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Futile Xylitol Cycle in *Lactobacillus casei*

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A futile xylitol cycle appears to be responsible for xylitol-mediated inhibition of growth of *Lactobacillus casei* Cl-16 at the expense of ribitol. The gratuitously induced xylitol-specific phosphoenolpyruvate-dependent phosphotransferase accumulates the pentitol as xylitol-5-phosphate, a phosphatase cleaves the latter, and an export system expels the xylitol. Operation of the cycle rapidly dissipates the ribitol-5-phosphate pool (and ultimately the energy supply of the cell), thereby producing bacteriostasis.

Those strains of *Lactobacillus casei* capable of growing at the expense of ribitol (rtl) are sensitive to xylitol (xtl) when the former is used as a source of energy (7). Previous studies have shown that growth at the expense of rtl results in the coincidental induction of an xtl-specific phosphoenolpyruvate (PEP)-dependent phosphotransferase system (PTS). The xtl PTS transports xtl into the cell to form xylitol-5-phosphate (xtl-5-P); however, the absence of an xtl-5-P dehydrogenase prevents the further metabolism of this intermediate, and it accumulates in the cell (7). Depending upon its external concentration, xtl can either completely inhibit growth (at >5 mM) or merely increase the generation time of the organism (at <2 mM). Although much is known about the metabolic events that occur during the xtl-mediated inhibition of *L. casei*, the actual mechanism responsible for producing the bacteriostatic effects is unknown. Thompson and Chassy (15) demonstrated that the inhibition of growth by 2-deoxy-D-glucose (2-DG) in cultures of *Streptococcus lactis* 133 growing on lactose or sucrose was due to the initiation and maintenance of a futile cycle. The 2-DG cycle catalyzed the import and export of the nonmetabolizable hexose at the expense of the energy reserves (PEP) of the cells, thereby inhibiting growth.

In this report, we demonstrate that the bacteriostatic effects of xtl are probably due to the futile cycling of the nonmetabolizable pentitol which (like the 2-DG-mediated inhibition) dissipates the energy reserves of *L. casei* Cl-16.

MATERIALS AND METHODS

Cultivation and maintenance of *L. casei* Cl-16. *L. casei* Cl-16 was obtained from the private collection of M. Rogosa, National Institute of Dental Research, Bethesda, Md. The organism was maintained and stored in fortified litmus milk medium (9). Lactobacillus-carrying medium (5) was used for all growth experiments and to prepare washed suspensions for resting-cell studies. All substrates were filter sterilized, and the microorganism was growth at 37°C in static culture.

Growth experiments. The fate of the metabolic intermediate products of rtl before and after the addition of xtl was monitored by periodic sampling of an exponential-phase culture. Replicate sterile screw-capped test tubes were filled with 15 ml of filter-sterilized lactobacillus-carrying medium containing 30 mM [1-^{14}C]rtl (specific activity, 1 μCi/μmol) and was adjusted to a density of 100 to 110 Klett units (660-nm filter) with a washed-cell suspension harvested from a mid-log-phase culture. Samples of 15 ml taken before and after the addition of 5 mM xtl were injected into 20 ml of distilled ice water and filtered through a 0.45-μm HA membrane (47-mm diameter; Millipore Corp., Bedford, Mass.). Cells retained on the filter were washed twice with 5 ml of cold (4°C) 0.05 M potassium phosphate buffer (pH 7.0 containing 0.01 M $MgCl_2$), immersed in 7 ml of boiling distilled water for 5 min, and cooled in ice, and the extracted components were separated from the cells by centrifugation for 30 min at 18,000 × *g*. Extracts were lyophilized and stored at −20°C until required for analysis.

