Carbohydrate Metabolism in Lactic Streptococci: Fate of Galactose Supplied in Free or Disaccharide Form

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Phosphorylation of free galactose by lactic streptococci was mediated by an adenosine triphosphate (ATP)-dependent kinase. The phosphoenolpyruvate (PEP) phosphotransferase system (PTS) was involved to a limited extent in transport of the sugar. The conversion of free galactose to glucose also was demonstrated, and uridine diphosphogalactose-4-epimerase was demonstrated to account for this change. Galactose, supplied as lactose, was phosphorylated during transport by means of the PTS with PEP as the phosphate donor. Data also indicated that galactose derived from lactose was catabolized by the glycolytic pathway. Results showed the participation of ATP or PEP, or both, in the phosphorylation of five growth sugars for lactose-streptococci, namely, lactose, glucose, lactate, maltose, and mannoe. Free galactose was phosphorylated exclusively by ATP except when cells were grown on galactose; in this case, slight involvement of PEP in phosphorylation also was noted. Lactose phosphorylation was much more effective with PEP except when cells were grown on lactose, in which case ATP was equally effective. Glucose was phosphorylated to about the same degree by either ATP or PEP.

Lactic streptococci (Streptococcus lactis, S. cremoris, and S. diacetilactis) are used as starter culture organisms in the manufacture of many fermented milk foods because of their ability to convert lactose to lactic acid. Although the metabolic fate of the glucose portion of lactose is well known, the metabolism of the galactose moiety in these bacteria is less well understood. Furthermore, no information is available concerning the ability of these bacteria to utilize galactose when glucose also is present, a situation they face when grown in milk. In this regard, Gilliland et al. (11) reported that, when fermenting milk is treated with exogenously added β-galactosidase to release more galactose and glucose from the lactose, the amount of free galactose which appeared in the whey increased with time. These facts inferred that galactose was either not metabolized or used at a much slower rate than glucose.

Work in this laboratory (26, 28) has shown that the lactic acid bacteria possess at least two mechanisms for the utilization of lactose. Citti et al. (6) showed that S. lactis 7962 possessed a typical β-galactosidase which was under the same type of metabolic control as the Escherichia coli enzyme system; Kashket and Wilson (16) confirmed this in noting that the lactose analogue thiomethylgalactoside accumulated in this strain in the unphosphorylated form. Certain lactobacilli also contain β-galactosidase, whereas others depend on β-D-phosphogalactoside galactohydrolase for lactose hydrolysis (28). Recently, the phosphoenolpyruvate (PEP) phosphotransferase system (PTS) (19, 20) for lactose utilization was demonstrated in all lactostreptococci except the 7962 strain (25, 26); it also was shown to be quite similar to the system in S. aureus (9, 12–15). Romano et al. (29) later reported on the distribution of this system in bacteria.

The lactose-splitting enzyme present in organisms possessing a PEP system is β-D-phosphogalactoside galactohydrolase, as first named by Laue and MacDonald (21, 22). The substrate hydrolyzed is a phosphorylated form of lactose, and the number six carbon of galactose is presumed to be the position of phosphorylation (13, 19). Under the influence of this enzyme, phosphorylated lactose is cleaved into one mole-
cule of glucose and a second molecule of galactose-6-phosphate (Gal-6-P). The metabolic fate of the Gal-6-P was unknown until recently, and for the lactic streptococci its further metabolism has not been demonstrated. Simoni and Roseman (31) suggested that the compound may be converted by S. aureus to Gal-1-P by a mutase and then metabolized through the Leloir pathway (23) or be converted to 6-phosphogalactonic acid and then enter the Entner-Doudoroff pathway. Recent findings of Bissett and Anderson (2), however, indicate that the compound is converted to tagatose-1,6-diphosphate, which is cleaved into glyceraldehyde-3-phosphate and dihydroxyacetone phosphate.

