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# Selective Utilization of Fructose to Glucose by *Candida magnoliae*, an Erythritol Producer

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## Abstract

*Candida magnoliae* isolated from honeycomb is an industrially important yeast with high erythritol-producing ability. Erythritol has been used as functional sugar substitute for various foods. In order to analyze the physiological properties of *C. magnoliae*, a study on sugar utilization pattern was carried out. The fermentation kinetics of glucose and fructose revealed that *C. magnoliae* has the discrepancy in glucose and fructose utilization when it produces erythritol. In contrast to most yeasts, *C. magnoliae* showed preference for fructose to glucose as a carbon source, deserving the designation of fructophilic yeast. Such a peculiar pattern of sugar utilization in *C. magnoliae* seems to be related to the evolutionary environment.

**Index Entries**: *Candida magnoliae*; fructophilic yeast; fructose utilization; erythritol.

## Introduction

Erythritol is a four-carbon sugar alcohol used as a food ingredient with taste and mouthfeel-enhancing properties. It is a naturally occurring sweetener in various fruits and fermented foods including grape, wine, beer, and soy sauce *(1)*. Erythritol has 60–80% sweetness relative to sucrose and is a good low-calorie sweetener *(2)*. Interestingly, more than 90% of ingested erythritol is not metabolized by the human body and is excreted in the urine without changing blood glucose and insulin levels *(3)*. Therefore, the four-carbon sugar alcohol might be advantageously used as a functional sugar substitute in special foods for people with diabetes and obesity *(4)*. Erythritol has been approved in the United States and used as

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a flavor enhancer, nutritive sweetener, and sugar substitute *(5)*. In Japan, it has been used since 1990 as a sugar substitute for various foods such as candies, soft drinks, chewing gum, jams, and yogurt *(6)*.

*Candida magnoliae* is a high erythritol-producing strain isolated from honeycomb (Deposition No.KFCC 11023). To improve the erythritol-producing ability, the parental wild strain was mutated by ultraviolet irradiation and nitrosoguanidine treatment to give a mutant strain *(7,8)*. The optimized fed-batch fermentation using the mutant strain of *C. magnoliae* resulted in 200 g/L erythritol concentration, 1.2 g/L/h productivity, and 0.43 g/g yield *(9)*. The genome of *C. magnoliae* was not sequenced yet and hence information was not sufficient to explore the erythritol production pathway at a gene level. In our previous reports, the expression levels of metabolic enzymes illustrated on two-dimensional electrophoresis (2-DE) served to infer the physiological properties of *C. magnoliae* at the protein level *(10–12)*. *C. magnoliae* showed unique physiological characteristics compared with typical yeasts including *Saccharomyces cerevisiae*. It is able to grow in a wide range of pH values in the presence of high concentrations of sugars *(8)*. Unlike *S. cerevisiae*, *C. magnoliae* does not produce ethanol, but produces erythritol and glycerol. The most interesting characteristic of *C. magnoliae* deserved by our attention is a peculiar preference of fructose to glucose as a carbon source. More investigations on the mode of sugar metabolism by *C. magnoliae* are desirable to use this strain for erythritol production in an industrial scale.

Not many studies on the preference for glucose or fructose in yeast strains have been performed. *Candida shellata* and *Zygosaccharomyces bailii* have a preference for fructose, whereas *S. cerevisiae* in general appears to be glucophilic *(13,14)*. The aim of this work was to investigate in more detail the sugar metabolism of *C. magnoliae* by analyzing fermentation kinetics under various sugar-containing media. Specifically, growth rate, biomass yield, and production of metabolites such as erythritol and glycerol were measured and compared.

## Materials and Methods

### *Yeast Strains and Culture Conditions*

*C. magnoliae* KFCC 11023 wild type was used for this study. The strain was isolated from honeycomb and is osmotolerant to produce erythritol as a major product. *C. magnoliae* was propagated and cultured under laboratory conditions at 30°C in medium, which contained 300 g/L glucose, 10 g/L yeast extract, and 20 g/L bactopeptone. Solid medium was supplemented with 20 g/L agar.

