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Increasing cellular respiration of fermented *S. Cerevisiae* using fuel with hydrolysis enzymes

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Abstract

The rate of cellular respiration for fermenting yeast can be manipulated by changing the concentration and chemical constitution of the carbohydrate source. While several studies have proposed the perfect ratio to use in fermentation for various carbohydrate sources, an analysis of hydrolysis enzymes versus no hydrolysis enzymes has yet to be investigated. Using honey and high fructose corn syrup in a yeast solution, the rate of fermentation was measured in ppm and analyzed using a One-Way ANOVA test. The findings suggest that fuel sources with hydrolysis enzymes, such as honey, do not have a significant effect on the fermentation of yeast because of the P-value (0.9940) determined through the One-Way ANOVA test did not suggest any correlation. While the hydrolysis enzymes, like a-amylase and invertase, found in honey should theoretically improve fermentation because they speed up the breakdown of starches into smaller monosaccharides, farther testing will be needed to determine whether this increases ethanol production.

Introduction

Due to the growing concern for fuel shortage and ethanol products like disinfectant, studying the industrial fermentation of ethanol has become paramount to the future of ethanol manufacturing (Hagerdal et al; 2006). The promises of ethanol as an effective biofuel has enticed many scientific studies to look into increasing ethanol and CO_2 yield during the fermentation process. Therefore, it is the goal of this lab to investigate avenues in which the fermentation process can be tailored to increase the efficiency of ethanol generation.

Fermentation is a process used by microorganisms when energy sources are scarce, and bears resemblance to aerobic respiration, by producing ATP through glycolysis. Therefore, the rate of cellular respiration can be monitored by measuring the concentrations of glycolysis outputs, including ethanol and CO_2. O-glycosidic bonds are the covalent linkage between two carbohydrates and form during dehydration reactions, where the hydroxyl group of one monomer and hydrogen from one monomer detach to form water. To remove a glycosidic bond, a polysaccharide must undergo

bond, a polysaccharide must undergo hydrolysis (Lehle et al; 1997).

For yeast fermentation, a variety of carbohydrate bases can be used. This experiment will explore the differences between honey and corn syrup in ethanol production. Honey, which contains large portions of sucrose, also has enzymes which aid in the hydrolysis of its disaccharide structure into smaller monomers (Rossano et al; 2012). Both enzymes present in honey, α-amylase and invertase of the proteolytic enzyme family, are responsible for the transformation of nectar into honey and honey into mead. A-amylase degrades carbohydrates, like starch, into sugar, while invertase can hydrolyze sucrose into glucose and fructose (Syu M and Chen Y;1997). The *Apis* and *Saccharomyces Cerevisiae* bee strains produce large amounts of the invertase enzyme. When the *Apis,* or honey bee, produces invertase, it is used to break down the sucrose found in nectar in order to create honey more easily (Vorlova L and Pridal A; 2002). This reaction also makes honey very acidic, because the sucrose is converted to fructose and gains an electron. Diatase, the amylase enzymes, are also found in large amounts in honey. These enzymes are catalysts for digesting starches into smaller compounds (Vorlova L and Pridal A; 2002). Since both of these are specifically intended for the breakdown of sugar molecules, it would be expected their presence should increase ethanol production. High fructose corn syrup, on the other hand, has no enzymes in its structure. It is made of a blending of glucose and fructose, but the two do not de-hydrogenate into disaccharides.

Previous studies have compared differences in the fermentation of just monosaccharides and disaccharides. For example, an experiment in 2013 regarding Italian grapevines showed grapes with higher sugar to total acid ratios were more effective in fermentation, and that grapes with only glucose, instead of fructose, seemed more efficient in regards to fermentation time and quality of the wine produced (Zinnai et al; 2013). A few cross-comparisons of monosaccharides and disaccharides have been conducted, such as in regards to natural lentil fermentation. However, the rate of production was not the concern of these studies, but rather the rate of fuel consumption for monosaccharides and disaccharides (Frias et al; 1997). This lab will look at the rate of ethanol production between a monosaccharide without enzymes and a disaccharide/monosaccharide blend with hydrolysis enzymes rather than the rate of fermentation differences between just monosaccharides and disaccharides.

The presence of hydrolysis enzymes leads to a greater degree of cellular respiration in *S. Cerevisiae* yeast during fermentation compared to no enzymes, because of the hydrolysis breakdown of sugar molecules aids in speeding up glycolysis, therefore producing more ethanol. If this hypothesis is supported, honey trials should have a greater ethanol output than high fructose corn syrup trials because the honey is a monosaccharide/disaccharide blend that contains hydrolysis enzymes. If the hypothesis is not supported, then it can be expected there will be no noticeable difference in ethanol output, or corn syrup will produce more ethanol than honey.

Methods

This laboratory utilized the procedure specifications provided by Authentic Research in Introductory Biology, including the recommendation for 0.6 grams of yeast, 10 milliliters of distilled water, and a fermentation time frame of three minutes.

The two chosen fuel sources for comparison during fermentation were high fructose corn syrup and honey, which possesses proteolytic enzymes. A third solution of 20 milliliters of water and 0.6 grams of yeast was also observed as a control for comparison to the high fructose corn syrup and honey yeast solutions that were manipulated in the experiment, with all three trials measuring the percentage of ethanol present per second in each of the solutions.

In order to activate the yeast before each trial could be conducted, a Minimag stir station was used for three minutes with just yeast and water, then the honey or high fructose corn syrup was added to the solution and then stirred for five minutes. The percentage of ethanol output per second as recorded by the Vernier LabQuest mini was measured in ppm of the desired variable. After the five minutes were recorded with the ethanol sensor for each trial, the slope of the line was collected and used to create the bar graph seen in the results section that compares the percentage of ethanol per second for each trial of all three solutions.

