Reactions of Oligosaccharides. IV. Fermentability by Yeasts[1]

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ABSTRACT

The anaerobic fermentation of a series of D-glucose polymers, namely G_2, G_3, G_4, G_5, G_6, G_7, and G_8 by brewers' yeast and bakers' yeasts, was examined. The oligosaccharides were isolated from corn (maize) starch hydrolysates by macro paper chromatography. Neither the bakers' nor brewers' yeast tested was able to ferment oligosaccharides higher than G_3, nor did the presence of glucose stimulate their fermentation. The presence of glucose did, however, stimulate all yeast samples to ferment G_2 (maltose) and G_3 (maltotriose). G_2 also promoted the fermentation of G_3. Fermentation of the glucose-maltose, glucose-maltotriose, and maltose-maltotriose sugar mixtures was incomplete. Reasons for this and for the inability of the yeasts to ferment oligosaccharides higher than maltotriose are discussed.

The fermentability of oligosaccharides by both bakers' and brewers' yeasts has been the subject of considerable study for many years, particularly in the brewing industry. Blish and Sandstedt (1) found that fresh bakers' compressed yeast was frequently deficient in its ability to ferment pure maltose alone. However, the addition of flour (especially from malted wheat) and dried yeast preparations greatly shortened the induction period and increased the fermentation rate. They attributed this increased rate to "accelerators" present in the flour and yeast additives. Subsequently Schultz and Atkin (2) substantiated the work of Blish and Sandstedt and also showed that the presence of glucose hastened the fermentation of maltose. They suggested that changes in cell wall permeability might account for this rate increase.

In 1957, Cook and Phillips (3), investigating the hydrolytic action of cell-free extracts of *Saccharomyces cerevisiae* (N.C.Y.C. 234) and *S. uvarum* (N.C.Y.C. 115), found that both maltose and maltotriose were rapidly attacked by enzyme preparations from *S. cerevisiae* but that only maltose was hydrolyzed by similar extracts from *S. uvarum*. Analysis indicated that a single enzyme from *S. cerevisiae* was responsible for the hydrolysis of both maltose and maltotriose, thus demonstrating that the two yeasts differed in their enzyme complements.

Blom and Schwarz (4) have shown that during the primary fermentation by *S. cerevisiae* of glucose, maltose, and trisaccharides in wort, the trisaccharides were fermented more slowly than the other two sugars. This was confirmed by Phillips (5), who showed that in fermentations with brewing yeasts carbohydrates were found to disappear from the wort in this order: Sucrose, monosaccharides, maltose, and maltotriose. It was suggested (6,7) that these variations in the relative fermentation rates of maltose and maltotriose were due to changes in the synthesis of permeases responsible for the transport of maltose and maltotriose.

Table I lists the yeasts of the genus *Saccharomyces* known to be maltotriose nonfermenting and maltotriose fermenting.

[1]Presented at the 55th AACC Annual Meeting, Minneapolis, October 1970.

The availability of a series of oligosaccharides with Degree of Polymerization greater than 3 prompted us to initiate a study on the fermentability of these sugars. The oligosaccharides, designated as G_2, G_3, G_4, G_5, G_6, G_7, and G_8, were previously isolated (12) in this laboratory from corn (maize) starch hydrolysates by using macro paper chromatography. Enzymatic and chromatographic evidence (13) showed these oligomers to be linear malto-oligosaccharides. This paper presents the results of fermentation studies with these oligosaccharides by brewers' and bakers' yeasts, both in the presence and absence of other sugars.

MATERIALS AND METHODS

Oligosaccharides

The oligosaccharides G_2, G_3, G_4, G_5, G_6, G_7, and G_8 were amorphous, chromatographically pure, and lyophilized to constant weight before use. Since G_2 was shown to be maltose, commercially available pure samples of maltose were substituted for G_2 in some cases. Thin-layer chromatography and "Glucostat" (Worthington Biochem. Co.) analysis of the reagent-grade maltose indicated a glucose content of less than 0.8%.

Yeast Samples

Fresh brewers' yeast (*S. carlsbergensis*), grown in wort for 48 hr., was obtained from the brewing division of Anheuser-Busch, Inc., St. Louis. Fresh bakers' yeast cake (*S. cerevisiae*) was obtained from the yeast plant, Anheuser-Busch, Inc., St. Louis. Another commercial sample of bakers' yeast was purchased in the supermarket.

