Adaptability of *Streptococcus thermophilus* to Lactose, Glucose and Galactose

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ABSTRACT

Significant differences in growth response to lactose, glucose and galactose were found among different strains of *Streptococcus thermophilus.* Some strains fermented all three carbohydrates (group A), whereas other strains utilized lactose and glucose only (group B) and one strain grew on lactose alone (group C). Characteristically, most lactose-grown strains in either group A or group B showed slow adaptation to either glucose or galactose after transfer to a medium containing either of these carbohydrates. Growth on glucose did not influence growth patterns following the transfer of either group A or group B strains to lactose broth. Lactose-grown group A and group B strains were restricted in growth following addition of galactose to the medium, whereas glucose-grown strains were not. The results suggested differences in carbohydrate transport mechanisms and utilization.

Lactic streptococci play a major role in the preparation of many dairy foods. Much information has accumulated on the metabolism of carbohydrates by this group of microorganisms (*5,7,13*). The evidence suggests that metabolism of lactose in group N streptococci (*Streptococcus cremoris, Streptococcus lactis,* and *S. lactis* subsp. *diacetylactis*) involves an energy-dependent group translocation mechanism catalyzed by a phosphoenolpyruvate (PEP)-mediated phosphotransferase system (PTS), and several membrane-bound, stereospecific catalytic proteins (*6*). Similar transport mechanisms had been described in several other genera of microorganisms (*11*). Group translocation involves the chemical modification of a carbohydrate during transmembrane transport, resulting in the conversion of lactose to glycosyl-β(1,4)-galactoside-6-phosphate (lactose-P), and the subsequent cleavage of the latter by β-D-phosphogalactoside galactohydrolase (β-Pgal) to glucose and galactose-6-phosphate (*9*).

Information is limited on carbohydrate transport systems and metabolism in *Streptococcus thermophilus*, a closely related thermotolerant microorganism that plays an essential role in the preparation of yogurt and certain cheese varieties. In a preliminary report, Reddy et al. (*10*) presented data on lactose transport in galactose- and glucose-grown cells of *S. thermophilus* and concluded that only galactose-adapted cells were induced to transport lactose, the transport of which was apparently dependent on a PEP-PTS system.

In our laboratory, adaptation tests with different strains of *S. thermophilus* showed that free galactose was not utilized by all strains, indicating possible differences in carbohydrate transport systems and(or) sugar metabolism. This paper reports on the growth characteristics of different strains of *S. thermophilus* in broth media, as influenced by preadaptation to lactose, glucose and galactose.

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MATERIALS AND METHODS

Cultures

Thirty one strains of *S. thermophilus* were obtained from colleagues and commercial sources (Table 1). The cultures were maintained in sterile 10% reconstituted nonfat dry milk and Hogg and Jago basal medium (*4*), containing 3% tryptone (Difco), 1% yeast extract, 0.2% beef extract and 0.5% KH_2PO_4, and having a final pH of 6.5 before sterilization. The basal medium was supplemented with lactose, glucose or galactose, as needed, at 0.5% concentration. The cultures were incubated at 37 C for 17-24 h, and stored at 4 C. Weekly transfers of all cultures were made.

The identity of the cultures was tested by several techniques. All strains grew at 45 C and yielded a negative test for arginine decarboxylase activity, according to the method of Mikolajcik (*8*). All strains gave negative results when tested for the presence of Lancefield's group N streptococcus antigen against Bacto Streptococcus Antiserum (Difco), using the capillary tube assay of Fung and Wagner (*3*). Antigen was prepared by the autoclave method (*2*). Maltose and mannitol fermentation tests, and the test for the ability to grow in the presence of 2% NaCl, were all negative, except for strains ST/AH and Is. These two strains were considered atypical.

Adaptation to carbohydrates and growth measurements

Carbohydrate adaptation experiments were carried out in lactose, glucose and galactose media, in which final carbohydrate concentration was 50 mM. Cultures initially grown in lactose medium were transferred (1%, v/v) to glucose and galactose media and incubated at 37 C for 24 h. Cultures showing growth after 24 h were subcultured daily for 5 days.

The growth characteristics of cultures were studied turbidometrically at 660 nm with Zeiss PMQ II spectrophotometer. The inoculum was prepared from a 14-h-old culture by centrifuging at 10,000 × *g* for 10 min and resuspending the pellet in 50 mM of K_2HPO_4-KH_2PO_4 buffer, pH 7.0. Appropriate dilutions of the cell suspensions were made so that a 0.1-0.2 ml aliquot added to 50 ml of fresh medium gave an initial absorbance of 0.01 at 660 nm. The carbohydrate concentration in these experiments was 10 mM, unless otherwise indicated. During incubation at 37 C, samples were withdrawn at convenient intervals for absorbance readings. Growth experiments were repeated three times.

