Fermentation of Glucose, Lactose, Galactose, Mannitol, and Xylose by Bifidobacteria

WYTSKE DE VRIES AND A. H. STOUTHAMER

Botanical Laboratory, Microbiology Department, Free University, Amsterdam, The Netherlands

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For six strains of Bifidobacterium bifidum (Lactobacillus bifidus), fermentation balances of glucose, lactose, galactose, mannitol, and xylose were determined. Products formed were acetate, L(+)-lactate, ethyl alcohol, and formate. L(+)-Lactate dehydrogenase of all strains studied was found to have an absolute requirement for fructose-1,6-diphosphate. The phosphoroclastic enzyme could not be demonstrated in cell-free extracts. Cell suspensions fermented pyruvate to equimolar amounts of acetate and formate. Alcohol dehydrogenase was shown in cell-free extracts. Possible explanations have been suggested for the differences in fermentation balances found for different strains and carbon sources. By enzyme determinations, it was shown that bifidobacteria convert mannitol to fructose-6-phosphate by an inducible polyol dehydrogenase and fructokinase. For one strain of B. bifidum, molar growth yields of glucose, lactose, galactose, and mannitol were determined. The mean value of Y (ATP), calculated from molar growth yields and fermentation balances, was 11.3.

In previous papers (21, 29, 30), it was shown that bifidobacteria ferment glucose via the fructose-6-phosphate phosphoketolase route. By enzyme determinations, the presence of the glycolytic system and hexose monophosphate pathway was ruled out (30). For one strain of Bifidobacterium (Lactobacillus bifidus), the fermentation pathway of glucose was confirmed by the determination of the fermentation balance of glucose in resting-cell suspensions. The fermentation balance found [glucose → L(+)-lactate + 1.5 acetate] could be explained by the operation of the fructose-6-phosphate phosphoketolase route and reduction of pyruvate to lactate. It was shown that lactate dehydrogenase (EC 1.1.1.27) of this strain had an absolute requirement for fructose-1,6-diphosphate. This explained the presence of small amounts of phosphofructokinase (EC 2.7.1.11), an enzyme otherwise not involved in the fermentation pathway, in cell-free extracts (29).

The present work investigated the pathways of pyruvate breakdown in other strains of Bifidobacterium. For that purpose, fermentation balances of glucose were determined in growing cultures of five strains of Bifidobacterium. In addition, fermentation balances of mannitol, lactose, xylose, and galactose were determined for some strains of Bifidobacterium. Acetate, lactate, ethyl alcohol, and formate proved to be the main fermentation products. Apparently, pyruvate, formed as an intermediate in the fructose-6-phosphate phosphoketolase route, was partly reduced to lactate by lactate dehydrogenase and partly split to acetyl phosphate and formate by the phosphoroclastic enzyme. Attempts were made to show alcohol dehydrogenase (EC 1.1.1.1) and the phosphoroclastic enzyme in cell-free extracts of one strain of Bifidobacterium.

By enzyme studies, the way by which bifidobacteria convert mannitol to fructose-6-phosphate was established.

Finally, molar growth yields of glucose, mannitol, lactose, and galactose are recorded in this paper. From molar growth yields and fermentation balances, Y (ATP) was calculated.

MATERIALS AND METHODS

Organisms and growth conditions. Of the stock collection of bifidobacteria, strains of different types were selected. The following strains were used: L. bifidus (S 128) obtained from K. C. Winkler (Laboratory of Microbiology, State University, Utrecht, The Netherlands); B. bifidum (S 200) obtained from J. de Waart (Central Institute of Nutrition and Food Research, Zeist, Holland); B. bifidum B5 (S 324) and B. bifidum B420 (S 327) obtained from H. Beerens (Institut Pasteur de Lille, France); B. lactentis 659 (S 332) and B. liberorum 76 (S 337) obtained from G. Reuter (Institut für Lebensmittelhygiene der Freien Universität, Berlin, Germany). Numbers in parentheses are the collection numbers of the strains men-
tioned. The strains were maintained as stab cultures in tomato-agar (Oxoid) containing 2% glucose and 0.2% cysteine. Mass cultures were grown in the medium previously described (30) to which 2% glucose was added. A McIntosh anaerobic jar was used to obtain anaerobic conditions. The gas phase was N₂ plus CO₂ (95:5). Cultures were incubated at 37 C.

