

Genetics of L-Sorbose Transport and Metabolism in *Lactobacillus casei*

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Genes encoding L-sorbose metabolism of *Lactobacillus casei* ATCC 393 have been identified on a 6.8-kb chromosomal DNA fragment. Sequence analysis revealed seven complete genes and a partial open reading frame transcribed as two units. The deduced amino acid sequences of the first transcriptional unit (*sorRE*) showed high similarity to the transcriptional regulator and the L-sorbose-1-phosphate reductase of the sorbose (*sor*) operon from *Klebsiella pneumoniae*. The other genes are transcribed as one unit (*sorFABCDG*) in opposite direction to *sorRE*. The deduced peptide sequence of *sorF* showed homology with the D-sorbitol-6-phosphate dehydrogenase encoded in the *sor* operon from *K. pneumoniae* and *sorABCD* to components of the mannose phosphotransferase system (PTS) family but especially to domains EIIA, EIIB, EIIC and EIID of the phosphoenolpyruvate-dependent L-sorbose PTS from *K. pneumoniae*. Finally, the deduced amino acid sequence of a truncated gene (*sorG*) located downstream of *sorD* presented high similarity with ketose-1,6-bisphosphate aldolases. Results of studies on enzyme activities and transcriptional analysis revealed that the two gene clusters, *sorRE* and *sorFABCDG*, are induced by L-sorbose and subject to catabolite repression by D-glucose. Data indicating that the catabolite repression is mediated by components of the PTS elements and by CcpA, are presented. Results of sugar uptake assays in *L. casei* wild-type and *sorBC* mutant strains indicated that L-sorbose is taken up by L-sorbose-specific enzyme II and that *L. casei* contains an inducible D-fructose-specific PTS. Results of growth analysis of those strains and a *man sorBC* double mutant suggested that L-sorbose is probably also transported by the D-mannose PTS. We also present evidence, from studies on a *sorR* mutant, suggesting that the *sorR* gene encodes a positive regulator of the two *sor* operons. Sequence alignment of SorR, SorC (*K. pneumoniae*), and DeoR (*Bacillus subtilis*) revealed that they might constitute a new group of transcriptional regulators.

In the enteric bacteria *Klebsiella pneumoniae* and *Escherichia coli*, the ketose L-sorbose is transported and phosphorylated through a phosphoenolpyruvate-dependent L-sorbose-specific phosphotransferase system (PTS) (36, 46, 47). The metabolism of L-sorbose involves the formation of D-fructose-6-phosphate, which enters the glycolysis pathway (Fig. 1A). During uptake of a PTS carbohydrate, the common PTS proteins, enzyme I (EI) and HPr, transfer a phosphoryl group from phosphoenolpyruvate to the substrate-specific EII complexes. The L-sorbose PTS EII consists of two membrane-bound proteins, EIIC^{Sor} and EIID^{Sor}, and two soluble components, EIIA^{Sor} and EIIB^{Sor} (47). Sequence analysis revealed that EIIS^{Sor} was homologous to D-mannose PTS elements of *E. coli* (10), EIIL^{Lev} of *Bacillus subtilis* (21), and the putative EIIA^{Ga} of *E. coli* (28). These four PTSs constitute the so-called mannose PTS class. In this class, HPr phosphorylates the EIIA domain at a conserved histidine, and the phosphoryl group is transferred in the next step to another histidyl residue in the EIIB domain. Sugar translocation occurs through EIIC and EIID, integrated in the membrane, and EIIB carries out the concomitant sugar phosphorylation (26, 29). The *K. pneumoniae* EIIS^{Sor} could also

transport D-fructose, and the *E. coli* EIIB^{Man} domain could complement the lack of the two soluble proteins EIIS^{Sor} and EIIB^{Sor} in *K. pneumoniae* (47). Similarly, EIIC^{Lev} and EIID^{Lev} elements of the levanase operon of *B. subtilis* were able to transport D-mannose in addition to D-fructose. Moreover, these two domains could substitute in *E. coli* for the function of EIIC^{Man} and EIID^{Man} for D-mannose uptake and act as λ bacteriophage receptors (23).

PTS elements are involved in sugar uptake and also in the regulation of chemotaxis, carbon metabolism, and modulation of gene expression (9, 20, 27). Many catabolic operons in bacteria are subject to carbon catabolite repression (CR) by rapidly metabolizable carbon sources, especially glucose (31, 37). The main CR mechanism identified in *E. coli* can be summarized as follows. EIIB^{Glc} acts as the sensor of extracellular glucose inducing adenylate cyclase. This increases the cyclic AMP pool, which acts as an effector of the true repressor, the catabolite-activating protein, that binds to the promoter region of the genes under CR (26). However, in gram-positive bacteria with low GC content, CR follows a completely different pattern. Besides the phosphorylation by EI-P on HPr residue histidine 15, HPr(His-P), required for PTS sugar transport, HPr can also be phosphorylated at the serine 46 residue, HPr(Ser-P), by a specific ATP-dependent protein kinase (7). Then, HPr(Ser-P) interacts with CcpA, which binds to a conserved sequence in the promoter regions, the catabolite-responsive element (*cre*), and negatively controls the expression of several carbohydrate catabolic enzymes (8, 16, 22). However, recent studies showed that there could be other mechanisms for the control of gene expression in the presence of readily metabolizable carbon sources. A family of regulator

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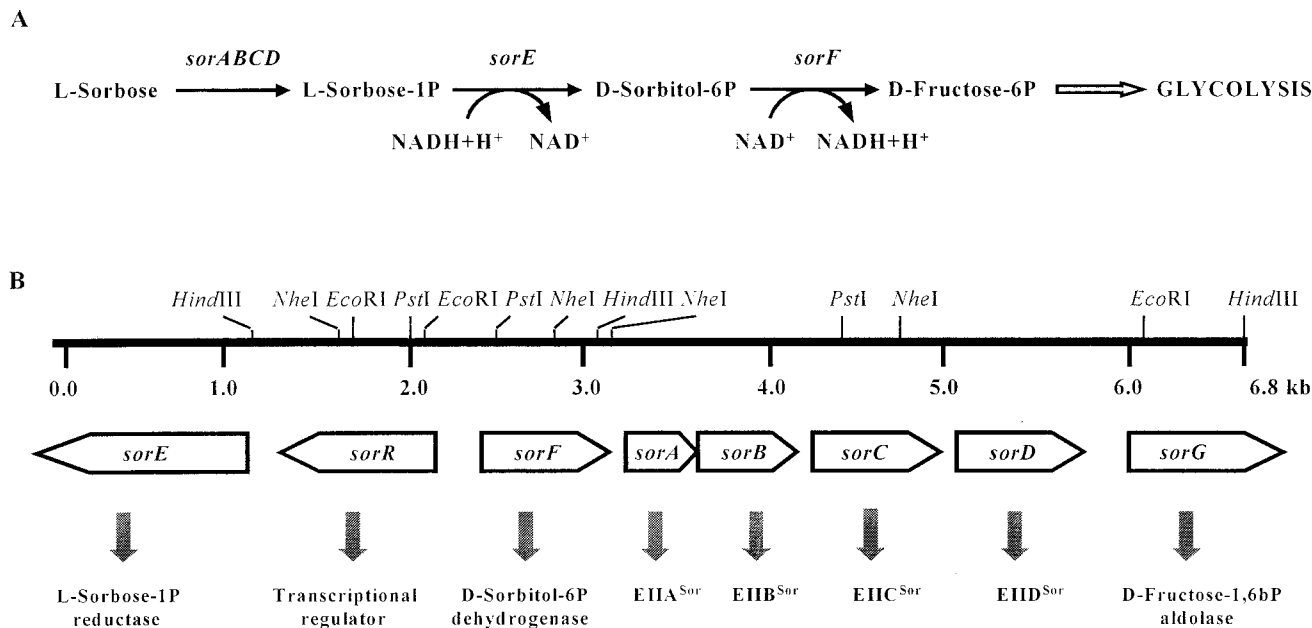


