Determination of sugar uptake in Saccharomyces

**Introduction**

The yeast Saccharomyces has been used for millennia for baking and brewing. Their ability to turn sugar into ethanol has been used to make food last longer, make food taste better, and allows for social lubrication at awkward office parties. Early brewers, bakers, and wine makers found that they would get a more reliable and predictable product by using a starting culturefrom their previous batch. This resulted in lineages of yeast that grew in man-made environments, which created the perfect opportunity for domestication[[1]](#footnote-1). The resulting genetic isolation of different strains allowed them to divergeinto five clades based on their geography and usage: Asian strains such as sake, wine strains, a mixed clade used in breadmaking, and two clades for beer. The first clade is geographically related to those beers from Germany, Britain, and the US (probably descended from the British lineage during colonization). The other beer clade is much more diverse geographically. Each clade has different phenotypes that have either been selected for or came about due to geographic isolation. One of these differences is how each clade processes sugar in their environment.

Brewer’s wort, the mixture used to make alcohol, contains maltose in 60%, maltotriose (15 - 20%), and glucose (10-15%)[[2]](#footnote-2). Glucose is preferentially used by Saccharomyces and acts as an inhibitor to maltose transporters[[3]](#footnote-3), [[4]](#footnote-4). After the glucose is gone, the Saccharomyces will begin to process maltose, and some will process the maltotriose if they are able to[[5]](#footnote-5) [[6]](#footnote-6) [[7]](#footnote-7). This process is not entirely efficient. Some strains are better at utilizing the maltose and/or maltotriose than others. Leftover sugar in the wort is a problem for brewers as that sugar could have been made into alcohol and can affect the final taste of the product. Understanding why some yeasts are more efficient in their maltose utilization and why some do not even use maltotriose is an important economic and culinary question to answer.

Sugar entering the cell is seen as the rate limiting reaction in fermentation[[8]](#footnote-8). Sugar enters through a proton symporter (Figure 1)[[9]](#footnote-9). Maltose transporter genes are called MALx1 where x stands for the chromosome that gene is located(For Example, MAL11 is the transporter gene on chromosome one). All MALx1 transporter genes share a high identity and have been shown to have a high affinity for maltose. AGT1 (alpha glucoside transporter) is an allele of MAL11 which can transport a wide range of sugars, including maltotriose[[10]](#footnote-10). MTT1 shares about 90% amino acid sequence identity with MALx1, transports maltose, maltotriose, trehalose, and turanose, and is unusual in that it has a higher affinity for maltotriose than maltose[[11]](#footnote-11) [[12]](#footnote-12). Different strains of yeast have different versions of these genes or none.

Figure 1. 
Figure 1. Proton Symporter. Sucrose is a large molecule that is unable to diffuse across the lipid membrane through diffusion. The cell creates a positive environment outside of the cell by pumping protons out of the cell and into the extracellular matrix. This electrical-chemical gradient of the protons outside of the cell powers the transport of the sucrose molecules across the lipid bilayer. (Retrieved from <http://bio1151b.nicerweb.com/Locked/media/ch07/07_19Cotransport_L.jpg>)

 Studies have focused on the performance of different transporters and their fermentation efficiency. To measure their alcohol efficiency is easy: measure the amount of alcohol in the wort over a given time. The techniques used to measure the sugar entering the cell are a bit more complicated. Sugar can be labelled with carbon-14. This radioactively labelled sugar is then added to a solution containing the yeast. After fifteen to twenty seconds, uptake is stopped by adding water at 0°C. By measuring the amount of radioactivity in the cell due to the carbon, the amount of sugar uptake can be determined. Yeast that have sugar transport proteins that had higher affinity for sugar would have a higher count of radioactivity inside the cell. This process of measuring the amount of sugar uptake through radioactive labelled carbon is called zero-trans uptake assay. For the zero-trans uptake to be accurate, less than 1% of the radioactive carbon should enter the cell and the uptake of any impurity can cause overestimation of how well a yeast specimen is uptaking sugar[[13]](#footnote-13) [[14]](#footnote-14).

Another technique that does not have this failing is measuring the rate of protons leaving the solution the yeast is suspended in before and after sugar is induced which will be called proton symport assays. Since large sugar molecules like maltose and maltotriose are taken into yeast cells through a proton symport mechanism, the protons leaving the solution would increase after sugar is added[[15]](#footnote-15). A pH meter is used to measure the amount of protons leaving the solution when no sugar is present in the solution. After sugar is added, the sugar-proton symport proteins would activate and take protons out of the solution. By comparing the rate of protons leaving the solution before sugar is added and after, the efficiency of sugar uptake can be measured without the risk of contamination causing overestimation of the zero-trans uptake assay.

