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Utilization of D-Ribitol by *Lactobacillus casei* BL23 Requires a Mannose-Type Phosphotransferase System and Three Catabolic Enzymes

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***Lactobacillus casei* strains 64H and BL23, but not ATCC 334, are able to ferment D-ribitol (also called D-adonitol). However, a BL23-derived *ptsI* mutant lacking enzyme I of the phosphoenolpyruvate:carbohydrate phosphotransferase system (PTS) was not able to utilize this pentitol, suggesting that strain BL23 transports and phosphorylates D-ribitol via a PTS. We identified an 11-kb region in the genome sequence of *L. casei* strain BL23 (*LCABL_29160* to *LCABL_29270*) which is absent from strain ATCC 334 and which contains the genes for a GlpR/IolR-like repressor, the four components of a mannose-type PTS, and six metabolic enzymes potentially involved in D-ribitol metabolism. Deletion of the gene encoding the EIIB component of the presumed ribitol PTS indeed prevented D-ribitol fermentation. In addition, we overexpressed the six catabolic genes, purified the encoded enzymes, and determined the activities of four of them. They encode a D-ribitol-5-phosphate (D-ribitol-5-P) 2-dehydrogenase, a D-ribulose-5-P 3-epimerase, a D-ribose-5-P isomerase, and a D-xylulose-5-P phosphoketolase. In the first catabolic step, the protein D-ribitol-5-P 2-dehydrogenase uses NAD^+ to oxidize D-ribitol-5-P formed during PTS-catalyzed transport to D-ribulose-5-P, which, in turn, is converted to D-xylulose-5-P by the enzyme D-ribulose-5-P 3-epimerase. Finally, the resulting D-xylulose-5-P is split by D-xylulose-5-P phosphoketolase in an inorganic phosphate-requiring reaction into acetylphosphate and the glycolytic intermediate D-glyceraldehyde-3-P. The three remaining enzymes, one of which was identified as D-ribose-5-P-isomerase, probably catalyze an alternative ribitol degradation pathway, which might be functional in *L. casei* strain 64H but not in BL23, because one of the BL23 genes carries a frameshift mutation.**

Many bacteria have the capacity to utilize a large number of sugars and sugar derivatives, including sugar alcohols (polyols). For example, hexitols such as mannitol or glucitol are well-established carbon sources for numerous bacteria, including the Gram-negative and Gram-positive model organisms *Escherichia coli* and *Bacillus subtilis*. They can either be taken up by ion symporters, such as GutP of *B. subtilis* (1), or by ABC transporters (2) or can be transported and concomitantly phosphorylated by the phosphoenolpyruvate (PEP):carbohydrate phosphotransferase system (PTS) (3, 4). The three pentitols arabinitol, xylitol, and ribitol are less frequently utilized by bacteria. The first pentitol is also known under the name arabitol and the last one as adonitol. D-Ribitol is present in plants (for example, in *Adonis vernalis*) (5), and D-ribitol-5-phosphate (D-ribitol-5-P) is also a constituent of teichoic and lipoteichoic acids of certain Gram-positive organisms. Evidence for a D-ribitol transporter and D-ribitol-specific metabolic enzymes was first provided for the bacterium *Enterobacter aerogenes* (previously called *Anaerobacter aerogenes* and *Klebsiella aerogenes*). The D-ribitol dehydrogenase of this organism was purified and characterized in the late 1950s (6, 7). The gene of this enzyme was cloned and its DNA sequence determined (8). It was detected in several other organisms, such as *Rhodobacter sphaeroides* (9), *Rhizobium trifolii* (10), *Klebsiella pneumoniae* (11), *Zymomonas mobilis* (12), *Sinorhizobium meliloti* (13), and *E. coli* (14). A second enzyme was found to be necessary for the metabolism of D-ribitol. It converts D-ribulose into D-ribulose-5-P and was therefore called ribulokinase. The two proteins D-ribitol dehydrogenase and ribulokinase are usually coordinately synthesized (15, 16). After its uptake via an ion symport protein (11, 14) or an ABC transporter (13), D-ribitol is first oxidized to D-ribulose with usually NAD^+ as an electron acceptor and subsequently phosphorylated in an ATP-dependent reaction to the pentose phosphate intermediate D-ribulose-5-P. In some Gram-positive organisms, D-ribulose-5-P is not metabolized via the pentose phosphate pathway but is reduced in an NADP-requiring reaction to D-ribitol-5-P and subsequently converted together with CTP to CDP ribitol, which is used as a building block for the synthesis of ribitol teichoic and lipoteichoic acids (17). In *K. pneumoniae*, D-arabinitol was found to be transported and metabolized by principally the same pathway. D-Arabinitol is transported by DalT (11), which resembles GlpT from *B. subtilis*, and catabolized by the enzymes DalD (D-arabinitol dehydrogenase, which converts D-arabinitol into D-xylulose) and DalK (xylulose kinase) (18).

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Transport of pentitols via the phosphoenolpyruvate:carbohydrate phosphotransferase system was first suggested when Jack London and his coworkers succeeded in purifying and characterizing NAD^+-dependent D-xylitol-5-P and D-ribitol-5-P dehydrogenases from *Lactobacillus casei* strains Cl83 and Cl16, respectively (19). The latter strain is more commonly known under the name 64H (20), and it lacks ribitol dehydrogenase activity. London and coworkers also succeeded in purifying a soluble D-xylitol-specific PTS protein (called III^{Xtl}, probably an enzyme IIA [EIIA] component; see below) from *L. casei* strain Cl83 (21). Finally, a *ptsH* mutant (*ptsH* encodes the PTS protein HPr) derived from *L. casei* 64H was reported to have lost the capacity to utilize D-ribitol (22). Together, these results suggested that another mode of transport and metabolism of the three pentitols might exist in certain bacteria, which was likely to resemble the utilization of hexitols transported by the PTS.

A functional PTS is usually composed of four soluble proteins or protein domains forming a phosphorylation cascade (23). In the first step, enzyme I (EI) autophosphorylates with PEP. P∼EI transfers the phosphoryl group to His-15 in HPr. These two proteins are called the general PTS components. In the next step, P∼His-HPr phosphorylates one of usually several EIIAs present in a bacterium, each being specific for a certain sugar. P∼EIIA transfers the phosphoryl group to an EIIB molecule with the same sugar specificity, which in the last step phosphorylates a carbohydrate molecule bound to the cognate membrane integral EIIC component or, for mannose/glucose-type PTS, to the EIIC and EIID heterodimer (see Fig. 3). Phosphorylation of the sugar is thought to lower its affinity for EIIC, and the phosphorylated carbohydrate is therefore released into the cytoplasm (24). EI, HPr, and the EIIAs as well as the EIIBs of all mannose/glucose-type PTS are phosphorylated at the Nε-1 or Nδ-2 position of a histidyl residue, whereas the EIIBs of all other PTS families are phosphorylated at a cysteyl residue.

