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Correlation between Depression of Catabolite Control of Xylose Metabolism and a Defect in the Phosphoenolpyruvate:Mannose Phosphotransferase System in *Pediococcus halophilus*

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Pediococcus halophilus **X-160 which lacks catabolite control by glucose was isolated from nature (soy moromi mash). Wild-type strains, in xylose-glucose medium, utilized glucose preferentially over xylose and showed diauxic growth. With wild-type strain I-13, xylose isomerase activity was not induced until glucose was consumed from the medium. Strain X-160, however, utilized xylose concurrently with glucose and did not show diauxic growth. In this strain, xylose isomerase was induced even in the presence of glucose. Glucose transport activity in intact cells of strain X-160 was <10% of that assayed in strain I-13. Determinations of glycolytic enzymes did not show any difference responsible for the unique behavior of strain X-160, but the rate of glucose-6-phosphate formation with phosphoenolpyruvate (PEP) as a phosphoryl donor in permeabilized cells was <10% of that observed in the wild type. Starved *P. halophilus* I-13 cells contained the glycolytic intermediates 3-phosphoglycerate, 2-phosphoglycerate, and PEP (PEP pool). These were consumed concomitantly with glucose or 2-deoxyglucose uptake but were not consumed with xylose uptake. The glucose transport system in *P. halophilus* was identified as a PEP:mannose phosphotransferase system on the basis of the substrate specificity of PEP pool-starved cells. It is concluded that, in *P. halophilus*, this system is functional as a main glucose transport system and that defects in this system may be responsible for the depression of glucose-mediated catabolite control.**

Pediococcus halophilus (soy pediococci), the group of gram-positive halophilic lactic acid bacteria occurring numerously in soy sauce moromi mash, were found to comprise a large number of strains heterogeneous in carbohydrate-fermenting ability (40).

Soy pediococci are industrially important microorganisms by virtue of their ability to ferment glucose in homo-lactic fashion to lactic acid (7, 8). At the earlier stage of fermentation, in which soy pediococci grow, soy moromi mash contains usually up to 400 mM of glucose, in addition to 30 mM of galactose, 30 mM of arabinose, and 20 mM of xylose as sugar components. In spite of their broad carbohydrate-fermenting abilities, most soy pediococci utilize glucose preferentially over the other sugars such as pentoses. Soy sauce contains high concentrations of amino acids from soybeans. Since pentoses (from the cell walls of crops such as soybeans or wheat used in brewing soy sauce) react easily with amino acids by the Maillard reaction and result in browning pigments, soy sauce manufacturers are eager to remove pentoses selectively from soy sauce moromi mash in the fermentation process.

We have attempted to isolate some mutants of *P. halophilus* soy pediococci which can preferentially utilize pentoses such as xylose and arabinose even in the presence of a large amount of glucose.

It is established, mainly in gram-negative enteric bacteria, that bacteria possess regulatory mechanisms which allow them to select preferred carbon sources when several are present in the growth medium simultaneously (catabolite control) (22, 28).

Catabolite control in gram-positive bacteria has also been reported, especially in streptococci. In *Streptococcus pyogenes*, an inducer expulsion mechanism was studied (24, 26). In lactic streptococci, sequential utilization of glucose, galactose, and lactose in relation to expulsion and exclusion mechanisms (35, 38) and that between glucose and arginine based on catabolite repression (3) have been studied. In *Streptococcus mutans*, some defects in the phosphoenolpyruvate (PEP):glucose phosphotransferase system (glc: PTS) could depress the glucose effect upon lactose fermentation (14–16). In *Streptococcus faecalis*, utilization of glycerol and dihydroxyacetone is regulated by a PTS (4, 5). Regulation of transport and metabolism of sugars in streptococci have been reviewed elsewhere (9, 11, 25, 31).

For soy pediococci, no information about glucose effect or glucose transport has been reported so far. In this report, catabolite control over xylose utilization and the mechanism of glucose transport in *P. halophilus* were studied.

MATERIALS AND METHODS

Organisms. *P. halophilus* I-13 and X-160 were obtained from the culture collection in this laboratory.

Culture maintenance. Unless otherwise stated, the organisms were grown and maintained statically at 30°C in a basal medium (BM) containing the following (in percent [weight/volume]): yeast extract (Difco Laboratories, Detroit, Mich.), 0.3; Polypepton (Daigo Eiyo Chemical Co., Osaka, Japan), 0.5; K_2HPO_4, 0.5; sodium thioglycolate, 0.1; and NaCl, 5. The medium was supplemented with 30 mM xylose as a carbon source (BM-X). The basal medium was sterilized at 120°C for 15 min, and the carbon source was sterilized by being passed through a membrane filter system (pore size, 0.22 μm; Millipore Corp., Bedford, Mass.) after the pH was adjusted to 7.0. The sterilized basal medium and carbon source were mixed just before use.

Growth of *P. halophilus* I-13 and X-160 in mixed-sugar medium. BM was supplemented with 0.4 M 2-(*N*-morpholino)ethanesulfonic acid (MES) buffer (pH 7.0) (BM-MES)

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and appropriate sugar concentrations (see Results), and cultures in BM-MES medium were grown without a static pH. The culture volume was 2 liters in a 3-liter jar fermentor or 10 ml in a screw-cap tube. In some cases, the complex medium containing 0.5% (wt/vol) each of beef extract (Difco), yeast extract (Difco), Polypepton (Daigo Eiyo), 0.1% sodium thioglycolate, and 5% NaCl (CM) was supplemented with the appropriate sugar concentration (see Results). Unless otherwise stated, 5% NaCl was used for BM and CM. Sterilization was as for BM-X. Pediococci were precultured in test tubes containing 5 ml of BM-X at 30°C for 30 h, were inoculated into 2 liters of CM or BM-MES in a stirred 3-liter Pyrex vessel, and were grown until stationary phase at 30°C. In 10 ml of screw-cap tube culture, the size of inoculation was 1% of cell density. An autoclavable pH electrode (W. Ingold, AG Industrie Nord, Urdorf, Switzerland) was incorporated into a jar fermentor, and a pH of 7.0 was maintained automatically with 5 N NaOH in CM. Duplicate samples were withdrawn from each culture at appropriate intervals; growth (optical density) was measured in the first sample with a Stasar III spectrophotometer (Gilford Instrument Laboratories, Inc., Oberlin, Ohio) operated at 530 nm or with a Klett-Summerson photoelectric colorimeter (Klett Manufacturing Co., Inc., New York, N.Y.) at 660 nm. Dry weights of cells were determined with washed cells after heating at 110°C for 20 h. Cells were removed from the second sample by Millipore filtration, and filtrates were assayed for residual sugars.

