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Transport kinetics of maltotriose in strains of *Saccharomyces*

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SUMMARY

Maltotriose transport was studied in two brewer's yeast strains, an ale strain 3001 and a lager strain 3021, using laboratory-synthesized ^{14}C-maltotriose. The maltotriose transport systems preferred a lower pH (pH 4.3) to a higher pH (pH 6.6). Two maltotriose transport affinity systems have been identified. The high affinity system has K_m values of 1.3 mM for strain 3021 and 1.4 mM for strain 3001. The low affinity system has K_m values of 46 mM for strain 3021 and 74 mM for strain 3001. The high affinity system was competitively inhibited by maltose and glucose with K_i values of 58 mM and 177 mM, respectively, for strain 3021, and 55 mM and 147 mM, respectively, for strain 3001. Cells grown in maltotriose and maltose had higher maltotriose and maltose transport rates, and cells grown in glucose had lower maltotriose and maltose transport rates. Early-logarithmic phase cells transported glucose faster than either maltose or maltotriose. Cells harvested later in the growth phase had increased maltotriose and maltose transport activity. Neither strain exhibited significant differences with respect to maltose and maltotriose transport activity.

INTRODUCTION

The utilization of wort sugars is one of the major determinants that control fermentation efficiency during the brewing process [21]. The rate and extent of sugar utilization is in turn controlled by the fermentation environment [17], the ability of yeast to transport sugars [6,8,18], and the rate of subsequent metabolism [14–16]. A common problem encountered by a number of breweries is the incomplete utilization of wort maltotriose, a trisaccharide composed of three glucose moieties. Since maltotriose is the second most abundant fermentable sugar in brewery wort, its incomplete fermentation results in a loss of fermentation material and atypical beer flavors [19]. To solve this problem, an understanding of both the influencing environmental factors and of the biochemical regulation of maltotriose uptake are important. This paper reports on the transport kinetics of maltotriose in two brewing strains of *Saccharomyces* and its relationship with maltose and glucose.

MATERIALS AND METHODS

Yeast strains. The yeast strains employed in this study were, with their Labatt Culture Collection numbers, *Saccharomyces uvarum* (*carlsbergensis*) brewing lager strain 3021, and *Saccharomyces cerevisiae* brewing ale strain 3001.

Fermentation. Yeast cells were inoculated into 100 ml of fresh wort or peptone–yeast extract–sugar media as indicated in the Results and Discussion section. Fermentations were conducted in 300-ml Erlenmeyer flasks, with shaking at 150 r.p.m. at 21 °C [10].

Synthesis of ^{14}C-maltotriose. The synthesis of ^{14}C-maltotriose was conducted according to the method of Pazur and Ando [20] with some modifications. A culture of *Bacillus macerans* (American Type Culture Collection 7069) was grown in sterilized medium containing the following materials per 100 ml: 10 mg potato slices, 3 g calcium carbonate, and 0.4 g ammonium sulfate. At the end of a two-week incubation period at 40 °C, the culture solution was centrifuged at 4000 × *g* for 10 min and the amylase activity was detected with starch and 0.1 N iodine/potassium iodide complex. One ml of this enzyme solution converted 60 mg starch to the end point (colorless) in 45 min. Samples of 50 mg D-[U-^{14}C]-glucose (1.5 mCi) and 300 mg α-cyclodextrin were dissolved in 2.5 ml of water and mixed with 2.5 ml *B. macerans* amylase solution. After three days incubation at 37 °C, the reaction was stopped by heating in a boiling water bath for 5 min. The samples were loaded as spots onto chromatography paper (20 × 20 cm, Whatman no. 3) and developed, in an ascending mode, in 1-butanol-pyridine-water (6:4:3, v/v) for 4 h. For satisfactory separation, four repeated runs were required. To determine the position of ^{14}C-maltotriose, pure maltotriose was loaded as a separate spot. At the end of the chromatography, a small piece of paper containing the pure maltotriose and some of the sample was cut and stained with silver nitrate/sodium hydroxide [12]. ^{14}C-maltotriose was located by reference to pure maltotriose, eluted from the paper with water, and identified by HPLC. Approximately 10 mg of ^{14}C-maltotriose was obtained and the specific radioactivity was 1.5 μCi mg^{-1}.

