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Glucose Repression Pathways in Saccharomyces Cerevisiae with Added Monosaccharides

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Saccharomyces cerevisiae, a type of yeast, uses alcoholic fermentation to take up and consume sugars, specifically glucose, for energy consumption thus producing carbon dioxide and ethanol gas in the process. *S. cerevisiae's* preferred energy source is glucose, causing other sugars to become repressed and unused because of glucose repression pathways. Our research shows ethanol levels when other monosaccharides besides glucose are added to the glucose and yeast solution. Saccharine, honey, and high fructose corn syrup were added separately to a solution of activated yeast and water. Ethanol levels were measured for each added substance. This research provides an insight into how glucose is used in the cells of yeast but could be applied to the fermentation of cancer cells and how certain protein pathways allows glucose into the cell.

Introduction

Baker's Yeast (*S. cerevisiae*) is a single celled organism that inhabits humid environments. Due to the variety in habitats, yeast does not have a system of controlling the saccharides to which they are exposed. Yeast undergoes varying rates of aerobic respiration and alcoholic fermentation according to the environmental conditions and concentrations of sugars. When glucose concentration exceeds 6 $x10^{-3}$ M, fermentation is the primary metabolic pathway that yeast uses to produce energy, a phenomenon known as the Crabtree Effect (DeDeken 1965). Even though alcoholic fermentation produces less energy than aerobic respiration, the ethanol the yeast produces restricts the growth of surrounding microorganisms, ensuring that yeast has more access to the surrounding saccharides (Verstrepen et. al 2004). This offers an explanation for the energetically unfavorable Crabtree Effect.

Yeast is unable to regulate the composition of the sugars in its environment, and has a preference for glucose over other saccharides and consumes glucose at a higher rate; additionally, glucose activates repression pathways that restrict the uptake of the less-preferred saccharides (Verstrepen 2004). Two main mechanisms govern the glucose preferential pathways in yeast. The first of which involves receptor proteins Snf3 and Rgt2 that signal the transcription of different hexose transport channels depending on the amount of

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glucose present outside the cell (Kayikci 2015). In an environment with a high concentration of glucose, low affinity transport channels (Hxt1 and Hxt6) are produced; while in low concentrations of glucose, high affinity transport channels (Hxt2 and Hxt4) are produced to maintain glucose equilibrium intra- and extracellularly (Kim *et. al* 2013). The six hexose transport proteins (Hxts) that *S. cerevisiae* possess each have different properties (Roy 2015). Hxt1, the low affinity glucose channel also transports fructose into the cell (Roy 2015). Fructose has been shown to initiate glucose repression in *S. cerevisiae* as well (Meijer 1998). However, galactose and xylose can never pass through glucose channels (Kim et. al 2012).

The second pathway governing saccharide intake by the cell involves gene expression catalyzed by Snf1 kinase (Carlson 1999). Snf1 kinase is a protein that responds to intracellular glucose concentration; Snf1 is active in low concentrations of glucose and inactive when glucose concentration is high (Kayikci 2015). When active, Snf1 phosphorylates Mig1, allowing it to leave the nucleus and allow transcription of GAL and MAL to occur; however, when Snf1 is inactivated in a high concentration of glucose, Mig1 is not phosphorylated and remains in the nucleus bound to the promoter regions of the genes that allow for the transcription of GAL and MAL (Shashkova *et.al* 2017). When these proteins are not produced, galactose and maltose cannot enter the cell to be metabolized (Shashkova *et. al* 2017).

In the present study we examined the effect of additional monosaccharides to a glucose containing solution on the fermentation of *S. cerevisiae*. We hypothesized that additional non-fructose monosaccharides present in a glucose containing yeast growth medium would have little effect on the metabolic rate of the yeast, because glucose is consumed first and repression pathways inhibit the uptake of other less-preferred monosaccharides. Fructose was expected to increase the rate of fermentation due to the fact that fructose can pass through the Hxt1 pathway intended for glucose transport into the cell. Our hypothesis will be supported if the glucose solution and the glucose plus non-fructose monosaccharide solutions have comparable rates of fermentation while the glucose and fructose solutions will have a greater rate of fermentation. A similarity in values among the glucose only group and the glucose and fructose group would indicate that fructose is not being utilized by the cell. Lastly, if the glucose only group has the lowest rate of fermentation of all the growth mediums then glucose repression is likely not occurring.

Methods

To examine the uptake of saccharides, we prepared four different yeast growth solutions and added different monosaccharides to them in a respiration chamber and measured the rate of ethanol production to determine which sugar solution was conducive of a greater rate of fermentation and consumed more efficiently.

A respiration chamber was prepared to measure the amount of fermentation occurring. In preparing the yeast solution, 0.6 grams of *S. cerevisiae* was obtained in a weigh boat and added to 10 mL deionized water in the respiration chamber. A magnetic stir bar was placed on the stirring plate and turned on to medium speed for five minutes, allowing the dormant yeast to activate. An ethanol sensor was utilized to examine the rate of fermentation, and the teflon tape covering the sensor was replaced before use. The sensor was connected to a labquest and calibrated for 5 minutes before use.