**Determination of fermentation balances.** The medium used had the following composition (per liter of water): tryptone (Oxoid), 10 g; yeast extract (Oxoid), 5 g; NaCl, 5 g; KH₂PO₄, 2 g; and K₂HPO₄, 3 g; the pH of the medium was 6.8. Exactly 10 μmoles of glucose, galactose, mannitol, or xylose, or 5 μmoles of lactose was added per ml of the medium. These concentrations were growth-limiting. When maximal optical density was reached, fermentation was assumed to be complete, and the culture was centrifuged. In the supernatant fluid, acetate, L(+)-lactate, ethyl alcohol, formate, pyruvate, and acetoin were determined. Except for glucose, residual substrate was not determined. Acetate was determined by means of the enzymatic method of Rose (20), which is based on the colorimetric determination of acetyl phosphate according to Lipmann and Tuttle (15). L(+)-Lactate, ethyl alcohol, and glucose were determined enzymatically by means of a Biochemistry Test Combination (C. F. Boehringer and Soehne GmbH, Mannheim, Germany). Formate was measured with formate dehydrogenase (EC 1.2.1.2) as described by Johnson et al. (11). Pyruvate was determined according to Friedemann and Haugen (8). Acetoin was determined according to Westerfeld (31). In all determinations, the medium was used as a blank. By adding known amounts of acetate, lactate, ethyl alcohol, formate, pyruvate, or glucose to the fermentation liquid, it was found that the medium did not influence the determination of these compounds. Intracellular carbohydrate was determined by the anthrone method (28) after the polysaccharides had been isolated from the cells as described by L. P. T. M. Zevenhuizen (Thesis, University of Amsterdam, Amsterdam, The Netherlands, 1966).

**Enzyme assays.** Cell-free extracts were prepared in 0.065 M potassium phosphate buffer (pH 6.8) to which 0.009 M reduced glutathione was added, as described previously (30). Protein was determined according to Lowry et al. (17).

Spectrophotometric assays were performed at 25 C in quartz cuvettes (1-cm light path) with an Unicam Sp 220 constant-wavelength scanner. The decrease or increase in absorbance was followed at 340 mÅ in the appropriate system.

The specific activity of lactate dehydrogenase and the influence of the concentration of fructose-1,6-diphosphate on the specific activity were determined as described previously (29). Mannitol kinase, mannitol-1-phosphate dehydrogenase (EC 1.1.1.17), and mannitol dehydrogenase (EC 1.1.1.67) were measured in a system containing, per 3 ml: potassium phosphate (pH 7.5), 190 μmoles; MgCl₂, 20 μmoles; nicotinamide adenine dinucleotide (NAD) or nicotinamide adenine dinucleotide phosphate (NADP), 0.6 μmole; adenosine triphosphate (ATP), 3 μmoles; mannitol or sorbitol, 5 μmoles; and cell-free extract, approximately 0.5 mg of protein. For the determination of mannitol dehydrogenase, ATP was omitted from the reaction mixture. Mannitol dehydrogenase and mannitol-1-phosphate dehydrogenase were also determined with fructose and fructose-6-phosphate, respectively, as substrates. In these instances, reduced NAD (NADH) was added instead of NAD. Controls to correct for NADH oxidase were included. Hexokinase (EC 2.7.1.1) and fructokinase (EC 2.7.1.4) were measured by the enzymatic method (23). Alcohol dehydrogenase was determined in a reaction mixture containing, per 3 ml: Na₃P₂O₇·10H₂O, 180 μmoles; semicarbazide hydrochloride, 180 μmoles; glucose, 52 μmoles; NaOH, 160 μmoles; NAD, 0.6 μ mole; ethyl alcohol, 300 μmoles; cell-free extract, 0.5 mg of protein (pH 8.7). Several attempts were made to show the phosphorolastic enzyme in cell-free extracts by measuring the formation of acetyl phosphate, acetate, and formate. The incubation mixture was the same as that described by Knappé et al. (14). However, 5'-adenosine-1-methyl-uracil was not tested as an activator. Instead, mixtures of L-methionine and ATP were used. In some experiments, fluoride and phosphate acetyltransferase (EC 2.3.1.8) were omitted from the reaction mixture.