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Sugar transport studies. Transport studies were conducted as described previously [10]. For maltotriose and maltose transport, cells were harvested at specified times during fermentation, washed twice with ice-cold distilled water and suspended in 100 mM tris-tartaric acid buffer (pH 4.3) or 100 mM potassium-phosphate buffer (pH 6.6) to a cell density of 40 mg wet weight ml^{-1}. For glucose transport, cells were washed twice with ice-cold water and suspended in 100 mM potassium-phosphate buffer (pH 6.6) to a cell density of 40 mg wet weight ml^{-1}. Transport was initiated by the addition of 0.1 ml of radioactive substrate (0.1 μCi ml^{-1}, with the desired concentration of the different sugars as described in the Results and Discussion section) to 0.9 ml of cell suspension and carried out at room temperature. At 30-s intervals, 0.2 ml of cell suspension was withdrawn, filtered through 0.45-μm pore size nitrocellulose filters, and washed twice with ice-cold buffer. The filters were solubilized in 10 ml scintillation fluid and the radioactivity determined using a liquid scintillation counter (Beckman 5000). In the studies of the low affinity transport system, employing high concentrations of substrate and sugar competition, where 50 or 100 mM inhibiting sugar was used, sorbitol was employed at equivalent concentrations as an osmotic control since sorbitol is an unfermentable sugar for both types of yeast strains studied. For the determination of biomass, 5-ml cell suspensions were sampled at the desired times and centrifuged at 4000 × *g* for 10 min. The pellets were washed twice with distilled water and dried in aluminum dishes at 80 °C overnight.

Calculations. The K_m and V_{max} values for maltotriose transport were determined by Lineweaver–Burke double reciprocal plots [7,13]. The K_i values for maltose and glucose were determined by replotting the K_m/V_{max} versus [I]. The data represent the average of three experiments with duplicates conducted on different days. The significant differences were tested at the $P = 0.05$ level.

RESULTS AND DISCUSSION

Determination of kinetic constants for maltotriose transport

Glucose and maltose are transported by two systems, a high affinity system with K_m values of 1.6 mM and 1.6 mM, respectively, and a low affinity system with K_m values of 21.2 mM and 40.4 mM, respectively [4,8]. Due to the lack of the availability of commercial ^{14}C-maltotriose, no information has been published on the kinetic constants for maltotriose transport. ^{14}C-maltotriose was synthesized in this laboratory using cyclohexamylose and ^{14}C-1-D-glucose through the coupling and redistribution activities of the *B. macerans* amylase [20]. Maltotriose transport was studied in both tris-tartaric acid (pH 4.3) and potassium-phosphate (pH 6.6) buffer. Approximately 2–3 times higher transport rates were observed in tris-tartaric acid buffer compared to potassium-phosphate buffer (Fig. 1), which demonstrates the relationship of maltotriose transport to proton uptake, as in the case for maltose transport [5,18]. All subsequent maltotriose transport studies were conducted in tris-tartaric acid buffer. Using various concentrations of maltotriose (0.025, 0.05, 0.1, 0.2, 5, 10, 20, 40 and 80 mM), two affinity systems were identified for maltotriose transport (Table 1). The K_m values of both strains for the high affinity system did not show significant differences and were similar to those for the high affinity maltose transport system. The K_m value of strain 3021 for the low affinity system was also similar to that for the low affinity maltose transport system, but was lower than that of strain 3001. The V_{max} values of both strains for the two affinity systems were similar.