Where the intracellular levels of xtl were monitored during growth at the expense of rtl, a similar procedure was followed up to the sampling step. After the addition of [1-^{14}C]xtl to a final concentration of 5 mM (specific activity, 1.2 μCi/μmol), 0.5 ml of culture was removed, filtered through a 0.45-μm HA membrane (22-mm diameter), and washed with 4 ml of 0.1 M potassium phosphate buffer (pH 7.0). The filters were dried and placed into glass vials containing 5 ml of Hydrofluor, and the radioactivity in the cells was measured with a Beckman model LS 6800 scintillation counter. To identify the xtl intermediate, larger quantities (1 to 2 ml of cell suspension) were treated as described above. The calculation of the concentration of intracellular metabolites was based on the amount of isotope extracted from 1 mg (dry weight) of cells and the specific activity of the substrate, assuming a volume of 2 ml/g (dry weight) of cells (determined by Mizushima et al. [10] for *Lactobacillus plantarum*).

Resting-cell transport studies. The procedure of Chassy and Thompson (1) was used to prepare cell suspensions harvested from mid-log-phase cultures for transport studies. Transport assays were performed in accordance with a procedure described by Thompson (14). The technique differed only in that [^{14}C]xtl or [^{14}C]rtl was used as substrate at concentrations between 0.5 and 5 mM (specific activity, 0.2 μCi/μmol).

Identification of intermediate products of pentitol utilization. Radiolabeled intermediate products present in lyophilized extracts of growing or resting cells were made up to a volume of 100 μl with distilled water (20,000 to 40,000 cpm/10 μl). Components in the extracts were separated by polyethyleneimine-cellulose thin-layer chromatography or descending-paper chromatography (Whatman no. 3) by previously published procedures (14). The intermediate prod-

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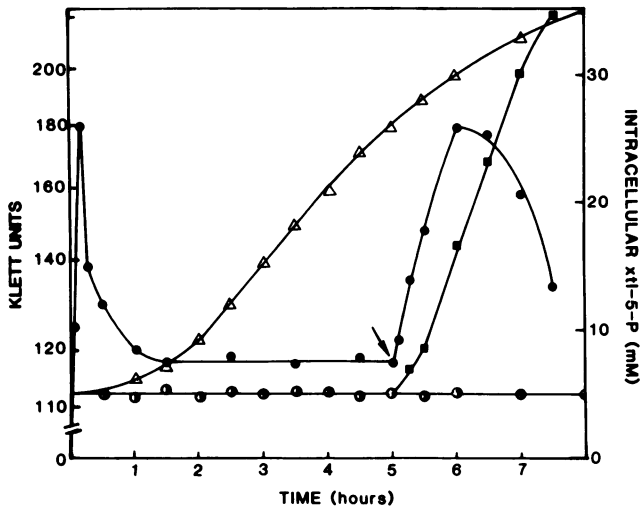

FIG. 1. Accumulation and expulsion of [^{14}C]xtl during the xtl-mediated inhibition of growth at the expense of rtl. At time zero, 5 mM xtl was added to one of several replicate mid-log-phase cultures of *L. casei* Cl-16 growing on rtl. Optical density of cultures are given in Klett units. Symbols: △, control culture growing on rtl; ◑, culture receiving 5 mM xtl; ■, culture receiving 10 mM glucose (arrow); ●, intracellular level of [^{14}C]xtl.

ucts were identified by cochromatography with the appropriate standards.

Phosphatase assay. Phosphatase activity was measured in cell extracts prepared by treating 2 to 4 g (wet weight) of cells suspended in 0.02 M MES [2-(*N*-morpholino) ethanesulfonic acid] (pH 6.0) containing 5 mM $MgCl_2$ and 10 mM β-mercaptoethanol with a Branson model 185 sonifier operating at 70% of total power output. Two treatments of 6-min duration were used to break the cells; the suspension was maintained at 4°C during the operation. After an initial centrifugation at 18,000 × *g* for 15 min to remove intact cells, the resultant supernatants were centrifuged at 190,000 × *g* for 90 min to separate the cytosol from the membrane fraction. Both fractions were tested for phosphatase activity with 2-deoxy-D-glucose-6-phosphate (2DG-6P), mannose-6-phosphate, fructose-1-phosphate, and xtl-5-P as substrates in the colorimetric phosphate assay of Horder (5). The composition of the reaction mixture was that described by Thompson and Chassy (16). Assays were conducted for 60 min, during which time reaction rates were linear and proportional to the protein concentration.

