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The yeast beast: a search for the best carbohydrate for the highest rate of fermentation in *S. cerevisiae*

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Abstract

The rate of fermentation of *Saccharomyces cerevisiae*, or yeast, can be impacted by the type of carbohydrate used. We wanted to explore the effect of glucose, lactose, and refined sucrose on the rate of ethanol production in yeast. Several studies have looked at the impact of temperature, pH, and salt concentration on fermentation, but this study will focus on the differences of ethanol production between monosaccharides and disaccharides. We conducted four, ten minute trials for the three types of sugar, measuring ethanol production with an ethanol probe. Our results suggest that disaccharides like lactose cannot undergo efficient fermentation but sucrose can in comparison to glucose. Future studies could involve testing how the size of the carbohydrate's chemical structures impacts its ability to enter the cell membrane and undergo fermentation.

Introduction

Saccharomyces cerevisiae, or baker's yeast, plays an essential role in the fermentation process that is used in a multitude of baked goods. Yeast must undergo anaerobic fermentation to release energy, or ATP, which is fundamental for a cell's survival (Bauer et al., 2016). Glucose, a monomer of carbohydrates, is the direct fuel for the glycolysis cycle that occurs in cells. It is a usable form for the cycle and requires no ATP to function. In disaccharides, however, molecules such as lactose and refined sucrose must be hydrolyzed into monomers of glucose and galactose or glucose and fructose, respectively, before undergoing fermentation (Yoon et al., 2003). Once glucose enters glycolysis and breaks down, ethanol and carbon dioxide are released as major products of yeast fermentation (D'Amore, 1992). Some sugars may increase the rate of metabolism and alter the taste of the alcohol produced. This adds variety in the types of products available to consumers. In our lab, we will be exploring the rate of fermentation on different carbohydrates, specifically looking at the differences between monosaccharides and disaccharides. We hypothesize that the rate of fermentation will

be higher in monosaccharides because glucose directly enters into glycolysis and does not have to spend ATP to convert the disaccharides into a usable form. Our hypothesis will be supported if glucose, a monosaccharide, produces more ethanol. However, our hypothesis will not be supported if the disaccharides, including lactose and refined sucrose, produce more or equal amounts of ethanol as compared to glucose.

Methods

We decided to test three different types of carbohydrates, including glucose, lactose, and refined sucrose and measure its effect on the rate of ethanol production. Four, ten-minute trials will be conducted for each of the variables, holding temperature and amount of carbohydrates constant. The yeast solution will be prepared by adding 0.6 g of yeast to 10 mL of deionized water and stirring it for three minutes (Shaw and French, 2018). After the ethanol sensor has warmed up for five minutes, we will start the experiment with glucose, a positive control group, to show what happens at the most basic level of glycolysis and fermentation. We will add 10 mL of 0.3 M glucose solution to the yeast solution and measure the rate of ethanol production (ppm/min) for ten minutes, which will be repeated over three more trials. The same procedure will be followed for 0.15 M lactose and 0.15 M refined sucrose solutions. LoggerPro will be used to measure ethanol production over time. An average of the trials for the different types of carbohydrates will be placed in a box and whiskers plot to determine their relative impact on ethanol production, with the types of carbohydrates on the x-axis and rate of ethanol production on the y-axis. A normality test showed that the data was not normally distributed, so a Kruskal-Wallis Test was conducted to compare the effect of the type carbohydrate on ethanol production in glucose, lactose, and refined sucrose solutions.

Results

The results show that glucose and sucrose demonstrated very similar box and whisker plots. Lactose showed a condensed box in comparison to the other two plots. One of our Lactose trials had a fermentation rate of 433.58 ppm/min, which was significantly higher than all the other trials, and thus skewed the data. We proceeded to throw out this point from our data set (Fig 1.1). Operating under the assumption that the data was not normally distributed, a Kruskal-Wallis test was conducted to compare the effect of the type of sugar on the rate of ethanol production in lactose, glucose, and sucrose. There was a significant effect of lactose on the rate of ethanol production [$p=0.03461$]. A Dunn's post hoc test revealed ethanol production was significantly lower in Lactose than in Glucose [$p= 0.01029$].

