**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 started from their previous batch. This resulted in lineages of yeast that grew in man-made environments which created the perfect opportunity for domestication[[1]](#footnote-0). This process caused yeasts to diverge into five clades based on their geography and useage: Asian strains such as saki, 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-1). Glucose is preferentially used by Saccharomyces and acts as an inhibitor to maltose transporters[[3]](#footnote-2), [[4]](#footnote-3). 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-4) [[6]](#footnote-5) [[7]](#footnote-6). 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-7). Sugar enters through a proton symporter[[9]](#footnote-8). Maltose transporter genes are called MALx1 where x stands for the locus of orign (1-4 or 6). All MALx1 transporter genes share a high identity and have been shown to have a high affinity for maltose (being maltose genes, this should not come as a surprise). AGT1 (alpha glucoside transporter) is an allele of MAL11 which can transport a wide range of sugars including maltotriose[[10]](#footnote-9). MTT1 shares about 90% of identity with MALx1 and transports maltose, maltotriose, trehalose, and turanose and is unusual in that it has a higher affinity for maltotriose than maltose[[11]](#footnote-10) [[12]](#footnote-11).

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. In zero-trans uptake assays, makes use of radiolabeled substrate which is uptaken by the cell. Less than 1% of the substrate should enter the cell and the uptake of any impurity can cause overestimation of how well a protein is uptaking sugar[[13]](#footnote-12) [[14]](#footnote-13). Another technique that does not have this failing is measuring the sugar-induced proton uptake soon after sugar is added to the sample[[15]](#footnote-14). A pH meter measures the basal limit of proton absorption then after the sugar is added, they subtract that limit from baseline to see how efficient the protein is at absorbing sugar.

