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Galactose Transport in *Streptococcus thermophilus*†

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Although *Streptococcus thermophilus* accumulated [^{14}C]lactose in the absence of an endogenous energy source, galactose-fermenting (Gal$^+$) cells were unable to accumulate [^{14}C]galactose unless an additional energy source was added to the test system. Both Gal$^+$ and galactose-nonfermenting (Gal$^-$) strains transported galactose when preincubated with sucrose. Accumulation was inhibited 50 or 95% when 10 mM sodium fluoride or 1.0 mM iodoacetic acid, respectively, was added to sucrose-treated cells, indicating that ATP was required for galactose transport activity. Proton-conducting ionophores also inhibited galactose uptake, although *N,N'*-dicyclohexyl carbodiimide had no effect. The results suggest that galactose transport in *S. thermophilus* occurs via an ATP-dependent galactose permease and that a proton motive force is involved. The galactose permease in *S. thermophilus* TS2b (Gal$^+$) had a K_m for galactose of 0.25 mM and a V_{max} of 195 μmol of galactose accumulated per min per g (dry weight) of cells. Several structurally similar sugars inhibited galactose uptake, indicating that the galactose permease had high affinities for these sugars.

In contrast to *Streptococcus thermophilus*, carbohydrate metabolism in the group N lactic streptococci has been well studied in recent years, and many of the basic processes relating to sugar transport, utilization, and regulation are known (8, 11, 12, 20). These microorganisms metabolize lactose and galactose via specific phosphoenolpyruvate (PEP)-dependent phosphotransferase systems (PTS) (2, 13, 14, 16, 24). In addition, galactose can also be transported via a galactose permease (galP) and further metabolized by enzymes of the Leloir pathway (2, 9).

Thompson and Thomas (26) and Thompson (23) have shown that the glycolytic reactions in *Streptococcus lactis* ML3 are regulated such that high internal concentrations of PEP and the PEP precursors, 3-phosphoglycerate and 2-phosphoglycerate, are maintained during starvation conditions. Starved cells are thus primed for sugar transport when PTS substrates do become available. In contrast to the PTS, the galP system in *S. lactis* ML3 and 133 (25) and the β-galactoside permease system in the atypical *S. lactis* 7962 (6) are ATP dependent, and active transport of β-galactosides by these systems occurs only when cells are energized with ATP. Furthermore, *S. lactis* 7962 lacks an endogenous energy reserve and requires ATP-generating substrates (glucose or arginine) for concentrative accumulation of β-galactosides (6).

Sugar metabolism in *S. thermophilus* differs from that observed for the group N lactic streptococci. Although these microorganisms ferment lactose, they lack PTS and phospho-β-galactosidase activity and instead appear to transport lactose via a lactose permease (17, 27). In addition, most strains of *S. thermophilus* are unable to ferment galactose, either as the free sugar or that generated intracellularly by lactose hydrolysis (15, 27). The recent isolation (21) of galactose-fermenting (Gal$^+$) variants derived from wild-type strains unable to ferment galactose (Gal$^-$) has provided the opportunity to examine and compare metabolic activities between Gal$^+$ and Gal$^-$ strains. In this communication we describe the properties of the galactose transport system in *S. thermophilus* and the role of an exogenous energy source in providing energy, as ATP, necessary for galactose transport activity.

MATERIALS AND METHODS

Microorganisms and growth conditions. Lactose-fermenting (Lac$^+$), Gal$^-$ parental strains of *S. thermophilus* TS2 and 821 and their Gal$^+$ derivatives, designated TS2b and 821b, respectively, were obtained from T. D. Thomas, New Zealand Dairy Research Institute. The procedures used for the selection of these derivatives and a description of their sugar-fermenting characteristics have been reported previously (21). All other strains were from the culture collection maintained in our laboratory.

Lac$^+$ Gal$^-$ strains were maintained in M17 medium (19) containing 14 mM lactose. Lac$^+$ Gal$^+$ strains were maintained in Elliker medium (3) containing 27 mM galactose. All cultures were transferred biweekly and grown at 42°C.