Manometric measurement of fermentation was by the direct Warburg method at 30°C. Each Warburg flask contained 33.5 mM potassium dihydrogen phosphate (pH 4.5), 5 mg. of yeast extract (Difco), and various amounts of sugars in the main compartment. The side arm contained 0.5 ml. yeast suspension (0.9 to 1.6 mg. dry weight). Carbohydrate was omitted in the control experiments. The total solution volume in each flask was 3.0 ml. and anaerobiosis was obtained by flushing for 10 min. with pure nitrogen. Yeast extract was added to each flask to increase the fermentation rate of maltose (Fig. 1). No significant fermentation of yeast extract by bakers' yeast was observed (Fig. 1).

TABLE I. MALTOTRIOSE FERMENTING AND NON-FERMENTING SACCHAROMYCES

Reference	Maltotriose Non-Fermenting	Reference	Maltotriose Fermenting
3, 4, 5, 8	S. uvarum	4, 5, 6, 8, and 9	S. crevisiae
8	S. pastorianus NCTC	5, 8	S. carlsbergensis[a]
8	S. cerevisiae var.	5	S. diastaticus (also ferments malto-tetraose)
8	S. cerevisiae strain ilicis	8	S. pastorianus C.B.S.
8	S. cerevisiae strain Sake	11	Distillers yeast NRRL Y-132
8	S. carlsbergensis var. polymorphus	11	Distillers yeast SC-1Y
5	S. chevalieri		

[a] S. carlsbergensis is now regarded as a synonym of S. uvarum (10).

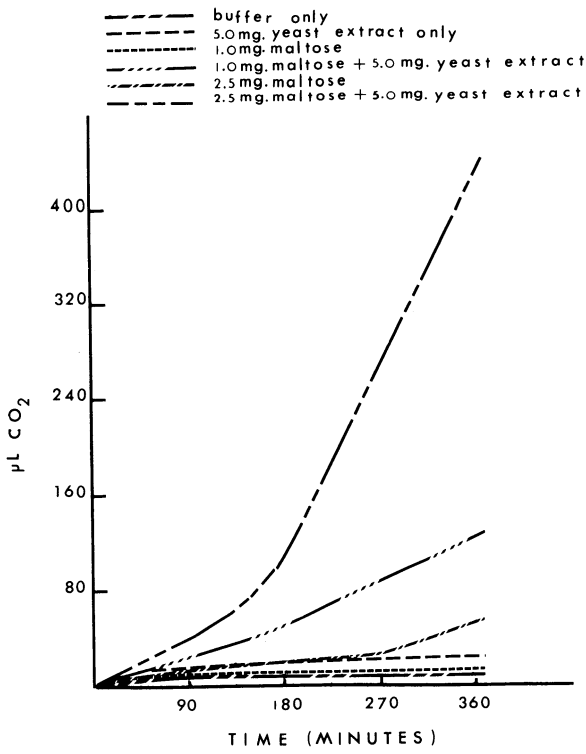

Fig. 1. Effect of yeast extract on the fermentation of maltose.

At the end of each experiment, the samples were checked microscopically to ensure the absence of bacteria.

A pure culture of *S. cerevisiae* was grown in 100 ml. of medium containing 0.1 g. of magnesium sulfate heptahydrate, 0.2 g. of potassium dihydrogen phosphate, 0.45 g. of yeast extract, 0.6 g. of peptone, and 4 g. (D.S.B.) of 75 D.E. corn syrup (containing approximately 20% maltose and 2% maltotriose) for 48 hr. at 30°C. The corn syrup was sterilized by passing through a sterile millipore filter (type HA, 0.45 μ; Millipore Corporation, Bedford, Mass.).

RESULTS AND DISCUSSION

The results of the fermentation of the oligosaccharides G_2, G_3, G_4, G_5, G_6, G_7, and G_8 by bakers' and brewers' yeasts are shown in Table II. Neither the bakers' nor brewers' yeast was able to ferment the oligosaccharides G_4 through G_8, nor did the presence of glucose stimulate their fermentation. The bakers' yeast (*S. cerevisiae*) was unable to ferment G_3 over a 5-hr. period but the same strain of yeast, after growing in 4% corn syrup used as a carbon source, did ferment G_3. It is apparent that the fermentability of G_3 by this yeast was induced during growth in the corn syrup. As expected, the brewers' yeast (*S. carlsbergensis*) was also able to ferment G_3. The presence of glucose stimulated both bakers' and brewers' yeasts to