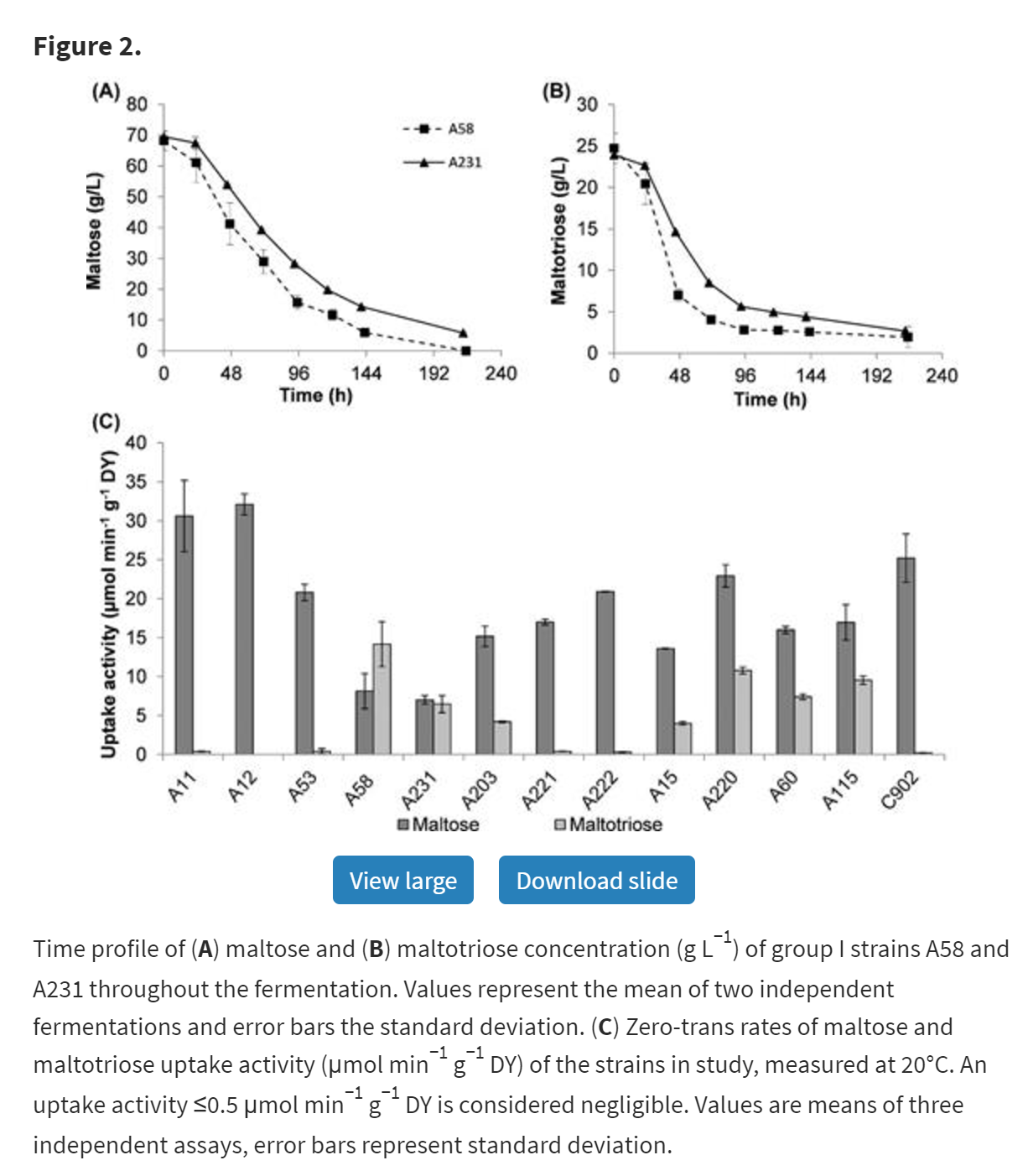
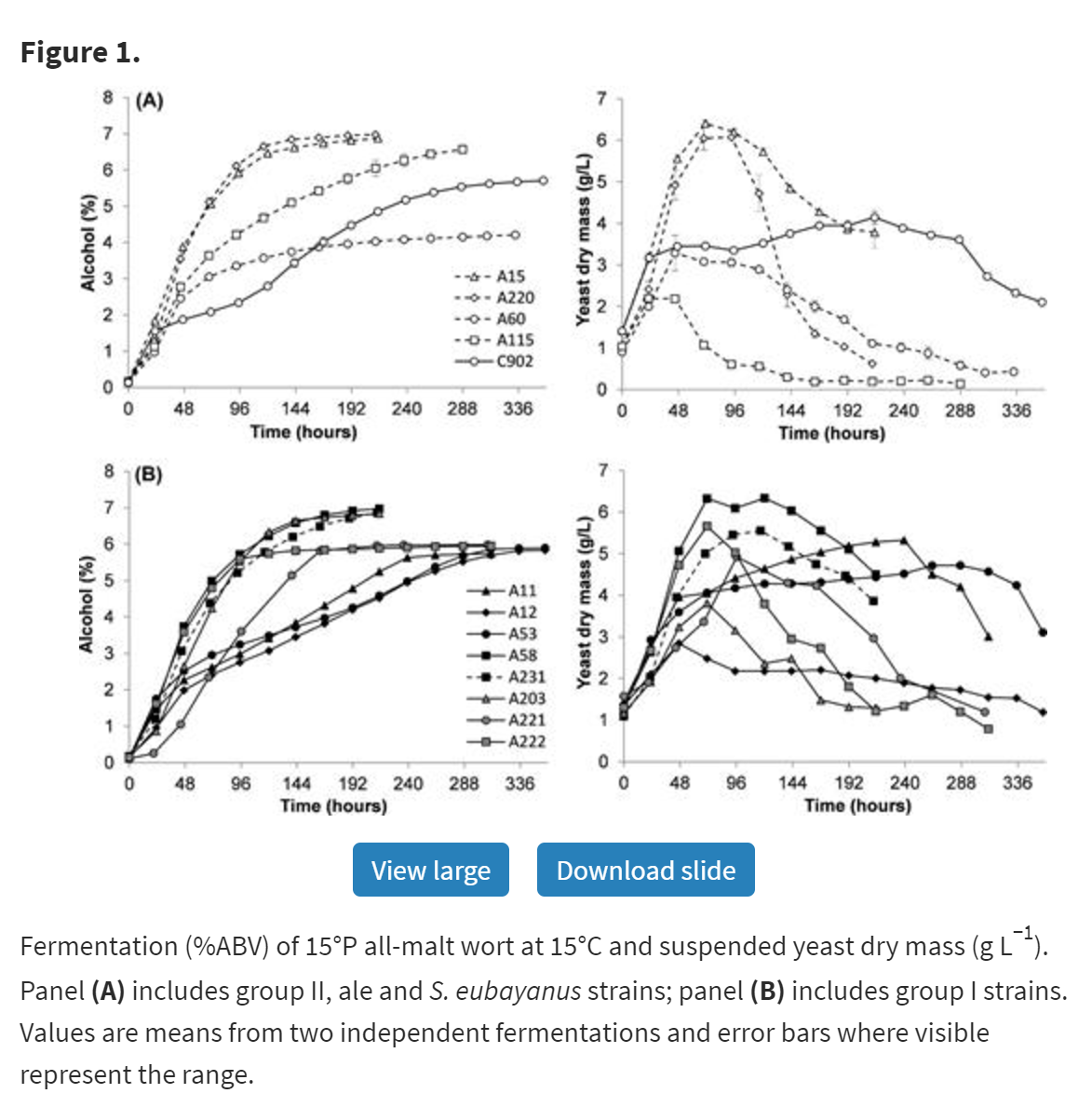
In recent study by Magalhaes et al. (2016)[[16]](#footnote-15), studied the relationship between alpha-glucoside transporters and fermentation performance of a set of brewing yeasts. They particularly wanted to know how lager yeast group I utilized maltotriose as little was known about that. During fermentation, they measured the alcohol content of the wort over time, the sugar content of the wort over time, and the efficiency of the alpha-glucoside transporters using zero-trans uptake assays. Even though some yeasts were observed to have high levels of maltose uptake, 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 to measure the sugar. 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 use the sugar co-transport measurement and the zero-trans measurement to determine uptake efficiency. Should both techniques agree that sugar uptake is not the rate limiting reaction, then we can look for another rate limiting reaction whether is it glycolysis or something else. Should they differ, then their results are no longer valid and would have to be revised.