In order to study the possibility that the low affinity transport is due to trapping of sugar in the periplasmic space as described by Benito and Lagunas [1], the cells of strain 3021 were treated with either 2 mM $HgCl_2$ or by boiling for 5 min, and then studied for their ability to transport maltotriose or maltose. As is shown in Fig. 2, 80 mM maltotriose or maltose could be accumulated by the untreated

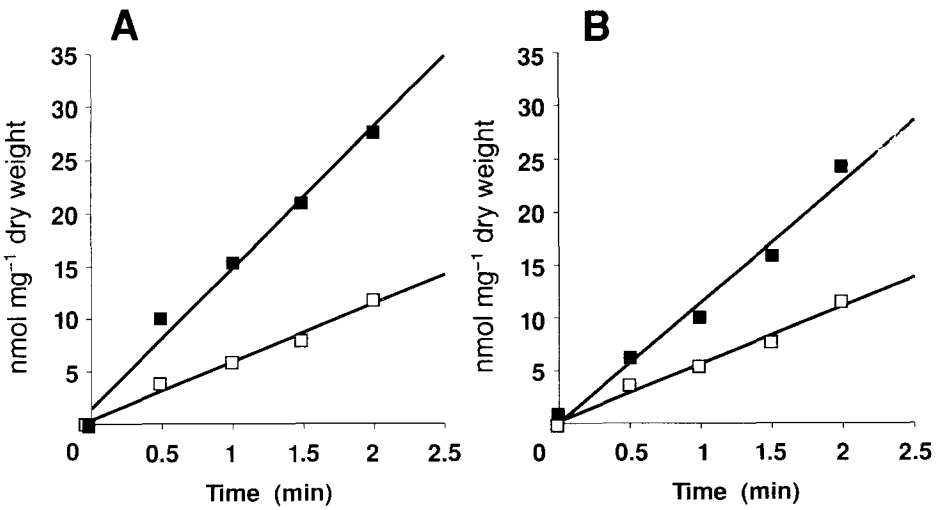

Fig. 1. Maltotriose (0.5 mM) transport in tris-tartaric acid buffer pH 4.3 (■) and potassium-phosphate buffer pH 6.6 (□). (A) Lager strain 3021; (B) ale strain 3001.

TABLE 1

Kinetic constants for maltotriose transport

Strains	Constants (mean ± SE)			
	High affinity		Low affinity	
	K_m (mM)	V_{max} (nmol min^{-1} mg^{-1} dry weight)	K_m (mM)	V_{max} (nmol min^{-1} mg^{-1} dry weight)
3021	1.3 ± 0.1	43.8 ± 2.8	45.6 ± 2.1	1049.5 ± 22.2
3001	1.4 ± 0.1	47.6 ± 5.2	73.9 ± 3.8	1138.2 ± 19.4

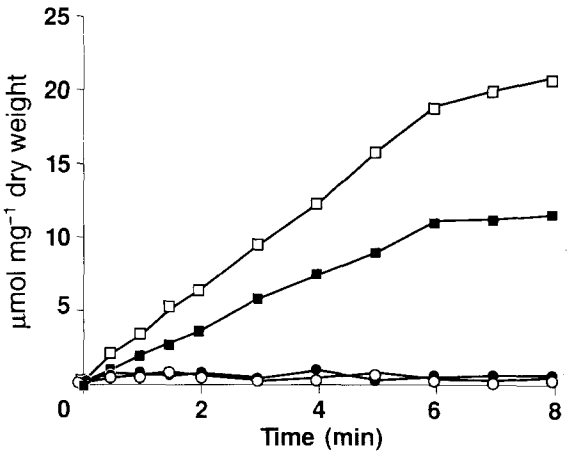

Fig. 2. Maltotriose (■) and maltose (□) transport by control cells and maltotriose (●) and maltose transport (○) by mercuric chloride-treated cells.

cells almost at a linearly increasing rate for up to 6 min. On the other hand, the $HgCl_2$-treated cells did not show obvious sugar transporting activity even though the transporting time had been extended to 8 min. Boiled cells (data not shown) were similar to the $HgCl_2$-treated cells. $HgCl_2$ disrupted the yeast cells within 20 s. Therefore, it was only added to the transport buffer and washing buffer. This treatment has an advantage over the boiling procedure. Boiling (usually takes about 5 min) may not only kill the cells but also destroy the physical structure of the cells. Changes in cellular structure may cause trapping of sugar in the periplasmic space and other artifacts. Our results demonstrated no evidence of significant maltotriose and maltose transport by the cells inactivated either with $HgCl_2$ or as a result of boiling. It appears that the low affinity transport activity discussed above is real in the strains studied, and is not due to an experimental artifact reported by Benito and Lagunas [1]. The fact that protein synthesis inhibitors affect both low and high affinity uptake provides insight into the fact that similar to the high affinity transporter the low affinity transporter includes a protein carrier [3].