FIG. 1. (A) Schematic representation of the transport and metabolism of L-sorbose. (B) Simplified restriction map and genetic organization of the sorbose operons from *L. casei*.

proteins that contain consensus PTS-regulated domains (PRD), such as the positive regulator LicR from *B. subtilis* (40), and antiterminators like BglG from *E. coli* (11), LicT, GlcT and SacY from *B. subtilis* (see reviews in references 37 and 41), and LacT from *Lactobacillus casei* (13), has been described. These regulators can activate the transcription of certain genes in the presence of the inducing molecule by dephosphorylation of a PRD, but their activity also can be modulated by HPr(His-P) phosphorylation of specific histidine residues in a second PRD in such a way that in the presence of rapidly metabolizable carbon sources, the regulators would be inactive. This mechanism has been considered an alternative form of CR mediated by HPr. A major interest of our laboratory includes carbohydrate-specific regulation of gene expression in *L. casei*, a facultative heterofermentative lactic acid bacterium frequently isolated from fermented food products and from human and other mammalian intestines. *L. casei* contains a D-mannose-specific PTS for glucose and mannose uptake. It was shown that the lack of a functional EII^{Man} leads to derepression of *lac* and *rbs* genes. In this strain, glucose can also enter the cell by a proton motive force-driven permease (44). Likewise, the chromosomal *ccpA* gene from *L. casei* was isolated and sequenced, and a mutant deficient in this gene was constructed (24).

Results of an investigation of the components of the EII^{Man} family in *L. casei* ATCC 393 cured of plasmid pLZ15 (strain BL23) prompted a study of the *sor* operons, the results of which are presented here. Sequence analysis revealed eight open reading frames (ORFs) whose products are involved in L-sorbose transport and its conversion to D-fructose-6-phosphate. These genes are organized in two clusters, *sorRE* and *sorFABCDG*, which are induced by L-sorbose and repressed by D-glucose. These findings constitute the first data on the genetics and metabolism of L-sorbose in gram-positive bacteria.

MATERIALS AND METHODS

Bacterial strains and culture conditions. *L. casei* wild-type strain BL23, *man* mutant BL23D (44), *ccpA* mutant BL71 (24), *man ccpA* double mutant BL72 (13), and *ptsI* mutant BL126 (R. Viana, V. Monedero, and G. Pérez-Martínez, unpublished data) were routinely grown at 37°C under static conditions on MRS medium (Oxoid), MRS basal medium (44), or MRS fermentation medium

(Adsa-Micro) supplemented with sugars at the concentrations indicated. *E. coli* DH5 α , *E. coli* DH10B, and *E. coli* XL1-Blue (Stratagene, La Jolla, Calif.) were used as hosts in cloning procedures. These strains were grown in Luria-Bertani medium at 37°C with vigorous shaking. Agar plates containing the same media were prepared. *E. coli* transformants were selected with ampicillin (50 μ g/ml) and 5-bromo-4-chloro-3-indolyl- β -D-galactoside (X-Gal) (40 μ g/ml); *L. casei* transformants were selected with erythromycin (5 μ g/ml).

Plasmids and transformation. The *L. casei* library (13) was constructed in pJDC9 (6). Plasmids pUC18 and pBluescript II SK+ (Stratagene) were used for subcloning and sequencing experiments. The integrative vector pRV300 (19) was used for insertional inactivation of genes in the *L. casei* chromosome. Transformation of *L. casei* and *E. coli* strains was performed by electroporation with a Gene Pulser apparatus (Bio-Rad Laboratories, Richmond, Calif.) as previously described (25) and as recommended by the manufacturer, respectively.

Cloning and DNA sequencing. Total DNA was isolated from *L. casei* as described before (25) and used as the template in a standard PCR with two primers, *man11* (5'-GGAGGSASSSCCATATAAYGC) and *man2* (5'-AGCYTRCCATGAATYAA), designed from the sequence of the *L. curvatus* mannose PTS genes (43). An amplified DNA fragment of the expected size was cloned in pUC18 and checked by DNA sequencing; then new internal primers were designed and used to screen by PCR a genetic library of *L. casei* in *E. coli* DH5 α (13). Isolation of plasmid DNA from *E. coli*, restriction endonuclease analysis, ligations, inverse PCR experiments, and construction of subclones in pBluescript II KS+ were performed by standard procedures (32). DNA sequencing was carried out by using an ABI PRISM dRhodamine Terminator Cycle Sequencing Ready Reaction kit with AmpliTaq DNA polymerase and an automatic ABI 310 DNA sequencer (Perkin-Elmer Corp.). M13 universal and reverse primers or primers that hybridize within the cloned DNA were used for this purpose. Alignment of sequences from different clones and analysis of the sequence for ORFs were carried out with version 8.1 of the Genetics Computer Group (Madison, Wis.) package. Sequence similarities were analyzed with the BLAST and FASTA programs.

Northern blot analysis. RNA was isolated from *L. casei* cells grown in MRS fermentation medium with 0.5% appropriate sugars to an optical density at 550 nm (OD_{550}) of 0.8. Cells were collected by centrifugation, washed with 50 mM EDTA (pH 8.0), resuspended in 1 ml of TRIZOL reagent (Gibco BRL, Grand Island, N.Y.), and then mechanically disrupted with glass beads in a cell disruptor (Savant Instruments, Holbrook, N.Y.). Total RNA was isolated according to the protocol of the TRIZOL manufacturer. Sample preparation, denaturing agarose gel electrophoresis, and RNA transfer were performed by standard methods (32). DNA probes for *sorE*, *sorR*, *sorF*, *sorC*, and *sorG* genes were synthesized in PCRs using *L. casei* chromosomal DNA as the template, deoxynucleoside triphosphate mix with digoxigenin-dUTP from the Boehringer digoxigenin-labeling kit, and TaqPlus Precision (Stratagene). Each probe was obtained by using two primers that hybridized within the appropriate gene: *sorE* probe with primers *sorE1* (5'-CCATAATCCAGCAGTACTTG) and *sorE2* (5'-CTTTCTTCATTG GGCCTGCG), *sorR* probe with *sorR1* (5'-AAGGCGTTGTTTCAATTGCC) and *sorR3* (5'-ACACGTGTCCACAGGCTAAG), *sorF* probe with *sorF1* (5'-A