Despite the remarkable work carried out by the group of Jack London on PTS-catalyzed pentitol metabolism, the detailed mechanism of D-ribitol utilization by certain *L. casei* strains remained obscure, because neither the PTS components involved in its transport nor the enzymes catalyzing the metabolism of D-ribulose-5-P could be identified. We noticed that *L. casei* strain BL23, the genome of which was sequenced in our laboratory (25), was able to ferment D-ribitol similarly to strain 64H, whereas strain ATCC 334 was not. We identified an operon present in strain BL23, but not ATCC 334, which we suspected to encode the enzymes necessary for D-ribitol transport and metabolism. We deleted the gene encoding one of the PTS components, which, similar to EI inactivation, led to the loss of D-ribitol fermentation. We also cloned four metabolic genes of the ribitol region into His tag expression vectors, expressed them in *E. coli*, purified the encoded enzymes, and determined their activity. These results allowed us to propose a detailed mechanism for D-ribitol transport and catabolism by *L. casei* strain BL23.

MATERIALS AND METHODS

Bacterial strains and growth conditions. *L. casei* strain BL23 as well as the *ptsI* and *rtlB* mutants derived from it were grown at 37°C under static conditions in MRS medium (Difco) or in MRS fermentation medium (Scharlau), which was supplemented with 0.5% D-ribitol. The fermentation medium contains chlorophenol red as a pH indicator, and acid production was determined by monitoring the color change of the medium from red (pH 6.9) to yellow (pH 5.1). For more quantitative assays, the pH was also measured by using a pH electrode. The *E. coli* strains NM522 (Stratagene) and BL23(DE3) were used for cloning experiments and for protein overproduction. NM522 and NM522(pREP4-GroES/EL) (26) transformed with plasmid pQE30 (Qiagen) harboring various genes of the ribitol region were grown at 37°C under agitation in Luria-Bertani (LB) medium supplemented with 100 μg/ml ampicillin and with 100 μg/ml ampicillin and 30 μg/ml kanamycin, respectively. *Lactococcus lactis* NZ9000, which was used as an intermediary cloning host for the vector pT1NX and its derivatives, was grown at 30°C under static conditions in M17 medium (Oxoid) containing 0.5% glucose. *L. lactis* transformants were selected with 5 μg/ml erythromycin.

Construction of the *rtlB* deletion mutant. In order to delete the *rtlB* gene of *L. casei* strain BL23, two DNA fragments corresponding to the regions upstream and downstream from *rtlB* were amplified by PCR by using chromosomal DNA of strain BL23 as the template and the primer pairs RtlAamForEco-RtlAamRevNot and RtlCavForNot-RtlCavRevSac (Table 1). The PCR product corresponding to the *rtlB* upstream region was cut with EcoRI and NotI and inserted into plasmid pRV300 (27) cut with the same enzymes. The second PCR product was inserted into the resulting plasmid after it was cut with NotI and SacI. The resulting plasmid, pRV300-deltaRtlB, was used to transform *L. casei* BL23. A single-crossover integrant was selected based on its resistance to erythromycin, and the correct insertion was confirmed by PCR using the reverse M13 universal primer and the oligonucleotide RtlBverifSac (Table 1). Subsequently, this integrant was grown in MRS medium without erythromycin for approximately 200 generations. Cells were plated on solid MRS medium and replica plated on MRS medium containing erythromycin. Antibiotic-sensitive clones were isolated, and one of them was selected (strain BL375) in which a second recombination event had caused the excision of the plasmid, leading to the deletion of *rtlB*. The correct deletion of the $EIIB^{Rtl}$-encoding *rtlB* gene was confirmed by PCR amplification using the primers RtlDhFor and RtlIICRev (Table 1) and DNA sequencing of the PCR product.

Complementation of the *rtlB* mutant with plasmid-encoded *rtlB*. The coding region of *rtlB* was amplified by PCR using *L. casei* BL23 chromosomal DNA as the template and primers RtlBbglII and RtlBspeI (Table 1). The amplified DNA fragment was digested with BglII and SpeI and cloned into the replicative vector pT1NX (28), which had been previously cut with the same enzymes. In the resulting plasmid, pT1-rtlB, the *rtlB* gene was expressed under the control of the lactococcal P1 constitutive promoter. This plasmid was used to transform the *L. casei rtlB* mutant BL375. One transformant was selected and named PL47. For control experiments, a transformant carrying empty pT1NX was also isolated.

Cloning of the different metabolic genes of the ribitol regulon into His tag expression vectors. The ribitol region present in strain BL23 contains six genes encoding presumed catabolic enzymes (Fig. 1). Five of these genes were amplified by PCR using chromosomal DNA of strain BL23 as the template and the appropriate primer pair (Table 1). Together, the *LCABL_29180* and *_29190* genes of strain BL23 encode a homologue of DeoC from *E. coli* and *B. subtilis* (Fig. 1). Owing to a frameshift mutation, this gene was disrupted into two open reading frames (ORFs). A DNA fragment with a sequence nearly identical to that of the *LCABL_29180* and *LCABL_29190* genes but without the frameshift mutation could be amplified by PCR using chromosomal DNA of *L. casei* strain 64H as the template and the primer pair 2D5PAldBamF-2D5PAldhinR (Table 1). The six PCR products corresponding to the various genes were cut with the appropriate restriction enzymes and cloned into the His tag expression vector pQE30 (Qiagen). The correct sequence of each gene was confirmed by DNA sequencing. The resulting plasmids carrying the *rtpD* gene of *L. casei* BL23 or the *deoC*-like gene amplified from strain 64H were used to transform *E. coli* strain NM522. The proteins encoded by the other four genes could not be obtained in soluble form because they formed inclusion bodies when produced in strain NM522. The pQE30-derived plasmids carrying the *rpiA*, *LCABL_29170*,

TABLE 1 Primers used in this study

Name	Sequence[a]	Use
RtlAamForEco	GTGG*GAATTC*TGTCTTAATGGGTGTAAGCGAAGAC	*rtlB* deletion
RtlAamRevNot	CCCC*GCGGCCGC*TCATGATTGCGGGTTACGTGATTTC	*rtlB* deletion
RtlCavForNot	TGAT*GCGGCCGC*AGACGTTAAAAATCTGCTGAAATAA	*rtlB* deletion
RtlCavRevSac	ATAA*GAGCT**C***CGAAAATGGCAATCGGCAAGATAGC	*rtlB* deletion
RtlBverifEco	CTGCAACCATGCAGTCATGACC	*rtlB* verification
RtlBverifSac	GTGCTTCTGATGATGAGAAAGC	*rtlB* verification
RtlDhFor	GCATTTGAAGCTATTGTCGC	*rtlB* deletion
RtlIICRev	GACTTGTTTAACTGGACCTG	*rtlB* deletion
RtlBbglII	CAAT*AGATCT*ACTGAGGGGGAAATTATAATGTC	*rtlB* complementation
RtlBspeI	TATC*ACTAGT*CCTCCATTTTTATTTCAGC	*rtlB* complementation
tdhForBamHI	AGGCT*GGATCC*ATGTTAAAGAACCCTGACGTATCAAG	RtpD purification
tdhRevPtsI	ATGTT*CTGCAG*TTACCATTCAAATTTAAGAACAG	RtpD purification
R5PepiBamF	GGA*GGATCC*ATGTCTATTGAGATTGCACCC	Rpe purification
R5PepiSalR	TAA***G****TCGA*CCCGTCTAGGAATTTGGTCATC	Rpe purification
PketolBglF	ATG*AGATCT*ATGGACCAAACAATTGAGAAAAC	Xpk purification
PketolhinR	ATG*AAGCTT*TTACATGTGCGATGACGTGCTG	Xpk purification
R5PIsoBamF	TGC*GGATCC*ATGAATCAAAATGACTTGAAGC	RpiA purification
R5PIsoHinR	TAA*AAGCTT*TTGAATATTATGACCCAGATAA	RpiA purification
2D5PAldBamF	TTG*GGATCC*TTGAGTAAATTGAGACGATTG	DeoC-like purification
2D5PAldhinR	ATG*AAGCTT*ACCCTCCTTATCTAGTCATTTG	DeoC-like purification
R5PDHBamF	GTG*GGATCC*ATGACAAAGATAAAAGCTGCTG	LCABL_29170 purification
R5PDHHinR	AGC*AAGCTT*GAATCCGTCTTTTCTTACTATTAG	LCABL_29170 purification