TABLE II. FERMENTATION OF OLIGOSACCHARIDES BY BAKERS' AND BREWERS' YEAST

Sugars[a] (Glucose)	Bakers' Yeast μl. CO_2/mg. cells[b]		Bakers' Yeast[c] μl. CO_2/mg. cells		Commercial Bakers' Yeast μl. CO_2/mg. cells		Brewers' Yeast μl. CO_2/mg. cells	
	4 hr.	5 hr.	4 hr.	5 hr.	4 hr.	5 hr.	4 hr.	5 hr.
G_1	199	...	206	207	174	174	173	200
G_2	374	...	836	905	43	93	557	728
$G_1 + G_2$	940	...	985	1,040	578	704	1,025	1,210
G_3	0	...	184	264	4	5	36	43
$G_1 + G_3$	500	...	625	860	282	379	248	276
G_4	0	...	7	7	1	2	13	13
$G_1 + G_4$	192	...	218	222	175	175	185	204
G_5	0	...	3	2	0	2	3	3
$G_1 + G_5$	213	...	222	222	180	178	164	186
G_6	0	...	0	0	15	14	0	0
$G_1 + G_6$	188	...	224	224	123	121	183	199
G_7	0	...	16	17	0	0	0	0
$G_1 + G_7$	191	...	224	225	167	165	194	211
G_8	0	...	0	0	15	15	8	6
$G_1 + G_8$	203	...	225	225	165	165	194	211

[a]The amount of each sugar is 5 mg. except G_1 (1 mg.), G_2 and G_3 (4 mg. each).

[b]Dry-weight basis.

[c]Bakers' yeast grown in 4% corn syrup as a carbon source.

ferment maltose and maltotriose. Table III shows that maltose was also able to stimulate fermentation of maltotriose. In the absence of maltose, maltotriose was not fermented over a 7-hr. period by the bakers' yeast. However, in the presence of maltose, a total of 83% of the mixed sugars was fermented.

There was about a 90-min. lag before the bakers' yeast started to ferment maltose (Fig. 2) and this, coupled with the fact that glucose stimulated the fermentation of maltose and maltotriose, suggested that the synthesis of some proteins is necessary before the yeast is able to ferment these two kinds of sugars. To confirm this, cycloheximide (1 mM), an inhibitor of protein synthesis (14), was added to the fermentation media. The fermentation of maltose and maltotriose in the presence and absence of glucose was observed. The results (Figs. 2 and 3) revealed that the presence of the antibiotic inhibited the fermentation of maltose by 82% and the stimulation by glucose of the fermentation of maltose and

TABLE III. FERMENTATION OF G_3 IN THE PRESENCE OF G_2

Sugars	Amount of Sugars mg.	μl. CO_2 Released by 1.1 mg. Dry-Weight Cells[a] hr.					Sugar Fermented after 7 hr. %
		1	2	4	6	7	
G_2	4.0	15	64	412	868	882	89
G_3	3.0	0	0	0	1.0	1	0
$G_2 + G_3$	4 + 3	7	55	503	1,377	1,489	83

[a]Fresh bakers' yeast. Data corrected for endogenous fermentation.