### *Fermentation Media and Condition*

Seed culture for fermentation was performed in a 500-mL baffled flask containing 50-mL growth medium at 30°C, 10*g*, and for 24 h. The growth medium was composed of 10 g/L yeast extract and 20 g/L bactopeptone

supplemented with one of the various carbon sources (glucose, fructose, sucrose, and a mixture of glucose and fructose). Batch culture in a fermentor was performed with 2.5-L jar fermentors (KoBioTech, Incheon, Korea) containing 1-L fermentation medium at 30°C with agitation at 40*g* and aeration at 1 vvm. Concentrations of sugars used as carbon source were 300 g/L glucose, 300 g/L fructose, 300 g/L sucrose, and a mixture of 150 g/L glucose and 150 g/L fructose. All experiments were done in duplicate under the same conditions.

### *Analytical Methods*

Dry cell weight was estimated using a calibration curve derived from the relationship between the absorbance at 600 nm and cell dry weight. The conversion factor of absorbance to dry cell weight was 0.26 for the *C. magnoliae* wild strain. Optical density was measured with a spectrophotometer (UltroSpec 2000, Pharmacia Biotech, NJ). Concentrations of glucose, fructose, sucrose, erythritol, and glycerol were determined by high-performance liquid chromatography (HPLC; Knauer, Berlin, Germany) equipped with the Aminex HPX-87H column (Bio-Rad, Richmond, CA) at 65°C. The flow rate of a mobile phase, 0.01*N* $H_2SO_4$, was set at 0.6 mL/min. Detection was done using a differential reflective index detector (Knauer, Germany).

## Results

### *Evaluation of Carbon Utilization Patterns*

Experimental results for batch fermentations with initial glucose or fructose concentration of 300 g/L were illustrated in Figs. 1 and 2, respectively. Glucose concentration in the medium declined at a constant rate, 0.8 g/L/h, after the lag phase of cell growth. Cells grew exponentially until 45 h and then grew up to 42 g/L; 4 g/L erythritol was produced and glycerol was not formed when grown in the glucose medium. Cells in the fructose medium grew exponentially until fructose was consumed about 30% of the initial concentration and then the biomass slightly increased, actively producing erythritol and glycerol during the stationary phase (Fig. 2). Finally, the cells grew up to 54 g/L and erythritol was produced at a constant rate of 3.0 g/L/h from the early exponential phase of cell growth until 100% of fructose initially added was depleted. This value represents a 3.8-fold increase in fructose consumption rate compared with glucose consumption rate. The final concentration of erythritol in the 300 g/L fructose medium was 85 g/L, which is about 21 times higher than that produced with 300 g/L glucose. Glycerol was produced in proportion to the cell mass during the exponential phase of cell growth but was not consumed later as a carbons source. The final concentration of glycerol produced was 77 g/L, which is almost the same amount of erythritol. The residual concentrations of sugars after the end of the fermentation were

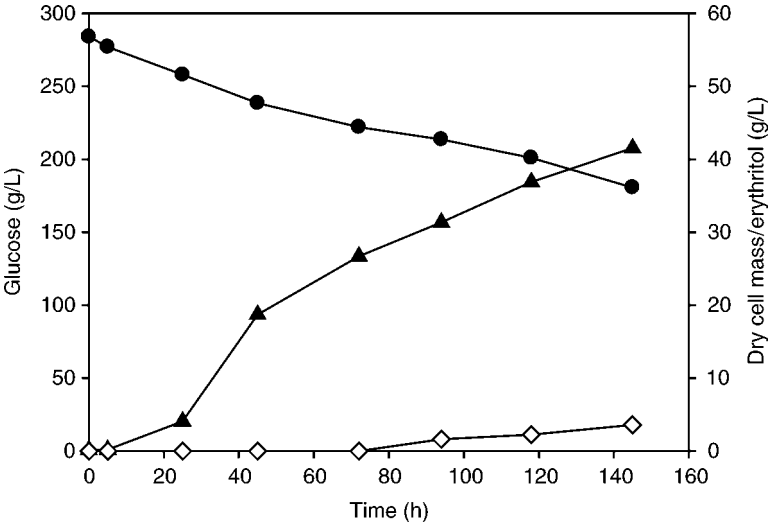


**Fig. 1.** Fermentation profiles of *C. magnoliae* wild type grown in 300 g/L glucose: dry cell mass (▲), glucose (●), and erythritol (◇).