[a] Restriction sites are in italics.

rpe, and *xpk* genes were therefore used to transform the *E. coli* strain NM522(pREP4-GroES/EL). This strain carries the plasmid pREP4 (Qiagen) containing the genes for the chaperone GroES/GroEL (26). Indeed, overproduction of three of the encoded proteins in strain NM522(pREP4-GroES/EL) rendered them soluble and allowed their purification (Fig. 2). Only the protein encoded by *LCABL_29170* remained exclusively present in inclusion bodies.

Production and purification of His-tagged enzymes. For the production of His-tagged proteins, *E. coli* NM522 strains carrying the pQE30-derived plasmids containing the genes *rtpD* or *deoC*-like were grown at

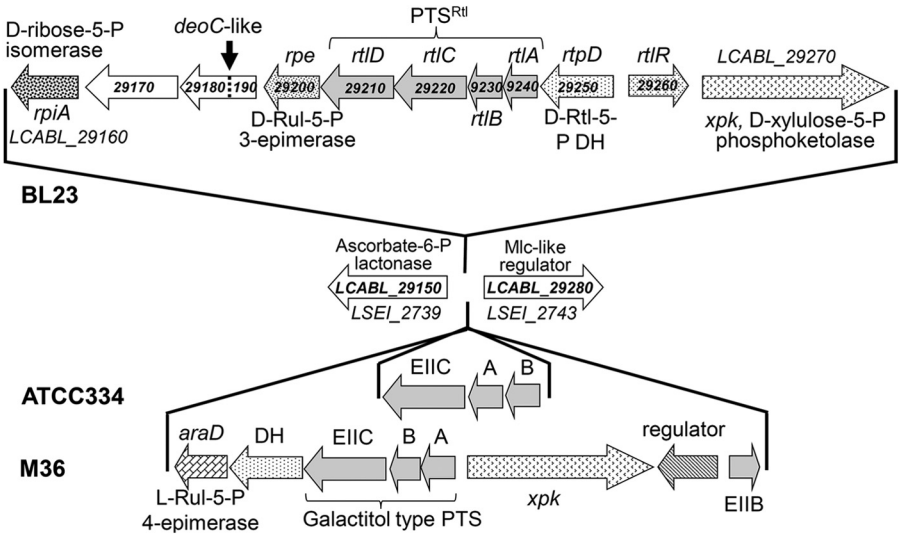

FIG 1 Schematic presentation of the ribitol region of *L. casei* strain BL23. A ribitol region identical to that of strain BL23 is also found in strains W56, 32G, CRF28, BD-II, and LC2W but not in ATCC 334. The region contains 11 genes, 4 of which code for a mannose-type PTS, 1 for a transcription regulator, and 6 for enzymes presumably involved in ribitol metabolism. The location of the frameshift mutation in the *deoC*-like gene of strain BL23 creating two ORFs (*LCABL_29180* and *LCABL_29190*) is indicated with an arrow (for details, see legend to Fig. 4). In strain BL23, the ribitol region is inserted between the genes *LCABL_29150* and *LCABL_29280*, which correspond to *LSEI_2739* and *LSEI_2743* in the genome sequence of strain ATCC 334, in which only three genes encoding a mannitol/fructose-type PTS are inserted at this locus. The *L. casei* strains 21/1, 12A, and Zhang have a gene arrangement identical to that of ATCC 334. In contrast, similar to BL23, strain M36 as well as Lpc-37, A2-362, and T71499 also contains a large inserted region coding not only for the components of a galactitol-type PTS specific probably for L-ribitol or L-arabinitol but also for the enzymes necessary for the metabolism of L-ribulose-5-P. In these strains, the insertion also occurred between homologues of *LCABL_29150* and *LCABL_29280*, and compared to strains ATCC 334 and BL23, the integration site is shifted by only 3 bp at the beginning and 7 bp at the end of the inserted region. "DH" indicates the presumed L-ribitol or L-arabinitol dehydrogenase present in strains M36, Lpc-37, A2-362, and T71499.

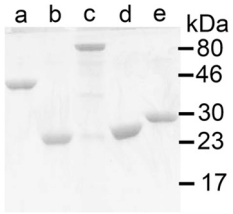

FIG 2 Electrophoretic separation of five His-tagged proteins encoded by genes of the ribitol region of *L. casei* strain BL23 (lanes a to d) or 64H (lane e) on a 0.1% SDS-12.5% polyacrylamide gel. For each protein, 5 μg was loaded on lanes a to e. Their calculated molecular weight (MW) (including the His tag) are as follows: ribitol-5-P 2-dehydrogenase (lane a), 41,330; D-ribulose-5-P 3-epimerase (lane b), 24,771; D-xylulose-5-P phosphoketolase (lane c), 91,165; D-ribose-5-P isomerase (lane d), 26,407; and presumed D-2-deoxyribose-5-P aldolase (lane e), 30,410. The D-xylulose-5-P phosphoketolase seemed to be partly degraded during purification, because several minor small fragments were coeluted with the enzyme during purification by ion chelate affinity chromatography on an Ni-NTA agarose column (lane c).

37°C to an optical density at 600 nm (OD_{600}) of 0.5 to 0.6 before 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG) was added. Growth was continued for 3 h before the cells were harvested by centrifugation. NM522(pREP4-GroES/EL) strains carrying the pQE30-derived plasmids containing the *rpiA*, *LCABL_29170*, *rpe*, and *xpk* genes were grown at 37°C to an OD_{600} of 0.5 to 0.6 before the temperature of the growth medium was lowered to 28°C and 1 mM IPTG was added. Growth continued at 28°C overnight before the cells were harvested by centrifugation. Preparation of crude extracts from the various transformants and purification of the His-tagged proteins by ion chelate affinity chromatography on nickel-nitrilotriacetic acid (Ni-NTA) agarose columns was carried out as previously described (29).

