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Molecular Cloning and Analysis of the *ptsHI* Operon in *Lactobacillus sake*

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The *ptsH* and *ptsI* genes of *Lactobacillus sake*, encoding the general enzymes of the phosphoenolpyruvate: carbohydrate phosphotransferase system (PTS), were cloned and sequenced. HPr (88 amino acids), encoded by *ptsH*, and enzyme I (574 amino acids), encoded by *ptsI*, are homologous to the corresponding known enzymes of other bacteria. Nucleotide sequence and mRNA analysis showed that the two genes are cotranscribed in a large transcript encoding both HPr and enzyme I. The transcription of *ptsHI* was shown to be independent of the carbon source. Four *ptsI* mutants were constructed by single-crossover recombination. For all mutants, growth on PTS carbohydrates was abolished. Surprisingly, the growth rates of mutants on ribose and arabinose, two carbohydrates which are not transported by the PTS, were accelerated. This unexpected phenotype suggests that the PTS negatively controls ribose and arabinose utilization in *L. sake* by a mechanism different from the regulation involving HPr described for other gram-positive bacteria.

In bacteria, the phosphoenolpyruvate:carbohydrate phosphotransferase system (PTS) is responsible for the uptake and phosphorylation of a number of carbohydrates (referred to here as PTS carbohydrates). A phosphorylation cascade is driven from the phosphoenolpyruvate through the general enzymes of the PTS, enzyme I and HPr, and then to the sugar-specific membrane-bound enzymes II (enzymes II^{sugar}) that concomitantly transport and phosphorylate the carbohydrates. The *ptsH* genes, encoding HPr, and *ptsI* genes, encoding enzyme I, of various bacteria have been cloned and sequenced. In several gram-positive and -negative bacteria, *ptsH* and *ptsI* are organized in an operon. The *ptsHI* operon is located downstream from *ptsG*, encoding enzyme II^{Glc}, in *Bacillus subtilis* (16) and upstream from the *crr* gene, encoding enzyme IIA^{Glc}, in *Escherichia coli* (6).

Besides its essential role in the entry of PTS carbohydrates into bacterial metabolism, the PTS is also involved in the regulation of various bacterial functions by different mechanisms, catabolite repression, inducer exclusion, and inducer expulsion (for reviews, see references 23, 24, and 27). In gram-positive bacteria, HPr plays a central role in these regulations. HPr is present in bacteria in the unphosphorylated form and can be phosphorylated at two different sites, at His 15 (P-His-HPr) by the phosphoenolpyruvate-dependent reaction catalyzed by enzyme I and at Ser 46 (P-Ser-HPr) by an ATP-dependent kinase. The P-Ser-HPr form can be dephosphorylated by a specific phosphatase. In *Enterococcus faecalis*, P-His-HPr activates the glycerol kinase, although glycerol is not a PTS carbohydrate (10). The same activation is suspected in *B. subtilis*, since several *ptsH* and *ptsI* mutants were described as unable to grow on glycerol as the sole carbon source (13, 16). This phenotype could be explained by the lack of glycerol kinase activation due to the absence of P-His-HPr; in *ptsI* mutants, the mutated enzyme I would not phosphorylate HPr on His 15, and in mutants harboring a *ptsH* deletion, all forms of HPr are missing. The second phosphorylated form of HPr, P-Ser-HPr, participates in the repression of transcription of several operons by interacting with the catabolite control protein CcpA, which binds to catabolite-responsive element sequences in operons sensitive to catabolite repression. The interaction of P-Ser-HPr and CcpA with catabolite-responsive element sequences results in transcription inhibition. The regulation by CcpA and P-Ser-HPr of the *B. subtilis gnt* operon, involved in gluconate utilization, has previously been investigated in some detail (8, 9, 12), and it was shown that a mutation at the Ser 46 residue of HPr confers resistance to catabolite repression to several catabolic genes. Although this mutation abolished glucose repression of the transcription of the gluconate kinase gene, it had no effect on gluconate uptake (9). In some gram-positive bacteria, P-Ser-HPr was shown to be involved in inducer expulsion, a mechanism by which the addition of metabolizable carbohydrates elicits the efflux of preaccumulated carbohydrates (27). In several gram-positive bacteria, the phosphorylation of HPr on the Ser 46 residue was detected, suggesting that the mechanisms of regulation by P-Ser-HPr are widespread in these microorganisms.