Protein concentrations in cell extracts or membrane preparations were measured by the Bio-Rad (Bio-Rad Laboratories, Richmond, Calif.) or biuret technique (3).

RESULTS

Uptake of [^{14}C]xtl during growth on rtl. The effect of the addition of 5 mM [^{14}C]xtl to a mid-exponential-phase culture of *L. casei* Cl-16 was twofold: (i) a rapid, initial uptake of the radiolabeled pentitol and (ii) an immediate cessation of growth (Fig. 1). Within 5 min, the intracellular pool of xtl reached a concentration of 26 mM; the pool size ultimately stabilized at a concentration of 8 mM. The addition of 10 mM glucose 5 h after the cessation of growth produced an immediate resumption of growth as seen in the increase in optical density. Thus, a major portion of the cells in the population apparently had retained viability during the period of bacteriostasis. Coincident with the resumption of growth, xtl import occurred, peaking at an intracellular concentration of 26 mM. This intracellular pool of xtl-5-P was maintained for 30 min but decreased markedly before the entry of the culture into the stationary phase of growth. Despite the rapid onset of exponential growth in the presence of glucose, the culture only achieved a doubling time of 150 min, a rate that is 80 to 90 min greater than the normal generation time. The cause of this protracted generation time and the fluctuations in the pool size of xtl-5-P will be discussed later.

Uptake and expulsion of [^{14}C]xtl by *L. casei* C1-16. Resting cells harvested from exponential-phase cultures growing at the expense of 30 mM rtl accumulated [^{14}C]xtl (5 mM) as xtl-5-P to a concentration of 80 to 100 mM over a 15-min period (Fig. 2A). The addition of 10 mM glucose to the cell suspension produced a rapid expulsion of xtl-5-P, reducing the internal concentration of the intermediate product to a

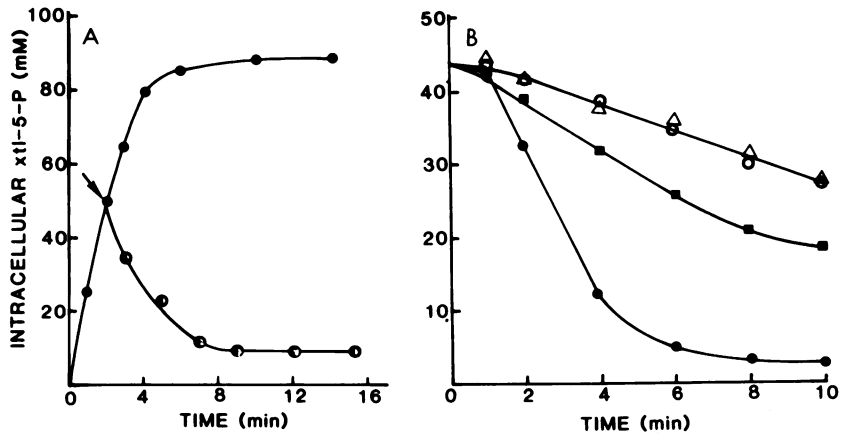

FIG. 2. Uptake and expulsion of [^{14}C]xtl by resting cells of *L. casei* Cl-16. (A) Kinetics of xtl uptake reported as the intracellular concentration of xtl-5-P. The arrow indicates the point at which 10 mM glucose was added to a duplicate flask. Symbols: ●, control (no glucose); ◑, 10 mM glucose. (B) Expulsion of xtl elicited by the addition of glucose or rtl to resting cells preloaded with [^{14}C]xtl-5-P. Symbols: ○, control (no substrate added); △, 10 mM 2-DG; ■, 10 mM rtl; ●, 10 mM glucose.