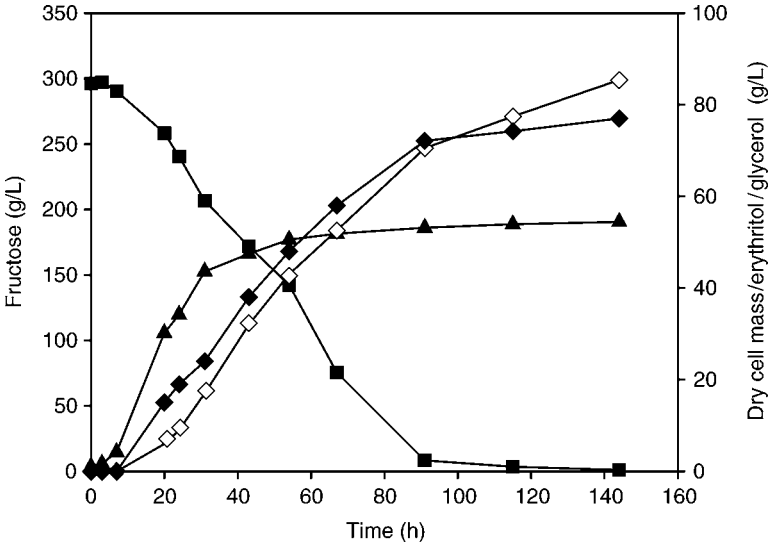


**Fig. 2.** Fermentation profiles of *C. magnoliae* wild type grown in 300 g/L fructose: dry cell mass (▲), fructose (■), erythritol (◇), and glycerol (◆).

181 g/L glucose for the glucose medium and 1 g/L fructose for the fructose medium in 144 h, indicating *C. magnoliae* was not able to utilize glucose completely, whereas it was able to use high concentration of fructose when it produced erythritol. Fermentation parameters obtained with 300 g/L glucose or fructose was summarized in Table 1. These results clearly showed that fructose favors erythritol production and cell growth in *C. magnoliae*.

Table 1
Summarized Results of Batch Fermentations by *C. magnoliae* Grown in Various Compositions of Sugars

| Sugar | Concentration (g/L) | Maximum dry cell mass (g/L) | Maximum erythritol concentration (g/L) | Specific growth rate, μ (/h) | Erythritol productivity (g/L/h) | Erythritol yield (g/g sugar) | Sugar consumption rate (g/L/h) |
|---|---|---|---|---|---|---|---|
| Glucose | 300 | 42 | 4 | 0.068 | 0.05 | 0.086 | 0.81 |
| Fructose | 300 | 54 | 85 | 0.097 | 1 | 0.29 | 3.24 |
| Glucose + Fructose | 150 + 150 | 50 | 50 | 0.19 | 1 | 0.29 | 2.96 (Fructose)<br>0.71 (Glucose) |
| Sucrose | 300 | 63 | 65 | 0.10 | 1 | 0.21 | 3.21 (Fructose)<br>0.18 (Glucose) |

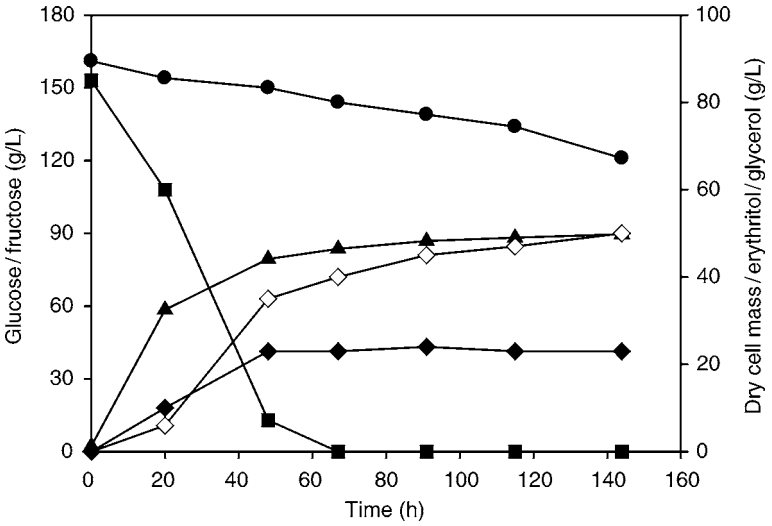


**Fig. 3.** Fermentation profiles of *C. magnoliae* wild type grown in a mixture of 150 g/L glucose and 150 g/L fructose: dry cell mass (▲), glucose (●), fructose (■), erythritol (◇), and glycerol (◆).