For use in transport experiments, cells were grown in Elliker broth containing the appropriate carbohydrate for 4 to 6 h at 42°C and were harvested by centrifugation at 10,000 × *g* for 10 min at 4°C. Cells were washed twice with 20 mM potassium phosphate buffer (pH 7.0) containing 10 mM $MgSO_4$ and 0.5 mM dithiothreitol. Cells were suspended in buffer to give an optical density corresponding to 50 to 100 μg (dry weight) of cells per ml.

Determination of intracellular volume. The intracellular volume of *S. thermophilus* TS2 was determined by the method of Winkler and Wilson (29). Two grams (wet weight) of a thick cell suspension was suspended in a 5.0 mM [^{14}C]inulin solution (10 mCi mmol^{-1}). The cells were centrifuged in a tared centrifuge tube for 15 min at 12,000 × *g*. The pellet was dried at 80°C until a constant weight was reached. The dried pellet was suspended in twice-distilled water, and the amount of retained [^{14}C]inulin was determined. Cell water volume was calculated from the difference between the wet and dry cell weight less the interstitial water volume (based on the inulin space). Periplasmic water was not considered significant (10) and was not determined. Duplicate determinations gave an average intracellular water volume of 1.70 ml per g (dry weight) of cells. This compares

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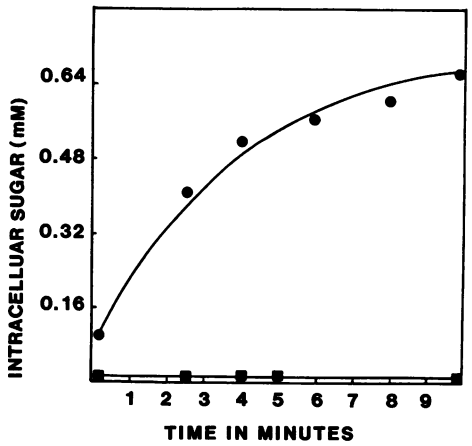

FIG. 1. Lactose (●) and galactose (■) uptake by resting cells of *S. thermophilus* TS2. Cells were suspended in phosphate buffer and incubated at 37°C. ^{14}C-sugars were added at a concentration of 5 μM.

to values of 1.45 and 1.67 obtained for *S. lactis* 7962 (6) and *S. lactis* ML3 (22), respectively.

Transport assays. Sugar uptake in resting cells was measured by adding ^{14}C-sugars to buffered cell suspensions at 42°C, followed by rapid filtration of 0.5-ml samples through 0.45-μm cellulose filters (Millipore Corp., Bedford, Mass.). Filters were washed with 5.0 ml of ice-cold phosphate buffer and dried in a 60°C oven. The filters were placed in vials with 10.0 ml of Aquasol-2 scintillation cocktail (New England Nuclear Corp., Boston, Mass.) and counted by liquid scintillation spectroscopy with a Beckman model LS 250 counter. All counts were corrected for quenching.

For kinetic determinations of sugar uptake, samples were taken after 10 s of incubation and used to calculate initial velocity values.

Other assays. Lactose and galactose were assayed enzymatically with kits (catalog no. 176303) purchased from Boehringer Mannheim Biochemicals, Indianapolis, Ind; sucrose and glucose were also assayed using enzyme kits (catalog no. 139041).

Chemicals. [1-^{14}C]galactose, thio-[*methyl*-^{14}C]galactose ([^{14}C]TMG), and carboxyl[^{14}C]inulin (for intracellular volume determinations) were obtained from the New England Nuclear Corp. D-Glucose-[1-^{14}C]lactose was obtained from Amersham Corp. (Arlington Heights, Ill.).

Purity of the [^{14}C]galactose was confirmed by thin-layer chromatography (18); all radioactivity migrated as a single spot corresponding to a galactose standard.

Carbonyl cyanide *m*-chlorophenylhydrazone, *N,N'*-dicyclohexylcarbodiimide (DCCD), and valinomycin were purchased from the Sigma Chemical Co., St. Louis, Mo. Pentachlorophenol was purchased from Aldrich Chemical Co., Milwaukee, Wis.