Maltotriose transport by cells grown in maltotriose, maltose and glucose

It is generally accepted that sugar transport by yeast cells involves plasma membrane-bound proteins, which are referred to as 'permeases' [5,21]. Two permease systems, a constitutive system and an inducible system (for maltose) and a phosphorylating enzyme related system (for glucose), have been reported [2,5,18]. After maltotriose and maltose enter the cells, they are rapidly cleaved by α-glucosidase into glucose [21]. It was expected that cells grown in a medium with maltotriose as sole sugar source would have enhanced maltotriose-transporting activity as a result of two possibilities. Maltotriose may induce maltotriose permease activity. Besides the induction of the permease, maltotriose may also induce the activity of α-glucosidase, which rapidly metabolizes maltotriose following its entry into the cells, thereby preventing 'feed-back inhibition' of the transport system by the accumulated intracellular maltotriose [10,11]. On the other hand, cells grown in medium with glucose as a sole sugar would have low maltotriose-transporting activity for the following reasons. One would be the repression of maltotriose permease by glucose [9,16,21]. Another, the inhibition of α-glucosidase, which results in the accumulation of maltotriose inside the cells and thereby tending to have a 'feed-back inhibition' on maltotriose transport. Maltose might act as an inducer similar to maltotriose or a repressor similar to glucose for maltotriose transport. As expected, maltotriose-grown cells had a significantly higher rate of maltotriose transport than cells grown in glucose (Table 2). The maltose-grown cells showed almost the same velocity of maltotriose transport as the maltotriose-grown cells. The similar action of maltotriose and maltose on the increase of maltotriose-transporting activity indicates two possibilities: maltose and maltotriose share the same permease and α-glucosidase, or maltose activates the maltotriose permease and α-glucosidase while it also activates its own. The data in Table 3 and the previous report [11] show that cells grown in maltotriose have maltose transport rates comparable to that of maltose-grown cells, which provides further evidence for the possibilities discussed above. Even though maltose-grown cells did not exhibit reduced maltotriose transport activity, cells cultured in wort with added maltose

TABLE 2

Maltotriose (0.5 mM) transport by lager strain 3021 and ale strain 3001 pre-grown in peptone–yeast extract medium with 3% (w/v) maltotriose or maltose or glucose

Growth phase[a]	Strains	Velocity (nmol min^{-1} mg^{-1} dry weight, mean ± SE)		
		Cells grown in maltotriose	Cells grown in maltose	Cells grown in glucose
Late-log (24 h)	3021	41.3 ± 0.7	39.2 ± 4.0	6.2 ± 0.3
	3001	40.0 ± 6.3	34.6 ± 5.6	7.9 ± 1.1
Stationary (48 h)	3021	19.3 ± 2.4	17.0 ± 1.1	11.8 ± 1.8
	3001	51.4 ± 3.9	48.6 ± 1.1	8.9 ± 0.6
Late-stationary (72 h)	3021	18.7 ± 2.2	17.0 ± 2.0	9.0 ± 1.3
	3001	34.4 ± 3.9	24.7 ± 3.0	11.4 ± 1.7

[a] Fermentations were conducted in 300-ml flasks at 21 °C with shaking and cells were harvested at indicated times for transport experiments.