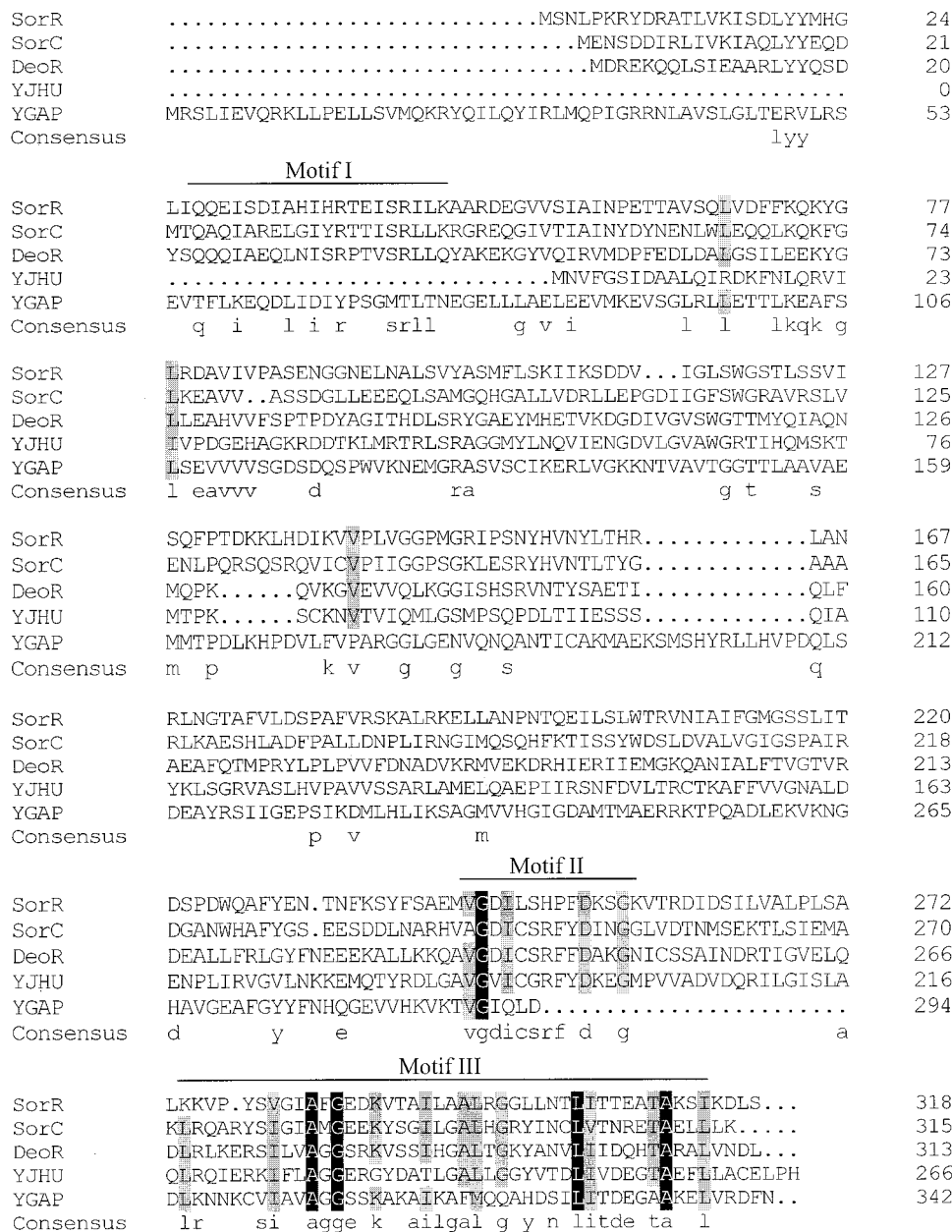


FIG. 3. Multiple amino acid sequence alignment of SorR from *L. casei* (this work), SorC from *K. pneumoniae* (accession no. P37078), DeoR from *B. subtilis* (accession no. P39140), YJHU from *E. coli* (accession no. P39356), and YGAP from *B. megaterium* (accession no. P35168). The residue number of each protein is indicated at the right. The consensus sequence (at least three residues conserved) is shown in lowercase letters. Residues conserved in all proteins are shown against a dark background. Residues that are similar in four sequences appear against a shaded background. Motif I indicates the position of the putative DNA-binding sites; motifs II and III indicate highly conserved regions.

Sor-PR and the rate of NAD⁺ reduction for Stol-PDh were determined by measuring the rate of absorbance change at 340 nm. Specific enzyme activities are given in nanomoles per minute per milligram of protein. Protein concentrations were determined by the method of Bradford (2).

Nucleotide sequence accession number. The nucleotide sequence data reported here have been submitted to the GenBank database under accession no. AF129168.

RESULTS

Cloning and sequence analysis of the sor genes. PCR amplification of *L. casei* DNA with two primers synthesized from the sequence of the *L. curvatus* D-mannose-specific PTS genes (*manA* and *manB*) (43) yielded a DNA fragment with the ex-

pected size (280 bp). This fragment was sequenced and used to screen by PCR a genetic library of *L. casei* in *E. coli* DH5α (13), and a positive clone containing a 3,639-bp insert in pJDC9 was obtained. Different restriction endonuclease fragments derived from this DNA insert were subcloned into pBlueScript II SK+ and sequenced. Sequence analysis revealed four ORFs. The highest homology was detected with Sor-PR (58% identical residues), the sorbose operon regulator (34% identical residues), and Stol-PDh (61% identical residues) of the *sor* operon of *K. pneumoniae* (45). The fourth ORF showed similarity with the EIIA component of the L-sorbose-specific PTS of *K. pneumoniae* (30% identical residues), the D-mannose-specific PTS

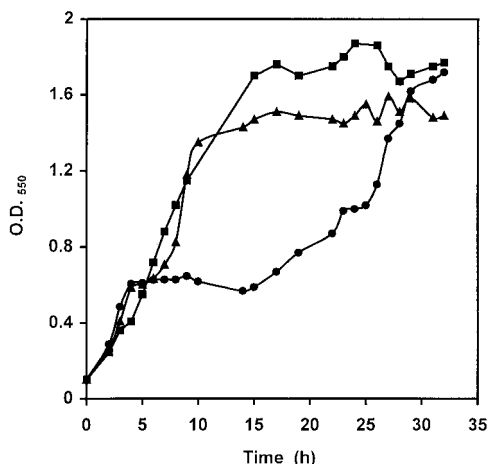


FIG. 4. Growth of *L. casei* on MRS basal medium containing 0.1% glucose plus 0.2% sorbose. ●, wild type (BL23); ▲, *ccpA* mutant (BL71); ■, *man* mutant (BL23D).

of *E. coli* (35% identical residues), and the D-fructose-specific PTS of the *lev* operon from *B. subtilis* (34% identical residues) (10, 21, 45).

Several attempts to obtain clones containing the upstream and downstream regions of the cloned DNA insert were unsuccessful. Reverse PCR experiments allowed us to sequence a 3,196-bp fragment downstream of the 3' end and a 300-bp fragment upstream of the 5' end. Sequence analysis revealed four additional ORFs. They showed high similarity to PTS proteins such as EIIB, EIIC, and EIID of the L-sorbose-specific PTS from *K. pneumoniae* (65, 70, and 72% identical residues, respectively) (45), EIIB, EIIC, and EIID of the D-mannose-specific PTS from *E. coli* (54, 61, and 57% identical residues, respectively) (10), and EIIB, EIIC, and EIID of the *lev* operon from *B. subtilis* (42, 51, and 54% identical residues, respectively) (21). The last ORF showed similarity to known ketose-bisphosphate aldolases, notably to the D-fructose-bisphosphate aldolase of *B. subtilis* (42% identical residues) (42).