RESULTS

Effect of energizers on uptake. Cells of *S. thermophilus* TS2 (Lac$^+$ Gal$^-$) used in transport experiments were harvested at the midlog phase and washed twice in phosphate buffer. These cells were metabolically active (as indicated by rapid growth after transfer into fresh growth medium) and could accumulate [^{14}C]lactose, but not [^{14}C]galactose, in the absence of an additional energy source (Fig. 1). The same was true for strain TS2b (Lac$^+$ Gal$^+$), even though this strain could grow on galactose, albeit more slowly than on lactose. Strains 821b and 19258, both Lac$^+$ Gal$^+$, as well as 15 other Lac$^+$ Gal$^-$ strains were unable to accumulate [^{14}C]galactose, although all could accumulate [^{14}C]lactose. In addition, none of these strains could accumulate the galactose analog [^{14}C]TMG.

Unlike *S. lactis*, *S. thermophilus* is unable to deaminate and produce ATP from arginine and depends exclusively on fermentation of carbohydrates to meet its energy needs. When sucrose, which is rapidly fermented by *S. thermophilus*, was added to cell suspensions before [^{14}C]galactose addition, galactose uptake in both Gal$^+$ and Gal$^-$ strains occurred (Fig. 2). Galactose-grown *S. thermophilus* TS2b transported galactose more rapidly and to greater intracellular concentrations than did the same strain grown in lactose. Strain TS2 (Gal$^-$) grown in lactose accumulated galactose to lower internal concentrations, but above the medium concentration, indicating that active transport was occurring. All other Gal$^-$ strains also transported galactose when energized with sucrose. Although TMG uptake occurred at a low rate in energized, galactose-grown cells of TS2b, no uptake was observed in energized lactose-grown cells of either TS2b or any of the Gal$^-$ strains.

Other potential energy sources were examined for their ability to promote galactose transport, although the narrow range of compounds which *S. thermophilus* ferments limited the number of possible energizers. Cells preincubated with sucrose and, to a lesser extent, lactose could accumulate galactose, but glucose and a 0.01% Casamino Acids (Difco) solution were not effective in providing transport energy. The slow rates of growth on glucose and uptake of [^{14}C]glucose in lactose-grown cells (data not shown) probably account for these results in cells preincubated with glucose. Although lactose is rapidly utilized by *S. thermophilus*, this sugar may compete with [^{14}C]galactose for uptake (Table 1), thereby reducing [^{14}C]galactose accumulation.

The dependence of galactose transport activity on an energy source was further indicated by correlation of galactose transport with the sucrose concentration in the medium. As long as sucrose was provided as a substrate for ATP generation, galactose uptake could occur. After about 40

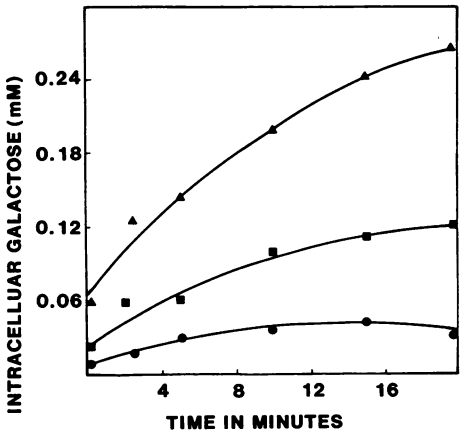

FIG. 2. Galactose accumulation in sucrose-energized cells of *S. thermophilus* TS2b (Gal$^+$) grown in galactose broth (▲) and lactose broth (■) and of *S. thermophilus* TS2 (Gal$^-$) grown in lactose broth (●). Samples (3.5 ml) of cells were preincubated with 0.20 mM sucrose for 5 min before the addition of [^{14}C]galactose (0.5 μM).