Even though galactose is a growth substate (30), definitive data have not been published showing how the lactic streptococci utilize free galactose; however, it has been assumed from the abstract of Shahani (J. Dairy Sci. 43:852, 1960) that the Leloir pathway (23) is operative. The present report presents data on the fate of galactose in cell-free extracts of S. lactis. The involvement of the PTS in transport of other sugars also is reported.

MATERIALS AND METHODS

Organisms. The lactic streptococci used were obtained from the stock culture collection maintained by the Department of Microbiology, Oregon State University.

Chemicals. d-[1-14C]galactose, specific activity 59 mCi/mmol and 3 mCi/mmol, and d-[U-14C] glucose, specific activity 3.0 mCi/mmol, were obtained from Amersham/Searle Corp., Arlington Heights, Ill. [14C]Lactose (d-[U-14C]galactose), specific activity 11.4 mCi/mmol, was obtained from New England Nuclear Corp., Boston, Mass. d-[U-14C]mannose, specific activity 180 mCi/mmol, was a gift from Ed Thompson, Oregon State University. [U-14C]maltose, specific activity 7.0 mCi/mmol, was obtained from Calbiochem, Los Angeles. Glucostat and galactostat reagents were purchased from Worthington Biochemical Corp., Freehold, N.J. The remainder of the chemicals used were obtained from Sigma Chemical Co., St. Louis, Mo.

Media and growth conditions. Cultures were routinely propagated in sterile skim milk at 32 C and stored frozen at -20 C. Before experimental use, cultures were transferred at least three times in a broth medium of the following composition in grams: desired carbohydrate, 10.0; tryptone (Difco), 10.0; yeast extract (Difco), 1.0; gelatin, 2.5; sodium acetate, 1.5; ascorbic acid, 0.5; and distilled water to 1 liter (1, 10). The pH of this medium was adjusted to 7.0 prior to autoclaving at 121 C for 15 min.

Disappearance of galactose. Cells of S. lactis C2 were grown for 6 h in 1 liter of galactose broth and harvested in the Sorvall refrigerated centrifuge at 6,000 x g. Initial inoculum was 1%, and cells were harvested in late log phase. The cells obtained were washed 2 times in 0.05 M sodium phosphate buffer at pH 7.0 and then suspended in 40 ml of the same buffer; 2 ml of a 1:9 (vol/vol) mixture of toluene- acetone was then added to the cells, and the mixture was shaken vigorously for 5 min at 25 C. These toluene-acetone-treated cells were used to study the disappearance of free galactose. At zero time, 5 ml of the treated cell mixture was added to each of the following solutions to a final optical density (OD) of 0.45 at 420 nm by using a Bausch and Lomb Sectronic 20 colorimeter: galactose (168 µg/ml); galactose (168 µg/ml) plus 0.03 M potassium fluoride (KF); galactose (168 µg/ml) plus 0.03 M KF plus 0.01 M PEP; galactose (168 µg/ml) plus 0.05 M KF plus 0.01 M adenosine triphosphate (ATP). The final volume of each solution was 20 ml. Four samples were removed at 5-min intervals and centrifuged at 12,000 x g to remove the cells. The supernatant was assayed for free galactose by using the galactostat assay.

Conversion of galactose to glucose. Cells of S. lactis C2 were grown in 1 liter of galactose broth for 5 h and harvested in the usual manner. The initial inoculum was 1% and cells grew until late log phase. Treatment of the cells with toluene-acetone was as described above. The resulting cell suspension (5 ml) was brought to a 40-ml final volume (OD of 0.35) in 0.05 M sodium phosphate buffer, pH 7.0, with 800 µg of added galactose per ml, 0.3 M KF, and 0.1 M ATP. This mixture was incubated at 37 C. Eight samples (5 ml each) were removed every 4 min and quickly frozen in acetone-dry ice. To assay the carbohydrate intermediates, the samples were thawed and centrifuged at 12,000 x g. The supernatants were assayed for glucose, glucose-1-phosphate (Glc-1-P), and Glc-6-P by using the glucostat assay and for galactose, Gal-1-P, and Gal-6-P by using the galactostat assay. To determine the Glc-1-P or Gal-1-P concentrations, the increase in free glucose or galactose was measured after 10 min of hydrolysis of the sample in 1 N HCl at 100 C. This technique measures only the sugar-1-P, for it was determined that none of the sugar-6-P was hydrolyzed by this time. To measure the Glc-6-P or Gal-6-P concentration, the further increase in free glucose or galactose was determined on the acid-hydrolyzed sample after an additional hydrolysis with 1.0 unit of Sigma type V alkaline phosphatase at pH 8.5 for 1 h at 37 C.