Selection of *P. halophilus* X-160. Moromi mash (1 g) was diluted appropriately with sterilized 15% sodium chloride solution, and 1 ml of diluted solution was transferred to 100 ml of CM supplemented with 1% (wt/vol) D-xylose as a carbon source, 50 mg of Kabicidin per liter (Daigo Eiyo) as an antifungal agent, 0.004% (wt/vol) bromocresol purple, and 15% (wt/vol) sodium chloride. Enrichment cultures of xylose-fermentable strains were done by stationary culture at 30°C anaerobically (in N_2 gas). The cultures whose bromocresol purple changed from purple to yellow by acid production were plated out on CM supplemented with 30 mM xylose, $CaCO_3$ (0.1% [wt/vol]), and agar (1.5% [wt/vol]) (X plate). Plate cultures were incubated anaerobically in GasPak (BBL Microbiology Systems, Cockeysville, Md.). The colonies which made halos on X plate were transferred into BM supplemented with 0.5% xylose–0.5% glucose–15% sodium chloride. *P. halophilus* X-160 was selected by residual sugar analysis as a strain which could utilize xylose in the presence of glucose.

Preparation of crude cell extracts. Cell extracts for assay of glycolytic enzymes were prepared as follows. *P. halophilus* X-160 and I-13 were precultured in 5 ml of CM-G (CM plus 30 mM glucose) at 30°C for 30 h, and the cells were collected by centrifugation (36,000 × *g* for 10 min) at 4°C. The supernatant fluid was removed, and the cell pellet was washed twice with 5 ml of sugar-free CM. The washed cells were suspended in 5 ml of sugar-free medium. One milliliter of each suspension was inoculated in 1,000 ml each of CM-G (30 mM glucose), CM-F (30 mM fructose), and CM-X (30 mM xylose). The cells were grown at 30°C for 50 h (late log or early stationary phase) and were then collected by centrifugation (12,000 × *g* for 10 min) at 4°C. The cell pellets were suspended in 100 ml of 20 mM potassium phosphate buffer (pH 7.4) containing 2 mM 2-mercaptoethanol–2 mM EDTA (KPB-1), and the cells were washed twice with the same buffer. The cells were resuspended to 5 ml in KPB-1, and the cells were disrupted by sonication. The suspensions were centrifuged (39,000 × *g* for 60 min) at 4°C, and the supernatants were assayed for enzyme activities.

Assay of enzymes. Activities of glycolytic enzymes were measured by spectrophotometric coupling to NAD^+- or $NADP^+$-linked reactions by methods described previously (1, 2, 18, 21). Enzyme activities are expressed as nanomoles of substrates consumed per minute at 30°C per milligram of protein.

For all assays of glycolytic enzymes, a buffer containing 50 mM triethanolamine hydrochloride (neutralized with KOH)–10 mM $MgCl_2$, pH 7.4 (TEA) in a total reaction volume of 3.0 ml was used.

D-Xylose isomerase activity was determined by incubating cell extract (25 to 100 μl) in a 0.95-ml reaction mixture which contained 50 μmol of Tris malate (pH 6.6)–1.0 μmol of $MnCl_2$–5 μmol of D-xylose. The assay was started by the addition of D-xylose and was stopped by the addition of 50% trichloroacetic acid to a final concentration of 2.5% after incubation for 5 min at 37°C. The precipitate was removed by centrifugation at 10,000 × *g* for 5 min, and the supernatant was analyzed for D-xylulose by the cysteine-carbazole method (6).

Transport studies. Cells of *P. halophilus* from 100 ml of a mid-log-phase culture in BM-G (30 mM glucose) were collected by centrifugation (12,000 × *g* for 10 min) at 4°C. The supernatant fluid was removed, and the cell pellet was washed twice with 50 ml of 100 mM potassium phosphate (pH 7.0) containing 5 mM $MgCl_2$–5% NaCl (KPB-2). The washed cells were finally suspended in 5 ml of KPB-2 to a concentration of 4 mg (dry weight) of cells per ml.

In the standard transport procedure, 50 μl of the cell suspension was added to 850 μl of 70 mM potassium phosphate (pH 7.0) containing 3.5 mM $MgCl_2$–5% NaCl (KPB-3) to obtain a final cell density of 200 μg (dry weight) of cells per ml. The suspension was gently agitated in a water bath at 30°C, and 0.1 ml of [^{14}C]glucose or [^{14}C]2-deoxyglucose (2DG) was added to this system (0.3 mM; specific activity, 1.7 μCi/μmol). The reaction was terminated with 5 ml of cold water (0°C) at a specified time, and immediately 2 ml of solution was removed for filtration through a membrane filter (Millipore type HA; pore size, 0.45 μm). The filters were washed twice by being passed through 5 ml of cold water (0°C), and after the filters were dried, radioactivity was determined by liquid scintillation spectrometry.