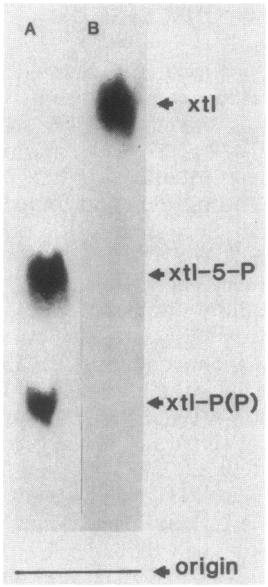

FIG. 3. Fluorographic identification of the intracellular xtl derivative and the expulsion product by paper chromatography. Lanes: A, boiling-water extract of resting cells incubated with [^{14}C]xtl; B, concentrate of extracellular medium after glucose-induced expulsion of the intracellular [^{14}C]xtl-5-P.

steady-state value of between 8 and 10 mM. At external concentrations of 0.5 mM xtl (a value near the K_m of the xtl PTS), the pentitol accumulates to an internal level 10-fold lower, i.e., 8 mM (data not shown).

To study the expulsion process, resting cells were preloaded with [^{14}C]xtl-5-P and exposed to the following compounds at a concentration of 1 or 10 mM; glucose, rtl, galactose, and 2-DG. Only glucose and rtl mediated the expulsion of [^{14}C]xtl-5-P, and the efflux rate with glucose was fourfold greater than that of rtl (Fig. 2B). Galactose did not cause the expulsion of radiolabeled xtl-5-P because the rtl-grown cells were not induced for the galactose PTS and the substrate could not be transported or metabolized by the cells (unpublished data). The nonmetabolizable glucose analog 2-DG is transported rapidly into resting cells of *L. casei* C1-16 by the glucose PTS to form 2DG-6P (data not shown). However, 2DG-6-P (like xtl-5-P) is a dead-end product and cannot generate energy for expulsion. To test whether energy production was a prerequisite for expulsion, cells preloaded with [^{14}C]xtl were preincubated with 5 mM iodoacetic acid, an inhibitor of glyceraldehyde-3-phosphate dehydrogenase (14), and exposed to 10 mM rtl or glucose. Under these conditions, neither substrate stimulated expulsion of radiolabeled xtl-5-P (data not shown). Since all energy derived from glycolysis occurs after oxidation of glyceraldehyde-3-phosphate, the expulsion of xtl appears to be an energy-dependent process.

Identification of intermediate products of xtl and rtl uptake. Polyethyleneimine-cellulose thin-layer chromatographic and fluorographic analyses of the contents of cells preloaded with [^{14}C]xtl, and the expulsion product in the medium, revealed that preloaded cells contained two radioactive components which migrated with R_fs of 0.64 and 0.15, respectively (data not shown). The more mobile compound, which constituted more than 90% of the total radioactivity applied to the plate, comigrated with the xtl-5-P standard. Analysis of the buffered reaction mixture after expulsion of the radioactive xtl intermediate revealed only a single radioactive substance, with a migration rate identical to the [^{14}C]xtl standard. Identification of xtl-5-P as the major intermediate product confirmed our earlier observations (7). Solutions of the intermediate products of [^{14}C]xtl uptake and expulsion were further concentrated and applied to paper chromatograms. Only xtl was present as the expulsion product (Fig. 3). Again, the more mobile component extracted from preloaded cells migrated with the xtl-5-P standard, and the less mobile material trailed the ribulose-1,5-diphosphate standard. Treating eluates of both materials with commercial alkaline phosphatase yielded products that migrated with the xtl standard. The chemical structure of this minor component is, as yet, unknown. However, it may be a diphosphate ester of xtl.

When strains of *L. casei* are grown at the expense of a hexose, several intermediate products, including fructose-1,6-diphosphate, triose phosphate, and PEP, are maintained at relatively high levels (1, 2). When [^{14}C]rtl was substituted for glucose as the energy source for growth, the pattern of intracellular metabolites changed dramatically (Fig. 4). Separation by thin-layer chromatography showed that only one major product (rtl-5-P) was detected, and triose phosphate and PEP were present at low levels. Repeating the separation by paper chromatography, which readily separates rtl-5-P from other intermediate products, yielded identical results (data not shown). Pool sizes of the intermediate products of rtl metabolism were estimated by analyzing replicate cultures before or shortly after the addition of the xtl (Fig. 4). During exponential growth on rtl, cells contained concentrations of rtl-5-P, triose phosphate, and PEP of 110, 4, and 2.7 mM, respectively. After the addition of xtl, the concentrations of these metabolites decreased to 9, 2.5, and 2.3