We investigated the PTS functions of *Lactobacillus sake*, a facultative heterofermentative lactic acid bacterium naturally occurring on meat, and found that several carbohydrates were transported by specific enzymes II of the PTS (19). The aim of the present study was to characterize the genes encoding the general enzymes of the PTS in *L. sake* and to investigate the putative involvement of HPr and enzyme I in the regulation of non-PTS carbohydrate utilization. Cloning of the *ptsHI* operon of *L. sake* allowed the construction of *ptsI* mutants. As expected, the disruption of *ptsI* abolished growth on several PTS carbohydrates. Surprisingly, it allowed bacteria to grow faster than did the parental strains on ribose and arabinose, two non-PTS carbohydrates. This observation suggests that in *L. sake*, the PTS negatively controls ribose and arabinose utilization by a mechanism different from those identified previously.

MATERIALS AND METHODS

Bacterial strains and plasmids. *E. coli* TG1 (14) and XL1Blue (Stratagene) were used for cloning and sequencing experiments. The *L. sake* strains used in this study are listed in Table 1.

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TABLE 1. *L. sake* strains

Strain	Genotype or characteristics	Source or reference
23K	Wild-type strain, plasmid cured, used as recipient strain	4
64F	Wild-type strain, partially plasmid cured	4
160X1K	Wild-type strain, plasmid cured	4
RV1000	23K *ptsI*::pRV10	This study
RV1001	23K *ptsI*::pRV11	This study
RV1002	23K *ptsI*::pRV12	This study
RV1004	23K *ptsI*::pRV16	This study

The phagemids pBluescript SK^+ and KS^+ (Stratagene) were used for cloning and subcloning experiments in *E. coli* strains. pRV300 is a pBluescript derivative containing an erythromycin resistance gene and has been used elsewhere as an integrative vector for the construction of *L. sake* mutants by single-crossover recombination (19a).

Media and growth conditions. *E. coli* strains were routinely grown on Luria-Bertani medium at 37°C (28). The complex MRS medium (Difco) described by De Man et al. (5) was used for *L. sake* propagation at 30°C. Growth rates were determined on chemically defined MCD medium (19) supplemented with various carbohydrates. The growth rates given are the means of at least three independent experiments. Ampicillin (50 mg · $liter^{-1}$ for *E. coli*) or erythromycin (5 mg · $liter^{-1}$ for *L. sake* and 200 mg · $liter^{-1}$ in *E. coli*) was added to the medium for the selection and propagation of transformants.

Transformations. *E. coli* and *L. sake* electrocompetent cells were prepared and transformed by the methods of Dower et al. (11) and Berthier et al. (3), respectively.

Oligonucleotides and PCR. Degenerated primers pts3, 5′-AA(A,G)GA(A,G)GT(C,G,T,A)GA(C,T)TT(C,T)TT(C,T)-3′, and pts5, 5′-CAT(G,A)CT(G,A)AA(C,T)TC(G,A)TC-3′, were used for PCR amplification of a DNA fragment of *ptsI* from *L. sake* 160X1K chromosomal DNA. PCR experiments were performed on a Perkin-Elmer 9600 apparatus, with *Taq* DNA polymerase from Promega. Reactions were carried out in 50 μl with 0.2 mM (each) deoxynucleoside triphosphates, 1 μg of chromosomal DNA template, 1.25% formamide, and 2.5 μM (each) primer for 30 cycles (94°C, 1 min; 55°C, 2 min; and 72°C, 3 min [for each cycle]). Formamide was omitted in subsequent experiments, when nondegenerated primers were used for amplification of various parts of the *pts* operon.

Nucleic acid extractions and hybridizations. Chromosomal DNA was prepared from *L. sake* by the method of Anderson and McKay (2). Extractions of total RNA from *L. sake* were performed with 50-ml exponential cultures in MCD medium. Bacteria were broken with glass beads in acidic phenol by the method of Anba et al. (1). Small-scale plasmid DNAs were prepared from *E. coli* by the alkaline lysis method (4). Large-scale preparations of plasmids were obtained by alkaline lysis and high-speed centrifugation in a cesium chloride gradient (28). Hybridizations were performed on Nytran-N nylon membranes (Schleicher & Schuell). Southern hybridizations were performed by enhanced chemiluminescence with a kit according to the protocol of the manufacturer (Amersham). Northern experiments and colony hybridizations with *E. coli* were performed with ^{32}P-radiolabelled probes by standard methods (28, 31).