Preparation of decryptified cells and assay of PTS activity. Decryptified cells were prepared by a modification of the method of Kornberg and Reeves (12) with a toluene-chloroform mixture (1:1) as a permeabilizing agent, and PTS activities were determined by the procedure of Keevil et al. (10). The reaction mixture (3 ml) contained 1.5 mM $NADP^+$, appropriate concentrations of phosphoryl donors (PEP, ATP, or both; see Results), 6 units of glucose-6-phosphate (G-6-P) dehydrogenase, 5 mM glucose, and decryptified cells (200 μg [dry weight] of cells per ml of reaction mixture with or without 10 mM iodoacetic acid [IAA]). The reaction was started by glucose addition, and NADPH production was monitored with a Stasar III spectrophotometer (Gilford) (340 nm) at 30°C. Preincubation at 30°C for 10 min was carried out to consume the endogeneous energy source. The initial velocity was expressed as nanomoles of G-6-P formed per minute per milligram (dry weight) of cells from NADPH production.

Preparation of cells containing [^{14}C]PEP pool intermediates. Glycolytic intermediates were determined qualitatively by a thin-layer autoradiography method (37). Washed *P. halophilus* I-13 cells (grown previously in 30 mM xylose

[BM-X]) were suspended in and thoroughly homogenized with 0.1 M piperazine-*N*,*N*'-bis(2-ethanesulfonic acid) (PIPES) buffer (pH 7.2) containing 0.5 mM $MgSO_4$–5% NaCl (PIPES-1) to a final volume of 8 ml (10 mg [dry weight] of cells per ml). [U-^{14}C]glucose solution (200 μl) was added to a final concentration of 10 mM (specific activity, 0.25 μCi/μmol). After 60 min of incubation at 30°C, all of the glucose had been metabolized, and the starved cells contained only the [^{14}C]PEP metabolites (3-phosphoglycerate [3-PG], 2-phosphoglycerate [2-PG], and PEP). IAA was then added to the suspension (10 mM final concentration) to block glyceraldehyde-3-phosphate dehydrogenase and subsequent glycolysis. The PEP-loaded cells were collected by centrifugation (24,000 × *g* for 30 min at 4°C), and the cell pellet was suspended to 8 ml with 0.1 M PIPES buffer (pH 7.2) containing 0.5 mM $MgSO_4$–5% NaCl–5 mM IAA. To monitor PEP utilization thereafter, 1-ml volumes of cell suspension were added to the appropriate system (see Fig. 6) containing the described sugars. After 5 min of incubation at 30°C, the tubes (1.5 ml of reaction mixture) were transferred to a boiling-water bath, and 5 ml of boiling water was added to each. The suspensions were boiled for 5 min, cooled, and clarified by centrifugation (24,000 × *g* for 30 min at 4°C). Supernatant fluids were removed and lyophilized, and the residue was reconstituted to 200 μl with distilled water. Radiolabeled metabolites and phosphorylated compounds were identified by thin-layer fluorography as described previously (29, 37).

Accumulation of sugars by starved cells. Starved cells, previously grown on BM-fructose (30 mM), containing endogeneous PEP potentials were prepared as described above. In the standard procedure, 1 ml of thick cell suspension (10 mg [dry weight] of cells per ml) was added to 1.375 ml of 0.1 M PIPES buffer (pH 7.0) containing 0.5 mM $MgSO_4$–5% sodium chloride. The suspension was incubated for 20 min at 30°C before IAA was added to a final concentration of 10 mM. Final cell density was 4 mg (dry weight) of cells per ml.

The 3-ml reaction mixture contained 0.1 M PIPES buffer (pH 7.0)–0.5 mM $MgSO_4$–5% NaCl–10 mM IAA and starved cells (400 μg [dry weight] of cells per ml). The reaction was started by ^{14}C-labeled sugar addition (0.3 mM; specific activity, 0.5 μCi/μmol). At specific times, 200 μl of reaction mixture was withdrawn, cells were collected by membrane filtration, and radioactivity was determined.

Effect of glucose or 2DG on [^{14}C]xylose uptake by nongrowing *P. halophilus* cells. Washed cells (grown previously on BM-X) were suspended at a density of 400 μg (dry weight) of cells per ml in PIPES-1. After 10 min of incubation at 30°C, [^{14}C]xylose (specific activity, 0.5 μCi/μmol) was added to the suspension to a final concentration of 1 mM. After 5 min of incubation, glucose or 2DG was added to the suspension to a final concentration of 5 mM. At appropriate intervals, 200-μl samples were withdrawn and filtered, and the accumulation of [^{14}C]xylose was determined.

Analysis of sugars. D-Glucose, D-galactose, D-xylose, and L-arabinose were determined by a post-column reaction high-pressure liquid chromatography-sugar analysis system by a modification of the methods of Mopper and Gindler (20). Xylose or arabinose was measured by the Bial reaction (13), and glucose was determined enzymatically by the glucose oxidase-peroxidase method (39).

Other methods. The protein concentration in the cell extract was estimated by the method of Lowry et al. (17) with bovine serum albumin as the standard.

Chemicals. All enzymes and substrates for assays of glycolytic enzymes were purchased from Boehringer GmbH, Mannheim, Federal Republic of Germany. D-[U-^{14}C]glucose (280 mCi/mmol), 2-deoxy-D-[U-^{14}C]glucose (300 mCi/mmol), methyl-(α-D-[U-^{14}C]gluco)pyranoside (150 mCi/mmol), D-[U-^{14}C]fructose (274 mCi/mmol), D-[1-^{14}C]mannose (55 mCi/mmol), and D-[U-^{14}C]xylose (76 mCi/mmol) were purchased from Amersham Japan Limited, Tokyo, Japan.