TABLE 3

Maltose (0.5 mM) transport by lager strain 3021 and ale strain 3001 pre-grown in peptone–yeast extract medium with 3% (w/v) maltotriose or maltose or glucose

Growth phase[a]	Strains	Velocity (nmol min^{-1} mg^{-1} dry weight, mean ± SE)		
		Cells grown in maltotriose	Cells grown in maltose	Cells grown in glucose
Late-log (24 h)	3021	67.8 ± 5.6	58.5 ± 3.5	6.2 ± 0.5
	3001	60.0 ± 6.0	53.2 ± 4.2	12.2 ± 0.9
Stationary (48 h)	3021	21.6 ± 1.4	9.8 ± 0.1	2.2 ± 0.1
	3001	96.6 ± 7.5	57.5 ± 4.7	9.0 ± 1.0
Late-stationary (72 h)	3021	11.2 ± 0.8	7.1 ± 0.7	2.2 ± 6.0
	3001	28.8 ± 1.9	24.1 ± 2.0	9.2 ± 0.9

[a] Fermentations were conducted in 300-ml flasks at 21 °C with shaking and cells were harvested at indicated times for transport experiments.

had a decreased maltotriose-utilizing ability which was similar to the situation with cells cultured in glucose-supplemented wort (Fig. 3). Sorbitol (8%) (a sugar not utilized by *Saccharomyces*) was added to a control wort in an attempt to 'mimic' the initial osmotic pressure. The explanation for the observations could be that maltotriose transport depends on both the absence or presence of other transport-inhibiting sugars, and the activity of the permease, which in turn depends on the action of an inducer and an inhibitor. While maltose is an inducer of maltotriose permease, it might also be an inhibitor for maltotriose transport (discussed in the next section). The maltotriose-consuming ability during a brewery wort fermentation is, therefore, controlled by a balance of the inducing action of maltose to the maltotriose permease and an inhibiting action of maltose on maltotriose transport.

Similar transport activities were observed for both strains 3021 and 3001 grown in different media and harvested in late-logarithmic growth phase. Nevertheless, as cells progressed further into stationary and late-stationary phase, strain 3021, grown in maltotriose and maltose had a significantly reduced maltotriose transport rate, but strain 3001 did not change its rate significantly. This phenomenon indicates that strain 3021 could not maintain its adapted high ability for maltotriose transport as long as strain 3001. Sugar analysis demonstrated that all three sugars in the medium had been depleted by late-logarithmic growth phase (data not shown).

Maltose and glucose inhibition of the high affinity maltotriose transport system

The previous section discussed maltotriose transport repression by glucose and induction by maltose. It did not provide information on how these two sugars compete with

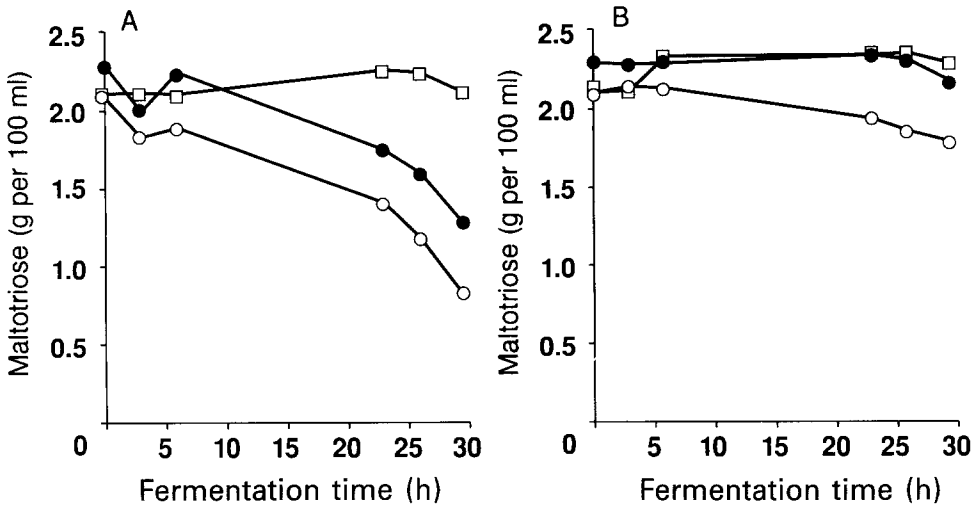

Fig. 3. Maltotriose utilization from wort containing added: 8% sorbitol (○), 8% glucose (□), or 8% maltose (●). (A) Lager strain 3021; (B) ale strain 3001.