Primer extension analysis. The 5′ end of RNA was mapped by the reverse transcriptase-directed primer extension method (31). The oligonucleotides used, PE1 (5′-ATACCTGTGTCTGCTACTACG-3′) and PE2 (5′-CCATGTTAATCGATCCCCTTT-3′), were complementary to positions 1188 to 1208 and 1151 to 1171, respectively, in the sequence of the *ptsHI* operon.

DNA sequence analysis. *E. coli* subclones were obtained by restriction enzyme subcloning or by generating overlapping deletions on the initial clones with an exonuclease III-mung bean nuclease kit (Stratagene). Single- and double-strand DNAs were sequenced according to the manufacturer's (Perkin-Elmer) protocols for cycle sequencing on a GenAmp PCR system 9600. Dideoxynucleotide chain termination sequencing reactions were performed with *Taq* DNA polymerase. Dye-coupled primers or synthetic primers and dye-coupled dideoxynucleotides (Applied Biosystems) were used. All sequences were determined on both strands. DNA sequences were analyzed with programs from the University of Wisconsin Genetics Computer Group software package.

Nucleotide sequence accession number. The *ptsHI* sequence has been deposited under GenBank accession no. U82366.

RESULTS AND DISCUSSION

Molecular cloning of the *ptsH* and *ptsI* genes. Alignments of enzyme I amino acid sequences of *E. coli*, *Salmonella typhimurium*, *B. subtilis*, *Staphylococcus carnosus*, and *Streptococcus salivarius* revealed several conserved regions (25). Two of these regions, KEVDFF and DEFSM, corresponding to amino acids 424 to 429 and 501 to 505, respectively, in *B. subtilis* enzyme I, were used to design degenerated primers pts3 and pts5. These were used for PCR amplification of chromosomal DNA from *L. sake* 160X1K. A 243-bp DNA fragment of the expected size was obtained. Its sequence revealed an open reading frame (ORF) homologous to amino acids 424 to 505 of *B. subtilis* enzyme I. This fragment was used as a probe to determine the restriction map of the *pts* region in *L. sake* by Southern hybridization experiments (Fig. 1A) and to clone by colony hybridization a 3.2-kb *Hin*dIII/*Eco*RV chromosomal DNA fragment of *L. sake* 160X1K, leading to plasmid pRV5 (Fig. 1B). A 254-bp *Hin*dIII/*Eco*RI fragment was subcloned and used as a probe to complete the restriction map and to clone a 3.1-kb overlapping *Pst*I/*Pst*I chromosomal DNA fragment, leading to plasmid pRV7 (Fig. 1B).

The inserts of pRV5 and pRV7 contained in pBluescript phagemid could be cloned in *E. coli* TG1 in only one orientation. *ptsH* and *ptsI* were oriented toward the *lacZ* gene. The *Pst*I/*Pst*I fragment could be cloned in both orientations in pBluescript (pRV7 and pRV8 [Fig. 1B]) in *E. coli* TG90 (15), a derivative of TG1 which carries the *pcnB80* mutation (20) and replicates plasmids at a low copy number.

Nucleotide sequence and deduced amino acid sequence of the *ptsH* and *ptsI* genes. The nucleotide sequence of 4,093 bp, starting at a *Sst*I site and ending at a *Rsa*I site, was determined. A 264-nucleotide ORF, corresponding to *ptsH* and encoding the 88-amino-acid HPr protein, was found. Ten nucleotides upstream from the ATG start codon, an AGGGGA motif, which could be a ribosome binding site, was found. The TAA stop codon of *ptsH* overlaps the ATG start codon of a second ORF, identified as *ptsI*. *ptsI*, encoding enzyme I, is 1,722 nucleotides (574 codons) long and stops at a TAG triplet. A putative ribosome binding site, AGGAAG, is located 13 nucleotides upstream from the start codon. A sequence resembling a transcription termination signal, which consists of a perfect palindromic sequence able to form a stem-loop structure ($\Delta G^{0'}$, −20.1 kcal mol^{-1}) followed by a stretch of seven T's, was observed 16 nucleotides downstream from the stop codon (T2; Fig. 1A). Another imperfect inverted repeat sequence which might resemble a transcription terminator-like structure (T1; Fig. 1A) was observed in the *ptsI* coding sequence ($\Delta G^{0'}$, −12.7 kcal mol^{-1}).