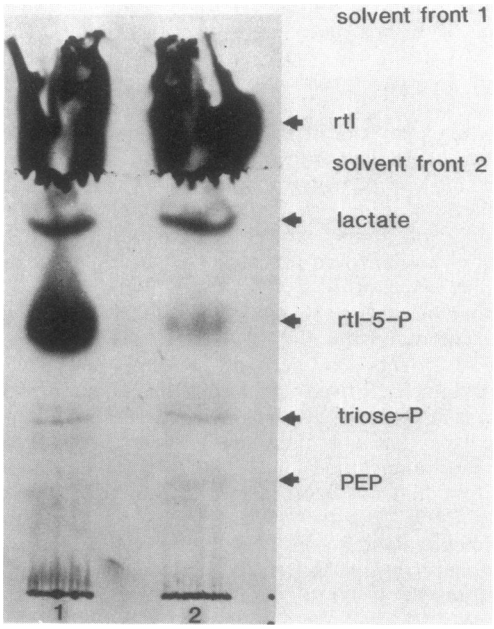

FIG. 4. Changes in the levels of intermediate products of [^{14}C]rtl fermentation produced by the addition of xtl. Lanes 1, intermediate product profile 10 min before the addition of xtl; 2, profile 10 min after the addition of 5 mM xtl.

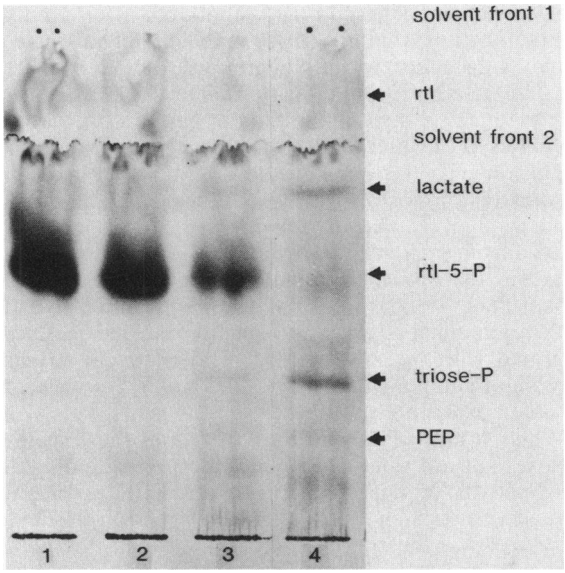

FIG. 5. Temporal sequence showing dissipation of intracellular pools of rtl-derived intermediates by incubating resting cells at 30°C. Lanes: 1, time zero; 2, 15 min; 3, 30 min; 4, 60 min.

mM, respectively. The sudden decrease in the metabolite pools should not be interpreted to mean that the cells have stopped metabolizing rtl after the addition of xtl. It was demonstrated previously that rtl continues to be transported and metabolized in the presence of xtl (7). Thus, in both resting and growing cells, the energy reserve that drives the initial uptake of xtl appears to be the pool of rtl-5-P. Indeed, resting cells appear to exchange the rtl-5-P reserve (at roughly 110 mM) for a pool of xtl-5-P (80 mM).

The stability of the rtl-5-P pool in resting suspensions of *L. casei* Cl-16 was determined by incubating cells grown on [^{14}C]rtl at 30°C and measuring the intracellular concentrations of [^{14}C]rtl-5-P over the course of time. In contrast to strains of *S. lactis* which maintain intracellular energy reserves for hours (18, 19) at ambient temperatures, *L. casei* Cl-16 depletes its pool of rtl-5-P within 1 h at 30°C (Fig. 5). Although the increase in the levels of triose phosphate and PEP partially compensated for the loss of the rtl-5-P pool, it represented less than 10% of the initial levels of radioactive intermediate products. To avoid losses such as these, cell suspensions were stored in an ice bath at 0°C until required for transport studies.