**Determination of fructose-1,6-diphosphate.** Fructose-1,6-diphosphate was determined in cell suspensions of strain S 324 fermenting glucose or lactose. To a cell suspension (approximately 12 mg (dry weight) of bacteria per ml of 0.067 M sodium potassium phosphate buffer, pH 7.5) 50 μmoles of glucose or 25 μmoles of lactose was added per ml. Fermentation took place at 37 C under N₂ plus CO₂ (95:5). When the pH had lowered to 6.0, fermentation was stopped by keeping the reaction vessel in a water bath at 80 C for 5 min. Cells were extracted with perchloric acid (final concentration, 0.5 M) at 20 C for 30 min. After centrifugation, the supernatant fluid was neutralized with 5 m K₂CO₃. The precipitated KClO₄ was removed by centrifugation (0 C). In the supernatant fluid, fructose-1,6-diphosphate was determined enzymatically with aldolase (EC 4.1.2.13), fructose-6-phosphate isomerase (EC 5.3.1.1), and glycerol-3-phosphate dehydrogenase (EC 1.1.1.8) (3). Positive controls were run in parallel.

**Determination of molar growth yields.** Growth yields were determined in the medium described above, to which growth-limiting concentrations of glucose, lactose, galactose, or mannitol were added. Growth yields were measured by filtration, with the use of a Stefl filter apparatus and membrane filters (MF 30) of constant weight (Sartorius Membranfilters GmbH, Göttingen, Germany). When growth was complete (maximal optical density), the culture was centrifuged. The supernatant fluid was passed through the filter which had previously been dried and weighed. Then the sediment was transferred to the filter quantitatively. After washing with water, the filter was dried to constant weight at 105 C.

**Chemicals.** NAD, NADP, NADH, ATP, and enzymes used in the determination of fructose-1,6-diphosphate and hexokinase, were obtained from C. F. Boehringer and Soehne GmbH, Mannheim, Germany.
RESULTS

Fermentation balances. In Table 1, fermentation balances of strains of Bifidobacterium belonging to different types are shown. Products were acetate, L(+)-lactate, ethyl alcohol, and formate. The proportions of the fermentation products varied with the strain used and with the substrate used with a given strain. Acetoin and pyruvate were not detected. D(-)-Lactate was not determined because in previous experiments with resting cell suspensions it was shown that the amount of L(+)-lactate formed equaled the total amount of lactate formed (unpublished data). Previously, it was shown that bifidobacteria did not form CO₂ from sugars (29; unpublished data).

Breakdown of glucose. From Table 1, it can be seen that products formed from glucose were acetate, L(+)-lactate, ethyl alcohol, and formate. Therefore, it seems that pyruvate formed from glucose via the fructose-6-phosphate phosphoketolase route was partly reduced to lactate and was partly split to acetyl phosphate and formate. In the latter case, half of the amount of acetyl phosphate, formed from pyruvate, must be reduced to ethyl alcohol to regenerate NAD from NADH formed by glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.12). If the pyruvate was converted completely into acetate, ethyl alcohol, and formate, the fermentation balance would be

\[
\text{glucose} \rightarrow 2 \text{acetate} + 0.5 \text{ethyl alcohol} + \text{formate}
\]