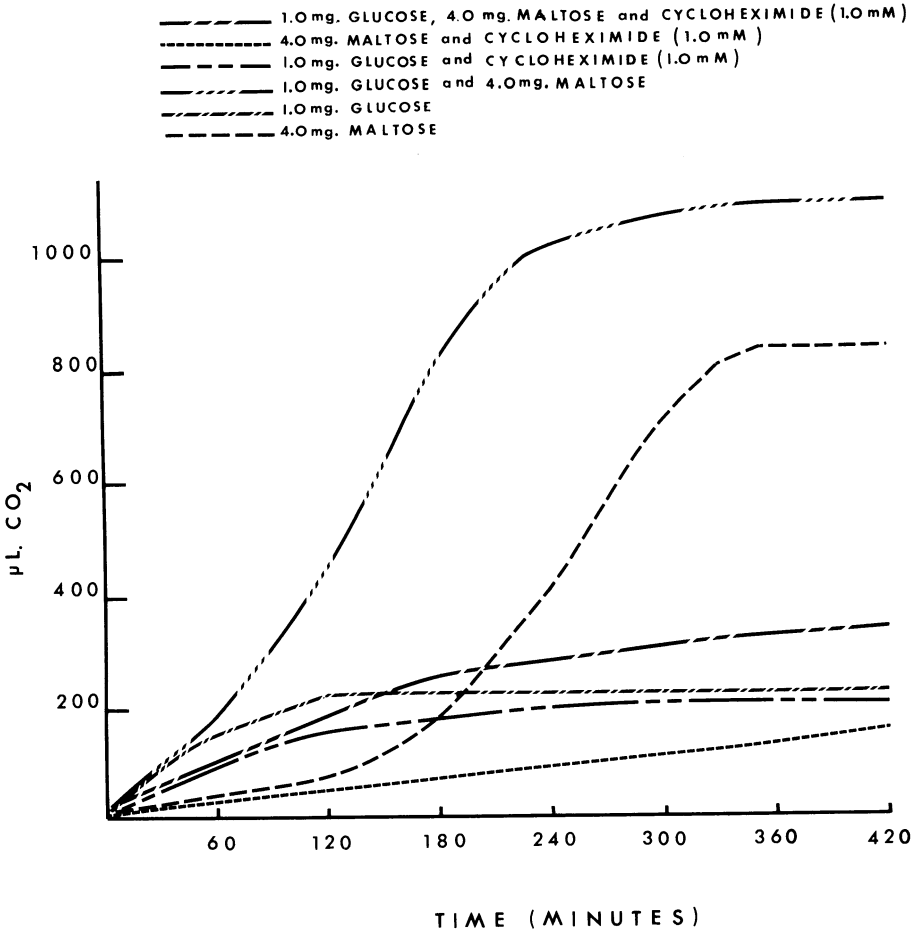

Fig. 2. Fermentation of maltose in the presence of glucose. Data corrected for endogenous fermentation.

maltotriose by 83 and 97%, respectively. The fermentation of glucose was decreased only by 7%. Some proteins involved in the fermentation of these sugars are clearly inducible. Because protein synthesis is an energy-consuming process, the major function of glucose in the fermentation of maltose and maltotriose may be to supply energy for the protein synthesis. It is reasonable to assume that these inducible proteins must be maltase, maltose, and maltotriose permeases (7). That the cycloheximide did not completely inhibit the fermentation of maltose and maltose-glucose mixed sugars may derive from the fact that the antibiotic is unable to inhibit protein synthesis completely at this level of concentration.

The fermentation of maltose and maltotriose in the presence of glucose reached a maximum after an incubation of 7 hr. Only 88% of glucose-maltose mixed sugars and 85% of glucose-maltotriose mixed sugars were fermented (Figs. 2, 3). The

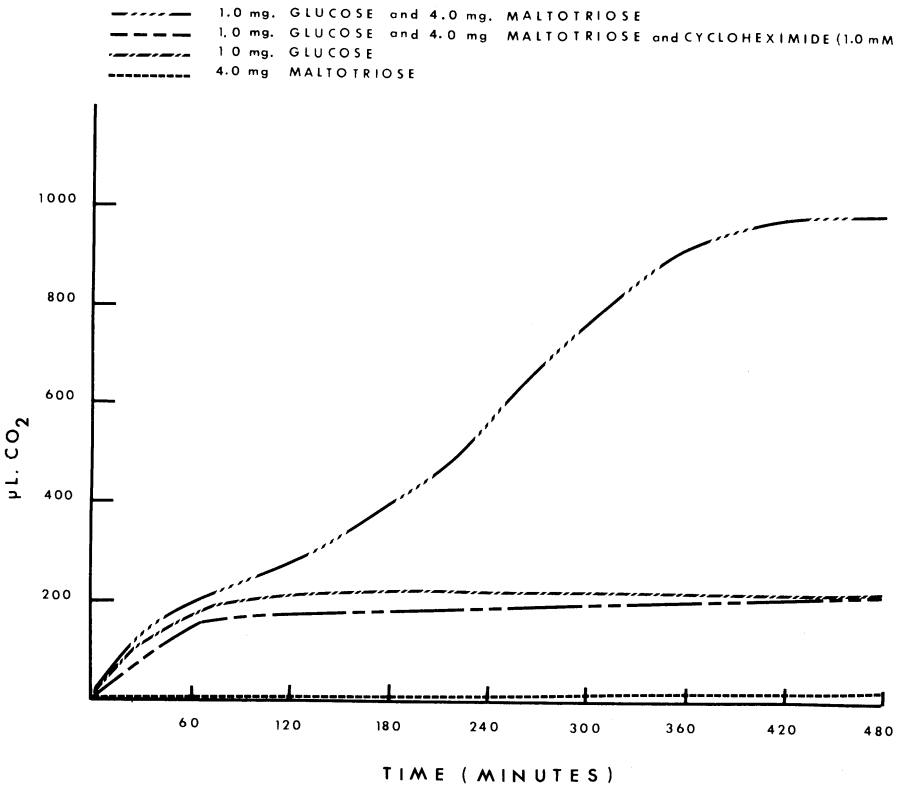

Fig. 3. Fermentation of maltotriose in the presence of glucose by bakers' yeast. Data corrected for endogenous fermentation.

incomplete fermentation of these sugars may be attributed to the following:

a) Some parts of the sugars are converted to other sugars or carbohydrates such as trehalose or glycogen (15), and stored in the cells.

b) Some glycolytic intermediates are converted to other cellular compounds and incorporated into the cells before the intermediates reach the final step to produce CO_2 and ethanol.

The fact that yeasts are unable to ferment oligosaccharides other than maltose and maltotriose may be genetically tied to defects in the transport system of these oligosaccharides.

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