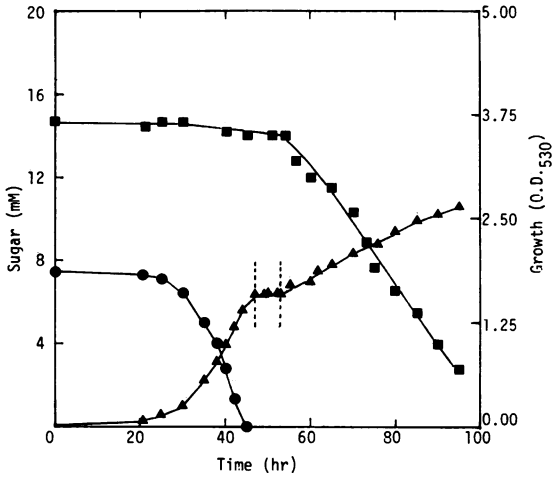

FIG. 1. Preferential utilization of glucose over xylose by *P. halophilus* I-13 in glucose-xylose BM-MES medium at 30°C. Symbols: ●, glucose (initial concentration, 7.5 mM); ■, xylose (initial concentration, 15 mM); ▲, cell growth. Dotted lines indicate transition points in sugar metabolism and growth rate.

RESULTS

Sequential or concurrent utilization of xylose in the presence of glucose. *P. halophilus* X-160 was isolated as a strain which could utilize xylose in the presence of glucose.

With *P. halophilus* I-13 growing on glucose (doubling time on glucose, ca. 6.1 h), the concentration of glucose dropped from an initial value of 15 mM to below 0.5 mM and then a time lag (~5 to 6 h) occurred before the very slow utilization of xylose was initiated (doubling time on xylose, ca. 12 h) (Fig. 1). In contrast to the sequential metabolism of glucose and xylose by *P. halophilus* I-13, both glucose and xylose were metabolized concurrently by *P. halophilus* X-160 (doubling time, 4.8 h) (Fig. 2). These results suggest that the glucose effect on xylose utilization might be due to repression of catabolic enzymes required for xylose metabolism.

When *P. halophilus* I-13 was grown in a glucose-fructose medium (CM-GF; each 30 mM), glucose and fructose were concurrently consumed in the early stage, and in the late stage, glucose was utilized rather faster than fructose (Fig. 3A). *P. halophilus* X-160 consumed glucose and fructose concurrently in log phase, but in stationary phase fructose was utilized faster than glucose (Fig. 3B).

In fructose-xylose medium (CM-FX; each 30 mM), both *P. halophilus* I-13 and X-160 utilized fructose preferentially over xylose (Fig. 4A and B).

Both strains produced approximately 2 mol of lactate from 1 mol of fructose (data not shown). From 1 mol of glucose consumed, *P. halophilus* I-13 produced substantially 2 mol of lactate, but *P. halophilus* X-160 formed 1.4 mol of lactate, 0.3 mol of acetate, and 0.3 mol of formate (data not shown).

These data suggested that glucose transport or metabolism by X-160 differed from that of wild-type strain I-13 at a point preceding phosphofructokinase 1 (EC 2.7.1.11) in the glycolytic pathway (EMP).

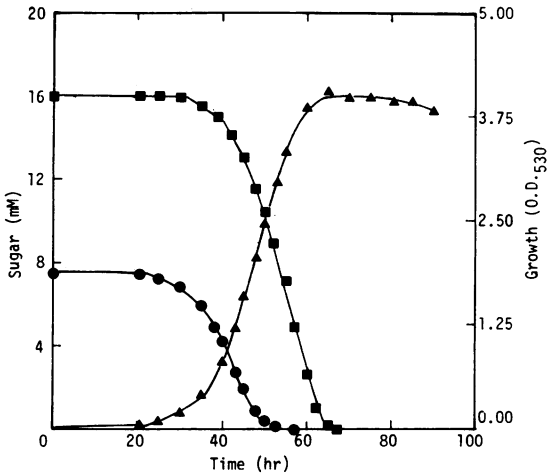

FIG. 2. Concurrent utilization of glucose and xylose and one-step growth of *P. halophilus* X-160 in glucose-xylose BM-MES medium at 30°C. Symbols: ●, glucose (7.5 mM); ■, xylose (16 mM); ▲, cell growth.

Xylose isomerase activity in the mixed-sugar medium with *P. halophilus* X-160 and I-13. Since strain I-13 showed diauxic growth in xylose-glucose culture, the catabolite control of enzyme synthesis for xylose metabolism was investigated by using xylose isomerase as a marker enzyme. *P. halophilus* I-13 and X-160 were cultured in CM-G (30 mM glucose), CM-GX (120 mM glucose, 30 mM xylose), and CM-X (30 mM xylose), and the xylose isomerase activity of each culture was measured in the late log phase (Table 1). With cells cultured in CM-G, both strains *P. halophilus* X-160 and I-13 showed no activity of xylose isomerase. With cells cultured in CM-GX, *P. halophilus* X-160 exhibited 50% of the xylose isomerase activity of cells cultured in CM-X. In contrast, strain I-13 showed no activity of xylose isomerase in CM-GX. When the cells were harvested from CM-GX, glucose remaining in the medium was confirmed in both cases (data not shown). With cells cultured in CM-X, both strains exhibited the same level of activity of xylose isomerase.

In conclusion, xylose isomerase activity was not induced in *P. halophilus* I-13 during growth in a medium containing both xylose and glucose. However, in *P. halophilus* X-160, isomerase activity was induced even in the presence of glucose. These results agreed well with the sugar consumption in culture experiments and showed that the glucose effect on xylose metabolism was characterized as a repression of synthesis of catabolic enzymes for xylose metabolism.