Assuming \(x\) mole of formate is formed per mole of glucose, the theoretical balance should be

\[
\text{glucose} \rightarrow (1.5 + 0.5x) \text{acetate} + (1 - x) \text{lactate} + 0.5x \text{ethyl alcohol} + x \text{formate}
\]

The balance of strain S 324, which was the mean of seven determinations, fitted the equation rather well. The fermentation balance found in growing cultures of strain S 128 agreed with the fermentation balance previously found (29) in resting-cell suspensions. Only small amounts of ethyl alcohol and formate were formed. In accordance with the theoretical balance, more lactate and less acetate was formed. It is very peculiar that strain S 327 formed no lactate at all from glucose.

Attempts to show the phosphorolastic enzyme in cell-free extracts of glucose-grown cells of strain S 324 failed. In a resting-cell suspension of this strain, the phosphorolastic split was demonstrated clearly. The fermentation balance of pyruvate was as follows:

\[
\text{pyruvate} \rightarrow 0.88 \text{acetate} + 0.79 \text{formate}
\]

The C recovery was 85%.

Breakdown of mannitol. The fermentation balances of mannitol, determined for strain S 324, S 200, and S 332, agreed with each other. There were only differences in the amount of lactate formed. As expected, more ethyl alcohol was formed from mannitol than from glucose. The theoretical balance, which is based on the formation of \(x\) mole of formate per mole of mannitol, is as follows:

\[
\text{mannitol} \rightarrow (1 + 0.5x) \text{acetate} + (1 - x) \text{lactate} + (0.5 + 0.5x) \text{ethyl alcohol} + x \text{formate}
\]

Comparing the balances found with the theoretical balances, it can be seen that the amount of ethyl alcohol formed was too low. Possibly, evaporation of ethyl alcohol took place during the incubation period, which lasted 2 days.

Further investigation on the pathway of breakdown of mannitol was accomplished by enzyme determinations. In Table 2, the specific activities of polyol dehydrogenase, glucokinase, fructokinase, and alcohol dehydrogenase in cell-free extracts of glucose- and mannitol-grown cells of strain S 324 are shown. NAD-specific polyol dehydrogenase and fructokinase were demonstrated in mannitol-grown cells. Because the specific activity of NADH oxidase was very small compared with that of polyol dehydrogenase, no correction was made for NADH oxidase. The polyol dehydrogenase showed no activity with NADP. The specific activity of polyol dehydrogenase was about the same for mannitol and fructose. The specific activity for sorbitol was somewhat less. The specific activity of fructokinase was much higher in mannitol-grown cells than in glucose-grown cells. Mannitol kinase and mannitol-1-phosphate dehydrogenase were absent from strain S 324. It can be concluded that mannitol was converted to fructose-6-phosphate by an inducible mannitol dehydrogenase and fructokinase. The same was found for two other strains of Bifidobacterium.

The specific activity of alcohol dehydrogenase was higher in mannitol-grown cells than in glucose-grown cells. The induction of alcohol dehydrogenase seems to be dependent on the amount of NADH generated from the growth substrate.

Fermentation of lactose, galactose, and xylose. Fermentation balances of lactose were determined for strains S 324, S 200, and S 327 (Table 1). The fermentation of lactose was different from that of glucose. Strain S 324 formed only trace amounts of ethyl alcohol and formate from lactose. Instead, much more L(+)-lactate was formed from 1 mole of lactose than from 2 moles of glucose, galactose, or mannitol. Strain S 200
TABLE 1. Fermentation balances in growing cultures of *B. bifidum*