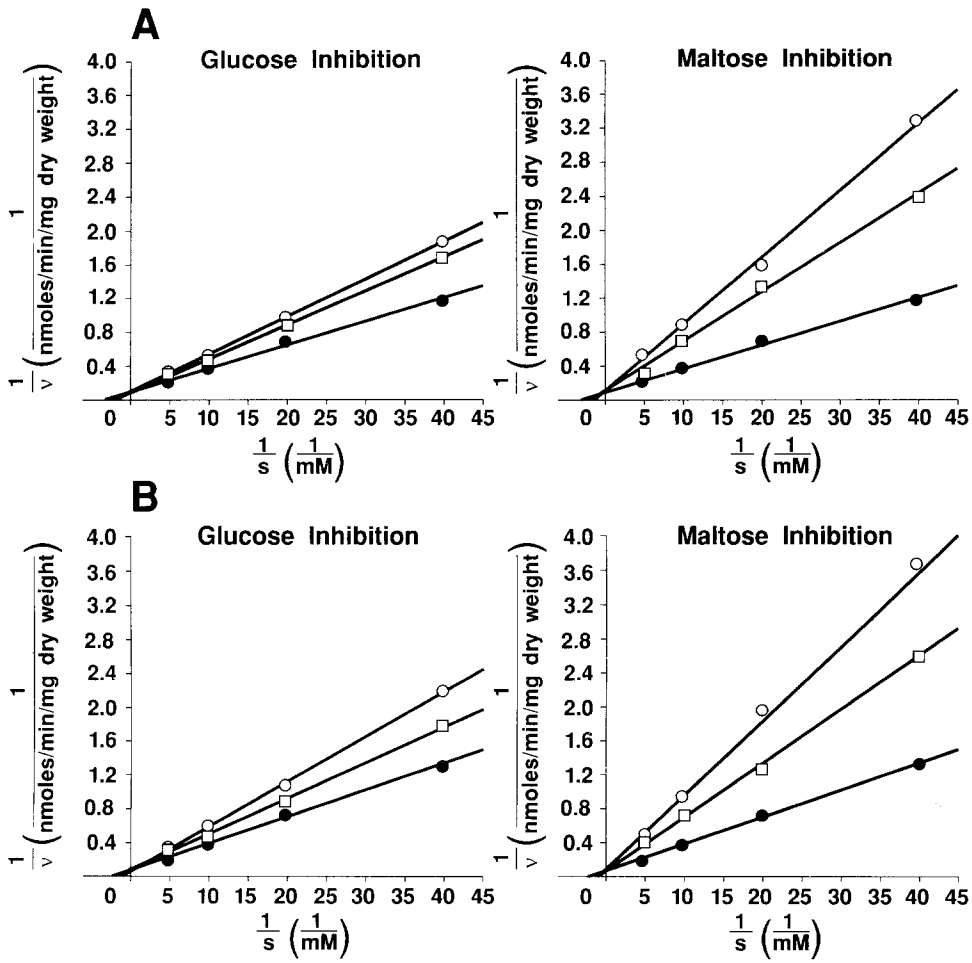

Fig. 4. Inhibition of maltotriose transport by glucose and maltose determined by Lineweaver–Burke double reciprocal plots. Maltotriose (0.025, 0.05, 0.1, 0.2 mM) transport was carried out in the presence of 50 mM (□) or 100 mM (○), maltose or glucose, or no sugar addition (●) by (A) lager strain 3021 and (B) ale strain 3001. The values in the plot (●) represent the average of the values for two plots in which 50 mM and 100 mM sorbitol were used as osmotic controls.