Inhibition of galactose uptake by fucose. Galactose uptake by whole cells of S. lactis C2 was measured in the presence of different concentrations of fucose (6-deoxygalactose). A 3.0 x 10^-3 M solution of galactose (containing 2.2 x 10^-2 µCi of [1-14C]galactose per ml) was prepared in 0.05 M sodium phosphate buffer at pH 7.0. Four equal samples were taken, and fucose was added to provide concentrations of 0.1, 0.01, 0.001, and 0.0001 M. At zero time whole cells of S. lactis C2 grown on galactose were introduced into the solutions to an OD of 0.28. At 2-min intervals, samples of 1 ml were removed and quickly filtered through membrane filters (HA 0.45 µm diameter; Millipore Corp.), and the filters were washed with 10 ml of the ice-cold phosphate buffer. The filters were transferred to scintillation vials and dried overnight at 45 C. The next morning, 10 ml of toluene-based scintillation fluid (2,5-diphenyloxo-
Sonic oscillator as the enzyme containing would with glucose assuming crude ruptured by diphosphogalactose at 60 min sodium phosphate buffer, pH 7.0, and the radioactivity of the samples was determined by liquid scintillation.

**Sugar phosphorylation studies.** Radioactive substrates were prepared (0.1 μCi/ml) in 0.05 M phosphate buffer, pH 7.0, which contained KF (0.03 M), MgCl₂ (0.01 M), and ATP (0.01 M) or PEP (0.01 M). At zero time, tolune-acetone-treated cells of *S. lactis* C2 were added, and every 2 min samples (2 ml) were removed and immediately frozen. To check for phosphorylation, the samples were thawed and 0.5 ml of each was placed on a Bio-Rad AG1-X2 (formate form) anion-exchange column (4.0 by 0.4 cm). Unphosphorylated sugars were washed from the column with 5 ml of water. Sugar phosphates were eluted from the column with 5 ml of 0.5 M ammonium formate in 0.2 M formic acid. Samples (0.1 ml) of the eluted compounds were placed in 10 ml of Bray fluid and counted by liquid scintillation. In experiments to evaluate the column procedure, about 70% of the total radioactivity applied to the column bed was recovered in the eluate, which approximated the counting efficiency of the liquid scintillation spectrometer.

The information for Tables 3 and 4 was obtained essentially the same way except the cells and substrates were mixed and allowed to remain together for 30 min, after which time the mixture was placed in boiling water for 5 min; the sugars and sugar phosphates were then separated by descending paper chromatography (4) and detected by p-anisidine spray.

**Uridine diphosphogalactose (UDP Gal)-4-epimerase assay.** The method provided by Sigma Chemical Co., St. Louis, Mo., to assay their enzyme preparation, was used. This method originally was published by Wilson and Hognes (33). One modification of the procedure of assay was employed; semicarbazide (0.2 M) was added to the glycine buffer. Lactic streptococci used were grown in galactose broth containing a low concentration of yeast extract (1 g/liter). Cells were grown for 6 h and harvested at 0°C by using the refrigerated Sorvall centrifuge at 5,000 × g. Cells were washed twice in 0.05 M sodium phosphate buffer at pH 7.0, suspended in 20 ml of the same buffer, and ruptured by sonic oscillation in a Raytheon 10-kc sonic oscillator for 30 min at maximum setting. This crude preparation was centrifuged for 10 min at 12,000 × g at 0°C, and the supernatant was used as the enzyme preparation. One unit of activity was defined as the amount of enzyme required to convert 1 μmol of uridine diphosphogalactose to uridine diphosphoglucone per min at pH 8.8 and 25°C.