Detection of xtl-5-P phosphatase activity in *L. casei* cell extracts. The expulsion of 2-DG from *S. lactis* cells required that 2DG-6-P (the dead-end intermediate product) be dephosphorylated before export across the cell membrane (15). This was accomplished by a hexose phosphate:phosphohydrolase that exhibited the highest level of catalytic activity with galactose-6-phosphate and 2DG-6-P (16); the phosphatase had not been tested, at that time, for activity with xtl-5-P. Cell extracts of *L. casei* Cl-16 were tested for a similar phosphatase activity. Cells harvested from cultures grown on either glucose or rtl were used to prepare the extracts, and these were examined for xtl-5-P phosphatase activity. Xtl-5-P phosphatase activity was detected in both extracts, but the preparation from rtl-grown cells contained a level of activity 3.5-fold greater than that found in glucose-grown cells (data not shown).

DISCUSSION

In previous publications, the xtl-mediated inhibition of growth of *L. casei* at the expense of rtl was shown to involve the uptake of xtl by a specific PTS and its intracellular accumulation as xtl-5-P (7). Growth and resting cell experiments reported here established that *L. casei* Cl-16 is capable of both importing and exporting xtl (Fig. 2) and that the two processes require energy for optimum efficiency. The PEP-dependent xtl PTS functions ultimately at the expense of the primary energy reserve of the cells (rtl-5-P), and the latter is rapidly dissipated when xtl is added to a growing-culture or resting-cell suspension of *L. casei* C1-16. Similarly, the rapid expulsion of xtl also appears to require a source of energy because resting cells preloaded with [^{14}C]xtl export the pentitol at a significant rate only when provided with a metabolizable substrate. In the presence of a nonmetabolizable substrate, e.g., 2-DG or an inhibitor of glycolysis such as iodoacetic acid (IAA) (which blocks sugar or pentitol metabolism before ATP and PEP production), the rate of xtl efflux was no greater than that observed with the control. The ability of *L. casei* Cl-16 to (i) import xtl via a specific PTS, (ii) dephosphorylate the transport product to xtl, and (iii) expel free xtl provides this organism with all of the components necessary to drive a cyclic mechanism that pumps xtl into and out of the cell. Thus, the potential exists for the cell to operate an energetically wasteful futile cycle during growth on pentoses or pentitols upon addition of xtl to the medium. Figure 6 summarizes how the futile xtl cycle might operate in conjunction with rtl metabolism and bears a number of similarities to the futile 2-DG cycle (15).

The question of whether the futile xtl cycle is actually responsible for the inhibition of growth on rtl requires further discussion. Although growing cells accumulate significantly less xtl-5-P (26 mM) than resting cells (80 mM), the former level may be sufficient to inhibit directly some essential step in rtl metabolism. Evidence from experiments presented here argues against that possibility. First, it was demonstrated that the expulsion of xtl from the 60 mM pool of xtl-5-P is accelerated by the addition of a metabolizable substrate (Fig. 2A). If xtl-5-P inhibited rtl metabolism, the latter could not have provided energy for the expulsion of xtl. Secondly, mid-log cultures of *L. casei* Cl-16 respond immediately to the addition of xtl and stop growing, yet expulsion of xtl continues for 1 h after growth has ceased. Since the cells do not synthesize storage products from rtl (unpublished data), energy for expulsion can only be derived from rtl metabolism. On the other hand, the operation of a futile cycle would divert only a portion of the energy supplies of the cells, and although it might prevent growth from occurring, rtl metabolism could continue at a level sufficient to meet the maintenance requirements of the organisms. This would explain how the cells continue to maintain a constant intracellular level of 8 mM xtl-5-P during the 5-h stationary period (Fig. 1) in the growth experiment and why the cells remained poised and capable of growth the moment glucose was added to the culture. The differential responses between glucose and rtl-mediated growth to xtl probably reflect the relative efficiencies with which the two substrates are metabolized to produce energy.