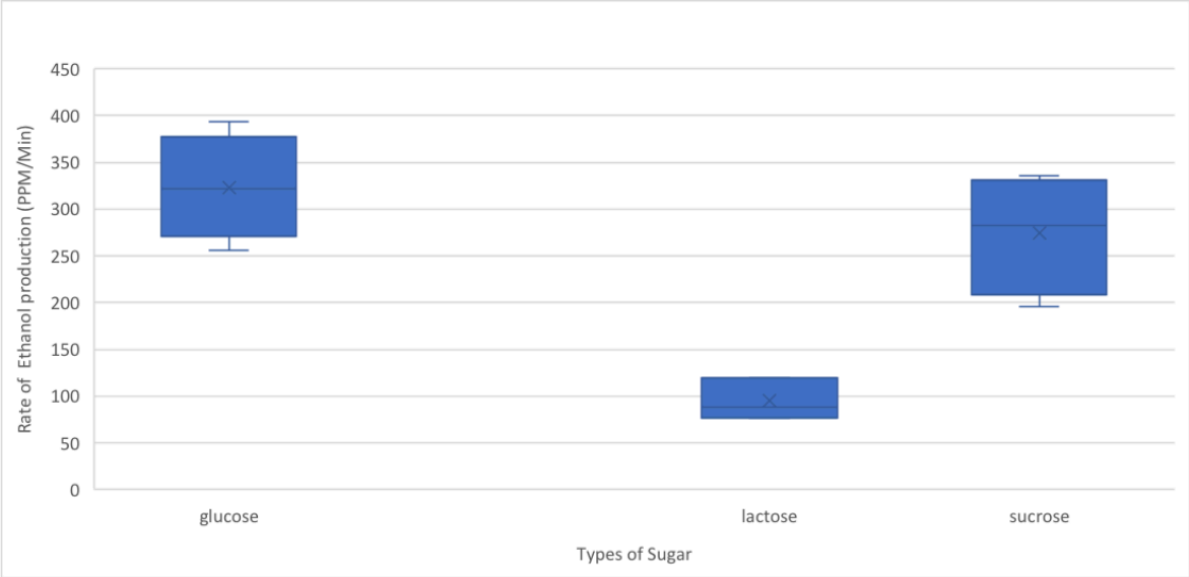

Figure 1.1| A comparison of the glucose, lactose, and sucrose and rate of ethanol production in ppm/min (N = 11). Lactose has a significantly lower rate of production whereas sucrose and glucose have comparable rates of ethanol production. This data is significant because of the difference in p-values found when a Kruskal-Wallis and Dunn's post hoc test was run [p=0.01029].

Discussion

The results from this experiment do not support our hypothesis due to the fact that glucose and sucrose had similar rates of ethanol production and were not statistically different from each other. However, there was a statistical difference between glucose and lactose. This is due to lactose digesting yeast before it can undergo fermentation rather than a difference between monosaccharides and disaccharides. According to Fig 1.1, there was only a slight decrease in the rate of ethanol production between glucose and sucrose. However, there was a significantly more substantial decrease between glucose and lactose. Therefore, the form in which carbohydrates enter glycolysis does not impact the rate of fermentation in Saccharomyces cerevisiae.

Overall, we had one outlier in the data, which was the first trial of lactose. We removed the trial from the data set because we believe that the graduated cylinder was not washed properly.

Alternatively, our hypothesis could be explained by the relative size differences and chemical structures of the carbohydrate molecules. Sugars cannot freely enter the membranes of cells, so transport proteins are necessary for the uptake of carbohydrates (Lagunas, 1993). In yeast cells, two transport proteins have been identified for the monosaccharides of glucose and galactose, so the transporters could have a higher affinity for glucose and sucrose as compared to lactose (Lagunas, 1993). Therefore, for future studies, we suggest looking at the molecular structures of carbohydrates to

determine their effect on the metabolism of yeast.

Additionally, while our data analyzes the best sugar for fermentation, we can use our knowledge of S. cerevisiae and contrast it to other colonies of yeast in terms of finding the best biofuels. For example, the coffee industry produces a lot of waste, and different strains of yeast are being used to convert the coffee waste into biofuels to be used in place of fossil fuels (Mussatto et al., 2011). Another study takes different yeast colonies and manipulates them to create specific chemicals such as 1-butanol which is used in biofuel production (de Jong et al., 2011). The scientists are looking to engineer the yeast to make it a key part in the cell factories, which mock animal cells and produce raw energy which can be used to create natural fuel with the organic waste products such as oxygen (de Jong et al., 2011). Such experiments provide broader implications in which we can take data from ethanol production in fermentation and seek out ways for genetic modification to create efficient factories that manufacture renewable, environmentally safe fuels.

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