RESULTS AND DISCUSSION

The results of a survey of different strains of *S. thermophilus* showed that growth responses to lactose, glucose and galactose were variable. Twelve strains fermented all three carbohydrates (group A), 18 strains fermented lactose and glucose but not galactose (group B) and one strain fermented lactose only (group C) under the experimental conditions employed. These observations suggested that not all strains of *S. thermophilus* had the potential to transport galactose or glucose to the interior of the cell. Failure to grow on galactose also may have resulted in some strains (group B) from lack of the D-tagatose-6-phosphate or the Leloir pathway, which are present in lactic streptococci (*1*).

The cultures were studied in detail to evaluate the effect of preadaptation on the initial growth response to fermentable carbohydrates. Group A lactose-grown strains appeared to be transiently cryptic with respect to glucose and/or galactose utilization and showed a prolonged adaptation phase, with the exception of strains Is, P-137 and 19258, following transfer to either glucose or galactose broth (Fig. 1). The adaptation phase, which was characterized by very slow growth, lasted up to 7 h or, in some instances longer, depending on the strain. However, following prolonged incubation (24-48 h) and repeated transfers in glucose and galactose media, the growth response to the hexoses became more rapid and, with glucose, became as immediate as that observed in the lactose medium. A similar phenomenon in *Lactobacillus bulgaricus* was observed by Snell et al. (*12*) who studied carbohydrate utilization in this microorganism.

In several cultures (strains 4, 7, L-225, 4074, 7132, and 9353), the effect of increased monosaccharide concentration on growth was also checked. Supplementation of the glucose with galactose (10 mM), or increasing the glucose concentration to 20 mM and 100 mM had only a minimal effect on the initial growth response of five strains. The exception was strain 7, which grew nearly as well in 20 mM glucose as in 10 mM lactose medium.

The initial growth response of group A strains to galactose, with the exception of the atypical strain Is, was also slow when glucose grown cells were transferred to galactose broth. Several galactose-grown group A strains (4, L-225, P-371, 4074, and 9353) showed slow initial growth when transferred to 10 mM glucose broth, while others remained unaffected or grew even more rapidly (strains Ds, Is, 7, 404, 7132, and 19258). On the other hand, following transfer to lactose broth, all glucose-grown group A strains of *S. thermophilus* showed growth patterns that were indistinguishable from those observed in cases of lactose-to-lactose transfer.

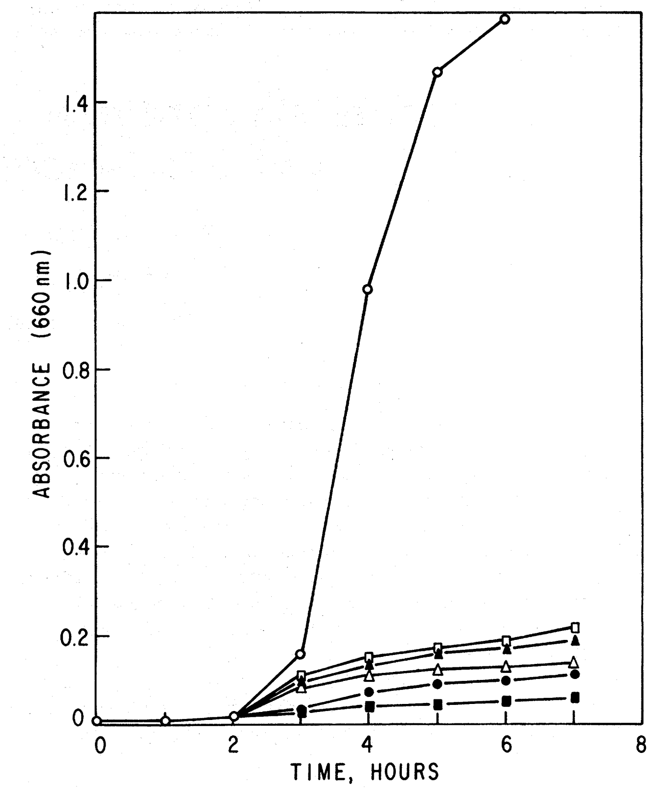

Figure 1. *Effect of preadaptation on the growth of* Streptococcus thermophilus *strain 9353 in lactose (L), glucose (G), and galactose (Ga) media.* ○, *L to L (10mM)*; ●, *L to G (10mM)*; △, *L to G (20mM)*; ▲, *L to G (100mM)*; □, *L to G (10mM) + Ga (10mM)*; ■, *L to Ga (10mM)*.

TABLE 1. *Adaptability of* Streptococcus thermophilus *to carbohydrates.*

Group A (L, G, GA)[a]		Group B (L, G)		Group C (L)	
Strain	Origin[b]	Strain	Origin	Strain	Origin
		4109	Hansen	406	INRA
4074	Hansen	6069	"		
7132	"	7024	"		
9353	"	6097	"		
7	Microlife	9	Microlife		
4	Miles	8	"		
19258	ATCC	YB/ST	"		
371	NIZO	3	"		
L225	"	33	"		
Is	"	ST/AH	"		
Ds	"	19987	ATCC		
MC	UN	14485	"		
404	INRA	3641	NRRL		
		ST_s	NIZO		
		EB-8	UN		
		SFi-1	Nestle		
		SFi-3	"		
		391	INRA		

[a]L: lactose, G: glucose, GA: galactose.