TABLE 1. Effect of sugars and sugar analogs[a] on the initial rate of [^{14}C]galactose uptake in *S. thermophilus* TS2b

Sugar or analog	% of initial rate[b]
Control (no addition)	100
Galactose	37
Lactose	33
o-Nitrophenyl-β-D-galactopyranoside	35
TMG	35
β-Methylgalactoside	28
α-Methylgalactoside	56
Thiodigalactoside	71
Sucrose	84
Glucose	107

[a] Competing sugars and sugar analogs were added at a concentration of 2.5 mM (five times the [^{14}C]galactose concentration) immediately before [^{14}C]galactose addition.

[b] The initial rate was determined to be 57 μmol of galactose accumulated per min per g (dry weight) of cells.

min, the sucrose concentration decreased from 0.165 to 0.005 mM, and galactose uptake was dramatically reduced. After 60 min, the sucrose in the medium was depleted, and cells had limited ability to accumulate galactose.

Effect of inhibitors on uptake. The amount of ATP produced in glycolyzing cells can be controlled in vivo by the addition of metabolic inhibitors. When 1.0 mM iodoacetic acid (IAA) was added to cell suspensions and preincubated with sucrose for 1 min before [^{14}C]galactose addition, very little galactose was accumulated (Fig. 3). NaF-treated cells accumulated galactose at a rate and to an intracellular steady-state concentration of about 50% of the control. Sodium azide had no effect on galactose uptake. When IAA or NaF was added after preincubation with sucrose, galactose accumulation was only slightly reduced, indicating little direct effect on the galactose transport system, although IAA at higher concentrations (>10 mM) inhibited transport activity in fully energized cells. *N*-Ethylmaleimide, also a sulfhydryl reagent, had no effect on transport activity at 5.0 mM.

Effect of ionophores. Inhibition of galactose uptake by the proton ionophores carbonyl cyanide *m*-chlorophenylhydrazone and pentachlorophenol added to fully energized cells indicated that transport was mediated via a proton motive force (Fig. 4). In addition, starved cells of *S. thermophilus* TS2b suspended in an artificially imposed gradient (prepared by adding valinomycin to cells in potassium-free buffer) accumulated galactose to more than 10 times the medium concentration. However, cells were resistant to DCCD, an ATPase inhibitor (data not shown).

Kinetics of galactose uptake. Kinetic plots of galactose transport were made from data obtained with both Gal$^+$ and Gal$^-$ strains, and typical saturation kinetics were observed, as shown for strain TS2b (Fig. 5). Galactose-grown TS2b had a K_m for galactose of 0.25 mM, compared with 0.39 for the lactose-grown Gal$^-$ parent strain, TS2. The V_{max} values were 195 and 20 μmol of galactose accumulated min^{-1} per g (dry weight) of cells, respectively.

Specificity of the galP system. The effect of competing sugars and sugar analogs on [^{14}C]galactose uptake was determined by measuring the initial rate of galactose accumulation in the presence of the test compound. According to the rationale of Kashket and Wilson (6) and Thompson (25), inhibition of galactose uptake by a competing sugar relates directly to the affinity of that sugar for the galP binding site. Lactose, galactose, TMG, *o*-nitrophenyl-β-D-galactopyranoside, and β-methyl galactoside, added at 5 times the [^{14}C]galactose concentration, reduced accumulation of the latter by 28 to 37%, whereas α-methyl galactoside and thiodigalactoside had less effect (Table 1). The addition of glucose and sucrose had no inhibitory effect on [^{14}C]galactose uptake.

DISCUSSION

S. thermophilus is unique among the lactic streptococci in that most wild-type strains are Gal$^-$ and, when grown in lactose-containing medium, utilize only the glucose portion of lactose and release free galactose into the extracellular

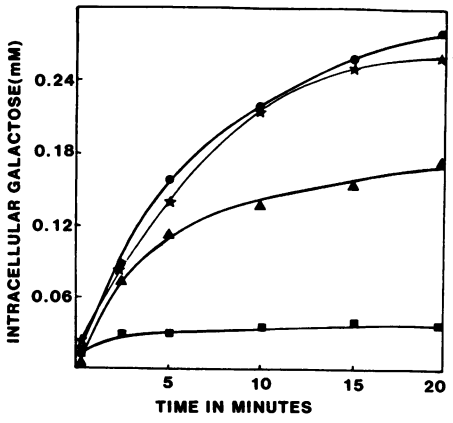

FIG. 3. Effect of IAA (1.0 mM) (■), sodium fluoride (10.0 mM) (▲), and sodium azide (10 mM) (★) on galactose uptake in *S. thermophilus* TS2b. For the control (●), no inhibitor was added. Inhibitors were added to 3.5 ml of cells immediately before sucrose addition and incubated for exactly 1 min before [^{14}C]galactose (0.5 μ) addition.