**Simultaneous use of glucose and galactose by galactose-adapted cells of Streptococcus diacetic- lactis ATCC 15346.** *S. diaceticlactis* was used for these experiments because it releases CO₂ from glucose (3). By assuming the absence of an unknown metabolic pathway, the detection of the radioactive gas produced from [1-¹⁴C]galactose would provide evidence for the conversion of galactose to glucose; also, determination of the specific activity of radioactive CO₂ with time under appropriate experimental conditions would indicate whether or not galactose and glucose were used simultaneously. Media were formulated in sodium phosphate buffer, pH 7.0, as follows in 30-ml capacity serum bottles: 33 mM galactose plus 0.2 μCi of [¹⁴C]galactose; 33 mM glucose plus 0.2 μCi of [¹⁴C]glucose; 33 mM galactose plus 0.2 μCi of [¹⁴C]glucose plus 33 mM galactose; 33 mM glucose plus 0.2 μCi of [¹⁴C]glucose plus 33 mM galactose. The specific activity of each radioactively labeled substrate was 3 mCi/mmol.

The media were inoculated with washed cells previously adapted and grown in galactose for 12 h. The optical densities at 420 nm of a 1:100 dilution of the inoculum varied from 0.28 to 0.32. Approximate cell dry weight values for the inoculum varied from 22.4 to 24.2 mg/ml. Incubation of the vials was performed under static conditions at 30°C for 30 min. The serum bottles were fitted with a small cup to hold filter paper on which to trap CO₂. To collect the CO₂, the pH was adjusted to 2.0 by adding 6 N HCl. The volume of HCl added was determined by using a duplicate vial not used in the experiment but containing the same materials as the experimental vials. Twenty minutes were allowed for the CO₂ to be evolved from the medium into the vial; at this time, 0.2 ml of phenylethylamine was injected onto the filter paper through the cap of the vial. One hour was allowed for absorption of CO₂ by the phenylethylamine. The filter paper was then placed in 10 ml of Bray scintillation fluid and counted in a Packard liquid scintillation spectrometer. Under identical conditions, the volume of CO₂ produced was determined by using a Gilson differential respirometer.

**RESULTS**

**Use of galactose by tolune-acetone-treated cells.** Typical data showing the effect of phosphate donor on galactose utilization appear in Table 1. It is clear from these data that ATP is the primary phosphate donor for galactose transport.

**Conversion of galactose to glucose.** Table 2 shows typical data for the utilization of galactose, suggesting that metabolism occurs via Gal-1-P to Glc-1-P and then to Glc-6-P. The exact enzymatic mechanism for this conversion is not known; however, a key Leloir pathway enzyme was shown to be present, and it appeared that galactose was first phosphorylated by ATP to form Gal-1-P. This appeared true because the level of free galactose declined very rapidly in the presence of ATP, whereas the level of Gal-1-P increased and later declined as it was converted to glucose intermediates. To obtain these results, it was necessary to block the glycolytic pathway with KF as indicated.