In a recent study, Magalhaes et al. (2016)[[16]](#footnote-16) studied the relationship between alpha-glucoside transporters and fermentation performance of a set of brewing yeasts. They particularly wanted to know how lager yeast group I utilized maltotriose as little was known about maltotriose uptake. They measured the alcohol content and the sugar content of the wort over the course of fermentation, and the efficiency of the alpha-glucoside transporters using sugars that were radioactively labelled with carbon-14. Even though some yeasts were observed to have high levels of maltose and maltotriose uptake by having large amounts of carbon-14 labelled sugars, those yeasts were not the fastest fermenters. This goes against the generally held belief that sugar transport is the rate limiting reaction in fermentation. In this study, they used the zero-trans uptake assay to measure the sugar. As mentioned earlier, this technique can be liable to overestimation of sugar uptake if a contaminant entered the solution. If the test was fault and some contaminant got in, then their results would be wrong.

 I purpose to rerun Magalahaes et al. experiment but measure the sugar uptake with the pH probe measuring proton uptake and the zero-trans uptake assay. Should both techniques agree that sugar uptake is not the rate limiting reaction, then we can look for another rate limiting reaction whether it is glycolysis or something else. Should they differ, then their conclusions are no longer valid and would have to be revised.

**Experiment**

To determine if uptake of sugars including maltose and maltotriose are the rate limiting reaction of fermentation, different yeasts will be tested. The sugar content of the wort over time and the alcohol in the wort over time will be measured. The sugar uptake will be measured using a combination of the zero-uptake assay and the proton symport assay.

Different strains of yeast from used for different types of beer brewing will be grown in a wort at 15° C. As they grow, the sugar and alcohol levels will be monitored over time (Figure 2). The levels of maltose and maltotriose will also be monitored over time (Figure 3). Once the levels of sugar and alcohol plateau showing the end of fermentation for that batch, the yeast will be collected from the wort. The yeast will be centrifuged and the supernatant analyzed for alcohol and sugar content. The yeast pellet will then be used to determine sugar uptake activity through zero-trans assays and through proton symport assays.

Figure 2.



Figure 2. The alcohol content of the wort of different strains over time. Each point of the graph represented the percentage of alcohol that was in the beer wort of different yeasts over time. The yeast was grown until the alcohol levels plateaued. Different strains were faster fermenter’s than others. The greater the slope of these lines, the faster that strain could convert sugars into alcohol. Strains A15 and A220 were faster fermenters than strain C902.

Figure 3.



Figure 3. The sugar content of the beer wort of different strains over time. (A) The starting content of Maltose was the same in each beer wort. A58 used maltose quicker than A231. (B) The starting content of maltotriose was the same in each beer wort. A58 used maltotriose faster than A231.

For the zero-trans assay, the yeast will be harvested from the beer wort. The yeast will then be washed with distilled water and suspended in a buffer. Radioactively labelled sugar will then be added to the solution. After 20 seconds, 10 ml of water at 0°C will be added to stop uptake. After filtering the solution through a Millipore filter, the cells and filters will be washed with chilled water and submerged in liquid scintillation cocktail and radioactivity counted.

For the proton symport assays, a pH meter will be used to determine the rate of protons leaving the solution and entering the yeast cells. Maltose or maltotriose will then be added. The rate of proton uptake by the yeast will be determined by measuring the pH after the sugar is added to the solution (<10s).

Both assays will be done to determine if the sugar uptake matches between the two assays and agree with the results of Magalhães et al. Should the sugar uptake rates agree i.e. the results of the zero-trans uptake assay and the proton symport assay say that a yeast has a slow or fast uptake of sugar, then we can determine if sugar uptake is the rate limiting reaction step in fermentation. The alcohol and sugar content of the wort will be compared with the sugar uptake rates. Should those yeast that have the highest sugar uptake also have the fast fermentation rates (faster alcohol production and fastest sugar consumption), then long-held belief that sugar uptake is the rate limiting step will be supported. Should those yeast that have the highest rates of sugar transport not have the highest rates of fermentation, then we would have evidence to suggest that sugar uptake is not the rate limiting step in fermentation.

**Discussion**

 Yeast has been used for its culinary and euphoric effects. The different strains of yeast tested have different sugar transporters. By understanding which strains can ferment different kinds of sugar present in the wort, the genes responsible for transportation of different sugars can be deduced. Understanding how to make yeasts efficient in how they uptake sugars will help create better yeasts for industry and for culinary pleasure.

 Earlier studies measured sugar uptake through zero-trans assays which can be proton to overestimation of sugar uptake should a contaminant enter the solution during tested or the solution incubated for a longer time than ~20 seconds. By using the zero-trans uptake assay and the proton symport assay, we can verify if yeasts that are identified as high transporters by one method are identified as high transporters by the other. Determining if the methods agree will allow us to determine if the idea of sugar uptake is the rate limiting reaction in fermentation is correct.

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