The portion of the futile cycle responsible for the uptake of xtl has been well characterized (6–8); however, little is known about the phosphatase and the xtl export system. Cell extracts possess an activity which causes the hydrolysis of xtl-5-P to xtl and P_i. This enzyme is presently being purified, and preliminary findings suggest that the phospha-

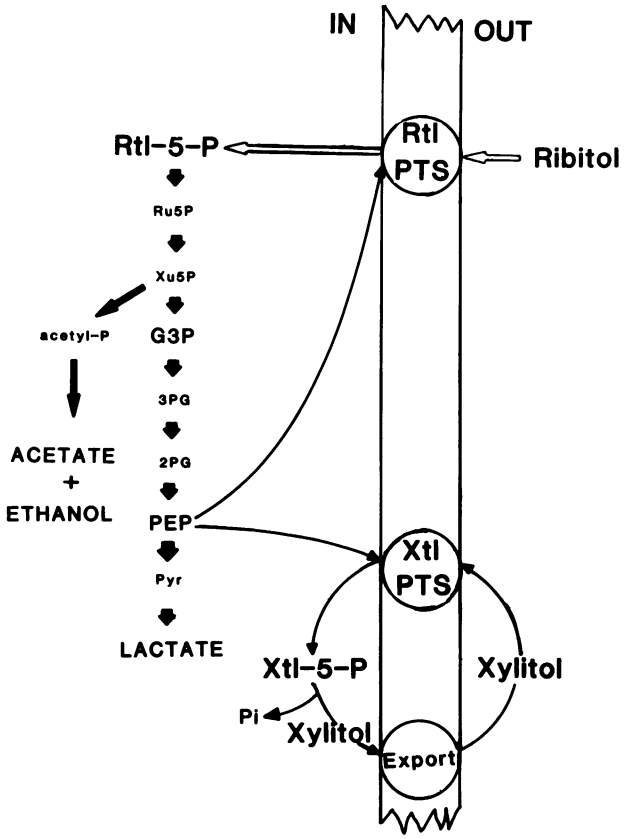

FIG. 6. Proposed operation of the three-step futile xtl cycle. The size of the lettering indicates the relative concentrations of the intracellular metabolite pools during rtl fermentation.

tase is a hydrophobic protein which may function near, or may be associated with, the cytoplasmic membrane (unpublished data). Exit of xtl is probably mediated by enzyme II^{Xtl} because this represents the only known transport system for this pentitol in *L. casei* Cl-16 (7).

After the accumulation of rtl-5-P by *L. casei* Cl-16 cells, the intracellular derivative was dephosphorylated, and efflux of free pentitol was observed (Fig. 2B). Furthermore, the rate of xtl expulsion from the cells was markedly stimulated by the addition of a metabolizable energy source (e.g., glucose, rtl) to the cell suspension. As mentioned earlier, this stimulation of xtl expulsion was abolished by metabolic inhibitors, such as IAA, and no stimulation of expulsion occurred in the presence of nonmetabolizable sugar analogs (e.g., 2-DG or 6-deoxyglucose [data not shown]). A striking parallel exists between the dephosphorylation and accelerated expulsion of the nonmetabolizable pentitol from *L. casei* and the previously described expulsion of the nonmetabolizable hexose-6-phosphate (methyl-β-D-thiogalactopyranoside-6-phosphate) from cells of lactic acid bacteria (12, 13, 17). Studies by Reizer et al. (11) and Thompson and Saier (17) suggest that energy (possibly ATP) and certain glycolytic intermediate products are involved in the increased rate of dephosphorylation and expulsion of thiomethylglucoside-6-phosphate in *Streptococcus pyogenes* and *S. lactis*, respectively. The xtl expulsion system of *L. casei* may operate in the same fashion.