Additional evidence that galactose was converted to glucose was obtained by growing *S. diaceticlactis* ATCC 15346 in 0.2% lactose broth supplemented with 0.1 μCi of radioactive lactose ([U-¹⁴C]Glc-Gal) and testing for ¹⁴CO₂ evolution and synthesis of ¹⁴C-labeled serine. Serine is synthesized from this organism from 3-phosphoglyceric acid which is derived from
Table 1. Influence of PEP and ATP, in the presence of KF, on the utilization of galactose by resting, toluene-treated, galactose-grown (6 h) cells of S. lactis C2

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Galactose</th>
<th>Galactose + KF</th>
<th>Galactose + KF + PEP</th>
<th>Galactose + KF + ATP</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>168</td>
<td>168</td>
<td>168</td>
<td>168</td>
</tr>
<tr>
<td>5</td>
<td>148</td>
<td>164</td>
<td>162</td>
<td>84</td>
</tr>
<tr>
<td>10</td>
<td>140</td>
<td>168</td>
<td>140</td>
<td>7</td>
</tr>
<tr>
<td>15</td>
<td>162</td>
<td>162</td>
<td>112</td>
<td>0</td>
</tr>
<tr>
<td>20</td>
<td>162</td>
<td>162</td>
<td>120</td>
<td>21</td>
</tr>
</tbody>
</table>

* Experimental conditions were as follows: galactose concentration, 168 μg/ml; KF, 0.03 M; and ATP or PEP, 0.01 M. A toluene-acetone-treated suspension (5 ml) of S. lactis cells was added to each solution to a final OD of 0.45.

* Data are expressed in μg of galactose per ml remaining in the supernatant.

Table 2. Compounds formed from galactose by toluene-treated, resting cells of S. lactis C2 previously grown (6 h) on galactose

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Glc</th>
<th>Gal</th>
<th>Gal-1-P</th>
<th>Glc-1-P</th>
<th>Glc-6-P</th>
<th>Gal-6-P</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>800</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>8</td>
<td>480</td>
<td>280</td>
<td>40</td>
<td>16</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>8</td>
<td>280</td>
<td>364</td>
<td>56</td>
<td>24</td>
<td>0</td>
</tr>
<tr>
<td>12</td>
<td>8</td>
<td>204</td>
<td>410</td>
<td>64</td>
<td>56</td>
<td>0</td>
</tr>
<tr>
<td>16</td>
<td>8</td>
<td>14</td>
<td>508</td>
<td>80</td>
<td>72</td>
<td>0</td>
</tr>
<tr>
<td>20</td>
<td>8</td>
<td>14</td>
<td>408</td>
<td>80</td>
<td>64</td>
<td>0</td>
</tr>
<tr>
<td>24</td>
<td>8</td>
<td>14</td>
<td>380</td>
<td>88</td>
<td>64</td>
<td>0</td>
</tr>
<tr>
<td>28</td>
<td>8</td>
<td>14</td>
<td>240</td>
<td>88</td>
<td>72</td>
<td>0</td>
</tr>
<tr>
<td>32</td>
<td>8</td>
<td>56</td>
<td>168</td>
<td>96</td>
<td>64</td>
<td>0</td>
</tr>
</tbody>
</table>

* Experimental conditions were as follows. S. lactis cells were toluene-acetone-treated and suspended in galactose solution (800 μg/ml) to an OD of 0.35 with 0.3 M KF and 0.01 M ATP. Incubation was at 37°C. Every 4 min, samples were removed, quickly frozen in acetone-dry ice, and later thawed and assayed with glucostat and galactostat as indicated in the Materials and Methods section.

If labeled serine were found, it would be direct proof that the [14C]galactose of lactose was converted to glucose. During such an experiment, CO₂ was evolved during 6 h of growth and 1,625 counts/min were counted on the filter paper due to the 14CO₂. Proof that galactose was entering mainline metabolic pathways was less definite, since the complex medium was required for growth and CO₂ evolution, and this medium apparently suppressed the synthesis of serine from glucose;

when a protein hydrolysate of the above cells was tested for radioactivity, serine was not labeled.

Chromatography of sugar phosphates. When the 16- through 32-min samples from Table 2 were tested chromatographically, the sugar phosphates detected were Glc-1-P with an Rf value of 0.32 compared to a known standard of 0.32 and Gal-1-P with an Rf value of 0.25 compared to a known standard of 0.25. Glc-6-P was not detected but was determined present enzymatically (Table 2).