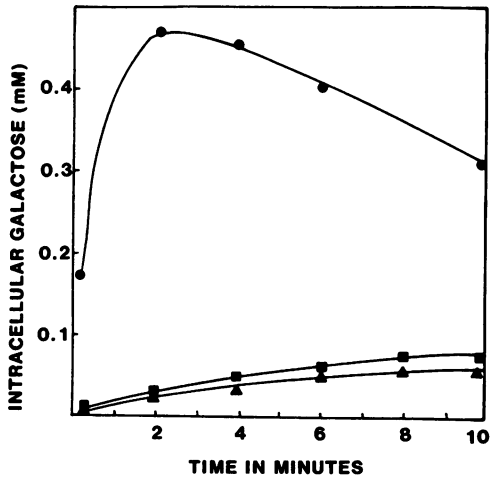

FIG. 4. Effect of proton-conducting ionophores on galactose uptake in *S. thermophilus* TS2b. Carbonyl cyanide *m*-chlorophenylhydrazone (5 μM) (■) or pentachlorophenol (5 μM) (▲) was added to cells after 5 min of preincubation with sucrose. For the control (●), no inhibitor was added. Both carbonyl cyanide *m*-chlorophenylhydrazone and pentachlorophenol were dissolved in 95% ethanol; ethanol was also added, in an equal volume, to the control.

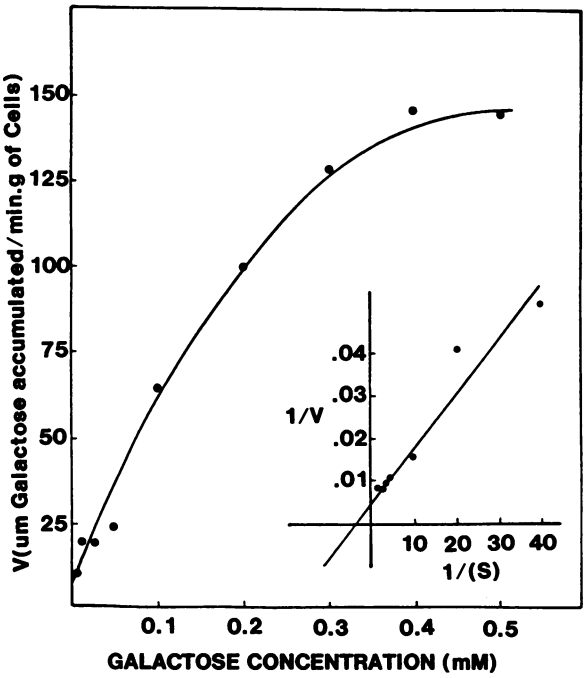

FIG. 5. Michaelis-Menton saturation curve of galactose uptake in *S. thermophilus* TS2b. Samples (1.0 ml) of resting cell suspensions were preincubated with 0.2 mM sucrose for 5 min at 42°C before the addition of galactose at various concentrations. Samples of 0.5 ml were removed at 10 and 20 s and filtered as described in the text. The insert shows a Lineweaver-Burk double-reciprocal plot of $1/v$ versus $1/[S]$, obtained by the least-squares method.

medium (15, 21, 27). The Gal^- phenotype in these strains has been thought to be due to either the absence of one or more catabolic enzymes, the absence of a specific galactose transport system, or both (15, 17). Hutkins et al. (5) have shown that both Gal^+ and Gal^- strains of *S. thermophilus* possessed the Leloir pathway enzyme, galactokinase and Thomas and Crow (21) recently described activities of galactokinase and two other Leloir enzymes, uridine diphosphate glucose-4-epimerase and galactose-1-phosphate uridyl transferase, in Gal^+ and Gal^- strains. The latter authors suggested that galactokinase may be rate limiting, assuming that *S. thermophilus* possessed a galactose transport system. However, the existence of such a system in these organisms has not been reported.