## *Selective Utilization of Fructose to Glucose*

To confirm clearly the selective utilization of fructose by *C. magnoliae*, the batch fermentations were performed with 300 g/L sucrose or a mixture of 150 g/L glucose and 150 g/L fructose. A fermentation profile in the sugar mixture was shown in Fig. 3. Even though the same amount of glucose and fructose was added initially, the selective utilization of fructose compared with glucose led to a discrepancy between the amount of glucose and fructose consumed (GF discrepancy) during the linearly erythritol-producing period of the fermentation. Right after inoculation, a significant amount of fructose was taken up at a constant rate of 2.9 g/L/h. This initial rapid uptake was followed by a period of almost depletion of fructose. Toward the end of fermentation, when glucose became more limiting, the GF discrepancy decreased. When the fermentation finally ceased, glucose was found at significantly higher concentrations than fructose. The GF discrepancy increased almost linearly during the fermentation (Fig. 4). The GF discrepancy determined after depletion of fructose was disregarded and focused on the fermentation stages when fructose was linearly consumed. A fermentation pattern for 300 g/L sucrose was displayed in Fig. 5. The results showed the *C. magnoliae* had the ability to hydrolyze sucrose to glucose and fructose by its invertase. *C. magnoliae* produced mainly extracellular invertase like other sucrose-consuming yeasts during the fermentation (data not shown). After the lag phase of cell growth, fast hydrolysis of sucrose was observed, resulting in accumulation of glucose and fructose. In the beginning of the fermentation, fructose also started to accumulate because its production by

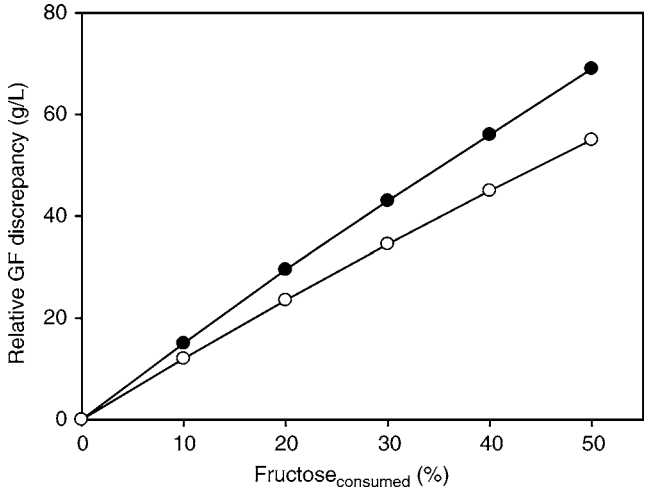


**Fig. 4.** Relative concentration of glucose to fructose consumed with fermentation time for the 300 g/L sucrose medium (○) and the mixture of 150 g/L glucose and 150 g/L fructose medium (●).

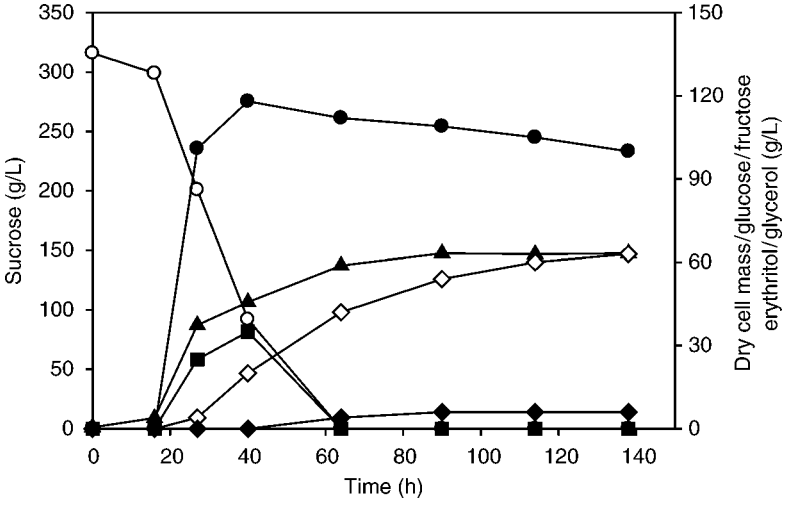


**Fig. 5.** Fermentation profiles of *C. magnoliae* wild type grown in 300 g/L sucrose: dry cell mass (▲), sucrose (○), glucose (●), fructose (■), erythritol (◇), and glycerol (◆).