The two *L. sake* proteins have high levels of identity to their respective counterparts from gram-positive bacteria. In the *L. sake* HPr protein, residues His 15 and Ser 46, as well as the flanking amino acids, are conserved. This suggests that as in other gram-positive bacteria, HPr can be phosphorylated on the Ser 46 residue by HPr kinase and thus be involved in catabolite repression. In *L. sake* enzyme I, the His 190 residue, known to be the phosphorylatable amino acid, is also conserved.

In *B. subtilis*, *E. coli*, and *S. typhimurium*, a small ORF (*orfK*) overlapping *ptsH* but in the opposite orientation was observed (7, 17, 29). Although it has been shown that *orfK* is expressed in *B. subtilis*, no evidence for its function has been obtained (17). No homology between *B. subtilis* and *E. coli* OrfKs was observed. In *L. sake*, an overlapping ORF of 85 amino acids is present. Its 55 C-terminal amino acids show 64% identity to *B. subtilis* OrfK; nevertheless, no experimental evidence for an *orfK* equivalent in *L. sake* was obtained. Furthermore, with the HPr protein of *L. sake* being 73% similar to *B. subtilis* HPr, the homologies between the two overlapping OrfKs might be due to chance.

In several other bacteria, the *ptsH* and *ptsI* genes are located near the genes encoding enzyme II^{Glc}. We have previously shown that in *L. sake*, glucose is not transported by enzyme