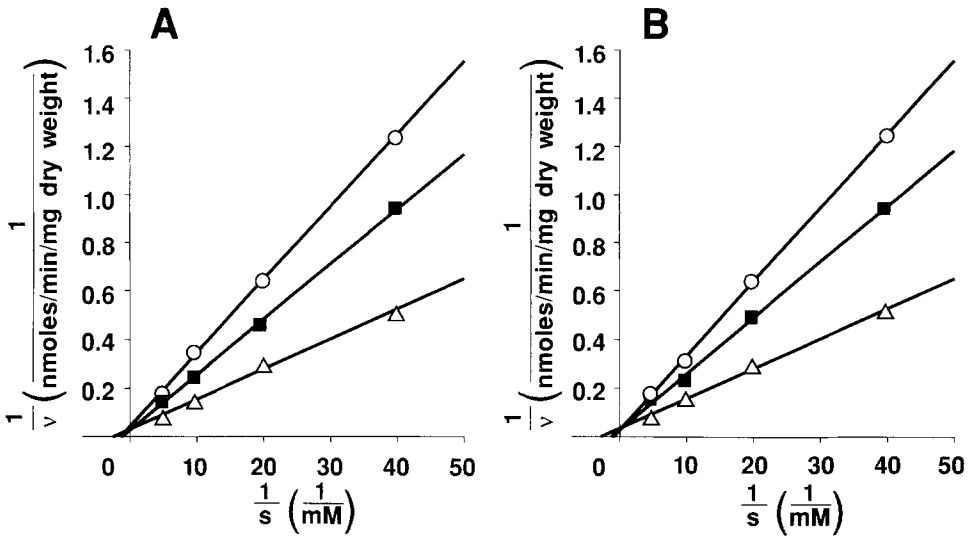

Fig. 5. Inhibition of maltose transport determined by Lineweaver–Burke double reciprocal plots. Maltose (0.025, 0.05, 0.1, 0.2 mM) transport was carried out in the presence of no added maltotriose (△), 50 mM maltotriose (■), and 100 mM maltotriose (○) by (A) lager strain 3021 and (B) ale strain 3001. The values in the plot (△) represent the average of the values for two plots in which 50 mM and 100 mM sorbitol were used as osmotic controls.

maltotriose for the permease. In order to investigate this, late-logarithmic phase cells grown in wort were examined for their maltotriose transport, with or without the presence of 50 or 100 mM glucose or maltose. As shown in Fig. 4, maltose and glucose inhibited the high affinity maltotriose transport system in an almost competitive manner although they are not typical competitive inhibitors of maltotriose transport. The K_i values of strain 3021 are 58.0 ± 3.2 mM for maltose and 167.2 ± 5.7 mM for glucose. The K_i values of strain 3001 are 55.0 ± 5.1 mM for maltose and 147 ± 8.1 mM for glucose. These values suggest a higher affinity of maltotriose permease for maltose than for glucose, i.e. higher competition of maltose than glucose for maltotriose. These results also explain the data discussed in the previous section, that is, cells fermenting a maltose-supplemented wort had lower maltotriose-consuming capacity. This is because of the competition of maltose with maltotriose for the permease and the greater maltose concentration in the wort (494.4 mM) than the K_i values of maltose. The result of approximately 40 times greater maltose K_i values than maltotriose K_m values may rule out the possibility that maltotriose and maltose share the same permease. The K_i values (70.4 ± 2.3 mM for strain 3021 and 71.5 ± 1.9 mM for strain 3001) of maltotriose for maltose transport (Fig. 5) and the K_m value (1.3 ± 0.1 mM for strain 3021 and 0.5 ± 0.1 mM for strain 3001) of maltose transport gives further evidence to this conclusion. In addition, it is also shown in Fig. 5 that maltotriose is a competitive inhibitor for maltose transport. Studies on a maltotriose-negative and maltose-positive yeast strain are currently ongoing in this laboratory. As a result, more information about the permease and α-glucosidase systems of maltotriose and maltose will be obtained.