Spectrophotometric enzyme activity assays. In order to measure the activities of the purified enzymes, we set up a series of spectrophotometric assays. For measuring the activity of D-ribitol-5-P 2-dehydrogenase (RtpD; EC 1.1.1.137), we used a 0.5-ml assay mixture containing 50 mM Tris-HCl (pH 7.4), 5 mM $MgCl_2$, 0.5 mM NADH (Sigma-Aldrich, Saint Quentin Fallavier, France), and 0.5 mM D-ribulose-5-P (Sigma-Aldrich). After preincubation for 10 min, the reaction was started by adding 9 μg D-ribitol-5-P 2-dehydrogenase. The disappearance of NADH was monitored by measuring the absorption at 340 nm by using an UVIKON 9X3 W double-beam spectrophotometer (Kontron Bio-Tek) with the "Autorate" program. In order to confirm the dependence of RtpD on divalent cations, EDTA was added at final concentrations between 5 and 50 mM and preincubated for 10 min before the reaction was started. To determine the activity of D-ribulose-5-P 3-epimerase (EC 5.1.3.1), we used D-xylulose-5-P (Sigma-Aldrich) as an initial substrate, which this enzyme transforms into D-ribulose-5-P. The subsequent NADH-requiring reduction of D-ribulose-5-P was measured as described above. The corresponding 0.75-ml assay mixture contained 50 mM Tris-HCl (pH 7.4), 5 mM $MgCl_2$, 0.5 mM NADH, 0.5 mM D-xylulose-5-P, and 18 μg D-ribitol-5-P 2-dehydrogenase, which corresponds to 1.3 enzyme units. The reaction was started by adding 15 μg D-ribulose-5-P 3-epimerase, and the disappearance of NADH was monitored by measuring the absorption at 340 nm. A similar assay was used to determine the activity of D-ribose-5-P isomerase (EC 5.3.1.6) by starting from D-ribose-5-P (Sigma-Aldrich). The 0.75-ml assay mixture contained 50 mM Tris-HCl (pH 7.4), 5 mM $MgCl_2$, 0.5 mM NADH, 0.5 mM D-ribose-5-P, and 18 μg (1.3 U) D-ribitol-5-P 2-dehydrogenase. The reaction was started by adding 22 μg D-ribose-5-P isomerase, and the disappearance of NADH was monitored by measuring the absorption at 340 nm. D-Xylulose-5-P phosphoketolase (EC 4.1.2.9) is a lyase that uses inorganic phosphate (P_i) to split D-xylulose-5-P into D-glyceraldehyde-3-P and acetylphosphate. Its activity was determined by measuring the formation of D-glyceraldehyde-3-P, which after its transformation into dihydroxyacetonephosphate by triosephosphate isomerase (Sigma-Aldrich) was reduced to glycerol-3-P in an NADH-requiring reaction catalyzed by glycerol-3-P dehydrogenase (Sigma-Aldrich). The 0.75-ml assay mixture contained 50 mM potassium phosphate buffer (pH 7.4), 5 mM $MgCl_2$, 0.5 mM NADH, 0.5 mM D-xylulose-5-P (Sigma-Aldrich), 50 μg triosephosphate isomerase (5 U), and 2.5 μg D-glycerol-3-P dehydrogenase (25 U). In order to demonstrate the importance of phosphate for the D-xylulose-5-P phosphoketolase reaction, the phosphate buffer was replaced with 50 mM Tris-HCl (pH 7.4). A possible effect of thiamine pyrophosphate was tested by including this compound in the assay mixture at concentrations between 0.15 and 0.5 mM. The reaction was started by adding 10 μg D-xylulose-5-P phosphoketolase, and the disappearance of NADH was monitored by measuring the absorption at 340 nm.

A similar assay based on the formation of D-glyceraldehyde-3-P was used to determine the activity of the presumed 2-deoxy-D-ribose-5-P aldolase (EC 4.1.2.4) of strain 64H, which was supposed to cleave 2-deoxy-D-ribose-5-P into D-glyceraldehyde-3-P and acetaldehyde. The 0.75-ml assay mixture contained 50 mM Tris-HCl (pH 7.4), 5 mM $MgCl_2$, 0.5 mM NADH, 0.5 mM D-2-deoxyribose-5-P, 5 μg triosephosphate isomerase (5 U), and 5 μg D-glycerol-3-P dehydrogenase (50 U). However, when various amounts of the presumed purified 2-deoxy-D-ribose-5-P aldolase derived from *L. casei* strain 64H were added, no decrease of the absorption at 340 nm was detected.

Nucleotide sequence accession number. The DNA sequence of the entire *deoC*-like gene of *L. casei* strain 64H has been deposited at the EMBL database under accession number HF562365.

RESULTS

***L. casei* strain BL23 can utilize D-ribitol and transports it via a PTS.** *L. casei* strain 64H has previously been reported to be able to utilize D-ribitol (19). When carrying out fermentation assays at 37°C under static conditions in MRS fermentation medium containing 0.5% D-ribitol, we observed that *L. casei* strain BL23 was also able to ferment this carbon source, whereas strain ATCC 334 was not. D-Ribitol was suggested to be transported in *L. casei* strain 64H via a specific PTS, because in contrast to other D-ribitol-utilizing bacteria, this organism contains a D-ribitol-5-P dehydrogenase and not a D-ribitol dehydrogenase (19). In addition, a *ptsH* mutant had lost the capacity to utilize ribitol (22). We therefore presumed that strain BL23 might also transport D-ribitol via a PTS. In order to test this hypothesis, we carried out fermentation studies with a *ptsI* deletion mutant (lacking the general PTS component enzyme I [Fig. 3]) derived from *L. casei* BL23 (30). Indeed, the *ptsI* mutant had lost the capacity to utilize D-ribitol as a carbon source. The pH in the ribitol-containing MRS fermentation medium dropped to about 5 during growth of the wild-type strain, whereas it remained close to the initial 6.9 for the *ptsI* mutant (Table 2), indicating that strain BL23 takes up the pentitol via a PTS.

We also carried out growth studies with the wild-type strain and the *ptsI* mutant. However, growth studies turned out to be difficult to perform because the cells grew very slowly and when the wild-type strain reached an OD_{595} of about 1, the cells started to lyse (see Fig. S1 in the supplemental material). Similar problems were encountered when *L. casei* strains were grown on other polyols such as *myo*-inositol (31). Because polyols require an additional oxidation step compared to sugars in order to be converted into glycolytic intermediates, a perturbation of the $NADH/NAD^+$ balance might be responsible for the growth difficulties. There was nevertheless a reproducible difference between the growth behavior of the wild-type strain and that of the *ptsI* mutant. While after