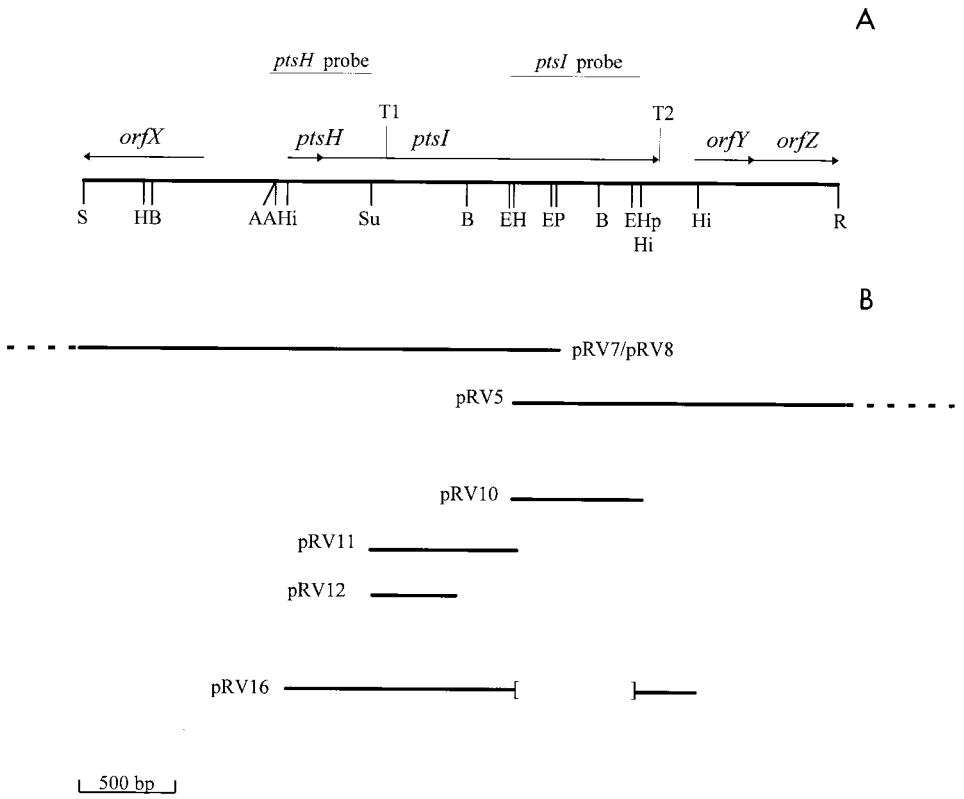

FIG. 1. Restriction map and gene organization of the *L. sake pts* locus. The following restriction sites are indicated: A, *Acc*I; B, *Bgl*I; E, *Eco*RI; H, *Hin*dIII; Hi, *Hin*cII; Hp, *Hpa*I; P, *Pst*I; R, *Rsa*I; S, *Sst*I; Su, *Stu*I; and V, *Eco*RV. Arrows indicate the directions of the *ptsH* and *ptsI* genes and of the three ORFs located upstream and downstream from the *pts* locus. T1 and T2 indicate the positions of two putative transcription terminators. The probes used for Northern experiments are shown above the restriction map. Plasmids used in this study are indicated below the restriction map. Plasmids pRV5, pRV7, and pRV8 contained inserts cloned in pBluescript that extend beyond the represented region (interrupted lines). Plasmids pRV10, pRV11, pRV12, and pRV16 contained inserts cloned into the pRV300 integrative vector. The deletion in pRV16 is indicated by brackets.

II^{Glc} but rather by enzyme II^{Man} and a non-PTS transport system (19). Two hundred ten nucleotides downstream from *ptsI*, a 438-nucleotide ORF was observed. This ORF, *orfY*, encodes a 146-amino-acid peptide that is 42% identical to CopY, the negative regulator of the *copYZAB* operon of *Enterococcus hirae*, which encodes copper ATPase (21). Two nucleotides downstream from the TAA stop codon of *orfY*, the 5′ end of *orfZ*, starting with an ATG (preceded by a GA-rich region), was observed. The protein deduced from the nucleic acid sequence of the 5′ end of *orfZ* (114 residues) is 53% identical to amino acids 68 to 183 of the N-terminal part of CopB, a P-type ATPase involved in copper efflux and encoded, as is CopY, by the *copYZAB* operon of *E. hirae* (22). *orfY* and *orfZ*, separated by only 2 nucleotides, should belong to a single operon since a unique promoter-like sequence was found upstream from *orfY*.

Five hundred sixty-eight nucleotides upstream from *ptsH*, we observed the 5′ end of a divergent ORF (*orf*X) encoding a protein with 40 to 50% identity to the ClpA, ClpB, and ClpC proteins of various bacterial species (30). Clp proteins have ATPase activity and possess two conserved domains separated by a spacer region, the size of which defines ClpA, ClpB, and ClpC proteins (30). The part of *orfX* cloned in pRV7 does not extend to the spacer region. Thus, we cannot conclude which Clp protein might be encoded by *orfX*. Whether the observation that the *ptsHI* operon of *L. sake* is flanked by genes encoding enzymes with ATPase activities is of physiological significance is not known at present.

Transcription of *ptsH* and *ptsI* genes. Primer extension experiments were carried out with total RNAs extracted from *L. sake* 23K cells grown on MCD medium containing glucose or ribose. Major and minor transcription starts were detected 78 and 50 nucleotides, respectively, upstream from *ptsH* (Fig. 2A). One putative promoter region containing −10 and −35 boxes is present upstream from the major transcription start point. The signal obtained with RNA isolated from bacteria grown on glucose was slightly stronger than that with extracts from ribose-grown bacteria.

Northern hybridization experiments were performed with total RNAs extracted from *L. sake* 23K cells grown on MCD media containing various carbohydrates. When the probe used was a 716-bp *Hin*dIII/*Hpa*I fragment (Fig. 1) internal to *ptsI*, a 2.1-kb transcript was detected (Fig. 2B). This size correlates with that of a transcript initiated at the site determined by primer extension experiments and ending at the palindromic sequence, T2, present downstream from *ptsI*. This transcript would thus encode both HPr and enzyme I. When the *Acc*I/*Stu*I DNA fragment (Fig. 1) containing *ptsH* and the 5′ end of *ptsI* was used, two transcripts, one of 2.1 kb and another of 0.6 kb, were detected (Fig. 2B). The short transcript might encode HPr only. However, it was expressed far less than was the larger transcript (Fig. 2B). The size of the smaller transcript fits initiation at the promoter and termination at the imperfect palindromic sequence, T1. The carbon source used for growth did not affect significantly the intensity of the hybridization signal, suggesting that the two transcripts are constitutively