<table>
<thead>
<tr>
<th>Collection no.</th>
<th>Biochemical type according to Dehnert (6)</th>
<th>Substrate</th>
<th>Products</th>
<th>C recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S 324</td>
<td>3</td>
<td>Glucose (7)</td>
<td>Acetate</td>
<td>1.85 (0.05)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lactose (4)</td>
<td>1.30 (0.01)</td>
<td>1.54 (0.02)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Galactose</td>
<td>1.82</td>
<td>0.30</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mannitol (5)</td>
<td>1.35 (0.03)</td>
<td>0.33 (0.02)</td>
</tr>
<tr>
<td>S 200</td>
<td>5</td>
<td>Glucose</td>
<td>1.87</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lactose</td>
<td>3.50</td>
<td>1.11</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mannitol</td>
<td>1.40</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Xylose</td>
<td>1.58</td>
<td>0.09</td>
</tr>
<tr>
<td>S 128</td>
<td>5</td>
<td>Glucose</td>
<td>1.52</td>
<td>0.62</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Xylose</td>
<td>1.23</td>
<td>0.40</td>
</tr>
<tr>
<td>S 327</td>
<td>1 or 2</td>
<td>Glucose</td>
<td>2.04</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lactose (6)</td>
<td>3.15 (0.04)</td>
<td>0.63 (0.07)</td>
</tr>
<tr>
<td>S 337</td>
<td>4</td>
<td>Glucose</td>
<td>1.50</td>
<td>0.35</td>
</tr>
<tr>
<td>S 332</td>
<td>4</td>
<td>Mannitol</td>
<td>1.39</td>
<td>0.15</td>
</tr>
</tbody>
</table>

* Except where otherwise indicated, two determinations were made with different fermentation supernatant fluids, and the values represent the mean, which was very close to the individual values.

b Number of determinations is given in parentheses.

C Standard error of the mean is given in parentheses.

and S 327 also formed more lactate from lactose than from glucose.

The fermentation balance of galactose determined for strain S 324 agreed with that of glucose.

Strain S 128 formed more lactate and less formate and ethyl alcohol from xylose than strain S 200. Unlike growing cultures, resting-cell suspensions of strain S 128 formed no ethyl alcohol or formate from xylose; instead, they formed acetate and lactate exclusively (29).

Determination of lactate dehydrogenase, fructose-1,6-diphosphate, and pyruvate. It was found that lactate dehydrogenase of 17 *Bifidobacterium* strains studied had an absolute requirement for fructose-1,6-diphosphate. Specific activities, expressed as micromoles of NADH converted per milligram of protein per minute, varied from 1 to 5. It must be emphasized that all determinations were carried out once and that no attention was paid to the growth phase in which the cells were harvested. Therefore, it is uncertain whether the differences in specific activities found reflected real differences between the strains or were influenced by the growth phase in which the cells happened to be harvested. For strain S 324, it was found that fructose-1,6-diphosphate at a concentration of $4 \times 10^{-3}$ mmole per ml of the reaction mixture caused 50% of maximal activity.

TABLE 2. Specific activity of some enzymes in cell-free extracts of *B. bifidum* S 324 grown on glucose or mannitol

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Substrate used in the enzyme assay</th>
<th>Specific activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Glucose-grown cells</td>
<td>Mannitol-grown cells</td>
</tr>
<tr>
<td>Polyol dehydrogenase*</td>
<td>0</td>
<td>0.09</td>
</tr>
<tr>
<td>Polyol dehydrogenase*</td>
<td>0</td>
<td>0.03</td>
</tr>
<tr>
<td>Polyol dehydrogenase*</td>
<td>0</td>
<td>0.08</td>
</tr>
<tr>
<td>Kinase*</td>
<td>0.06</td>
<td>0.44</td>
</tr>
<tr>
<td>Kinase*</td>
<td>0.12</td>
<td>0.09</td>
</tr>
<tr>
<td>Alcohol dehydrogenase*</td>
<td>0.03</td>
<td>0.10</td>
</tr>
</tbody>
</table>

* Specific activity expressed as micromoles of NADH formed per milligram of protein per minute.

b Specific activity expressed as micromoles of NADH converted per milligram of protein per minute.

c Specific activity expressed as micromoles of reduced nicotinamide dinucleotide phosphate formed per milligram of protein per minute.