Comparison of maltotriose transport with maltose and glucose transport

In order to compare the transport rates of maltotriose, maltose and glucose of cells grown for different time periods, both strains were grown in brewery wort and harvested in early-logarithmic, late-logarithmic and stationary growth phase. As shown in Fig. 6, glucose was transported faster than maltotriose and maltose in cells harvested in early-logarithmic phase. As the cells grew further to late-logarithmic phase, the maltotriose and maltose transport rates increased, with a higher transport rate of maltose than of maltotriose. The glucose transport rate remained similar to that in cells harvested in early-logarithmic phase. The cells harvested in stationary phase did not change their maltotriose transport velocities significantly, but had a lower maltose transport velocity compared to the cells harvested earlier. The glucose transport rate for the lager strain also decreased. These data agree with the sugar utilization profiles during a brewery wort fermentation where the glucose is completely utilized in early logarithmic phase, followed by maltose and then maltotriose [18,21]. There were no obvious differences between the strains with regard to their maltotriose and maltose transport. On the other hand, strain 3001 had higher glucose-transporting activity than strain 3021. We have found that strain 3021 utilized more maltotriose than strain 3001 from wort during fermentation. It was anticipated that strain 3021 might have a higher affinity and maximum velocity for maltotriose transport than strain 3001. However the data in Table 1 do not show any significant difference with respect to their K_m and V_{max} values. Furthermore, as discussed above, the cells of both strains from different growth phases displayed similar maltotriose transport rates (Table 2). In addition, the subsequent maltotriose hydrolysis

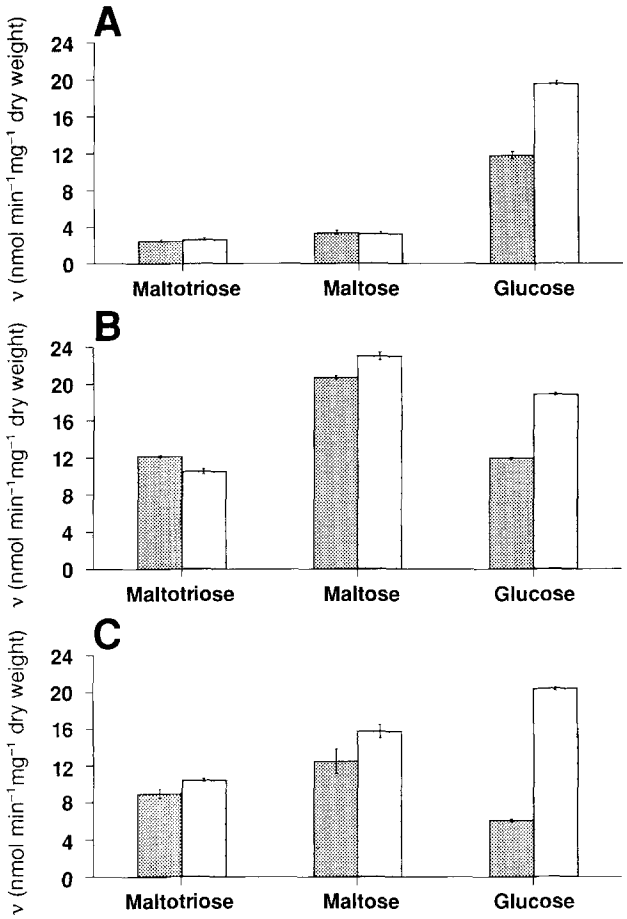

Fig. 6. Comparison of maltotriose, maltose and glucose (0.5 mM) transport by lager strain 3021 (■) and ale strain 3001 (□), grown in wort at 21 °C with shaking, and harvested in different growth phases. (A) Early-logarithmic phase – 7 h; (B) late-logarithmic phase – 24 h; (C) stationary phase – 48 h.

by α-glucosidase also plays a role in contributing to the cell's capacity to utilize maltotriose. Experiments on this are currently ongoing in this laboratory.

CONCLUSIONS

Similar to maltose and glucose, maltotriose has both low and high affinity transport systems. The K_m values of the two maltotriose transport systems are similar to those of the two maltose transport systems. Maltose competitively inhibits the high affinity maltotriose transport system. The higher K_i value of maltose over the K_m value of maltotriose suggests that maltotriose and maltose probably do not share the same permease system. On the other hand, the results indicate that cells grown in maltotriose and maltose have similar maltotriose- and maltose-transporting activity and that the two sugars are the inducers of each other's permease and/or α-glucosidase. The two strains do not have significant differences in maltotriose transport rate except that the ale strain (3001) could maintain the high maltotriose transport activity longer than the lager strain (3021) when they were pre-grown in maltotriose or maltose. The kinetic constants for maltotriose transport have not given the answer to the question: 'Why does the lager strain utilize more maltotriose than the ale strain during a brewery wort fermentation?'.

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