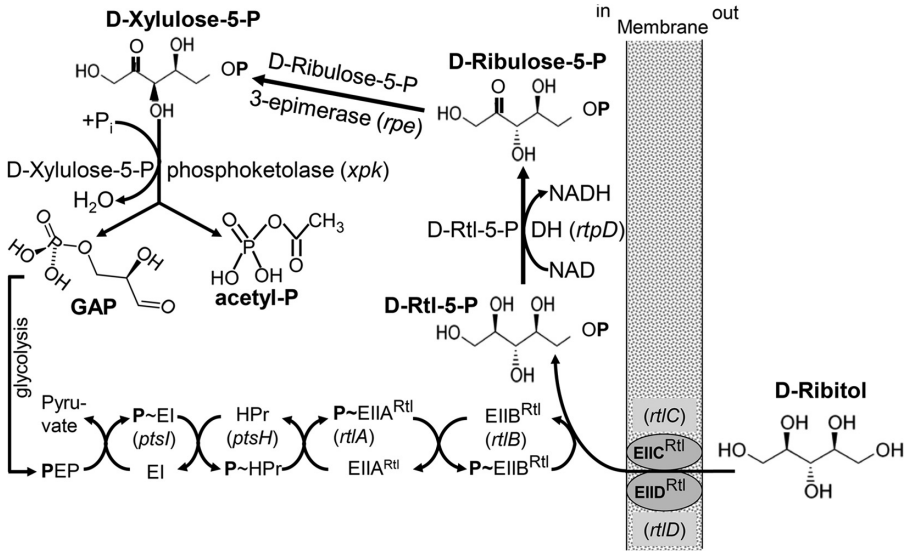

FIG 3 Schematic presentation of D-ribitol transport and catabolism in *L. casei* BL23. D-Ribitol is transported and phosphorylated by a mannose-type PTS (PTS^{Rtl}). The phosphoryl group is provided by PEP and is transferred to D-ribitol bound to the integral membrane proteins $EIIC^{Rtl}$ and $EIID^{Rtl}$ via a phosphorylation cascade formed by the proteins EI, HPr, $EIIA^{Rtl}$, and $EIIB^{Rtl}$. In the first metabolic step, intracellular D-ribitol-5-P is oxidized to D-ribulose-5-P in an NAD^+-requiring reaction catalyzed by the enzyme D-ribitol-5-P 2-dehydrogenase. The enzyme D-ribulose-5-P 3-epimerase converts D-ribulose-5-P into D-xylulose-5-P, which is subsequently cleaved in a P_i-requiring reaction catalyzed by the enzyme D-xylulose-5-P phosphoketolase into D-glyceraldehyde-3-P (GAP) and acetyl-P. D-Glyceraldehyde-3-P metabolized via glycolysis is converted into PEP used for ribitol phosphorylation, which closes the cycle. The gene designations of the different proteins are indicated in italics.

about 28 h of growth the wild-type strain transiently reached an OD_{595} of more than 1, the *ptsI* mutant barely exceeded an OD_{595} of 0.6 (see Fig. S1).

The *LCABL_29210-LCABL_29240* genes encode the D-ribitol-specific mannose-type PTS components. D-Ribitol-5-P 2-dehydrogenase of *L. casei* strain 64H had been purified to homogeneity (19). It was reported to possess a molecular mass around 40 kDa when migrating on an SDS-polyacrylamide gel. *Streptococcus pneumoniae* contains a protein belonging to the medium-chain dehydrogenase/reductase/zinc-dependent alcohol dehydrogenase family. This enzyme was reported to use NADPH to reduce D-ribulose-5-P to D-ribitol-5-P, which is subsequently converted into CDP-ribitol, used for the synthesis of ribitol-containing teichoic and lipoteichoic acids of this organism (17). This enzyme also has a molecular mass of about 40 kDa, and we therefore suspected that the streptococcal anabolic D-ribitol-5-P 2-dehydrogenase and the catabolic *L. casei* enzyme might be related. We carried out a BLAST search with the amino acid sequence of the streptococcal protein in the genome sequence of *L. casei* BL23 (25) in order to possibly detect the D-ribitol-specific genes of this organism. We indeed picked up one *L. casei* BL23 protein (LCABL_29250) that exhibits 35% sequence identity to the streptococcal protein. The *L. casei* protein was annotated as threonine dehydrogenase (Tdh). It is part of an 11-kb region that is absent from strain ATCC 334 (Fig. 1). In BL23, the *tdh*-like gene is followed by 9 other genes transcribed in the same direction and presumably forming an operon. Four of them encode the sugar-specific EIIA, -B, -C, and -D components of a PTS of the mannose/glucose family. In order to test whether the mannose-type PTS is indeed involved in D-ribitol transport, we deleted the gene encoding the EIIB component (LCABL_29230) as described in Materials and Methods. The resulting mutant, BL375, had indeed lost its capacity to utilize D-ribitol as a carbon source. During growth in ribitol-containing MRS fermentation medium, the pH remained close to 7 (Table 2), indicating that the genes *LCABL_29240* to *LCABL_29210* code for the D-ribitol-specific PTS (PTS^{Rtl}), and they were therefore renamed *rtlA*, *rtlB*, *rtlC*, and *rtlD* (Fig. 1).

TABLE 2 Acidification of the fermentation medium after 48 h of growth of the *L. casei* wild-type strain BL23 and mutants derived from it

Strain (genotype)	pH value reached after growth for 48 h[a]
BL23	5.05 ± 0.12
BL126 (*ptsI*)	6.77 ± 0.09
BL375 (*rtlB*)	6.89 ± 0.02
PL47 (*rtlB*) complemented	5.33 ± 0.04

[a] The mean values of three independent experiments together with the calculated standard deviations are presented.

Complementation of the *rtlB* mutant restores ribitol fermentation. In order to complement the *rtlB* mutant BL375 with the *rtlB* gene, the latter was amplified by PCR and inserted into vector pT1NX (28). In the resulting plasmid, pT1-rtlB, the *rtlB* gene is expressed under the control of the constitutive lactococcal P1 promoter (see Materials and Methods). Transformation of the *rtlB* mutant with pT1-rtlB indeed restored ribitol fermentation to about the same level as was observed for the wild-type strain BL23 (Table 2), whereas the *rtlB* mutant transformed with empty pT1NX was not able to ferment ribitol (data not shown). We also carried out growth studies with the *rtlB* mutant BL375 and the complemented strain PL47. While the *rtlB* mutant behaved similarly to the *ptsI* mutant, the complemented strain PL47 grew almost identically to the wild-type strain, with a transient increase of the OD_{595} to more than 1 (see Fig. S1 in the supplemental material). These results further established that the PTS encoded by the

TABLE 3 Specific activities of purified *L. casei* BL23 enzymes related to the metabolism of ribitol in this organism

Sp act (μmol substrate formed/min and mg protein)			
RtpD, ribitol-5-P 2-dehydrogenase	Rpe, ribulose-5-P 3-epimerase	Xpk, xylulose-5-P phosphoketolase	RpiA, ribose-5-P isomerase
76.8	24.5	10.4	18.2

rtlA, *rtlB*, *rtlC*, and *rtlD* genes is essential for ribitol uptake by *L. casei* strain BL23.