Initial findings indicated that lactose transport activity was present in resting cells of strain TS2b, but that these cells were unable to accumulate galactose (Fig. 1). These results suggested that lactose uptake could occur in the absence of an exogenous energy source, presumably because ATP was rapidly produced from passive or facilitated diffusion and subsequent metabolism of lactose, thus fueling additional active transport by the lactose permease.

In contrast, galactose is metabolized more slowly than lactose, and the slow rate of ATP generation from galactose may have limited subsequent accumulation of galactose. Transport of galactose occurred only when an additional metabolizable energy source was provided (Fig. 2). Similarly, in *S. lactis* ML3, galactose uptake by the galP system also required an exogenous energy source, arginine or glucose (25). These results suggest that resting cells of *S. thermophilus* are essentially energy depleted.

Both sucrose and lactose are rapidly fermented by TS2b with generation times of 20 to 25 min (21), and both sugars would be expected to energize cells. However, cells preincubated with lactose accumulated galactose at a significantly slower rate than sucrose-treated cells. Lactose, which is structurally similar to galactose in that both sugars are β-D-galactosides, may compete for the galP binding site (Table 1) and reduce galactose entry into the cells. Sucrose, however, is structurally distinct from galactose and had little affinity for the galP (Table 1). The difference in galactose uptake activity in sucrose- and lactose-treated cells may have been due to this competitive effect.

The glycolytic inhibitors NaF and IAA have been used to control the rate of glycolysis and formation of ATP and PEP in *S. lactis* in vivo (13). In *S. lactis* C2, NaF effectively blocked PTS activity, since PEP could not be produced (13). NaF had no effect on lactose uptake in *S. lactis* 7962, however, since this system was not dependent on PEP (13). In *S. thermophilus* cells preincubated with sucrose and 10 mM NaF, the initial rate of galactose uptake was reduced by about 50%, indicating that enough ATP was produced to fuel the galactose transport system, but to a lesser degree than in control cells not treated with NaF. IAA blocks all glycolytic activity and essentially prevented ATP formation in *S. thermophilus*, as indicated by the absence of galactose accumulation as well as lactose uptake (data not shown). The absence of PEP-dependent phosphorylation of galactose by cell extracts of *S. thermophilus* (5) further indicated that these organisms lacked a PTS that was specific for galactose.

Kashket and Wilson (6) and Thompson (25) used proton-conducting ionophores to demonstrate the involvement of a proton motive force in the galactose transport system in *S. lactis*. Galactose uptake by *S. thermophilus* was sensitive to both carbonyl cyanide m-chlorophenylhydrazone and pentachlorophenyl at 5 μM; at higher concentrations (1 mM), galactose uptake was abolished. The data indicate that galactose transport in *S. thermophilus* is dependent on a proton motive force similar to that in *S. lactis* 7962 (7).

Although the ATPase inhibitor DCCD had no effect on galactose uptake in *S. thermophilus* TS2b, it may be that this organism is resistant to the action of this compound. Resistance to DCCD has been reported in *Streptococcus faecalis* (1) and *Escherichia coli* (4). Fillingame (4) suggested that ATPase insensitivity to DCCD may occur in organisms that are not dependent on ATPase activity for ATP-driven energy transducing reactions. The mechanism by which *S. thermophilus* is able to maintain a proton motive force via a DCCD-resistant process is not known.