It might be possible that the differences between the fermentation balances of glucose and lactose could be correlated with the concentration of fructose-1,6-diphosphate and pyruvate in the
cells. Attempts to show fructose-1,6-diphosphate in strain S 324, growing with an excess of glucose or lactose, were unsuccessful. Probably trace amounts of fructose-1,6-diphosphate, present in the cells, were metabolized during the centrifugation procedure required to concentrate the cells into a small volume. In cell suspensions in which glucose or lactose were being fermented, trace amounts of fructose-1,6-diphosphate were present [0.0005 to 0.003 µmole per mg of bacteria (dry weight)]. Differences in fructose-1,6-diphosphate content between glucose- or lactose-fermenting cells could not be detected. However, it must be emphasized that the determination was not very accurate, because of the small amounts present. Pyruvate was determined in the supernatant fluids of cultures growing with an excess of glucose or lactose. The concentrations, expressed as micromoles of pyruvate per milliliter of supernatant fluid, were 0.60 ± 0.08 (10 determinations) or 0.29 ± 0.05 (8 determinations), respectively.

**Growth yields.** Strain S 324 was selected for the determination of molar growth yields of lactose, galactose, and mannitol (Table 3). The growth yield per mole of glucose was 37.4. According to Table 1, 1.85 moles of acetate was formed from 1 mole of glucose. The fructose-6-phosphate phosphoketolase route yielded 1.5 moles of acetate and 2.5 moles of ATP. Thus, 0.35 mole of acetate was formed by the phosphoroclastic reaction, yielding 0.35 mole of ATP. The value of Y (ATP), defined by Bauchop and Elden (2) as grams (dry weight) of cells formed per mole of ATP, was 13.1. In the same way, Y (ATP) was calculated for lactose, galactose, and mannitol. Previously (unpublished data), it was found that lactose is split to galactose and glucose by hydrolysis, and not by phosphorolysis. The high molar growth yield for glucose cannot be explained by formation of internal polysaccharides from glucose. Not more than 1% internal polysaccharides was shown to be present in this strain when it was growing with an excess of glucose.

**DISCUSSION**

In the present work, fermentation balances of glucose, lactose, galactose, mannitol, and xylose were determined in growing cultures of bifidobacteria. C recoveries were between 81 and 97%. Except for glucose, residual substrate was not determined. Mostly, glucose was fermented completely. For one strain (S 327), the amount of residual glucose was 17% of the amount of glucose added. In this case, a correction was made for residual glucose. In calculating the balances of the other substrates, it was assumed that they had been fermented completely. However, the possibility of incomplete substrate utilization cannot be excluded, because some strains grew very slowly with limiting concentrations of sugars, and cells might have been harvested before the point of maximal growth. Possibly, this factor explains the low C recovery obtained for strain S 327 growing on lactose. Other factors, which could have led to low C recoveries, might be the formation of a few other products, such as mannitol, glycerol, or succinate, and ethyl alcohol volatility.

From the results presented in this paper, it can be concluded that bifidobacteria convert pyruvate, formed as an intermediate in the fructose-6-phosphate phosphoketolase route, by two paths. The first path is a reduction of pyruvate to L(+)-lactate. L(+)-Lactate dehydrogenase of all strains studied specifically requires fructose-1,6-diphosphate for activity, as was found previously for Streptococcus bovis by Wolin (33). The second path is a cleavage of pyruvate into acetol phosphate and formate by the phosphoroclastic enzyme. Part of acetol phosphate formed is reduced to ethyl alcohol. Previously, Dittman (7) reported the formation of ethyl alcohol from sugars by bifidobacteria. However, he did not test for the presence of formate.