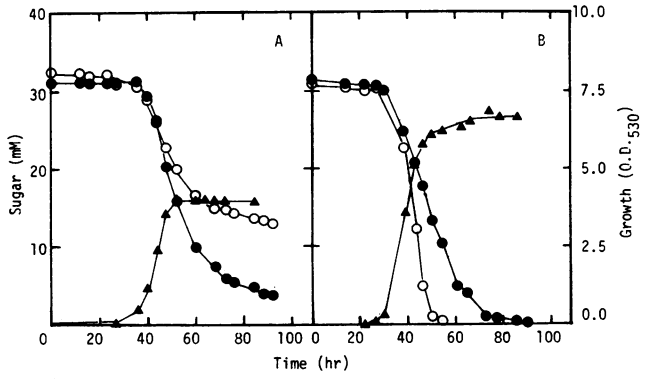

FIG. 3. Utilization of glucose and fructose and growth of *P. halophilus* I-13 (wild-type) (A) and X-160 (B) in BM with static pH. Symbols: ●, glucose; ○, fructose; ▲, cell growth.

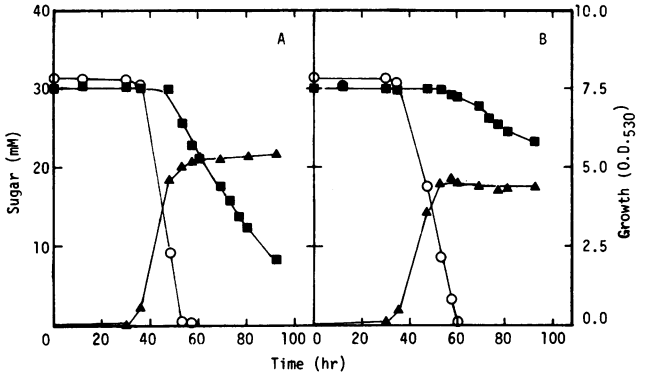

FIG. 4. Preferential utilization of fructose over xylose, and growth of *P. halophilus* I-13 (A) and X-160 (B) in BM with static pH. Symbols: ○, fructose; ■, xylose; ▲, cell growth.

Comparison of activities of glycolytic enzymes. The unusual metabolism of glucose in strain X-160 might be attributable to some defects in the upper stream of the glycolytic pathway. Glycolytic enzyme activities were compared for strains I-13 and X-160 grown on various media (Table 2).

Only the activity of phosphoglucose isomerase (EC 5.3.1.9) in strain X-160 was found to be lower (~40 to 50%) than that observed in strain I-13.

Effect of 2DG on growth of *P. halophilus* I-13 and X-160 and their glucose transport abilities. The nonmetabolizable glucose analog 2DG, which can be transported but not metabolized through the EMP, was investigated in the two strains (Fig. 5).

In glucose-xylose medium (BM-GX) and xylose medium (BM-X), both strains grew at almost the same rate and pattern. In 2DG-xylose medium (BM-2DGX). *P. halophilus* X-160 grew at the same rate as in BM-X (Fig. 5B) while the growth of *P. halophilus* I-13 was suppressed and occurred only after a long time lag (ca. 60 h) (Fig. 5A). This means that the difference in glucose metabolism between the two strains depends on their transport systems.

From comparative analysis of sugar transport with intact cells of the two strains, glucose transport and 2DG transport by *P. halophilus* X-160 cells were found to be <10% of those observed in strain I-13 (data not shown).

TABLE 1. Effect of sugar composition in growth medium on xylose isomerase activities of *P. halophilus* I-13 and X-160

Strain[a]	Carbon source (mM)		Activity[b] (nmol/min per mg of protein)
	Glucose	Xylose	
I-13	30	0	ND (0)
	120	30	ND (0)
	0	30	38 (100)
X-160	30	0	ND (0)
	120	30	17 (48)
	0	30	35 (100)

[a] Cells were grown in CM containing sugars as listed above.
[b] Activities were expressed as nanomoles of xylulose formed per minute per milligram of protein. Values in parentheses were expressed as percentage relative to that for CM-X (100%) in each strain. ND, Not detected.

TABLE 2. Activities of glycolytic enzymes in *P. halophilus* I-13 and X-160

Strain	Carbon source[a]	Activity (nmol/min per mg of protein) of[b]:										
		HXK	ZWF	PGI	PFK	ALD	TPI	GLD	PGK	PGM	ENO	PYK
I-13	Glc	21	ND[c]	1,768	201	143	449	1,768	111	247	229	1,447
	Fru	37	12	1,363	187	152	511	1,485	131	273	227	1,424
	Xyl	25	ND	1,376	158	118	298	1,749	105	218	161	1,138
X-160	Glc	32	14	856	166	128	409	1,375	164	222	179	1,929
	Fru	19	6	921	190	117	406	1,662	84	180	199	1,808
	Xyl	16	ND	846	142	108	307	1,086	125	207	175	1,361

[a] CM was supplemented with 30 mM of glucose (Glc), fructose (Fru), or xylose (Xyl).
[b] Enzyme abbreviations: HXK, hexokinase; ZWF, G-6-P dehydrogenase; PGI, phosphoglucose isomerase; PFK, phosphofructokinase; ALD, fructose-1,6-bisphosphate aldolase; TPI, triose phosphate isomerase; GLD, glyceraldehyde 3-phosphate dehydrogenase; PGK, phosphoglycerate kinase; PGM, phosphoglycerate mutase; ENO, enolase; PYK, pyruvate kinase.
[c] ND, Not detected.

It has been confirmed that the difference in glucose metabolism by the two strains was due to their glucose transport activities.

Glucose phosphorylation activities in decryptified cells. To determine the type of glucose transport mechanism in *P. halophilus*, the intracellular sugar phosphorylation step was investigated by measuring the rate of phosphorylation of glucose in decryptified cells in the presence of PEP or ATP or both as the phosphoryl donor(s). The rate was estimated from recovery of added glucose as sugar phosphate (Table 3).

When the phosphoryl donor was PEP, the rate of G-6-P formation in *P. halophilus* X-160 was <10% of the rate in I-13. When ATP was used as the phosphoryl donor, G-6-P-forming activities were almost the same in the two strains. When both PEP and ATP were added simultaneously, the activities of G-6-P formation were as high as the sum of the individual phosphorylation activities for PEP and ATP.