Inhibition of galactose uptake by fucose. The uptake of [14C]galactose was measured in S. lactis C2 in the presence of different amounts of fucose. Figure 1 shows the results.

Sugar phosphorylation studies. Figure 2 shows typical results when toluene-acetone-treated cells of S. lactis C2 were used to
phosphorylate galactose and lactose with ATP or PEP in the presence of Mg2+ and KF. It may be seen that ATP was the effective phosphate donor for galactose, whereas PEP was the effective phosphate donor for lactose.

Table 3 shows typical data obtained when *S. lactis* was induced on different carbon sources and the toluene-acetone-treated cells were used to phosphorylate five different sugars. Results are expressed as percent phosphorylation, and these data further indicate which substrates are transported through the PTS; lactose, glucose, and mannose were phosphorylated by this system, but very little phosphorylation of galactose or maltose was mediated by PEP. ATP also was inefficient in stimulating phosphorylation of maltose.

Table 4 gives data on the percent phosphorylation of four growth substrates by *S. cremoris* HP and *S. cremoris* HP Lac+ mutant. These results support the data in the previous table. Maltose was not studied since it is not a substrate for *S. cremoris*.

**UDP-Gal-4-epimerase assay.** Control enzyme from Sigma Chemical Co., St. Louis, Mo., showed 0.16 units of protein per mg. *S. lactis* C2 gave 0.38 units of protein per mg, and *S. cremoris* 459 showed 0.31 units of protein per mg; no activity was noted in any of these preparations without added semicarbazide.

**Simultaneous use of galactose and glucose.**

To check the ability of the lactic streptococci to utilize glucose and galactose simultaneously, 0.05 M phosphate buffer was prepared in which both glucose and galactose were added as carbon sources. Control flasks were also used in which either galactose or glucose was the sole carbon source. Table 5 shows the total CO2 produced as well as the specific activity in a 30-min time period. It may be seen that the presence of glucose reduced the specific activity of the CO2 produced from galactose, and the presence of galactose reduced the specific activity of CO2 produced from glucose. These data indicated that galactose-induced cells simultaneously utilized glucose and galactose.

**DISCUSSION**

The rapid decline of free galactose in the presence of ATP and the concomitant increase of Gal-1-P which subsequently decreased indicated that phosphorylated intermediates were formed and metabolized when cells were provided with this sugar. Since PEP did not cause the same response as ATP, it appeared that galactose was transported primarily by a system other than the PTS. Inhibition of galactose uptake in whole cells of *S. lactis* C2 (Fig. 1) by fucose (6-deoxygalactose) also supported this idea. The sixth carbon of galactose is believed (13, 19) to be the position of phosphorylation of the hexose when the PTS operates; however, since fucose lacks an oxygen on the sixth carbon, it is not phosphorylated. If the 1PTS were functional, fucose would not be expected to inhibit the uptake of galactose, unless there were some competition for the initial binding of the enzyme to the substrate due to the structural similarity of the two molecules.

The enzyme responsible for phosphorylation of galactose apparently is galactokinase, the first enzyme of the Leloir pathway (23) which initiates the conversion of galactose to glucose. By blocking the glycolytic pathway with fluoride and supplying the cells with galactose and ATP, it was possible to show conversion of this sugar to Gal-1-P and subsequently to Glc-1-P and Glc-6-P, providing direct evidence for galactose conversion to glucose. Leloir pathway enzymes were believed functioning in these conversions as substantiated by the presence of UDP Gal-4-epimerase. Although first attempts to assay for this enzyme were not successful,

### Table 3. Role of ATP and PEP in phosphorylation of various sugars by *S. lactis* C2 induced on five different carbon sources