[b]Hansen, Chr. Hansen's Laboratory, Inc., courtesy of R. L. Sellars; Microlife, Microlife Technics, courtesy of E. R. Vedamuthu; Miles, Miles Laboratories, Inc.; ATCC, American Type Culture Collection; NIZO, Netherlands Institute for Dairy Research, Ede, The Netherlands; UN, University of Nebraska, courtesy of K. M. Shahani; INRA, National Agricultural Research Institute, Jouy-Eu-Josas, France, courtesy of J. Auclair; NRRL, Northern Regional Research Center, U.S. Department of Agriculture; Nestle, Nestle Products Ltd., Lausanne, Switzerland, courtesy of T. Sozzi.

Strains of *S. thermophilus* that could not ferment free galactose (group B) also appeared to be cryptic with respect to glucose metabolism, and showed slow initial growth, with the exception of strains 14485 and 19987, when lactose-grown cells were transferred to glucose broth. Strains in this group showed normal growth patterns when glucose-grown cells were transferred to lactose broth, similar to the phenomenon observed with group A strains.

The results showed that most group A strains had difficulty metabolizing glucose and galactose, and most group B strains were slow to initiate growth on glucose, when a lactose-grown culture was inoculated into a medium with a monosaccharide as the carbon source. Since streptococci generally rely on glycolysis to ferment sugars (5), it was unlikely that group A and group B lactose-grown strains of *S. thermophilus* lacked the enzymic potential to metabolize glucose and/or galactose. It was more likely that in the transiently cryptic cells, one or more stereospecific components of monosaccharide transport systems were not induced when cells were growing in a lactose medium. On the other hand, glucose- and most galactose-grown group A strains, as well as all glucose-grown group B strains, initiated growth in lactose broth without showing a long adaptation phase. This suggested that transmembrane transport of lactose was, at least in part, constitutively expressed in most group A and group B strains of *S. thermophilus*, regardless of previous growth on glucose or galactose.

Lactose-grown strains of all three groups (A, B and C), except the atypical strains Is in group A and ST/AH in group B, grew more slowly than the control, when galactose (25 mM or 50 mM) was added to lactose broth at the start of incubation (Fig. 2). Glucose and the nonmetabolizable sugar alcohol mannitol failed to induce a similar change in growth pattern. The growth patterns of glucose-grown strains of either group A or group B, however, were not influenced when glucose medium was supplemented with galactose.

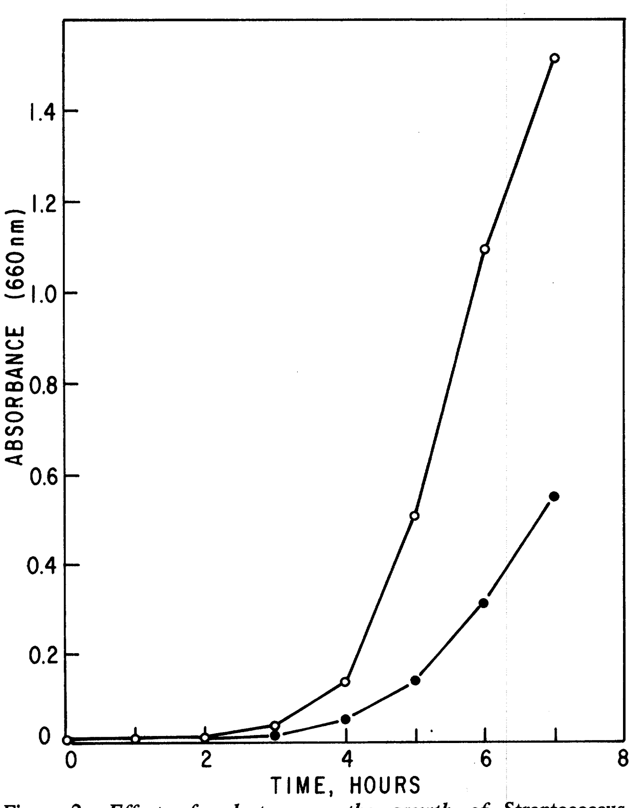

Figure 2. *Effect of galactose on the growth of* Streptococcus thermophilus *strain Ds in lactose broth.* ○, *L (10mM);* ●, *L (10mM) + Ga (25mM).*

The data suggested that in broth culture galactose interfered with lactose but not glucose metabolism in *S. thermophilus*. Group A strains, which fermented galactose, were not as susceptible as group B strains. The growth-suppressive effect of galactose is possibly the result of the inihibition of one or more catalytic proteins involved in the transmembrane transport of lactose.

Most strains in both groups A and B apparently have inducible transport systems specific for glucose or galactose, or both, whereas the transport of lactose is constitutively expressed. The inducible nature of hexose transport systems was supported by the finding that most lactose-grown strains required 6-7 h before initiation of rapid growth following transfer to media containing glucose or galactose.

Carbohydrate uptake studies with strains adapted to lactose, glucose and galactose are in progress.

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