After 5 minutes, 5 ml of 0.3M glucose and 5 ml of DI water--diluting the glucose solution to 0.15M--were added and the ethanol sensor was immediately placed over the opening of the chamber. The mixture was stirred for 10 minutes, recording ethanol concentration in ppm every ten seconds. Results were recorded in LoggerPro (LoggerPro3 2016). Trial 1 was started with a clean and empty chamber. The DI water and yeast were mixed for 5 minutes and then 5 ml of glucose and 5 ml of saccharine were added to the solution. The ethanol sensor was placed in the opening, and left for 10 minutes. The yeast solution was prepared the same way as in previous trials for trial 2. After the solution was activated 10 ml of 0.3M honey was added. 10 ml were used instead of 5 ml because honey is made with 0.15M glucose and 0.15M fructose, which makes up for the 5 ml of 0.3M glucose originally used in other trials. Trial 3

consisted of the same methods, and 10 ml of 0.3M high fructose corn syrup was added to the yeast solution for the same reason as the honey. Each trial was completed two times. Our data was put into a bar graph to show the relationship between added monosaccharides and ethanol levels. A one-way ANOVA test was also performed to find the difference between means of our trials. These can be found in the results.

Results

The rate of fermentation had no significant difference in any of the trials we conducted (X^2 =2056, df = 3, P = 0.3377). The metabolic rate did not differ when alternative monosaccharides were added to a glucose-containing solution (See Figure 1, Appendix). The addition of the secondary monosaccharides showed no difference in rate of fermentation from glucose alone, resulting in no trend among the experimental groups.

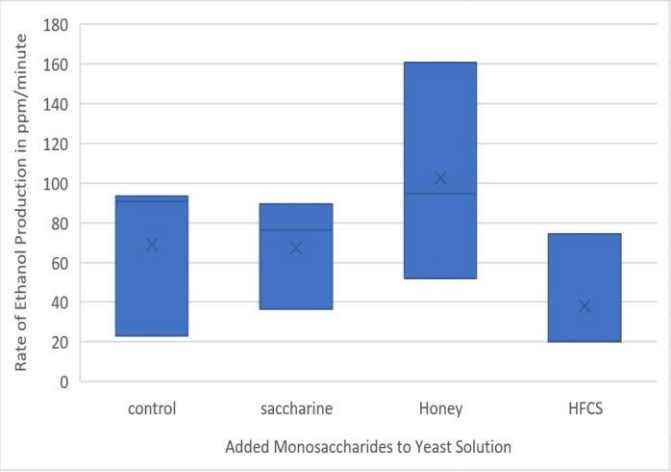

Figure 1. Comparison of different monosaccharides added to an *S. Cerevisiae* yeast solution, measured in rate of ethanol production in ppm/minute. Each Group had a total of three trials.

Discussion

Consistent with our hypothesis, no significant difference existed in the rate of respiration between the glucose solution and the glucose and saccharine solution. Contrary to our hypothesis, the glucose and fructose solutions--honey and high fructose corn syrup--did not have a significantly different rate of fermentation than the glucose-only solution.

Since our glucose concentration of 0.15 M exceeded the 6 x 10^{-3} M threshold for the Crabtree Effect, fermentation was the primary metabolic pathway the yeast used. Our data demonstrates strongly the prevalence of glucose repression pathways active in *S. cerevisiae*. The glucose concentration of 0.15 M was constant in all four of our groups. Since there was no significant difference in rate of ethanol production, there was also no significant difference in fermentation and the amount of monosaccharide consumed. This demonstrates that the additional monosaccharides were not utilized by the yeast due to preferential glucose pathways, with fructose being a monosaccharide that's consumption was repressed. This result is not consistent with Meijer's or Roy's work that suggest that fructose is consumed and utilized in the same way as glucose (Meijer 1998, Roy 2015). Future research on the effects of glucose and fructose on fermentation rates of yeast can lend insight into *S. cerevisiae*'s ability to repress or utilize fructose as an energy source in the presence of glucose.

On our third day of experimentation, the rate of ethanol production was significantly lower than previously observed. Multiple ethanol probes and different containers of yeast were used, however the ethanol production remained low. It was later found that the temperature of the lab influenced our results. *S. Cerevisiae* is highly sensitive to temperature changes and has an optimal rate of fermentation near 75 degrees Fahrenheit. The lab temperature was approximately 69 degrees Fahrenheit, low enough to profoundly alter the rate of fermentation. Despite the temperature fluctuations, the relative rates of respiration among the saccharides remained fairly consistent. Due to the low rates of fermentation measured on the third day of trials, the range in fermentation rates was large. Specifically, the range in fermentation rates in honey solution is large. This may appear to be a significant difference, but due to the difference in temperature on the trial dates, the highly elevated value was unable to be ruled out as an outlier. Thus, there was no significant statistical difference among our four growth mediums.

Our found results could have implications for cancer research. Cancer cells have higher levels of aerobic glycolysis, which is the conversion of glucose into pyruvate. This means that cancer cells have an increased aerobic glycolysis, and a greater uptake of glucose which can lead to an increased level of tumor aggression (Gatenby, 2004). If glucose repression pathways can be greater understood, it may be possible to slow down the rate of glycolysis, which could slow tumor growth.

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