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LITERATURE CITED

1. **Chassy, B. M., and J. Thompson.** 1983. Regulation of lactose-phosphoenolpyruvate-dependent phosphotransferase system and β-D-phosphogalactoside galactohydrolase activities in *Lactobacillus casei*. J. Bacteriol. **154**:1195–1203.
2. **Chassy, B. M., and J. Thompson.** 1983. Regulation and characterization of the galactose-phosphoenolpyruvate-dependent phosphotransferase system in *Lactobacillus casei*. J. Bacteriol. **154**:1204–1214.
3. **Gornall, A. G., C. J. Bardawill, and M. M. David.** 1949. Determination of serum proteins by means of the biuret reaction. J. Biol. Chem. **177**:751–766.
4. **Hansen, P. A. (ed.).** 1968. Type strains of *Lactobacillus* sp., p. 16. American Type Culture Collection, Rockville, Md.
5. **Horder, M.** 1972. Colorimetric determination of orthophosphate in the assay of inorganic pyrophosphatase activity. Anal. Biochem. **49**:37–47.
6. **London, J., and N. M. Chace.** 1979. Pentitol metabolism in *Lactobacillus casei*. J. Bacteriol. **140**:949–954.
7. **London, J., and S. Hausman.** 1982. Xylitol-mediated transient inhibition of ribitol utilization by *Lactobacillus casei*. J. Bacteriol. **150**:657–661.
8. **London, J., and S. Z. Hausman.** 1983. Purification and characterization of the III^{Xtl} phospho-carrier protein of the phosphoenolpyruvate-dependent xylitol:phosphotransferase found in *Lactobacillus casei* Cl83. J. Bacteriol. **156**:611–619.
9. **London, J., and K. Kline.** 1973. Aldolase of lactic acid bacteria: a case history in the use of an enzyme as an evolutionary marker. Bacteriol. Rev. **37**:453–478.
10. **Mizushima, S., Y. Machida, and K. Kitahara.** 1963. Quantitative studies on glycolytic enzymes in *Lactobacillus plantarum*. I. Concentration of inorganic ions and coenzymes in fermenting cells. J. Bacteriol. **86**:1295–1300.
11. **Reizer, J., M. J. Novotny, C. Panos, and M. H. Saier, Jr.** 1983. Mechanism of inducer expulsion in *Streptococcus pyogenes*: a two-step process activated by ATP. J. Bacteriol. **156**:354–361.
12. **Reizer, J., and C. Panos.** 1980. Regulation of β-galactoside phosphate accumulation in *Streptococcus pyogenes* by an expulsion mechanism. Proc. Natl. Acad. Sci. U.S.A. **77**:5497–5501.
13. **Reizer, J., and M. H. Saier, Jr.** 1983. Involvement of lactose enzyme II of the phosphotransferase system in rapid expulsion of free galactosides from *Streptococcus pyogenes*. J. Bacteriol. **156**:236–242.
14. **Thompson, J.** 1978. In vivo regulation of glycolysis and characterization of sugar:phosphotransferase systems in *Streptococcus lactis*. J. Bacteriol. **136**:465–476.
15. **Thompson, J., and B. M. Chassy.** 1982. Novel phosphoenolpyruvate-dependent futile cycle in *Streptococcus lactis*: 2-deoxy-D-glucose uncouples energy production from growth. J. Bacteriol. **151**:1454–1465.
16. **Thompson, J., and B. M. Chassy.** 1983. Intracellular hexose-6-phosphate:phosphohydrolase from *Streptococcus lactis*: purification, properties, and function. J. Bacteriol. **156**:70–80.
17. **Thompson, J., and M. H. Saier, Jr.** 1981. Regulation of methyl-β-D-thiogalactopyranoside-6-phosphate accumulation in *Streptococcus lactis* by exclusion and expulsion mechanisms. J. Bacteriol. **146**:885–894.
18. **Thompson, J., and T. D. Thomas.** 1977. Phosphoenolpyruvate and 2-phosphoglycerate: endogenous energy source(s) for sugar accumulation by starved cells of *Streptococcus lactis*. J. Bacteriol. **130**:583–595.
19. **Thompson, J., and D. A. Torchia.** 1984. Use of ^{31}P nuclear magnetic resonance spectroscopy and ^{14}C fluorography in studies of glycolysis and regulation of pyruvate kinase in *Streptococcus lactis*. J. Bacteriol. **158**:791–800.