The bar graph was chosen because the bars for each trial clearly display the significant differences in ethanol percentage between the honey, high fructose corn syrup, and water yeast solutions. To determine the degree of difference in ethanol production between the three, a One-way ANOVA was performed with Excel and analyzed in the results.

Results

As shown by the graph below, the collected data shows that honey, which contains the enzymes α-amylase and invertase, produces more ethanol during fermentation than the high fructose corn

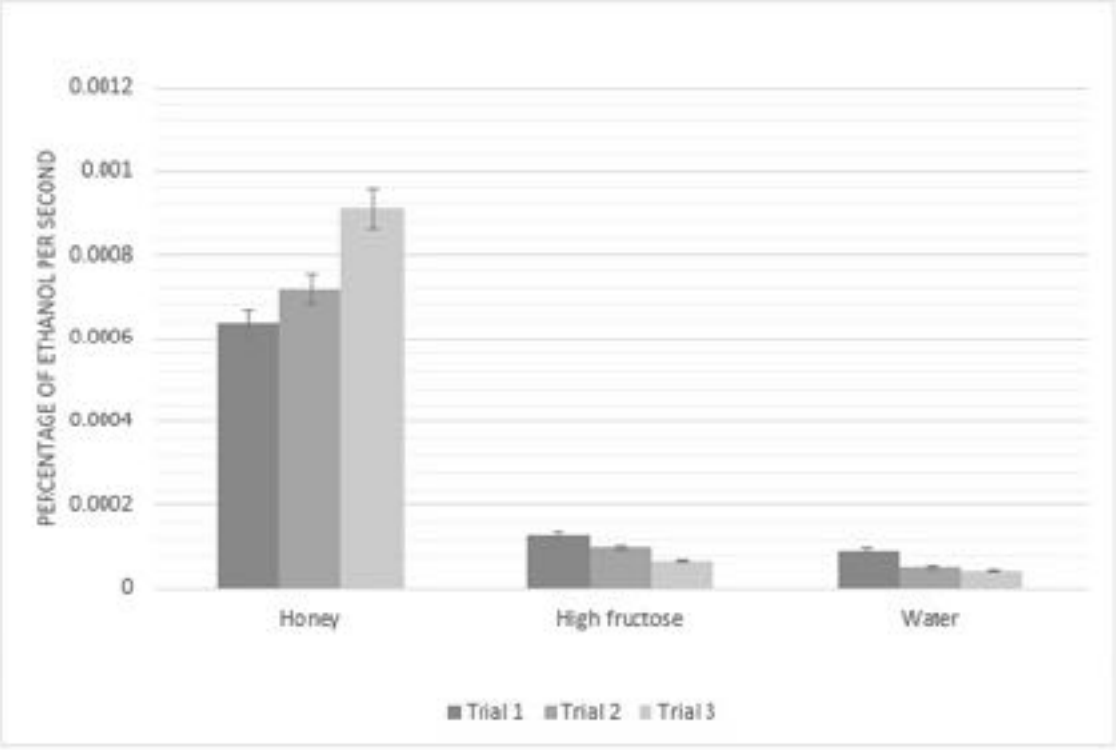

Figure 1 - Shows three trials for honey, high fructose, and water measurements. The graph displays the amount of ethanol produced per second over a span of five minutes, with error bars displaying a standard 5% uncertainty.

syrup or water trials, at a rate of around .00065% to .0009% per second over a span of five minutes. High fructose has a reduced rate of ~.0001% in comparison, and water trials, which have neither enzymes nor a sugar fuel source, were all less than .0001%.

A One-Way ANOVA was conducted to compare the effect of different sugars on ethanol production in honey, high fructose corn syrup, and water conditions. There was not a significant effect of sugar type on ethanol production between the three conditions; ($p=.9940$). As there was no significant P value, a Tukey's pairwise test was not conducted.

Discussion

Overall, the hypothesis that honey produced more ethanol during the fermentation of *S. Cerevisiae* when compared to high fructose corn syrup because of its hydrolysis enzymes appears to be incorrect, because the One-way ANOVA test produced an insignificant P-value of 0.9940. Although the data collected and displayed in the graph seems to support the hypothesis originally stated, the insignificant P-value means that more research will be needed in order to conclude whether honey, which contains enzymes that break apart bonds between monomer residues, is actually responsible for increased ethanol and CO_2 production during glycolysis. High fructose corn syrup does not contain these hydrolysis enzymes and it should, therefore, require the yeast to use more energy to convert fructose into useable fuel, which explains why the honey possessed higher levels of ethanol percentage per second in all trials conducted; however, since the P-value was insignificant, there could be other reasons why honey had higher levels of ethanol compared to the high fructose corn syrup.

One alternative interpretation of the data could be that honey is a monosaccharide and disaccharide blend, while high fructose is made of only monosaccharides (Rossano et al; 2012). It is possible that the blend of monosaccharides and disaccharides is only responsible for the increased production of ethanol. Further experimentation will be needed to determine whether hydrolysis enzymes increase fermentation production.

For future research, it is recommended that larger amounts of yeast, honey, high fructose corn syrup, and water should be measured for a greater amount of time with more trials. This way, the results might provide a more conclusive explanation of the hypothesis, and the P-value would be more representative of the data. The lab also recommends that future experiments isolate the enzymes present in honey, and test in monosaccharide, disaccharide, and monosaccharide/disaccharide blend solutions to see whether these enzymes actually increase production, and in which sugar structure they are most effective at producing ethanol in. Concluding the most effective method for ethanol production could greatly increase the prospects of ethanol as a viable biofuel.

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