We have named the *sor* genes of *L. casei* according to sequence homology of the proteins that they encode. Hence the gene for the transcriptional regulator is *sorR*, those encoding the sorbose-specific PTS elements are *sorA*, *sorB*, *sorC*, and *sorD*, and then the metabolic genes, named in alphabetical order, are *sorE*, *sorF*, and *sorG*, for the genes encoding Sor-PR, Stol-PDh, and D-fructose-1,6-bisphosphate aldolase, respectively. Schematic representation of the L-sorbose pathway and a restriction endonuclease map of the L-sorbose operons of *L. casei* are illustrated in Fig. 1. A putative promoter region was identified in each DNA strand of the intergenic region between *sorR* and *sorF* (Fig. 2). Both regions are AT rich and have potential -10 and -35 boxes. A *cre*-like sequence consisting of a perfect palindrome of 14 bp was also found in that region (17).

sorE (412 amino acids) has two potential ATG start codons separated by four codons, but only one putative ribosome-binding site (35) was detected in front of the second ATG. Downstream of this gene, a potential *rho*-independent terminator was found. The second ORF, *sorR*, was found upstream of *sorE*. The deduced amino acid sequence corresponded to a protein of 318 amino acids (M_r , 34,913). An intergenic region of 136 bp separates the third ORF, *sorF*, which is transcribed in the direction opposite that of *sorR* and *sorE* and encodes a protein of 266 amino acids (M_r , 28,459). *sorA* encodes the first protein of the L-sorbose-specific PTS system, EIIB^{Sor}, with 138 amino acids (M_r , 14,985). The stop codon of *sorA* overlapped

in one base with the start codon of *sorB*. This ORF was predicted to encode EIIB^{Sor} (164 amino acids; M_r , 18,304). EIIB^{Sor} and EIIB^{Sor} contain histidyl residues at positions 10 and 14, respectively, which align with the phosphorylation sites of the homologous IIA and IIB proteins. Thirteen base pairs from the *sorB* we found the start codon of *sorC*. Its predicted amino acid sequence corresponds to a protein, EIIC^{Sor}, of 277 amino acids (M_r , 29,002). Downstream we found *sorD*, the deduced product of the ORF corresponds to a 282-amino-acid protein, EIID^{Sor} (M_r , 30,788). After a stretch of 191 bp, an incomplete ORF, *sorG*, truncated at amino acid 276, was found. Ribosome-binding sites were found always between 4 and 10 bp upstream of the start codons of all ORFs.

Nature of SorR. When the predicted amino acid sequence of SorR was used in a Blast search, the most homologous proteins were the *sor* operon regulator (SorC) from *K. pneumoniae* (45), the deoxyribonucleoside regulator (DeoR) from *B. subtilis* (33), the hypothetical transcriptional regulator in the *fecI-fimB* intergenic region (YJHU) from *E. coli* (3), and the hypothetical transcriptional regulator of the *gap* operon (YGAP) from *Bacillus megaterium* (34). Alignment of the SorR sequence with those sequences is shown in Fig. 3. The amino-terminal region contains the hypothetical DNA-binding helix-turn-helix motif, except for the YJHU sequence, which suggests that it could be a sugar kinase rather than a transcriptional regulator (39). The carboxy-terminal region reveals two highly conserved motifs, motifs II and III, of uncertain function. However, motif III showed homology with the carboxy-terminal region of the ROK family, which includes transcriptional repressors, ORFs of unknown function, and sugar kinases (39), suggesting that it could be involved in sugar binding. Based on the unique features just mentioned, *L. casei* SorR, *K. pneumoniae* SorC, and *B. subtilis* DeoR could be representative of a new group of transcriptional regulators.

Growth pattern on D-glucose and L-sorbose of *L. casei* strains. When the wild type was grown on a basal MRS medium supplemented with 0.1% D-glucose and 0.2% L-sorbose, growth stopped for about 10 h, possibly after D-glucose was consumed (Fig. 4). However, the *man* mutant, BL23D, displayed no diauxic growth, and in the *ccpA* mutant, BL71, the plateau of diauxic growth was reduced to only 3 h. These results indicated that the *sor* operon is repressed by glucose and that the repression is mediated by PTS elements and by the CcpA protein.

Transcription analysis of the *sor* genes. Northern blot experiments were performed with internal DNA fragments of the coding regions of *sorE*, *sorR*, *sorF*, *sorC*, and *sorG* as probes. The results with the *sorE* and *sorF* probes are shown in Fig. 5.

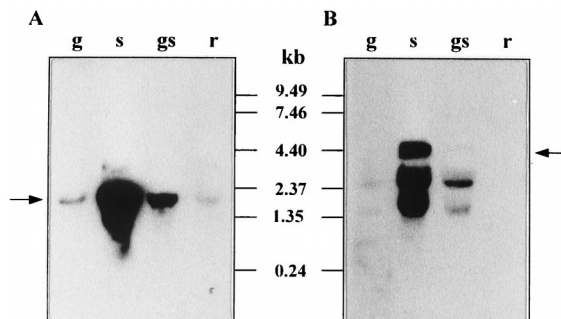


FIG. 5. Northern blot analysis of *sor* mRNA, using an internal DNA fragment of *sorE* (A) or *sorF* (B) as the probe. RNA was isolated from *L. casei* grown with 0.5% glucose (g), 0.5% sorbose (s), 0.5% glucose plus 0.5% sorbose (gs), or 0.5% ribose (r). Arrows indicate transcripts of about 2,200 and 4,400 nucleotides; positions of size standards are marked in the center.

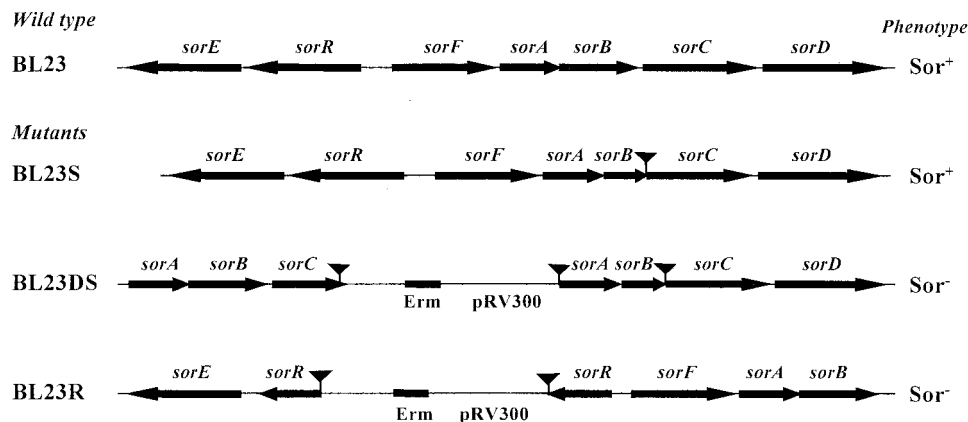


FIG. 6. Schematic representation of the genetic organization of the sorbose operons in wild-type and mutant strains of *L. casei*. The strategy for disrupting the *sor* genes is described in Materials and Methods. Arrows indicate ORFs and their orientations. Deleted DNA fragments are represented by triangular symbols. The erythromycin gene (Erm) and DNA from plasmid pRV300 are also indicated. Phenotypes were tested by streaking single colonies on MRS fermentation medium with 0.5% L-sorbose. + and - indicate fermentation and no fermentation, respectively.

The same data were obtained with the other probes assayed: a strong signal was found with RNA isolated from *L. casei* cells grown in the presence of L-sorbose, weaker bands were detected when D-glucose was present in the growth medium, and weak or no signals were observed when D-ribose was used as the carbon source. These results suggested that transcription of the *sor* genes was induced by the addition of L-sorbose in the culture and repressed by D-glucose.