***LCABL_29250* encodes an NADH-dependent D-ribitol-5-P 2-dehydrogenase.** The similarity of LCABL_29250 to the *S. pneumoniae* D-ribitol-5-P 2-dehydrogenase suggested that this protein might have the same catalytic function. We therefore cloned the gene in the His tag expression vector pQE30, purified the protein (Fig. 2, lane a), and carried out a spectrophotometric assay using D-ribulose-5-P as the substrate as described in Materials and Methods. LCABL_29250 indeed reduced D-ribulose-5-P to D-ribitol-5-P, thus confirming that this protein is a D-ribitol-5-P 2-dehydrogenase. A specific activity of 76.8 μmol per min and mg enzyme (Table 3) was calculated for LCABL_29250, which was renamed RtpD. However, in contrast to the streptococcal enzyme, LCABL_29250 used exclusively NADH as a reducing cofactor. No reaction occurred with NADPH. Absolute specificity for NADH has also been reported for D-ribitol-5-P 2-dehydrogenase isolated from *L. casei* strain 64H (19). The enzyme of strain BL23 was also very specific for its sugar substrate and could not reduce D-xylulose-5-P, a stereoisomer of D-ribulose-5-P, to D-xylitol-5-P. Although the enzyme is a member of the Zn^{2+}-dependent alcohol dehydrogenase family, adding Zn^{2+} ions at various concentrations did not increase the activity of the enzyme. It is possible that RtpD binds Zn^{2+} with high affinity, thus preventing a significant loss of the cofactor during purification. This assumption is in agreement with the observation that it required relatively high concentrations of EDTA (25 mM) to see an about 3-fold inhibition of the activity of RtpD (data not shown). The RtpD activity could be restored by adding 25 mM Zn^{2+}. In contrast, no increase in enzyme activity was observed when 25 mM Mg^{2+} or Mn^{2+} ions was added to the EDTA-containing assay mixture. Complete inhibition of RtpD occurred at 50 mM EDTA. The enzyme therefore behaves similarly to previously characterized members of the Zn^{2+}-dependent alcohol dehydrogenase family (32).

A D-ribulose-5-P 3-epimerase catalyzes the second step of D-ribitol catabolism. The protein encoded by *LCABL_29200*, located downstream from the genes coding for the D-ribitol-specific PTS components, was annotated as D-ribulose-5-P 3-epimerase, an enzyme of the pentose phosphate pathway that catalyzes the interconversion of D-ribulose-5-P and D-xylulose-5-P. In order to test whether this protein exhibits indeed the predicted activity, we cloned the *LCABL_29200* gene into the His tag expression vector pQE30, purified the protein (Fig. 2, lane b), and set up a coupled spectrophotometric assay as described in Materials and Methods. LCABL_29200 was indeed able to convert D-xylulose-5-P into D-ribulose-5-P, which was subsequently reduced to D-ribitol-5-P in an NADH-consuming reaction. A specific activity of 24.5 μmol per min and mg enzyme (Table 3) was calculated for LCABL_29200. The gene encoding this protein was therefore renamed *rpe*, for D-ribulose-5-P 3-epimerase (Fig. 1).

A gene encoding a repressor is located upstream from *rtpD*. *LCABL_29260*, the gene located upstream from *rtpD* and oriented in the opposite direction, encodes a putative repressor of the DeoR family resembling, among others, GlpR from *E. coli* and IolR from *B. subtilis* (48% sequence similarity). Binding of repressors of this family to often two or more imperfect direct repeats is usually prevented by the interaction with a phosphorylated carbohydrate or catabolic intermediate, as has been shown for the *E. coli glp* (33, 34) and the *B. subtilis iol* regulons (35). A perfect 10-bp direct repeat (***TTTGCAAACT***TTTGCTTTAACCATT***TTTGCAAACT***) containing an 8-bp perfect palindrome (in italics) is located in the middle of the noncoding region between the divergently oriented *rtpD* gene and the presumed regulator gene. It is therefore likely that in response to the presence or absence of ribitol, the protein encoded by *LCABL_29260* binds to the direct repeats and thereby controls the expression of the divergently oriented genes of the ribitol region, including *LCABL_29260* and *LCABL_29270* (Fig. 1). We therefore renamed the *LCABL_29260* gene *rtlR*.

A D-xylulose-5-P phosphoketolase catalyzes the third step of D-ribitol catabolism. *LCABL_29270*, the gene downstream from *rtlR*, encodes a protein exhibiting significant sequence similarity to D-xylulose-5-P phosphoketolase (EC 4.1.2.9). Although the gene encoding the presumed D-xylulose-5-P phosphoketolase in *L. casei* BL23 is oriented in opposite direction to the ribitol genes, it probably belongs to the D-ribitol regulon because it is part of the about 11-kb region present in strain BL23 but absent from strain ATCC 334 (Fig. 1). This enzyme uses inorganic phosphate (P_i) to cleave D-xylulose-5-P into D-glyceraldehyde-3-P and acetyl-P. D-Xylulose-5-P phosphoketolase was first described for heterofermentative lactobacilli (36, 37), but it is present also in other organisms, such as *Fibrobacter* (38) and *Clostridium acetobutylicum* (39). This phosphoketolase is involved in the metabolism of at least part of the xylulose taken up and phosphorylated by these organisms. It was therefore likely that in *L. casei* BL23, this enzyme cleaves D-xylulose-5-P formed by the D-ribulose-5-P 3-epimerase. In order to test this hypothesis, the *LCABL_29270* gene was cloned into plasmid pQE30, the His-tagged protein was purified (Fig. 2, lane c), and its presumed D-xylulose-5-P phosphoketolase activity was measured by setting up a coupled spectrophotometric assay (see Materials and Methods). LCABL_29270 indeed functions as D-xylulose-5-P phosphoketolase, with a specific activity calculated to be 10.4 μmol per min and mg enzyme (Table 3). D-Xylulose-5-P phosphoketolases use thiamine pyrophosphate as a cofactor (40), which is usually covalently bound to the enzyme, and a loss during purification was therefore unlikely. We nevertheless included this cofactor in the assay mixture at various concentrations (0.15 to 0.5 mM), which, however, did not increase the activity of D-xylulose-5-P phosphoketolase (data not shown). As expected, the activity drastically dropped when the phosphate buffer in the assay mixture was replaced with 50 mM Tris-HCl. No activity at all was observed when D-xylulose-5-P was replaced with D-ribulose-5-P. However, when both D-ribulose-5-P and D-ribulose-5-P 3-epimerase were present in the assay mixture, formation of D-glyceraldehyde-3-P could be detected again. In summary, these results suggest that D-ribitol transported by the PTS and converted into D-ribitol-5-P is first intracellularly oxidized to D-ribulose-5-P by the enzyme D-ribitol-5-P 2-dehydrogenase (RtpD) (Fig. 3). D-Ribulose-5-P is subsequently transformed into D-xylulose-5-P by the enzyme D-ribulose-5-P 3-epimerase. In the last step, D-xylulose-5-P phosphoketolase cleaves D-xylulose-5-P into the glyco-