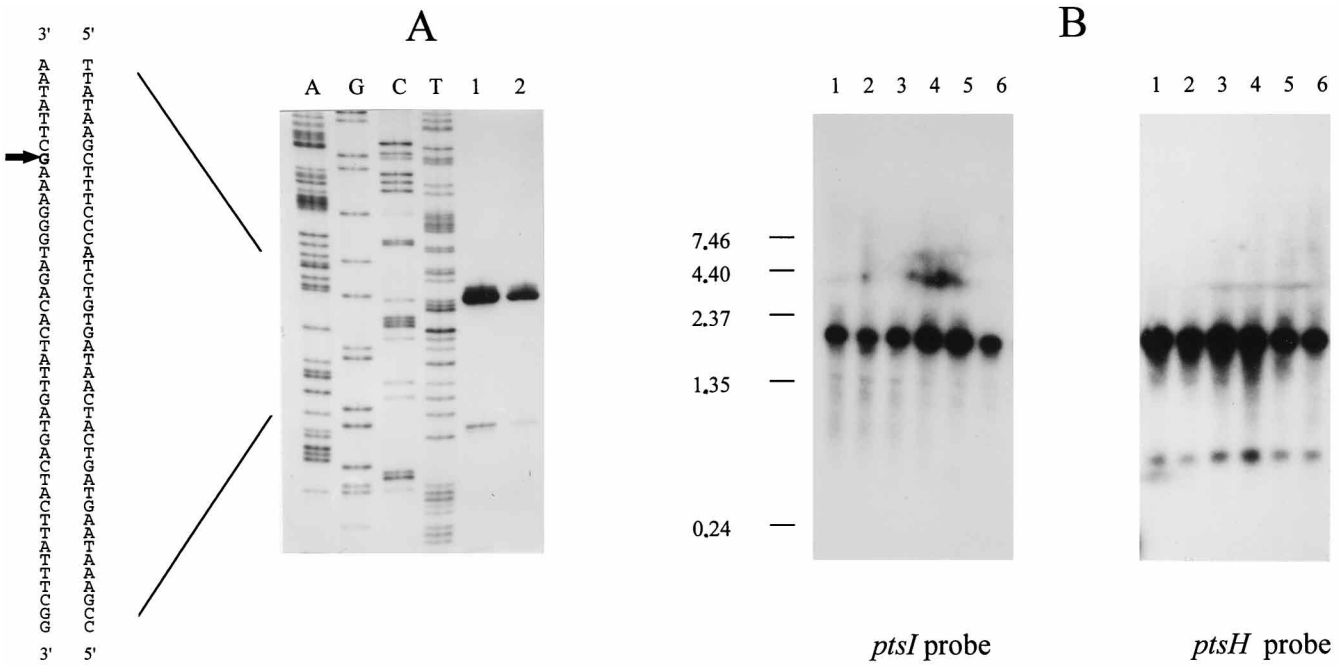

FIG. 2. Transcription analysis of the *L. sake ptsHI* genes. (A) Primer extension analysis performed with primer PE1. The sequence ladder (lanes A, G, C, and T) was generated with the same primer. RNA was isolated from strain 23K cells grown on glucose (lane 1) or ribose (lane 2). The arrow indicates the major transcription start site. (B) Northern blot analysis. Total RNAs were extracted from *L. sake* 23K cells grown on MCD medium containing glucose (lanes 1), glucose without thiamine (lanes 2), sucrose (lanes 3), fructose (lanes 4), mannose (lanes 5), or ribose (lanes 6). The probes used were a *Hin*dIII/*Hpa*I DNA fragment in *ptsI* and an *Acc*I/*Stu*I DNA fragment corresponding to *ptsH*, as indicated. Size standards (in kilobases) are given on the left.

expressed. However, the signal intensity decreased significantly when RNAs were prepared from late-exponential-phase cells, suggesting that the expression of *ptsHI* depends on the growth phase (data not shown).

Sequence data and the results obtained from RNA analyses showed that *ptsH*, encoding HPr, and *ptsI*, encoding enzyme I, are organized in an operon. Both genes are cotranscribed, and their transcription is not regulated by the carbon source used for growing bacteria. Such cotranscription of *ptsH* and *ptsI* was shown in *E. coli* (6) and *B. subtilis* (17). A small transcript weakly expressed and possibly encoding only HPr was detected in *L. sake*. Such a transcript was observed in *E. coli* and suspected in *S. carnosus* because of the presence of a transcription terminator-like structure in *ptsI*, found also in *E. coli* and *B. subtilis* (17, 18, 26). In structure, the *ptsHI* operon of *L. sake* thus seems similar to the *ptsHI* operons of other bacteria.