A signal corresponding to a transcript of about 2,200 nucleotides was obtained when *sorE* and *sorR* fragments were used as probes. This mRNA size could correspond to the size of *sorR* plus *sorE*. A transcript of about 4,400 nucleotides was detected with *sorF* and *sorC* probes. This size suggested that *sorFABCDG* are transcribed as one unit. These probes also detected two smaller bands that possibly are an artifact due to the position of rRNA, as has been reported by others (15, 38).

The transcriptional start sites of the *sor* operons were determined by primer extension experiments (Fig. 2). Two primer extension products were obtained when we performed the analysis with primer *sorF3*, which anneals at the 5' end of *sorF*. The initiation sites correspond to guanine and adenine residues, 54 and 70 bp upstream of the *sorF* ATG start codon, respectively. A transcription initiation site, 62 bp upstream of the *sorR* ATG start codon, was identified when we used primer *sorR4*, which anneals at the 5' end of *sorR* (Fig. 2).

Disruption of the *L. casei* L-sorbose-specific PTS genes. To test the biological function of the L-sorbose-specific PTS genes, a *sorBC* mutant was constructed as described in Materials and Methods. This strain, named BL23S, had a deletion of 117 amino acids at the carboxy-terminal region of *sorB* and 28 amino acids at the amino-terminal region of *sorC*. This deletion also introduced a frameshift mutation at the fusion point between *sorB* and *sorC* (Fig. 6). The mutant exhibited a sugar fermentation pattern identical to that of the wild type (BL23), with the difference that when grown on MRS basal medium with 0.5% L-sorbose as the carbon source, the mutant's doubling time (206 min) was greater than that of the wild type (157 min). When the same culture medium contained 0.1 or 0.05% L-sorbose, the mutant did not grow. A *ptsI* mutant did not ferment L-sorbose (Viana et al., unpublished data), suggesting that L-sorbose was probably transported by another PTS with less efficiency. To determine if L-sorbose was also taken up by the D-mannose-specific PTS, we disrupted the L-sorbose-specific PTS genes in the *man* mutant chromosome (Fig. 6). The

resulting strain, named BL23DS, barely grew on MRS fermentation medium with 0.5% sorbose, presenting a very high doubling time of 301 min (the *man* mutant has a doubling time on sorbose of 168 min). These experiments indicated that with high L-sorbose concentrations, this sugar could also be taken up through the EII^{Man} elements.

Insertional inactivation of *sorR*. To provide evidence for the functional importance of *SorR* for L-sorbose metabolism in *L. casei*, plasmid pRV300, containing a DNA fragment internal to *sorR*, was integrated into the chromosome of *L. casei* (Fig. 6). The integrants obtained were analyzed for the ability to ferment L-sorbose on MRS fermentation medium. All integrants exhibited an L-sorbose-negative phenotype; we picked one, named BL23R, for further analysis.

Fructose transport activity in wild-type *L. casei* and the *sorBC* mutant. Since EII^{Sor} components have been shown to transport the analogue D-fructose (47), we tried to determine the uptake rate of D-fructose in *L. casei* wild-type and *sorBC* and *ptsI* mutant strains pregrown on D-fructose or L-sorbose. There have been no previous reports on D-fructose transport in *L. casei*. The *ptsI* mutant (Viana et al., unpublished data) showed no D-fructose uptake (data not shown). The wild-type and *sorBC* mutant strains pregrown on D-fructose can efficiently incorporate D-fructose (apparent K_m s of 12 and 16 μ M, respectively). The *sorBC* mutant pregrown on L-sorbose showed very little D-fructose uptake; however, the wild type was able to transport D-fructose at a lower rate (apparent K_m of 61 μ M) than when it was pregrown on D-fructose (Fig. 7A). These data suggest that the D-fructose transport activity detected in the D-fructose-pregrown *sorBC* mutant was due to a fructose-inducible PTS, possibly a D-fructose-specific PTS. The existence of an inducible EII^{Fru} would explain the difference between the transport activity of *L. casei* wild-type cells pregrown on D-fructose and L-sorbose. The remaining activity of the *L. casei* wild type pregrown on L-sorbose would account for D-fructose translocation through EII^{Sor}. To confirm that D-fructose is indeed transported through EII^{Sor}, D-[¹⁴C]fructose uptake was measured with increasing amounts of L-sorbose (Fig. 7B). When the *L. casei* wild type was pregrown on L-sorbose, there was a competitive inhibition of D-fructose uptake (estimated K_i of 104 μ M) by as little as 10 μ M L-sorbose. However, no inhibition of D-fructose uptake by L-sorbose was detected in the *sorBC* mutant pregrown on D-fructose (data not shown).

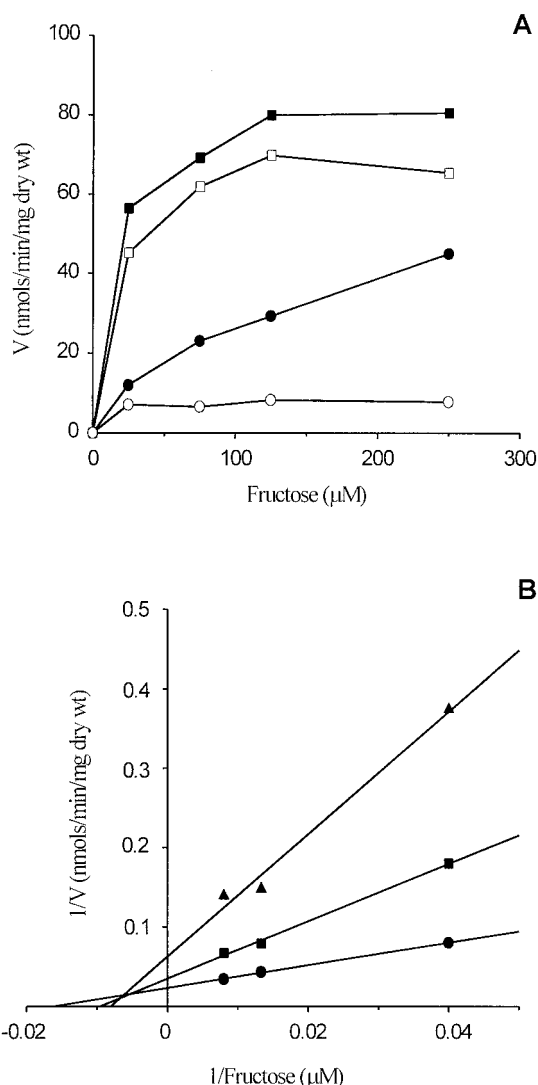


FIG. 7. (A) D-[^{14}C]fructose uptake rate in *L. casei* wild-type (BL23) and *sorBC* mutant strains. Cells were pregrown on MRS fermentation medium with 0.5% fructose (■, wild type; □, *sorBC* mutant) or 0.5% sorbose (●, wild type; ○, *sorBC* mutant). (B) Lineweaver-Burk plot of D-[^{14}C]fructose uptake rate without sorbose (●) or with sorbose (■, 10 μM ; ▲, 100 μM) by the wild type pregrown on MRS fermentation medium with 0.5% sorbose.

These results suggested that the *sorBC* mutant was impaired in EII^{Sor} .