hydrolysis of sucrose was faster than its consumption. However, glucose continuously accumulated up to approx 120 g/L until the residual fructose was almost consumed. After depletion of fructose, glucose was used and the fermentation profile is similar to that in the medium containing glucose alone. Erythritol increased to 65 g/L, with an erythritol yield of 0.21 g/g of fructose and glucose consumed. Glycerol was produced as well. Fermentation results of *C. magnoliae* in batch cultures were summarized in Table 1. The rate of fructose consumption was

higher than that of glucose in both sucrose and a mixture of glucose and fructose. In both fermentations, fructose was first used up followed by consumption of glucose by *C. magnoliae* (Figs. 3 and 5). Interestingly, the diauxic growth was not observed in disagreement with the cases typically in which two carbon sources were present in the same growth medium. *C. magnoliae* showed preference to fructose when using a mixture of glucose, fructose, and sucrose as carbon source. The GF discrepancy in a mixture of sugars was also higher than that in the sucrose medium (Fig. 4). These results are consistent with fructophilic behavior described previously for other strains such as *C. shellata* and *Z. baillii* (*13*,*14*).

## Discussion

The experimental results with *C. magnoliae* isolated from honeycomb have shown clearly the selective utilization of fructose relative to glucose, indicating *C. magnoliae* to be fructophilic. The exact reason for such an observation needs to be characterized. In *S. cerevisiae*, fructose is used concomitantly with glucose, the latter is used first, which gives rise to a discrepancy between glucose and fructose (*15*,*16*). The metabolic pathway of fructose utilization is very similar to the glucose assimilation pathway. The transporters are shared although their affinity for glucose is higher than for fructose, but $V_{max}$ for the two sugars is similar. Hence, the transport step could be a reason for selective utilization of glucose in *S. cerevisiae*. For *C. magnoliae*, however, glucose was not consumed when fructose was present in the growth medium. Research efforts to characterize hexose transporters in yeasts usually deal with glucose as substrate, but most transporters characterized so far do not discriminate between glucose and fructose. Recently, the fructose-specific transporter of *Z. bailii* was cloned and characterized by functional complementation of *S. cerevisiae* incapable of growth on hexoses (*17*). Like *C. magnoliae*, *Z. bailii* consumed fructose faster than glucose (*14*). In *Z. bailii*, fructose promoted the inactivation of the glucose transporter, preventing the utilization of glucose when fructose was also available. Moreover, fructose crosses the plasma membrane through fructose-specific transporter at a higher rate than glucose and can be metabolized faster when fructose is present in high concentrations. After transport, glucose is phosphorylated by glucokinase and hexokinases 1 and 2, whereas fructose is only phosphorylated by the latter two enzymes in *S. cerevisiae* (*18*). The affinity of the hexokinases is higher for glucose. Hence, the phosphorylation step could be another reason for the cause of the discrepancy in glucose and fructose fermentation. Fructose is a ketose sugar, nearly 30% of which is present in the furanose form in solution (*19*), whereas glucose is an aldose, nearly 99.9% of which is present in the pyranose form (*20*). Because glucose and other sugars are transported in the pyranose form rather than in the furanose form, the actual

transport-competent concentration of fructose is below its total concentration (21). Differences in physicochemical properties like these may explain the lower affinity for fructose of the transport system (22) and the hexokinases (23,24). After phosphorylation, fructose-6-phosphate readily enters glycolysis by conversion into fructose-1,6-bisphosphate, whereas glucose-6-phosphate still has to be converted first into fructose-6-phosphate by phosphogluco-isomerase. The cause of the GF discrepancy therefore appears to be located in the transport and/or phosphorylation steps of the fermentation pathway.

In conclusion, the selective utilization of fructose by *C. magnoliae* was observed especially when the yeast produced erythritol from sucrose or a mixture of glucose and fructose. More research has to be done to elucidate the fructophilic behavior of *C. magnoliae* at a gene or protein level.

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