The addition of IAA to the reaction systems did not affect significantly the G-6-P formation rate in either strain. IAA was thought not to inhibit G-6-P formation.

These results, together with the results of transport experiments with intact cells, lead to the conclusion that a defect in PTS activity for glucose transport in *P. halophilus* X-160 is responsible for the derepressible utilization of xylose in the presence of glucose.

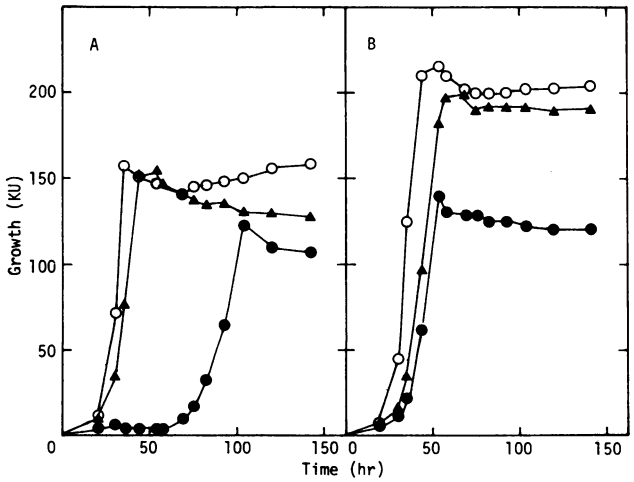

FIG. 5. Effect of 2DG on growth of *P. halophilus* I-13 (A) and X-160 (B) in BM. Symbols: ○, glucose (15 mM) and xylose (15 mM) (BM-GX); ▲, xylose (30 mM) (BM-X); ●, 2DG (15 mM) and xylose (15 mM) (BM-2DGX).

Consumption of PEP concomitant with glucose or 2DG transport in *P. halophilus* I-13. PTS as the glucose transport mechanism in *P. halophilus* was additionally confirmed with an investigation on in vivo PEP consumption concomitant with glucose or 2DG transport. Suspensions of starved *P. halophilus* I-13 cells containing a ^{14}C-labeled PEP pool were incubated with ^{14}C-labeled or nonlabeled (cold) sugars (glucose, 2DG, or xylose) (Fig. 6).

When [^{14}C]glucose (Fig. 6B), cold glucose (Fig. 6E), [^{14}C]2DG (Fig. 6C), or cold 2DG (Fig. 6F) was transported, the [^{14}C]PEP pool in the starved cells disappeared. Then, [^{14}C]G-6-P or [^{14}C]fructose-6-phosphate and [^{14}C]fructose-1,6-diphosphate were generated in [^{14}C]glucose-transporting cells (Fig. 6B), and [^{14}C]2DG-6-P was generated in [^{14}C]2DG-transporting cells (Fig. 6C).

In contrast to the glucose- or 2DG-transporting cells, the [^{14}C]PEP pool remained in cells after incubation with xylose (Fig. 6G) or [^{14}C]xylose (Fig. 6D). The [^{14}C]fructose-1,6-diphosphate band in [^{14}C]xylose transport (Fig. 6D) was thought to be derived from a reverse reaction of fructose-1,6-biphosphate aldolase (EC 4.1.2.13) with glyceraldehyde-3-phosphate which might be a part split from xylulose-5-phosphate by phosphoketolase (EC 4.1.2.9), according to the inhibition experiments on glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.12) by IAA (data not shown).

[^{14}C]PEP consumption occurred simultaneously with glucose or 2DG transport via the glc:PTS.

TABLE 3. Glucose phosphorylation activities in decryptified *P. halophilus* I-13 and X-160

Phosphoryl donor[a]	Activity[b] (nmol/min per mg of protein)	
	X-160	I-13
PEP	0.91 (11)[c]	8.34 (100)
ATP	3.03 (116)	2.60 (100)
PEP + ATP	4.66	8.94
PEP + IAA	0.61 (10)	5.84 (100)
ATP + IAA	2.88 (97)	2.96 (100)
PEP + ATP + IAA	3.64	7.84

[a] Each test tube contained phosphoryl donors or IAA as listed above and 200 μg (dry weight) of cells in 3 ml of KPB-4. The reactions were carried out at 30°C. Concentrations were as follows: PEP, 5 mM; ATP, 5 mM; IAA, 10 mM.
[b] Activities were expressed as nanomoles of G-6-P formed per minute per milligram of protein. Values in parentheses were the percentage of activities relative to that of *P. halophilus* I-13 as 100%.

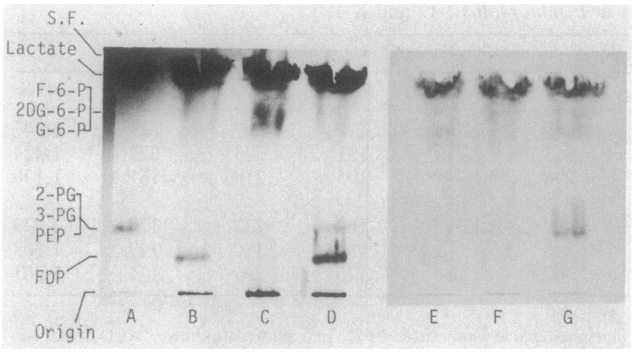

FIG. 6. Demonstration by ^{14}C fluorography of in vivo consumption of PEP concomitant with glucose and 2DG transport in *P. halophilus* I-13. Starved cells grown previously on CM-X containing [^{14}C]PEP-pool intermediates (2-PG, 3-PG, and PEP) were suspended at a density of 6.6 mg (dry weight) per ml in 0.1 M PIPES buffer (pH 7.2) containing 0.5 mM $MgSO_4$–5% NaCl–5 mM IAA and (lane A) no sugar (control), (lane B) [^{14}C]glucose, (lane C) [^{14}C]2DG. (lane D) [^{14}C]xylose, (lane E) nonlabeled (cold) glucose, (lane F) cold 2DG, or (lane G) cold xylose (sugar concentration, 13.3 mM; specific activity, 0.25 μCi/μmol). After 5 min of incubation at 30°C, the cells were extracted and ^{14}C-metabolites were detected by fluorography as described in the text. S.F., second solvent front.