Enzymatic activities in *L. casei* wild-type and *sor* mutant strains. When the wild-type and mutant strains were grown on D-ribose as the sole carbon source, Sor-PR and Stol-PDh activities were below the detection level. The presence of L-sorbose in the culture medium induced both activities (Table 1). Activities in the *sorBC* mutant, BL23S, were similar to those in the wild type. However, the PTS transporter double mutant, BL23DS, had very low activities. These results confirmed that L-sorbose could also be taken up by the D-mannose-specific PTS, hence inducing the operon. Neither Sor-PR nor Stol-PDh activity was detected in *sorR*::pRV300 mutant BL23R crude extracts. Finally, both activities in the *man* mutant were higher than in the wild type, and Stol-PDh activity was higher than in the *sorBC* mutant. These activity levels are comparable to those in the wild type grown on L-sorbose (without D-ribose) (Table 2). Differences in activities due to the presence of ri-

biose were consistently observed in other experiments, but an explanation could not be found.

Influence of EII^{Man} and CcpA on *sorE* and *sorF* expression. Glucose repression exerted on Sor-PR and Stol-PDh was tested in the wild type (BL23), in the *man* mutant (BL23D), in the *ccpA* mutant (BL71), and in the *man ccpA* double mutant (BL72) (Table 2). The wild type showed the strongest repression with the addition of glucose, followed by the *ccpA* mutant, with only a 2% residual activity. The *man* mutant in a glucose-plus-sorbose mix exhibited a 16 and 19% residual activities of Sor-PR and Stol-PDh, respectively. This partial loss of glucose repression explains the lack of diauxic growth of this strain (Fig. 4). The major loss of glucose repression was observed in the *man ccpA* double mutant, which showed 54 and 64% residual activities of Sor-PR and Stol-PDh, respectively. However, the activity levels of the fully induced wild type were not recovered. This degree of glucose repression suggests that an additional mechanism of CR could still exist.

DISCUSSION

The genes encoding PTS proteins and enzymes involved in L-sorbose metabolism in *L. casei* have been sequenced, and some of them have been characterized by inactivation through Campbell-like recombination. D-Fructose was used as analog of L-sorbose in uptake experiments. It was transported by EII^{Sor} , as this activity was missing in the *sorBC* mutant (BL23S), and evidence for an inducible D-fructose-specific PTS was found. Growth experiments with the wild type and a *man* mutant indicated that although L-sorbose is mainly taken up via EII^{Sor} , EII^{Man} also could play a secondary role in L-sorbose transport. In fact, this multivalent sugar specificity could be expected from members of the mannose PTS family (26). Assays of the metabolic enzymes showed that in the *sorBC* mutant (BL23S), Sor-PR and Stol-PDh activities were induced by L-sorbose as in the wild type. The *sorBC*::pRV300 double mutant *man* (BL23DS) showed almost no Sor-PR activity and a low, but significantly higher, Stol-PDh activity (Table 1), suggesting that a PTS element (EII^{Man} or EII^{Sor}) or the translocation product, L-sorbose-1-phosphate, is an essential effector for the SorR-mediated induction of the *sor* operons.

In *K. pneumoniae*, all *sor* genes are expressed from a single promoter, for which SorC would act as a repressor in its normal, uninduced state and as an inducible activator in the presence of L-sorbose (48). In *L. casei*, insertional inactivation of *sorR* led to no expression of *sorF*, indicating that *sorR* encodes a positive regulator. Its inactivation also abolished the expression of *sorE*; however, this might be due to a polar effect of the

TABLE 1. Enzyme activities in *L. casei* wild-type and *man*, *sorBC*, and *sorR* mutant strains

Strain ^b	Activity (nmol/min/mg of protein) ^a			
	L-Sorbose-1-P reductase		D-Sorbitol-6-P dehydrogenase	
	Rib	Rib + Sor	Rib	Rib + Sor
BL23 (wild type)	<10	760 ± 68	<10	775 ± 23
BL23S (<i>sorBC</i>)	<10	669 ± 106	<10	1,083 ± 66
BL23R (<i>sorR</i>)	<10	<10	<10	<10
BL23D (<i>man</i>)	<10	1,263 ± 68	<10	992 ± 129
BL23DS (<i>man sorBC</i>)	<10	26 ± 25	<10	138 ± 19

^a Data represent averages ± standard deviations from at least three experiments.

^b *L. casei* strains were grown on MRS fermentation medium supplemented with 0.5% D-ribose (Rib) or with 0.5% D-ribose plus 0.5% L-sorbose (Rib + Sor).

TABLE 2. Enzyme activities in *L. casei* wild-type and regulatory mutant strains

Strain ^b	Activity (nmol/min/mg of protein) ^a					
	L-Sorbose-1-P reductase			D-Sorbitol-6-P dehydrogenase		
	Glu	Sor	Glu + Sor	Glu	Sor	Glu + Sor
BL23 (wild type)	<10	1,275 ± 117	<10	<10	1,135 ± 105	<10
BL23D (<i>man</i>)	<10	1,359 ± 221	223 ± 20	<10	1,182 ± 66	229 ± 33
BL71 (<i>ccpA</i>)	<10	1,070 ± 55	27 ± 3	<10	1,956 ± 258	46 ± 10
BL72 (<i>man ccpA</i>)	<10	696 ± 96	378 ± 41	<10	1,481 ± 100	952 ± 169

^a Data represent averages ± standard deviations from at least three experiments.

^b *L. casei* strains were grown on MRS fermentation medium supplemented with 0.5% D-glucose (Glu), with 0.5% L-sorbose (Sor), or with 0.5% D-glucose plus 0.5% L-sorbose (Glu + Sor).

mutation, as *sorR* and *sorE* were cotranscribed. Alignment of the deduced amino acid sequence of SorR with sequences of the most similar proteins found in database revealed three highly conserved motifs, a putative DNA-binding helix-turn-helix at the amino terminus (motif I), and two other conserved areas (motifs II and III) for which no clear function could be deduced. However, homology of motif III with a region in proteins of the ROK family (39) suggested a sugar-binding function. The conclusions drawn from the alignments must be considered carefully because although SorC from *K. pneumoniae* was included in the alignment (Fig. 3), other authors reported that SorC showed homology to other sugar regulators (14) which are not shared by *L. casei* SorR or the other proteins shown in Fig. 3.

Many observations indicated that expression of the *sor* operons in *L. casei* was subject to CR. As mentioned earlier, adenylate cyclase, cyclic AMP, and a catabolite-activating protein-like protein are not related to CR in AT-rich gram-positive bacteria; instead, this effect depends on the phosphorylation of the HPr Ser46 residue and the association of HPr(Ser-P) with CcpA to promote its binding to the *cre* sequences. In this respect, a *cre* site has been found in the promoter region of the *sor* operons which matches the consensus sequence for gram-positive bacteria (17). It overlaps the putative -10 region of the first likely promoter, as well as the -35 region of the second promoter of the *sorFABCDG* cluster and the -10 region of the *sorRE* promoter (Fig. 2). Indirect evidence suggested that CcpA and elements of the D-mannose-specific PTS are directly or indirectly related to the CR of the *sor* operons. The diauxic growth exhibited by the wild type in L-sorbose plus D-glucose was abolished in the *man* mutant, which is consistent with the partial relief of glucose repression on Sor-PR and Stol-PDh in this strain. The activities of the sorbose catabolic enzymes were less sensitive to the presence of glucose in the *man ccpA* double mutant (showing only 1.6- to 2-fold repression). However, the *ccpA* mutant still displayed a remarkable CR of these activities. These data also indicate that yet another CR mechanism may operate in regulation of the *sor* operon, reminiscent of results of earlier studies on regulation of the *L. casei lac* operon (1, 12). In future work, the roles of SorR and other elements in this residual CR will be investigated. Additionally, when the *L. casei* strains were grown in the presence of L-sorbose, the level of Stol-PDh activity was significantly higher in the *ccpA* mutant than in the wild type. This may indicate that a metabolite derived from L-sorbose such as fructose-1,6-bisphosphate can stimulate CcpA, thus exerting some CR effect over the *sorFABCDG* promoter. A similar self-repressing effect has also been found for lactose in the *lac* operon of *L. casei* (12).

