, volumes, and concentrations would be necessary for experiment. Not only this, she helped us better understand how to get our desired initial cell concentrations with the materials given. Also, we appreciate our T.A. Katelyn Tovey being available in the lab in case assistance was needed.

Finally, we would like to thank the University of British Columbia for offering the Biology 342 course and providing the facilities and equipment that made this experiment possible.

Literature Cited

Bard, M., Neuhauser, J., and Lees, N. 1980. Caffeine resistance of *Saccharomyces Cerevisiae.* J. Bacteriol, **141**(2): 999-1002.

Homann, O.R., Dea, J., Noble, S.M., and Johnson, A.D. 2009. A phenotypic profile of the *Candida albicans* regulatory network. PLoS Genetics, **5**(12): 1-12.

James, J.E. 2011. Addiction medicine, B.A. Johnson edition. Springer Science + Business Media, New York. NY.

Johnston, G. C., Pringle, J. R., and Hartwell, L, H. 1977. Coordination of growth with cell division in the yeast *Saccharomyces cerevisiae.* Experimental Cell Research, **105**(1): 79-98.

Kapitzky, L., Beltrao, P., Berens, T.J., Gassner, N., Zhou, C.S., Wuster, A., Wu, J., Babu, M.M., Elledge, S.J., Toczyski, D., Lokey, R.S., and Krogan, N.J. 2010. Cross-species chemogenomic profiling reveals evolutionarily conserved drug mode of action. Molecular Systems Biology, **6**(451): 1-13.

Karathia, H., Vilaprinyo, E., Sorribas, A., and Alves, R. 2011. *Saccharomyces cerevisiae* as a model organism: a comparative study. PLoS one, **6**(2): e16015.

Kuranda, K., Leberre, V., Sokol, S., Palamarczyk, G., and Francois, J. 2006. Investigating the caffeine effects in the yeast *Saccharomyces cerevisiae* brings new insight into the connection between TOR, PKC, Ras/cAMP signaling pathways. Molecular Microbiology, **61**: 1147-1166.

Ostergaard, S., Olsson, L., and Nielsen, J. 2000. Metabolic engineering of *Saccharomyces cerevisiae*. Microbiology and Molecular Biology Reviews, **64**: 34-50.

Powers, T. and Walter, P. 1999. Regulation of ribosome biogenesis by the rapamycin-sensitive TOR-signaling pathway in *Saccharomyces cerevisiae*. Mol. Biol. Cell. **10**(4): 987-1000.

Pronk, J., Steensmays, Y., and Van Dijkent, J. 1996. Pyruvate metabolism in *Saccharomyces cerevisiae.* Yeast, **12**: 1607-1633.

Reinke, A., Anderson, S., McCaffery, J.M., Yates, J., Aronova, S., Chu, S., Fairclough, S., Iverson, C., Wedaman, K.P., and Powers, T. 2004. TOR complex 1 includes a novel component, Tco89p (YPL180w), and cooperates with Ssd1p to maintain cellular integrity in *Saccharomyces cerevisiae*. J. Biol. Chem. **279**(15): 14752-14762.

Saccharomyces Genome Database. [ ]. A website with *Saccharomyces cerevisiae* gene information. Available from http://www.yeastgenome.org/ [accessed 8 November 2013].

Taylor, S.S., Yang, J., Wu, J., Haste, N.M., Radzio-Andzelm, E., and Anand, G. 2003. PKA: a portrait of protein kinase dynamics. Biochimica et Biophysica Acta., **1697**(1-2):259-269.

Tortora, P., Burlini, N., Hanozet, G. H., and Guerritore, A. 1982. Effect of caffeine on glucose-induced inactivation of gluconeogenetic enzymes in *Saccharomyces cerevisiae.* European Journal of Biochemistry, **126**: 617-622.