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64H        181  ACG TAT GAA CAT GAA TTG ACA AAG GTG ACG ACC ATT CTG CGA GTT GAT GCG AGC ACT GAT 289
                 T   Y   E   H   E   L   T   K   V   T   T   I   L   R   V   D   A   S   T   D
                 T   Y   E   H   E   L   T   -   -   -   T   F   C   E   L   M   R   A   L   I
BL23   2879299  ACG TAT GAA CAT GAA TTG ACA A-- --- --- --CA TTC TGC GAG TTG ATG CGA GCA CTG AT  2879250
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FIG 4 Frameshift-creating deletion in the *deoC*-like gene of strain BL23 encoding a presumed D-2-deoxyribose-5-P aldolase. The deletion of 10 bp in the *deoC*-like gene of strain BL23 compared to the *deoC*-like gene of strain 64H occurred at position 200 and led to the formation of two ORFs (*LCABL_29180* and *LCABL_29190*). Five other *L. casei* strains also possess a ribitol region containing a *deoC*-like gene followed by a homologue of *LCABL_29170* and an *rpiA*-like gene (see Fig. 1). In three of these strains (W56, BD-II, and LC2W), the same frameshift mutation is found in *deoC*, suggesting that these strains are closely related to BL23. In contrast, the frameshift mutation is absent from strains 32G and CRF28, which contain an intact *deoC* gene identical to that of strain 64H. The numbers refer to the position in the *deoC*-like gene of strain 64H and to the position in the genome sequence of strain BL23 (25).

lytic intermediate D-glyceraldehyde-3-P and acetyl-P. This allows *L. casei* to synthesize one PEP and two ATP molecules when metabolizing D-ribitol, with the PEP molecule being used for the uptake and phosphorylation of the pentitol.

Three additional genes are associated with the ribitol utilization region of BL23. The D-ribitol region encoding the D-ribitol PTS components contains at its end four additional ORFs (*LCABL_29160* to *LCABL_29190*) which possibly encode the enzymes for an alternative pathway for D-ribitol catabolism. However, this pathway is not operative in *L. casei* strain BL23, because one of the genes appeared to be split into two ORFs (*LCABL_29180* and *LCABL_29190*). The two protein fragments exhibit significant sequence similarity (65%) to aldolases of the DeoC/LacD family present in, for example, *E. coli*, lactobacilli, and clostridia. DeoC is a D-2-deoxyribose-5-P aldolase (41), which cleaves D-2-deoxyribose-5-P into D-glyceraldehyde-3-P and acetaldehyde. Because *L. casei* strain 64H is also able to utilize D-ribitol (14), we PCR amplified the corresponding gene from this organism. The resulting PCR product exhibited a DNA sequence nearly identical to that of *LCABL_29180* and *LCABL_29190*, except that a deletion of 10 bp had occurred in strain BL23, which introduced a frameshift leading to the two ORFs observed in strain BL23 (Fig. 4). The same deletion was found in *L. casei* strains W56 (42), BD-II (43), and LC2W (44), whereas the two strains 32G and CRF28 contained an intact *deoC*-like gene. However, the two latter strains are probably not able to utilize ribitol, because both contain a frameshift mutation in the D-ribitol-5-P 2-dehydrogenase-encoding *rtpD* gene. In order to test whether the DeoC-like enzyme functions indeed as a D-2-deoxyribose-5-P aldolase, we cloned the amplified 64H gene into the His tag plasmid pQE30 and purified the encoded protein (Fig. 2, lane e). However, when D-2-deoxyribose-5-P was incubated with the *L. casei* 64H-derived protein, no formation of D-glyceraldehyde-3-P could be detected in a coupled spectrophotometric assay. One possible explanation for this negative result might be that the enzyme becomes inactivated during purification. It is also possible that the gene encoding this protein in strain 64H carries a mutation leading to the inactivation of the enzyme or, finally, that it does not possess the presumed D-2-deoxyribose-5-P aldolase activity.

The protein encoded by the last gene of this operon, *LCABL_29160*, exhibits significant similarity to the pentose phosphate pathway enzyme D-ribose-5-P isomerase. This enzyme transforms D-ribulose-5-P into D-ribose-5-P. In order to determine whether LCABL_29160 exhibits indeed the presumed D-ribose-5-P isomerase activity, the corresponding gene was cloned into plasmid pQE30. The encoded protein was subsequently purified (Fig. 2, lane d), and its activity was determined by using a coupled spectrophotometric assay (see Materials and Methods). LCABL_29160 was indeed able to convert D-ribose-5-P into D-ribulose-5-P, and its specific activity was determined to be 18.2 μmol per min and mg enzyme. Finally, the protein encoded by the penultimate gene of the region from *LCABL_29250* to *LCABL_29160* is annotated as Zn-containing alcohol dehydrogenase. When cloned into various His tag expression vectors, this gene was only barely expressed in *E. coli* and formed inclusion bodies. We therefore were not able to purify this protein in an active form and to determine its function. However, it is likely that this protein reduces D-ribose-5-P formed from D-ribulose-5-P by the enzyme D-ribose-5-P isomerase (LCABL_29160) to D-2-deoxyribose-5-P, which might subsequently be cleaved by the presumed D-2-deoxyribose-5-P aldolase (LCABL_29180 and LCABL_29190) into D-glyceraldehyde-3-P and acetaldehyde.

DISCUSSION

While the hexitols mannitol, glucitol (also called sorbitol), and galactitol are often transported via a PTS, pentitols seem to be less frequently taken up via this transport system. The occurrence of PTS-catalyzed ribitol transport was suggested by the identification of several pentitol phosphate dehydrogenases. The first pentitol phosphate dehydrogenase characterized was a D-ribitol-5-P 2-dehydrogenase from *Lactobacillus plantarum* (45). However, genome sequencing revealed that in several *L. plantarum* strains, the gene encoding a D-ribitol-5-P 2-dehydrogenase is located in the teichoic acid biosynthesis region, and it probably catalyzes the anabolic conversion of ribulose-5-P into ribitol-5-P, which is subsequently integrated into teichoic acids, similar to what has been reported for *S. pneumoniae* (17). The first strong support for PTS-catalyzed ribitol transport was obtained for *L. casei* strain 64H, because this strain was able to ferment ribitol and contains a ribitol-5-P 2-dehydrogenase (19), and a *ptsH* mutant derived from it had lost the capacity to ferment ribitol (22). A xylitol-specific PTS component (21) and a xylitol-5-P dehydrogenase (19) were purified from *L. casei* Cl83, confirming that this organism transports xylitol via a PTS. Finally, evidence for PTS-catalyzed transport of D-arabinitol, the third pentitol, was obtained for *Listeria monocytogenes*. A transposon mutant had been isolated which was no longer able to utilize D-arabinitol and which had the transposon integrated into a gene encoding an EIIC component of a PTS of the galactitol family (46). The genes encoding the three PTS components are followed by two genes encoding potential D-arabinitol-5-P dehydrogenases. A D-arabinitol-phosphate dehydrogenase oxidizing D-arabinitol-1-P to L-xylulose-5-P and D-arabinitol-5-P to ribulose-5-P was also detected in *Enterococcus avium* (47). This enzyme uses NAD^+ as well as $NADP^+$ as cofactors and depends on Mn^{2+} and not Zn^{2+}. The gene encoding this enzyme is located in an operon containing also the genes for the components of a galactitol PTS (47). In addition, the enterococcal enzyme exhibits 64% sequence identity to one of the presumed

listerial D-arabinitol-phosphate dehydrogenases (Lmo2664) and 31% identity to the enzyme encoded by the neighboring gene. In conclusion, there was strong evidence that all three pentitols can be transported via a PTS. However, in no case has an in-depth study of PTS-catalyzed pentitol transport and subsequent catabolism of the pentitol phosphate been carried out.