Construction of *ptsI* mutants. To confirm that the cloned genes encode the general enzymes of the PTS and to investigate the putative involvement of these enzymes in non-PTS carbohydrate utilization, we constructed *ptsI* mutants by chromosomal integration. We recently studied homologous recombination in *L. sake* and constructed an integrative vector, pRV300, carrying a resistance gene for erythromycin (19a). To construct a series of *L. sake ptsI* mutants, a series of inserts internal to *ptsI* (a 716-bp *Hin*dIII/*Hpa*I fragment, an 816-bp *Stu*I/*Hin*dIII fragment, and a 441-bp *Stu*I/*Bgl*I fragment [Fig. 1B]) were cloned in pRV300, leading to plasmids pRV10, pRV11, and pRV12, respectively. A fourth plasmid, pRV16, containing a 1,068-bp *Hin*cII/*Eco*RI fragment and a 288-bp *Hin*cII/*Hin*cII fragment cloned in pRV300 was also constructed (Fig. 1B). This plasmid contained the 3′ end of *ptsI*, including transcription terminator T2, with a 759-bp deletion in *ptsI*. In pRV10 and pRV16, the *ptsI* fragments were oriented toward the erythromycin and ampicillin resistance genes. In pRV11 and pRV12, the *ptsI* fragments were in the same orientation as the antibiotic resistance genes. *L. sake* 23K was transformed with pRV10, pRV11, pRV12, and pRV16 for erythromycin resistance, leading to mutant strains *L. sake* RV1000, RV1001, RV1002, and RV1004, respectively. The integration of plasmids pRV10, pRV11, and pRV12 should lead to the inactivation of *ptsI*, truncated at different locations. The integration of pRV16 by recombination within the *Hin*cII/*Eco*RI fragment should lead to a mutated *ptsI* gene containing a 759-bp deletion. Recombination of pRV16 at the 288-bp *Hin*cII/*Hin*cII fragment was not expected since we have recently shown that the smallest size required for recombination with integrative vector pRV300 in *L. sake* is about 300 bp (19a). The integration of plasmids at the *pts* locus by a single-crossover event was verified by PCR experiments with chromosomal DNA extracted from transformants and with primers located upstream and downstream from the inserts cloned in pRV300 (data not shown).

Further evidence for inactivation of the *ptsI* gene in *L. sake* mutant strains RV1000, RV1001, RV1002, and RV1004 was obtained by Northern experiments with RNAs isolated from ribose-grown bacteria and hybridized to the *Acc*I/*Stu*I probe (Fig. 3). In RV1001 and RV1002, the large transcript was not detected. In RV1004, the 2.1-kb transcript was shortened to 1.3 kb. This size was consistent with the deletion of part of *ptsI*. In RV1000, a larger transcript was observed; its size is consistent with the presence of a transcription terminator, located in pRV300, at the 3′ end of the erythromycin resistance gene. The amounts of the large transcript in RV1000 and RV1004 were similar to the wild-type level. The small transcript, encoding HPr, was detected in the mutants at the same low level as it was in 23K. We thus concluded that RV1000 and RV1004 lack enzyme I but synthesize normal amounts of HPr and that

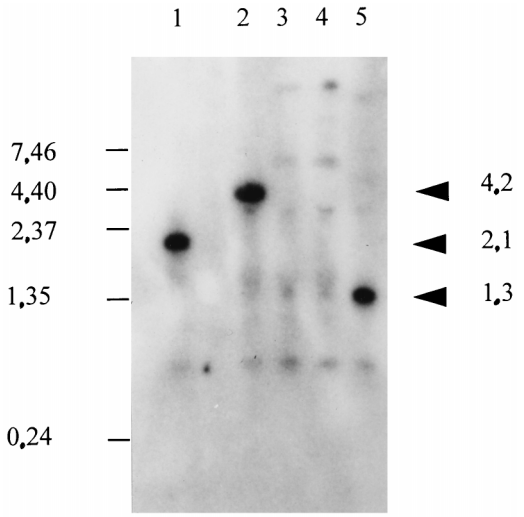

FIG. 3. Northern blot analysis of *pts* transcripts in *ptsI* mutants. The *ptsH* probe used was the same as that used in Fig. 3B. Lanes: 1, 23K; 2, RV1000; 3, RV1001; 4, RV1002; 5, RV1004. The positions of major transcripts are shown on the right. Size standards (in kilobases) are given on the left.

the mutations in RV1001 and RV1002 lead to the lack of enzyme I and to a dramatic decrease in HPr expression.