Processes such as inducer exclusion or inducer expulsion are very important and could indeed occur in this system. How-

ever, because the experiments in this work were designed to study the genetic induction or CR of the genes in the *sor* operons, sugar uptake and L-sorbose catabolic activities (Sor-PR and Stol-PDh) were always quantified in fully induced or repressed cells, for which inducer expulsion or exclusion effects could not be studied.

Among the genes characterized, a distal gene (*sorG*) possibly encoding a D-fructose-1,6-bisphosphate aldolase was found. After D-fructose-1,6-bisphosphate is formed by the activity of Sor-PR and Stol-PDh (18, 36) (Fig. 1), it seems likely that a phosphofructokinase yields D-fructose-1,6-bisphosphate, which then is hydrolyzed to dihydroxyacetone phosphate and glyceraldehyde phosphate by a D-fructose-1,6-bisphosphate aldolase. If SorG is confirmed to be an aldolase and since *sorG* is cotranscribed and coordinately regulated with *sorFABCD* genes, it would exclusively function during L-sorbose metabolism in *L. casei*. This would be expected in a highly specialized pathway when genes normally used for sugar catabolism are not fully induced. Previous studies showed that in the heterofermentative *Lactobacillus brevis*, the genes encoding a complete PTS, a D-fructose-1-phosphate kinase, and a D-fructose-1,6-bisphosphate aldolase are inducible by D-fructose (30). The presence of this pathway in *L. casei* may be related to its ecophysiology in the intestinal tract, as it would provide this bacterium an excellent machinery for scavenging vegetable cells and cell wall hydrolysates.

In this study, transport and metabolism of L-sorbose were analyzed for the first time in a gram-positive bacterium. The genetic organization of these operons is significantly different from those in *K. pneumoniae* and *E. coli*, where the gene encoding the transcriptional regulator (*sorC*) precedes the rest of the genes and all are expressed from a single promoter (46, 47). In *L. casei*, however, *sorRE* and *sorFABCDG* are divergently transcribed, and their expression is subject to SorR-mediated L-sorbose induction, with a strong CR, occurring primarily through the PTS/CcpA signal transduction pathway.

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REFERENCES

- Alpert, C.-A., and U. Siebers. 1997. The *lac* operon of *Lactobacillus casei* contains *lacT*, a gene coding for a protein of the BglG family of transcriptional antiterminators. *J. Bacteriol.* **179**:1555-1562.
- Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**:248-254.