The amount of pyruvate converted via either pathway varies with the strain used. The reduction of pyruvate to lactate seems to be dominant in strain S 128. On the other hand, strain S 327 does not form lactate from glucose. In the other strains studied, both routes are involved in the conversion of pyruvate. The amount of pyruvate converted via either pathway turns out to be influenced by the growth substrate. For instance, three strains studied form much more L(+)-lactate from lactose than from glucose. Three factors which may influence the choice between the two pathways can be mentioned. The first is the relative amount of the phosphoroclastic enzyme and lactate de-
hydrogenase. This factor cannot be tested, because all attempts to show the phosphoroclastic enzyme in cell-free extracts failed. Most probably, this enzyme is very unstable and is inactivated during the preparation of the cell-free extract. A second factor, the amount of fructose-1,6-diphosphate present in the cell, might explain why strain S 327 does not form lactate from glucose. This strain grows very slowly on glucose, and the intracellular concentrations of fructose-6-phosphate and ATP may remain too low to form the concentration of fructose-1,6-diphosphate required to activate lactate dehydrogenase. For strain S 324, no difference in fructose-1,6-diphosphate content between lactose- and glucose-fermenting cell suspensions was found, although more lactate was formed from lactose than from glucose. In both cell suspensions, trace amounts of fructose-1,6-diphosphate were present. Assuming that the water content of bifidobacteria is 80% and the density is 1.3, the amounts of fructose-1,6-diphosphate vary from 0.1 to 0.5 μmole per ml of undried bacteria. Comparison of this value with the amount of fructose-1,6-diphosphate (4 × 10^-3 μmole per ml of reaction mixture) required to yield 50% of maximal activity of lactate dehydrogenase makes it evident that the low concentration of fructose-1,6-diphosphate found in the cells is sufficient to account for activation of lactate dehydrogenase. A third factor which might regulate the conversion of pyruvate could be a different affinity of lactate dehydrogenase and the phosphoroclastic enzyme toward pyruvate. In cultures of strain S 324 growing with glucose, the stationary concentration of pyruvate was about twice as large as that in cultures growing with lactose. The difference between the fermentation balances of glucose and lactose found for this strain might be explained by supposing that the phosphoroclastic enzyme has a lower affinity for pyruvate than does lactate dehydrogenase.

Two pathways for converting mannitol are known for bacteria. Escherichia coli, Aerobacter aerogenes, Bacillus subtilis, Staphylococcus aureus, and L. plantarum convert mannitol to fructose-6-phosphate by means of mannitol phosphotransferase, utilizing ATP or phosphoenolpyruvate as a phosphate donor, and mannitol-1-phosphate dehydrogenase (4, 5, 10, 13, 16, 26, 27, 32). The conversion of mannitol to fructose by a dehydrogenase has been described for L. brevis (19), Gluconobacter oxydans (12, 24), Acetobacter suboxydans (1), Azotobacter agilis (18), and Pseudomonas fluorescens (22). Mostly, the dehydrogenase is a polyol dehydrogenase which converts both mannitol and sorbitol. In this paper, it is shown that the first steps in the degradation of mannitol by bifidobacteria are dehydrogenation to fructose by an inducible, NAD-specific polyol dehydrogenase and subsequent phosphorylation of fructose to fructose-6-phosphate by an inducible fructokinase.

Bauchop and Elsdon (2) defined Y (ATP) as grams (dry weight) of cells formed per mole of ATP. They found Y (ATP) = 10 for Strep tococcus faecalis and Saccharomyces cerevisiae. Other investigators (9, 25) found about the same value for several other bacteria. It was suggested that Y (ATP) is a constant for different bacteria. In the present investigation, molar growth yields of glucose, lactose, galactose, and mannitol were determined for one strain of Bifidobacterium. From the molar growth yields and fermentation balances, Y (ATP) was calculated. The values of Y (ATP) were 13.1, 10.4, 9.9, and 11.8 for glucose, lactose, galactose, and mannitol, respectively. The mean value of Y (ATP) is 11.3 ± 0.7 (4x). This value agrees with the value of 10 initially reported by Bauchop and Elsdon (2) and since found for several other bacteria.

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LITERATURE CITED