The apparent K_ms of the lactose permeases for lactose in *S. thermophilus* 19258, 6097, and TS2b were previously reported as 0.48, 0.20, and 0.24 mM, respectively [R. Hutkins, H. A. Morris, and L. L. McKay, J. Dairy Sci. 67(Suppl. 1):49, 1984]. These values are very near the K_m for galactose determined in this study (ca 0.25 mM). The high affinities of the lactose permease for galactose (unpublished data) and the galactose permease for lactose (Table 1) suggest that these galactosides may share a common carrier system. Several lines of evidence argue against this hypothesis, however. First, Gal^- and Gal^+ strains transported galactose at significantly different rates, whereas lactose transport in these strains occurred at identical rates, indicating that galactose and lactose transport systems were distinct systems. Furthermore, a UV-induced Lac^- mutant of strain 19258 (Gal^+) was unable to accumulate lactose, but galactose carrier activity was equal to that of the parental strain, indicating that lactose was transported via a separate system. The galactose permease possessed high affinity for several other sugars, including *o*-nitrophenyl-β-D-galactopy-

ranoside and TMG (Table 1); however, these sugars were actually transported into the cell at very slow rates in the presence of lactose (data not shown). Although the galactose and lactose permease may share common affinities for galactose and lactose, the data indicate that only the true substrates are transported by the respective carrier. The data presented here and in a previous study (R. Hutkins, H. A. Morris, and L. L. McKay, J. Dairy Sci. **66**(Suppl. 1):55, 1983) suggest the possibility that some sugars may be bound, but not transported, by the sugar transport systems in *S. thermophilus*.

The inability of many commercial strains of *S. thermophilus* to utilize galactose has practical implications in a number of fermented dairy products. Free galactose in cheese, for example, can participate in undesirable browning reactions (N. Olson, Dairy Rec. **84**:112–113, 1983) and can be used as a substrate by heterofermentative bacteria to produce undesirable end products (28). Thomas and Crow (21) and Hutkins et al. (5) have shown that enzymes of the Leloir pathway are either not inducible or permanently repressed in Gal^- strains of *S. thermophilus*. The results reported currently show that galactose transport occurred via a galP, which was also present in Gal^- strains, but whose activity was very low and was dependent on an exogenous energy source. These results also indicate that the phenotypic difference between Gal^+ and Gal^- strains of *S. thermophilus* is due not only to differences in Leloir pathway enzyme activities, but also to differences in galactose transport activities in these strains.