3. Burland, V., G. Plunkett III, H. J. Sofia, D. L. Daniels, and F. R. Blattner. 1995. Analysis of the *Escherichia coli* genome VI: DNA sequence of the region from 92.8 through 100 minutes. *Nucleic Acids Res.* **23**:2105–2119.
4. Chassy, B. M., and J. Thompson. 1983. Regulation of lactose-phosphoenolpyruvate-dependent phosphotransferase system and β -D-phosphogalactosidase galactohydrolase activities in *Lactobacillus casei*. *J. Bacteriol.* **154**:1195–1203.
5. 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.
6. Chen, J.-D., and D. A. Morisson. 1988. Construction and properties of a new insertion vector, pJDC9, that is protected by transcriptional terminators and useful for cloning of DNA from *Streptococcus pneumoniae*. *Gene* **64**:155–164.
7. Deutscher, J., and M. H. Saier, Jr. 1984. ATP-dependent protein kinase-catalyzed phosphorylation of a seryl residue in HPr, a phosphate carrier protein of the phosphotransferase system in *Streptococcus pyogenes*. *Proc. Natl. Acad. Sci. USA* **80**:6790–6794.
8. Deutscher, J., E. Küster, U. Bergstedt, V. Charrier, and W. Hillen. 1995. Protein kinase-dependent HPr/CcpA interaction links glycolytic activity to carbon catabolite repression in Gram-positive bacteria. *Mol. Microbiol.* **15**:1049–1053.
9. Deutscher, J., C. Fischer, V. Charrier, A. Galinier, C. Lindner, E. Darbon, and V. Dossionnet. 1997. Regulation of carbon metabolism in Gram-positive bacteria by protein phosphorylation. *Folia Microbiol.* **42**:171–178.
10. Erni, B., B. Zanolari, and H. P. Kocher. 1987. The mannose-permease of *Escherichia coli* consists of three different proteins. Amino acid sequence and function in sugar transport, sugar phosphorylation, and penetration of phage λ DNA. *J. Biol. Chem.* **262**:5238–5247.
11. Görke, B., and B. Rak. 1999. Catabolite control of *Escherichia coli* regulatory protein BglG activity by antagonistically acting phosphorylations. *EMBO J.* **18**:3370–3379.
12. Gosalbes, M. J., V. Monedero, and G. Pérez-Martínez. 1999. Elements involved in catabolite repression and substrate induction of the lactose operon in *Lactobacillus casei*. *J. Bacteriol.* **181**:3928–3934.
13. Gosalbes, M. J., V. Monedero, C.-A. Alpert, and G. Pérez-Martínez. 1997. Establishing a model to study the regulation of the lactose operon in *Lactobacillus casei*. *FEMS Microbiol. Lett.* **148**:83–89.
14. Heuel, H., A. Shakeri-Garakani, S. Turgut, and J. W. Lengeler. 1998. Genes for D-arabitol and ribitol catabolism from *Klebsiella pneumoniae*. *Microbiology* **144**:1631–1639.
15. Huang, F. G., G. Coppola, and D. H. Calhoun. 1992. Multiple transcripts encoded by the *ihvGMEDA* gene cluster of *Escherichia coli* K-12. *J. Bacteriol.* **174**:4871–4877.
16. Hueck, C. J., and W. Hillen. 1995. Catabolite repression in *Bacillus subtilis*: a global regulatory mechanism for the Gram-positive bacteria? *Mol. Microbiol.* **15**:395–401.
17. Hueck, C. J., W. Hillen, and M. H. Saier, Jr. 1994. Analysis of a *cis*-active sequence mediating catabolite repression in Gram-positive bacteria. *Res. Microbiol.* **145**:503–518.
18. Kelker, N. E., R. A. Simkins, and R. L. Anderson. 1972. Pathway of L-sorbose metabolism in *Aerobacter aerogenes*. *J. Biol. Chem.* **247**:1479–1483.
19. Leloup, L., S. D. Ehrlich, M. Zagorec, and F. Morel-Deville. 1997. Single-crossover integration in the *Lactobacillus sake* chromosome and insertional inactivation of the *ptsI* and *lacL* genes. *Appl. Environ. Microbiol.* **63**:2117–2123.
20. Lux, R., K. Jahreis, K. Bettenbrock, J. S. Parkinson, and J. W. Lengeler. 1995. Coupling the phosphotransferase system and the methyl-accepting chemotaxis protein-dependent chemotaxis signaling pathways of *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* **92**:11583–11587.
21. Martin-Verstraete, I., M. Debarbouille, A. Klier, and G. Rapoport. 1990. The levanase operon of *Bacillus subtilis* includes a fructose-specific PTS regulating the expression of the operon. *J. Mol. Biol.* **214**:657–671.
22. Martin-Verstraete, I., J. Stülke, A. Klier, and G. Rapoport. 1995. Two different mechanisms mediate catabolite repression of the *Bacillus subtilis* levanase operon. *J. Bacteriol.* **177**:6919–6927.
23. Martin-Verstraete, I., V. Michel, and A. Charbit. 1996. The levanase operon of *Bacillus subtilis* expressed in *Escherichia coli* can substitute for the mannose permease in mannose uptake and bacteriophage lambda infection. *J. Bacteriol.* **178**:7112–7119.
24. Monedero, V., M. J. Gosalbes, and G. Pérez-Martínez. 1997. Catabolite repression in *Lactobacillus casei* ATCC 393 is mediated by CcpA. *J. Bacteriol.* **179**:6657–6664.
25. Postma, M., R. J. Leer, N. van Luijk, M. J. F. van Giezen, P. T. H. M. Heuvelmans, B. C. Lokman, and P. H. Pouwels. 1991. Incompatibility of *Lactobacillus* vectors with replicons derived from small cryptic *Lactobacillus* plasmids and segregational instability of the introduced vectors. *Appl. Environ. Microbiol.* **57**:1822–1828.
26. Postma, P. W., J. W. Lengeler, and G. R. Jacobson. 1993. Phosphoenolpyruvate:carbohydrate phosphotransferase systems of bacteria. *Microbiol. Rev.* **57**:543–594.
27. Reizer, J., A. H. Romano, and J. Deutscher. 1993. The role of phosphorylation of HPr, a phosphocarryer protein of the phosphotransferase system, in the regulation of carbon metabolism in Gram-positive bacteria. *J. Cell. Biochem.* **51**:19–24.
28. Reizer, J., T. M. Ramseier, A. Reizer, A. Charbit, and M. H. Saier, Jr. 1996. Novel phosphotransferase genes revealed by bacterial genome sequencing: a gene cluster encoding a putative N-acetylglucosamine metabolic pathway in *Escherichia coli*. *Microbiology* **142**:231–250.
29. Saier, M. H., Jr., and J. Reizer. 1994. The bacterial phosphotransferase system: new frontiers 30 years later. *Mol. Microbiol.* **13**:755–764.
30. Saier, M. H., Jr., J.-J. Ye, S. Klinke, and E. Nino. 1996. Identification of an anaerobically induced phosphoenolpyruvate-dependent fructose-specific phosphotransferase system and evidence for the Embden-Meyerhof glycolytic pathway in the heterofermentative bacterium *Lactobacillus brevis*. *J. Bacteriol.* **178**:314–316.
31. Saier, M. H., Jr., S. Chauvaux, G. M. Cook, J. Deutscher, I. T. Paulsen, J. Reizer, and J.-J. Ye. 1996. Catabolite repression and inducer control in Gram-positive bacteria. *Microbiology* **142**:217–230.
32. Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. *Molecular cloning: a laboratory manual*, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
33. Saxild, H. H., L. N. Andersen, and K. Hammer. 1996. *dra-nupC-pdp* operon of *Bacillus subtilis*: nucleotide sequence, induction by deoxyribonucleosides, and transcriptional regulation by the *deoR*-encoded DeoR repressor protein. *J. Bacteriol.* **178**:424–434.
34. Schlapfer, B. S., and H. Zuber. 1992. Cloning and sequencing of the genes encoding glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate kinase and triosephosphate isomerase (*gap* operon) from mesophilic *Klebsiella megaterium*: comparison with corresponding sequences from thermophilic *Bacillus stearothermophilus*. *Gene* **122**:53–62.
35. Shine, J., and L. Dalgarno. 1974. The 3' terminal sequence of *Escherichia coli* 16S ribosomal RNA: complementarity to nonsense triplets and ribosome binding sites. *Proc. Natl. Acad. Sci. USA* **71**:1342–1346.
36. Sprenger, G. A., and J. W. Lengeler. 1984. L-Sorbose metabolism in *Klebsiella pneumoniae* and Sor⁺ derivatives of *Escherichia coli* K-12 and chemotaxis toward sorbose. *J. Bacteriol.* **157**:39–45.
37. Stülke, J., M. Arnaud, G. Rapoport, and I. Martin-Verstraete. 1998. PRD—a protein domain involved in PTS-dependent induction and carbon catabolite repression of catabolic operons in bacteria. *Mol. Microbiol.* **28**:865–874.
38. Sulavik, M. C., and D. B. Clewel. 1996. Rgg is a positive transcriptional regulator of the *Streptococcus gordonii* *gtfG* gene. *J. Bacteriol.* **178**:5826–5830.
39. Titgemeyer, F., J. Reizer, A. Reizer, and M. H. Saier, Jr. 1994. Evolutionary relationships between sugar kinases and transcriptional repressors in bacteria. *Microbiology* **140**:2349–2354.
40. Tobisch, S., J. Stülke, and M. Hecker. 1999. Regulation of the *lic* operon of *Bacillus subtilis* and characterization of potential phosphorylation sites of the LicR regulator protein by site-directed mutagenesis. *J. Bacteriol.* **181**:4995–5003.
41. Tortosa, P., S. Aymerich, C. Lindner, M. H. Saier, Jr., J. Reizer, and D. Le Coq. 1997. Multiple phosphorylation of SacY, a *Bacillus subtilis* antiterminator negatively controlled by the phosphotransferase system. *J. Biol. Chem.* **272**:17230–17237.
42. Trach, K., J. W. Chapman, P. Piggot, D. LeCoq, and J. A. Hoch. 1988. Complete sequence and transcriptional analysis of the *spoA* region of the *Bacillus subtilis* chromosome. *J. Bacteriol.* **170**:4194–4208.
43. Veyrat, A., M. J. Gosalbes, and G. Pérez-Martínez. 1996. *Lactobacillus curvatus* has a glucose transport system homologous to the mannose family of phosphoenolpyruvate-dependent phosphotransferase systems. *Microbiology* **142**:3469–3477.
44. Veyrat, A., V. Monedero, and G. Pérez-Martínez. 1994. Glucose transport by the phosphoenolpyruvate:mannose phosphotransferase system in *Lactobacillus casei* ATCC 393 and its role in carbon catabolite repression. *Microbiology* **140**:1141–1149.
45. Wehmeier, U. F., and J. W. Lengeler. 1994. Sequence of the *sor*-operon for L-sorbose utilization from *Klebsiella pneumoniae* KAY2026. *Biochim. Biophys. Acta* **1208**:348–351.
46. Wehmeier, U. F., B. M. Wöhrli, and J. W. Lengeler. 1995. Molecular analysis of the phosphoenolpyruvate-dependent L-sorbose:phosphotransferase system from *Klebsiella pneumoniae* and of its multidomain structure. *Mol. Gen. Genet.* **246**:610–618.
47. Wehmeier, U. F., B. Nobemann, and J. W. Lengeler. 1992. Cloning of the *Escherichia coli* *sor* genes for L-sorbose transport and metabolism and physical mapping of the genes near *metH* and *iclR*. *J. Bacteriol.* **174**:7784–7790.
48. Wöhrli, B. M., U. F. Wehmeier, and J. W. Lengeler. 1990. Positive and negative regulation of expression of the L-sorbose (*sor*) operon by SorC in *Klebsiella pneumoniae*. *Mol. Gen. Genet.* **224**:193–200.
49. Wöhrli, B. M., and J. W. Lengeler. 1990. Cloning and physical mapping of the *sor* genes for L-sorbose transport and metabolism from *Klebsiella pneumoniae*. *Mol. Microbiol.* **4**:1557–1565.