<table>
<thead>
<tr>
<th>Inducer</th>
<th>Substrate phosphorylated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Galactose</td>
</tr>
<tr>
<td></td>
<td>ATP</td>
</tr>
<tr>
<td>Galactose</td>
<td>95</td>
</tr>
<tr>
<td>Glucose</td>
<td>21</td>
</tr>
<tr>
<td>Lactose</td>
<td>92</td>
</tr>
<tr>
<td>Maltose</td>
<td>80</td>
</tr>
<tr>
<td>Mannose</td>
<td>13</td>
</tr>
</tbody>
</table>

*Data are expressed as percent phosphorylation, which was calculated by dividing the total counts eluted from the column into the counts in the phosphate fraction. These data were obtained after the substrates and toluene-acetone-treated cells were mixed and held for 30 min.*
this was remedied when oxidation of reduced nicotine adenine dinucleotide was prevented by trapping pyruvate with semicarbazide.

Lactose or galactose-induced cells of lactic streptococci would phosphorylate and use galactose and glucose simultaneously once these sugars were in the cell. Evidence is taken from Table 3 on the influence of carbon source on phosphorylation of sugars. Apparently this was not true of glucose-grown cells because, under these conditions, the phosphorylation of galactose was repressed. Carbon dioxide evolution by *S. diacetilactis* cells grown in galactose, when supplied glucose and galactose simultaneously, revealed the ability of lactic streptococci to use both sugars at the same time. The cells may not continue this process throughout growth, however, since it is possible that galactose-adapted cells, when provided both sugars, would degrade galactose enzymes and preferentially use glucose.

Figure 2 indicates that the effective phosphate donors for lactose and galactose are different. Galactose phosphorylation required ATP, whereas lactose phosphorylation required PEP. These data also showed phosphorylation of lactose with ATP but at a much lower level, suggesting that two systems of transport exist in these organisms. The transport of galactose in bacteria is known to occur by various mechanisms (5, 17, 32), and lactic streptococci systems for transport of lactose have been studied in detail (7, 24, 25, 27). One system involves the PEP-dependent PTS in which the sugar is phosphorylated as it enters the cell. Also, lactose may be hydrolyzed to component monosaccharides, which are phosphorylated by an ATP-dependent kinase, as is the case for *S. lactis* 7962 (6, 16).

In experiments on the effects of carbon source on phosphorylation (Table 3), it was seen that galactose was phosphorylated by ATP, whereas glucose was phosphorylated by either ATP or PEP. Lactose was more effectively phosphorylated by PEP; however, at least in the case of lactose-grown cells, lactose was also phosphorylated by ATP. Apparently galactokinase was affecting this latter phosphorylation. Glucose, maltose, and mannose greatly reduced phosphorylation of lactose by ATP, perhaps by catabolic repression. Maltose was not effectively phosphorylated by either phosphate donor, which raises the question of how it may be transported; this sugar may be hydrolyzed by maltase, and the resulting glucose molecules phosphorylated. In such a case, one would expect both ATP and PEP to be effective in phosphorylating maltose as glucose. Mannose was more effectively phosphorylated by PEP in most cases, but glucose also repressed the phosphorylation of this sugar as well as galactose. In this regard, Wilkins (32) showed that, whereas galactose-grown cells of *S. faecalis* would accumulate phosphorylated galactose, glucose-grown cells lacked this ability.

The phosphorylation studies using *S. cremoris* HP and *S. cremoris* HP Lac− revealed why this mutant was unable to grow on lactose: it lacked the ability to phosphorylate the substrate. This

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**Table 4. Phosphorylation of four growth substrates by *S. cremoris* HP and an *S. cremoris* HP Lac− mutant**

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Organism</th>
<th>Wild type</th>
<th>Lac− mutant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ATP</td>
<td>PEP</td>
<td>ATP</td>
</tr>
<tr>
<td>Galactose</td>
<td>52</td>
<td>23</td>
<td>77</td>
</tr>
<tr>
<td>Glucose</td>
<td>94</td>
<td>94</td>
<td>64</td>
</tr>
<tr>
<td>Lactose</td>
<td>48</td>
<td>94</td>
<td>8</td>
</tr>
<tr>
<td>Mannose</td>
<td>89</td>
<td>87</td>
<td>35</td>
</tr>
</tbody>
</table>

* Data are expressed as percent phosphorylation; the wild type was lactose grown, whereas the mutant was induced and grown in galactose.