PEP-dependent sugar uptake by starved cells. It was further investigated whether the type of PTS for glucose transport in *P. halophilus* was similar to the man:PTS of *Streptococcus lactis* (29, 35) or to the glc:PTS of *Escherichia coli* (23).

IAA inhibits the EMP on the point of glyceraldehyde-3-phosphate dehydrogenase but not the PTS. After starvation, glycolysis of the cells was halted by IAA addition, and the subsequent supply of PEP potential was inhibited (Table 3 and Fig. 6). Patterns of sugar uptake of the two strains are illustrated in Fig. 7.

Starved *P. halophilus* I-13 cells (grown previously on fructose), which contained a high PEP potential (3-PG, 2-PG, and PEP) as observed in Fig. 6A and data not shown,

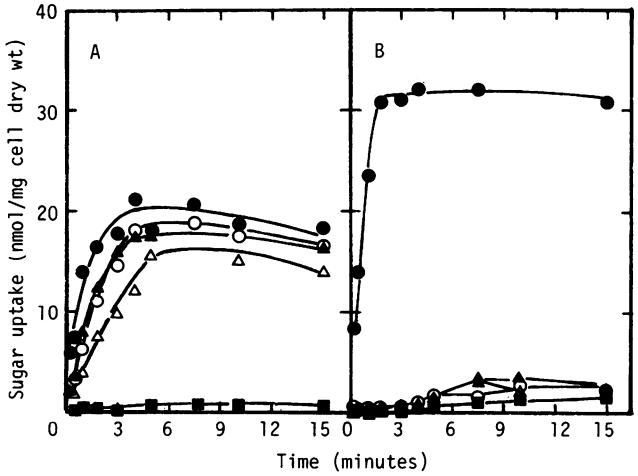

FIG. 7. PEP-dependent sugar uptake by starved cells of *P. halophilus* I-13 (A) and X-160 (B). The cells, grown previously on fructose, were suspended at 400 μg (dry weight) per ml in PIPES-1 buffer (pH 7.2) containing 10 mM IAA–0.3 mM radiolabeled sugar (specific activity, 0.5 μCi/μmol). Symbols: ●, fructose; ○, glucose; ▲, mannose; △, 2DG; ■, α-MG. Uptake of radiolabeled sugar was monitored as described in the text.

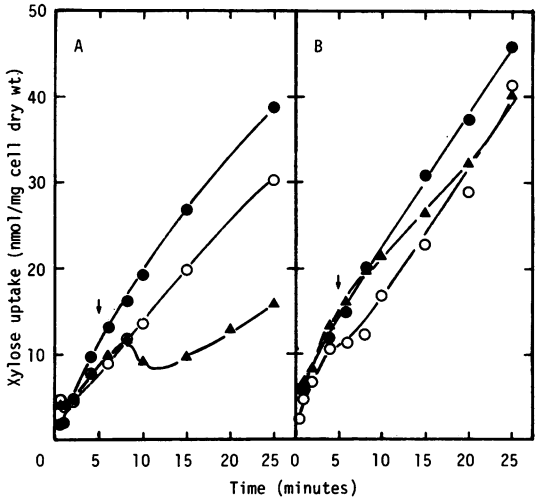

FIG. 8. Effect of addition of glucose and 2DG to pediococcal cells transporting xylose. (A) *P. halophilus* I-13. (B) *P. halophilus* X-160. The cells were suspended at a final concentration of 400 μg (dry weight) per ml in 0.1 M PIPES buffer (pH 7.0) containing 3.5 mM $MgCl_2$–5% NaCl. After 10 min of preincubation, radiolabeled xylose (0.5 μCi/μmol) was added, and then at 5 min (↓) glucose (○), 2DG (▲), or nothing (control) (●) was added to the reaction mixture at a final concentration of 5 mM. [^{14}C]xylose accumulation was monitored as described in the text.

accumulated glucose, mannose, 2DG, or fructose up to a maximum amount of ca. 15 to 20 nmol per mg (dry weight) of cells but not methyl-α-D-glucopyranoside (Fig. 7A). In contrast, fructose-grown *P. halophilus* X-160 cells were unable to accumulate significant levels of any of these PTS sugars except fructose (Fig. 7B).

Consequently, the main glucose transport system in *P. halophilus* is similar to the II^{man}/III^{man} system (man:PTS) of *S. lactis* (29, 35) rather than to the II^{glc}/III^{glc} system (glc:PTS) found in *E. coli* (23). Since *P. halophilus* X-160, whose PTS activity for PEP-dependent glucose phosphorylation was less than that of the wild type (Table 3), was unable to uptake significant levels of man:PTS substrates (glucose, mannose, and 2DG), neither strain could accumulate α-MG (glc:PTS-specific substrate).

The fact that the PEP-loaded cells of both strains showed approximately the same level of fructose uptake suggests that fructose is independently transported through a fructose-specific PTS other than man:PTS.

Glucose-mediated catabolite inhibition in *P. halophilus*. Glucose-mediated catabolite repression of synthesis of enzymes required for xylose metabolism was described above. We next considered whether or not the catabolite inhibition of xylose metabolism would occur upon the addition of glucose to the cultures of *P. halophilus* growing on xylose. Strains I-13 and X-160 were harvested in BM-MES-xylose (30 mM), and at exponential phase (120 Klett units) glucose was added to a final concentration of 15 mM. It was observed that, in wild-type strain I-13, xylose metabolism of the cells growing on xylose was weakly inhibited by glucose addition whereas in *P. halophilus* X-160 (man:PTS mutant) xylose metabolism was scarcely inhibited by glucose addition (data not shown).