The results obtained during this study confirm that *L. casei* strain BL23 is able to take up the pentitol D-ribitol via a PTS of the mannose/glucose family composed of four ribitol-specific proteins. In strain BL23, catabolism of D-ribitol-5-P formed during PTS-catalyzed transport is achieved by the three additional enzymes D-ribitol-5-P 2-dehydrogenase, D-ribulose-5-P 3-epimerase, and D-xylulose-5-P phosphoketolase (Fig. 3). A BLAST search revealed that the genes encoding the seven proteins required for D-ribitol uptake and metabolism are absent not only from strain ATCC 334 but also from most of the other 18 *L. casei* strains for which the genome sequence has been determined (25, 42–44, 48–50). In fact, in addition to BL23, the entire ribitol region is present in only five other *L. casei* strains: W56, 32G, CRF28, BD-II, and LC2W. These five strains also possess the three additional genes located upstream from the *LCABL_29250-LCABL_29160* gene cluster. Three of them contain a deletion of a small sequence in the presumed *deoC* gene identical to the deletion detected in BL23 leading to a frameshift mutation (Fig. 4). Only the two recently sequenced strains, CRF28 and 32G (49), possess a *deoC*-like gene resembling *LCABL_29180* and *LCABL_29190*, which however lacks the frameshift-inducing deletion; they therefore contain a gene nearly identical to the presumed *deoC* present in strain 64H. In these three strains the entire ORF is translated, but it remains nevertheless questionable whether the encoded protein is functional. Using a coupled spectrophotometric assay, we failed to demonstrate that the *L. casei* 64H protein possesses the presumed D-2-deoxyribose-5-P aldolase activity.

Two *L. casei* strains used for the production of marketed probiotic drinks, *L. casei* Shirota (Yakult) and *L. casei* defensis (Danone), were recently also shown to ferment ribitol (51). However, although this work has been published, the genome sequence data are not yet accessible. Surprisingly, in the same study, *L. casei* strain BL23 was also tested and was found not to ferment ribitol when using the API CH 50 kit (bioMerieux, Marcy l'Etoile, France), although in our assays with this kit, BL23 always gave a positive fermentation signal for ribitol. We have no explanation for this discrepancy. Homologues of the proteins encoded by the genes from *LCABL_29260* to *LCABL_29200* are present in the same order in several *Lactobacillus salivarius* strains. Interestingly, in *L. salivarius* strains UCC1118 and ECT 5713, the ribitol genes are present on plasmids pMP118 and pHN3, respectively, but in each organism, the last three genes corresponding to *LCABL_29250-LCABL_29160* are lacking. This observation further supports the assumption that these three genes are not essential for the metabolism of D-ribitol but possibly constitute a second pathway for ribitol catabolism.

Two genes exhibiting significant sequence similarity to either *LCABL_29270* (*xpk*), which encodes the enzyme D-xylulose-5-P phosphoketolase, or *LCABL_29240* (*rtpD*), which codes for D-ribitol-5-P 2-dehydrogenase, are also found in *L. casei* strains M36, Lpc-37, A2-362, and T71499. The corresponding operon in these four organisms also contains the gene for an L-ribulose-5-P 4-epimerase (EC 5.1.3.4), which resembles AraD of *E. coli* and *B. subtilis* (more than 55% amino acid sequence identity). AraD converts L-ribulose-5-P into D-xylulose-5-P during L-arabinose utilization (52, 53). The presence of a gene in the *xpk-araD* region of the four *L. casei* strains that encodes an enzyme resembling D-ribitol-5-P 2-dehydrogenase of strain BL23 suggests that this enzyme might form L-ribulose-5-P from either L-ribitol or L-arabinitol transported and phosphorylated by a PTS. The three genes located at the beginning of this region encode indeed the EIIA, -B, and -C components of a galactitol-type PTS (Fig. 1), and the gene located at the end of the inserted region encodes a second galactitol-type EIIB component. It is therefore tempting to assume that this PTS transports and phosphorylates either L-ribitol or L-arabinitol, which would subsequently be oxidized to D-ribulose-5-P. Indeed, the dehydrogenase of the *xpk-araD* region shows the most significant similarity to the *E. avium* arabinitol-phosphate dehydrogenase (47). Pentitols with the L-configuration are very rare in nature (54), but at least L-arabinitol has recently been shown to be utilized by plant-symbiotic bacteria (13). In the last catabolic step, D-xylulose-5-P formed from L-ribulose-5-P by the enzyme L-ribulose-5-P 4-epimerase is probably cleaved by the D-xylulose-5-P phosphoketolase into D-gylceraldehyde-3-P and acetyl-P. The catabolic pathway would therefore largely resemble the one operative in strain BL23 for the utilization of D-ribitol (Fig. 3). A region of identical gene composition is also present in the two *Lactobacillus rhamnosus* strains LRHMPDP2 and LRHMPDP3. In addition to being part of the D- or L-ribitol or L-arabinitol region, D-xylulose-5-P phosphoketolase is sometimes also associated with an arabinose region. This is the case in several *L. plantarum* strains, in which the *xpk* gene is located next to an L-arabinose region composed of four genes encoding an L-arabinose transporter, an L-arabinose isomerase, a ribulokinase, and an L-ribulose-5-P 4-epimerase.

Interestingly, identical to the ribitol region of strain BL23, the presumed L-ribitol or L-arabinitol operon in the four *L. casei* strains M36, Lpc-37, A2-362, and T71499 is also inserted between homologues of *LCABL_29150* (encoding an L-ascorbate-6-phosphate lactonase belonging to a presumed ascorbate-specific PTS operon) and *LCABL_29280* (encoding a sugar kinase-like transcription regulator). As outlined in Fig. 1, in the various strains of *L. casei*, at least three different PTS-related regions have been inserted at this specific locus. BL23 and related strains contain a mannose-type PTS specific for D-ribitol, M36 and its related strains a galactitol-type PTS presumed to transport L-ribitol or L-arabinitol, and ATCC 334 and like strains a mannitol/fructose-type PTS. The insertion points in the three types of strains are nearly identical and differ only by 3 bp at the beginning and by 7 bp at the end of the inserted fragment. It is interesting to note that the region comprising about 35 kb upstream and 80 kb downstream from the D-ribitol region contains a very large number of carbohydrate transport and utilization operons. However, the gene composition of these operons suggests that the specificity of these carbohydrate transporters exhibits a great variability. This entire region therefore seems to be a hot spot for the integration of carbohydrate transport and metabolism operons, which might have been one factor that allowed *L. casei* strains in the course of evolution to efficiently adapt to the carbon sources prevailing in the changing environments to which they are exposed (25, 42–44, 48–50).

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