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LITERATURE CITED

1. **Abrams, A., J. B. Smith, and C. Baron.** 1972. Carbodiimide-resistant membrane adenosine triphosphatase in mutants of *Streptococcus faecilis*. I. Studies of the mechanism of resistance. J. Biol. Chem. **247**:1484–1488.
2. **Bisset, D. L., and R. L. Andersen.** 1974. Lactose and D-galactose metabolism in group N streptococci: presence of enzymes for both the D-galactose-1-phosphate and D-tagotose-6-phosphate pathways. J. Bacteriol. **117**:318–320.
3. **Elliker, P. R., A. W. Anderson, and G. H. Hannesson.** 1956. An agar culture medium for lactic acid streptococci and lactobacilli. J. Dairy Sci. **39**:1611–1612.
4. **Fillingame, R. H.** 1975. Identification of the dicyclohexyl carbodiimide-reactive protein component of the adenosine-5-triphosphate energy transducing system of *Escherichia coli*. J. Bacteriol. **124**:870–883.
5. **Hutkins, R., H. A. Morris, and L. L. McKay.** 1985. Galactokinase activity in *Streptococcus thermophilus*. Appl. Environ. Microbiol. **50**:777–780.
6. **Kashket, E. R., and T. H. Wilson.** 1972. Role of metabolic energy in the transport of β-galactosides by *Streptococcus lactis*. J. Bacteriol. **109**:784–789.
7. **Kashket, E. R., and T. H. Wilson.** 1974. Proton motive force in fermenting *Streptococcus lactis* 7962 in relation to sugar accumulation. Biochem. Biophys. Res. Commun. **59**:879–886.
8. **Lawrence, R. C., T. D. Thomas, and B. E. Terzaghi.** 1976. Reviews of the progress of dairy science: cheese starters. J. Dairy Res. **43**:141–193.
9. **LeBlanc, D. J., V. L. Crow, L. N. Lee, and C. F. Garon.** 1979. Influence of the lactose plasmid on the metabolism of galactose by *Streptococcus lactis*. **137**:878–884.
10. **Maloney, P. C., E. R. Kashket, and T. A. Wilson.** 1975. Methods for studying transport in bacteria. Methods Membr. Biol. **5**:1–49.
11. **Mason, P. W., D. P. Carbone, R. A. Cushman, and A. S. Waggoner.** 1981. The importance of inorganic phosphate in regulation of energy metabolism of *Streptococcus lactis*. J. Biol. Chem. **356**:1861–1866.
12. **McKay, L. L.** 1980. Regulation of lactose metabolism in dairy streptococci. *In* R. Davis (ed.) Developments in food microbiology. Applied Science Publishers, Ltd., England.
13. **McKay, L. L., A. Miller III, W. E. Sandine, and P. R. Elliker.** 1970. Mechanisms of lactose utilization by lactic streptococci: enzymatic and genetic analyses. J. Bacteriol. **102**:804–809.
14. **McKay, L. L., A. Walter, W. E. Sandine, and P. R. Elliker.** 1969. Involvement of phosphoenolpyruvate in lactose utilization by group N streptococci. J. Bacteriol. **99**:603–610.
15. **O'Leary, V. S., and J. H. Woychik.** 1976. Utilization of lactose, glucose and galactose by a mixed culture of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* in milk treated with lactase enzyme. Appl. Environ. Microbiol. **32**:89–94.
16. **Park, Y. H., and L. L. McKay.** 1982. Distinct galactose phosphoenolpyruvate-dependent phosphotransferase system in *Streptococcus lactis*. J. Bacteriol. **149**:420–425.
17. **Somkuti, G. A., and D. J. Steinberg.** 1979. Adaptability of *Streptococcus thermophilus* to lactose, glucose, and galactose. J. Food Prot. **42**:885–887.
18. **Stahl, E.** 1969. Thin layer chromatography, 2nd ed. Springer-Verlag, New York.
19. **Terzaghi, R. E., and W. E. Sandine.** 1975. Improved medium for lactic streptococci and their bacteriophage. Appl. Microbiol. **29**:807–813.
20. **Thomas, T. D.** 1976. Regulation of lactose metabolism in group N streptococci. Appl. Environ. Microbiol. **32**:474–478.
21. **Thomas, T. D., and V. L. Crow.** 1984. Selection of galactose-fermenting *Streptococcus thermophilus* in lactose-limited chemostat cultures. Appl. Environ. Microbiol. **48**:186–191.
22. **Thompson, J.** 1976. Characteristics and energy requirements of an L-amino isobutyric acid transport system in *Streptococcus lactis*. J. Bacteriol. **127**:719–730.
23. **Thompson, J.** 1978. In vivo regulation of glycolysis and characterization of sugar: phosphotransferase systems in *Streptococcus lactis*. J. Bacteriol. **136**:465–476.
24. **Thompson, J.** 1979. Lactose metabolism in *Streptococcus lactis*: phosphorylation of galactose and glucose moieties in vivo. J. Bacteriol. **140**:774–785.
25. **Thompson, J.** 1980. Galactose transport systems in *Streptococcus lactis*. J. Bacteriol. **144**:683–691.
26. **Thompson, J., and T. D. Thomas.** 1977. Phosphoenolpyruvate and 2-phosphoglycerate: endogenous energy sources for sugar accumulation by starved cells of *Streptococcus lactis*. J. Bacteriol. **130**:583–595.
27. **Tinson, W., A. J. Hillier, and G. R. Jago.** 1982. Metabolism of *Streptococcus thermophilus*. I. Utilization of lactose, glucose, and galactose. Aust. J. Dairy Technol. **37**:8–13.
28. **Tinson, W., M. F. Ratcliff, A. J. Hillier, and G. R. Jago.** 1982. Metabolism of *Streptococcus thermophilus*. III. Influence on the level of bacterial metabolites in cheddar cheese. Aust. J. Dairy Technol. **37**:17–21.
29. **Winkler, H. H., and T. H. Wilson.** 1966. The role of energy coupling in the transport of β-galactosides by *Escherichia coli*. J. Biol. Chem. **241**:2200–2211.