**Table 5. Specific activity of CO2 evolved by galactose-induced cells of *S. diacetilactis* 15346 over a 30-min period at 30 C in different carbohydrates**

<table>
<thead>
<tr>
<th>Carbohydrate</th>
<th>Counts/min§</th>
<th>μliters of CO2 per mg per 30 min§</th>
<th>Sp act</th>
</tr>
</thead>
<tbody>
<tr>
<td>33 mM Gal plus 0.2 μCi of [14C]Gal</td>
<td>26,879</td>
<td>6.74</td>
<td>0.081</td>
</tr>
<tr>
<td>33 mM Glc plus 0.2 μCi of [14C]Glc</td>
<td>25,373</td>
<td>6.29</td>
<td>0.081</td>
</tr>
<tr>
<td>33 mM Glc and Gal plus 0.2 μCi of [14C]Gal</td>
<td>21,205</td>
<td>7.81</td>
<td>0.054</td>
</tr>
<tr>
<td>33 mM Gal and Glc plus 0.2 μCi of [14C]Glc</td>
<td>20,499</td>
<td>7.81</td>
<td>0.052</td>
</tr>
</tbody>
</table>

* Specific activity is expressed as micromoles of labeled CO2 per micromoles of total CO2 per milligrams of cell dry weight per 30 min.

§ Counts per minute are an average of two determinations, and counts were corrected to 100% efficiency by the channel ratio method.

The total volume of gas was determined by using a Gilson differential respirometer and was employed under identical conditions as when the labeled compound was used. The data are expressed as microliters of CO2 per milligram of cell dry weight at a total time of 30 min and is the average of three determinations.
could be due to a lack or impairment of enzyme II or factor III, or both, of the PTS. It was hoped that this mutant would phosphorylate lactose and thereby provide a source of Gal-6-P. This mutant also lacked both of the lactose cleavage enzymes. Since the lac marker in lactic streptococci is believed to be carried on plasmids (24), mutations in this region probably represent a complete loss of the episome; hence, lac- mutants lack all enzymes of the PTS for this carbohydrate.

The fact that glucose and lactose were effectively phosphorylated by PEP confirmed operation of the PTS in lactic streptococcal cells. This would suggest that a correlation exists between sugar transport and PTS activity as reported recently (18) in *E. coli*. A concern in the early phases of this work was that PEP could be converted into ATP by action of pyruvate kinase. Evidence to the contrary comes from the fact that galactose was phosphorylated by ATP, not PEP, in lactose-grown cells. If PEP were converted to ATP, then PEP would also appear to be an effective phosphate donor for galactose.

Current research attention is being given the enzymes of the Leloir pathway and the induction of UDP Gal-4-epimerase. Since this pathway in now known to be present in the lactic streptococci, it becomes important to determine how Gal-6-P enters the scheme. Regarding the suggestion of Simoni and Roseman (31) that Gal-6-P may be oxidized to 6-phosphogalactonic acid, recent experiments in our laboratory have indicated this is not the case; Gal-6-P (Sigma Chemical Co., St. Louis), which by chromatography was found to contain 0.2% Glc-6-P, was oxidized (nicotinamide adenine dinucleotide phosphate reduction), but the reaction was felt due to the contaminating Glc-6-P. It is likely that Gal-6-P in these organisms will be found to be metabolized through tagatose as in *S. aureus* (2).

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LITERATURE CITED

22. Laue, P., and R. E. MacDonald. 1968. Studies on the relation of thiomethyl β-D-galactoside accumulation to...