Phenotypic analysis of *L. sake pts* mutants. *L. sake* mutant strains were tested for their ability to grow on various carbon sources on MCD plates containing erythromycin. As expected, none of the mutants grew on sucrose, fructose, mannose, or *N*-acetylglucosamine, previously shown to be PTS carbohydrates in *L. sake* (19). The growth of the mutants on glucose was partially impaired compared to that of the wild-type strain. This result is in agreement with the previous observation that in *L. sake*, glucose is transported by both a PTS permease and a non-PTS permease (19). Surprisingly, the growth of the mutant strains on ribose and arabinose, two non-PTS carbohydrates, was faster than that of the wild-type strain. In MCD liquid medium containing 0.1% ribose, the doubling time of strains RV1000 and RV1004 was 164 ± 8 min, while it was over 360 min for the parental strain. Such improved growth of *pts* mutants on non-PTS carbohydrates had not been observed previously in any bacteria. This prompted us to test that this unexpected phenotype was not caused by secondary mutations selected during construction of the mutants, by isolating revertants that lost the integrative plasmid by recombination between the flanking repeated sequences. For that purpose, strain RV1000 was grown in complex MRS medium without erythromycin for 30 to 40 generations, plated on MRS, and replica plated on MRS with or without erythromycin. The lack of selective pressure allowed the loss of pRV10 integrated into the chromosome, characterized by the loss of erythromycin resistance. Four erythromycin-sensitive clones were tested for their ability to grow on various carbohydrates on MCD plates. All were again able to grow on PTS carbohydrates and grew slowly on ribose and arabinose, as did the initial wild-type strain. These results show that the unexpected phenotype observed in RV1000, RV1001, RV1002, and RV1004 is indeed the consequence of the *pts* mutation and suggest a new regulation mechanism involving the PTS.

In order to test that the observation for *L. sake* 23K transformants was not strain specific, plasmid pRV10 was used to transform *L. sake* 64F in order to construct a *pts* mutant in a different genetic background. On MCD plates, the transformants obtained were unable to grow on mannose, fructose, and sucrose but grew on arabinose and ribose faster than did 64F. This shows that the involvement of the PTS in arabinose and ribose utilization is not strain specific but is a property of the *L. sake* species.

These results suggest that in wild-type *L. sake* strains, the general enzymes of the PTS negatively regulate the utilization of ribose and arabinose, directly or indirectly. From the experimental data presented here, we cannot yet conclude on which function, gene, or protein this regulation is exerted. Although no extensive studies have been done of *L. sake* metabolic pathways, we have previously shown that thiamine is required for the utilization of these two carbon sources, suggesting that the phosphoketolase pathway is used for both of them (19). It is thus possible that negative regulation of the PTS acts on a catabolic enzyme of this pathway, common to the degradation of ribose and arabinose, or that the regulation occurs earlier in the pathway on two functions, each specific for ribose or arabinose. Further investigations of the genes involved in the utilization of these two carbohydrates are required to determine the target of this new regulation and to establish the mechanism by which the disruption of *ptsI* can result in a phenotype not described previously for other bacteria. In two of the *L. sake* mutants reported here, the transcript encoding HPr and a truncated enzyme I was expressed at the same level as in the wild-type strain. In these mutants, unphosphorylated HPr, and possibly P-Ser-HPr, might thus be present, as in the parental strain, whereas these two forms might be lacking or expressed at very low levels in the two other mutants, in which transcription of *ptsHI* was not detected. Since the phenotypes of the two classes of mutants were similar, the involvement of HPr and P-Ser-HPr in this regulation seems unlikely. In all mutant strains, P-His-HPr, as well as phosphorylated and unphosphorylated forms of enzyme I, should be lacking. Thus, we propose that one of these proteins might be the regulatory molecule. P-His-HPr or P-enzyme I might exert in wild-type *L. sake* cells negative regulation of a function responsible for ribose and arabinose utilization. Negative regulation would thus be inefficient in *pts* mutants. A role for unphosphorylated enzyme I is unlikely, since its concentration in bacteria growing on non-PTS carbohydrates is expected to be low.

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