We also investigated the effects of glucose and 2DG on [^{14}C]xylose uptake by nongrowing cells of *P. halophilus*. The addition of glucose to washed cells of strain I-13 (grown previously on xylose) resulted in a reduced rate of xylose

uptake but not in complete inhibition. The addition of 2DG led to a rapid inhibition of xylose uptake for about 10 min, after which period xylose uptake recovered. But the velocity of xylose uptake with 2DG remained slower than that in the control without 2DG (Fig. 8A). With *P. halophilus* X-160, glucose addition resulted also in a slight depression of xylose uptake. However, 2DG addition did not have such a serious inhibitory effect as that observed in strain I-13 (Fig. 8B).

These data show that (i) xylose uptake of pediococcal cells grown previously on xylose was inhibited only slightly by glucose addition and (ii) reduced man:PTS activity leads to resistance to inhibition by glucose or 2DG.

DISCUSSION

man:PTS is a functional glucose transporter in soy pediococci. The results of the culture experiments on the growth of the two strains in fructose-glucose medium and analysis of the organic acids produced suggest that either transport or metabolism of glucose might be different in the two strains (Fig. 3). The specificity of the glucose metabolism in strain X-160 compared with that of strain I-13 is more likely explained by a decrease of the activity of glucose transport rather than by any defect in the EMP (Table 2; Fig. 5 and 7).

Our results suggest that (i) the man:PTS (not the glc:PTS) is the main functional glucose transport system in soy pediococci and (ii) that a defective man:PTS is responsible for the difference(s) in glucose metabolism by the wild-type (I-13) and mutant strains (X-160) which were isolated from nature. Furthermore, (i) the rates of sugar uptake of glucose, mannose, and 2DG (but not of fructose) in intact or IAA-treated *P. halophilus* X-160 cells were <10% of those observed in strain I-13 (Fig. 7). This simultaneous decrease of the rates of sugar uptake for man:PTS substrates observed in strain X-160 means that the substrate specificity of the glucose transporter in soy pediococci is similar to that of the man:PTS found in *S. lactis* as one of the main glucose transport systems of lactic streptococci (34, 35). (ii) PEP-dependent G-6-P-forming activity in decryptified *P. halophilus* X-160 cells was <10% of that observed in strain I-13 (Table 3). This agreed well with the rates of glucose uptake observed with intact or IAA-treated cells as described above. (iii) In vivo consumption of the PEP pool concomitant with glucose or 2DG transport and the consequent G-6-P or 2DG-6-P formation were observed (Fig. 6). These observations were similar to those reported for *S. lactis* (19, 29, 30, 36, 37). (iv) The two strains showed no accumulation of α-MG, which is a typical substrate of the glc:PTS in enteric bacteria (23) or *S. mutans* (14–16) (Fig. 7). These findings show that the PTS for glucose uptake by soy pediococci is similar to the man:PTS of *S. lactis* (34, 35). Furthermore, the cells of strain I-13 grown previously on xylose or fructose showed the same activities of both glucose transport and G-6-P formation as the cells grown previously on glucose (data not shown). In soy pediococci, the man:PTS is thought to be constitutive.

PEP-loaded starved *P. halophilus* X-160 cells scarcely accumulated glucose (Fig. 7B), but the man:PTS-defective mutant X-160 could still grow on glucose (Fig. 2). This residual ability of glucose metabolism in strain X-160 might mean that glucose is transported by a glucose permease and is subsequently phosphorylated by glucokinase with ATP similar to those observed in *S. lactis* (Tables 2 and 3) (31, 34). Further studies are needed to confirm this speculation.

Another suggestion is that fructose might be independently transported by a fructose:PTS.

In substrate specificity, PTSs in soy pediococci are very similar to the man:PTS or fructose:PTS found in lactic streptococci (35). To our knowledge, there was only one report on the PTS of pediococci, by A. H. Romano et al., but it was not detailed (27).

It was observed in the experiment on the effect of 2DG on growth that strain I-13 became resistant to 2DG after a 60-h time lag (Fig. 5A). Thompson and Chassy reported that *S. lactis* K1 had the ability to grow on many sugars, including sucrose and lactose, in the presence of a high concentration (>500 mM) of 2DG (32, 33). The phenomenon observed in *P. halophilus* I-13 may be similar to that observed in *S. lactis*, whose man:PTS was regulated by short-term control (decrease of man:PTS activity) and long-term control (decrease of synthesis of man:PTS) of the 2DG treatment. Further study is needed to understand the phenomenon.

Catabolite control and man:PTS in soy pediococci. In xylose utilization by strain X-160, depression of both glucose repression and glucose inhibition was observed (Fig. 2 and 8B; Table 1). It is suggested that the release of glucose catabolite control might be attributed directly or indirectly to a defect in the man:PTS of strain X-160 (Fig. 5B and 7B; Table 3). Possible mechanisms are as follows. (i) The decrease of man:PTS activity in strain X-160 may cause decreases in concentrations of glycolytic intermediates and, subsequently, a defect in catabolite control (if glycolytic intermediates are involved in glucose catabolite control). (ii) A component of the man:PTS may participate directly to effect catabolite inhibition. Further studies are necessary to confirm or refute the two hypotheses.

Our aim is to breed a special halophilic lactic acid bacterium which can selectively utilize xylose and arabinose in the presence of a high concentration of glucose (>400 mM). This requires overcoming the glucose effect.

In this report, we propose the hypothesis that, in soy pediococci, the man:PTS plays a key role for expression of glucose catabolite control and that some defects of the man:PTS lead to depression of the catabolite control. To evaluate whether or not the hypothesis is correct, the introduction of some defects in the man:PTS in the wild-type *P. halophilus* I-13 and subsequent observations of its glucose effect are in progress.

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