**Experiment**

[This experiment will make use of the materials and methods in Magalhães, Vidgren, Ruohonen, & Gibson (2016). [Maltose and maltotriose utilisation by group I strains of the hybrid lager yeast *Saccharomyces pastorianus*](https://academic.oup.com/femsyr/article/16/5/fow053/2465898/Maltose-and-maltotriose-utilisation-by-group-I#78578961). FEMS Yeast Res 16(5). Doi: <https://doi.org/10.1093/femsyr/fow053>]

In the original experiment, yeast strains were obtained from VTT culture collection consisting of group I strains, group II strains, Ales, and a S. eubayranus strain. The yeasts were grown in a wort at 15° C. Samples were collected and spun down in the centrifuge. The supernatant was used for wort and beer analysis. The pellet was washed and used for yeast analysis. The amount of alcohol (Fig. 1). The concentration of maltose (Fig. 2A) and Maltotriose (Fig. 2B) were measured over time. The sugar uptake activity was also measured for each yeast strain using zero-trans uptake assays (Fig. 2C). Comparing the alcohol production in Fig. 1 and Fig. 2., Magalhaes et al. concluded, “Higher activity did not necessarily ensure faster fermentation. In fact, strains with highest maltose uptake activity (Fig. 2C) were relatively slow fermenters (A11, A12, A53 and C902; Fig. 1).”



The process for the zero-trans uptake assay is listed below.

“For maltose and maltotriose uptake measurement, the yeast strains were grown at 20°C in liquid YP medium containing maltose (4% w/v) to an OD600 nm between 4 and 8. The cells were harvested by centrifugation (10 min, 5000 rpm, 0°C), washed with ice-cold water and 0.1 M tartrate-Tris (pH 4.2) and resuspended in the same buffer to a concentration of 200 mg fresh yeast mL−1. Zero-trans rates of [U-14C]-maltose and [U-14C]-maltotriose uptake at 20°C, 10°C or 0°C were determined with 5 mM of substrate as described by Lucero et al. (1997). Two incubation times were tested to ensure linearity with respect to time with t2 corresponding to at least 90% of t1 value. Statistical analysis was performed with R (http://www.rproject.org/; v3.3.0) by using one-way ANOVA and Tukey's test.” (direct passage from article)

For my proposal, I want to also measure the uptake using the technique outlined in Alves et al. (2008) which is listed below.

“The -glucosidase activity in cells collected by centrifugation (2,500 g for 3 min) at the exponential phase of growth was determined in situ with permeabilized yeast cells as described previously (31) by using 100 mM MOPS (morpholinepropanesulfonic acid)-NaOH (pH 6.8) buffer and 2 mM p-nitrophenyl-D-glucopyranoside (pNPG) or 100 mM maltose or maltotriose as substrates. The activity of the AGT1 permease was determined with a specific colorimetric assay (17) using 5 mM pNPG in 100 mM succinate-Tris, pH 5.0. All assays were done in triplicate, and controls with previously boiled yeast cells were used. The rates and kinetics of active H-trehalose, H-maltose, or H-maltotriose symport were determined as previously described (32) using a PHM84 research pH meter attached to a TT1 Servograph (Radiometer, Copenhagen). Initial rates of sugar-induced proton uptake were calculated from the slope of the initial (10-s) part of the curve obtained in the recorder by subtracting the basal rate of proton uptake observed before the addition of 0.1 to 100 mM sugar. All determinations were done at least in duplicate, and assays were monitored so that no more than 5% of the substrate was depleted. All activities were expressed as nanomoles of the substrate transported (or hydrolyzed) per milligram (dry weight) of cells per minute.” (direct quote from article)

Using these two measurements for sugar utilization and uptake, I would verify the previous results and see if their claim that “Higher activity did not necessarily ensure faster fermentation.” The final results would be presented much like the ones shown above. Maltose and maltotriose levels in the residual wort would be measured and the alcohol level in the wort. The two measurements of the sugar upkeep would also be measured and the two techniques would be compared to see if they returned similar results.

**Discussion**

Yeast has been used for its culinary and euphoric effects. Understanding how to make yeasts efficient in how they uptake sugars will help create better yeasts for industry and in the kitchen.

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