Molecular Cloning and Functional Expression in *Lactobacillus plantarum* 80 of *xylT*, Encoding the D-Xylose–H\(^+\) Symporter of *Lactobacillus brevis*

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Lactobacillus brevis* is a ubiquitous microorganism that can be isolated from various biotopes, such as milk, fermented vegetables, and the intestinal tracks of animals and that is often found as a spoilage contaminant in beer production (7, 11, 26). Fermented plant materials, one of the predominant ecological niches of *L. brevis*, are usually rich in hemicellulose fibers and therefore represent an abundant source of D-xylose, from which *L. brevis* can derive energy for growth. The fermentation of D-xylose is not a common property among *Lactobacillus* species. Besides *L. brevis* and *Lactobacillus pentosus*, most of the other lactobacilli are unable to utilize D-xylose as an energy source. The ability to ferment D-xylose, however, could improve the properties of heterofermentative lactic acid bacteria that are commonly used in fermented-food technology. *Lactobacillus plantarum*, for instance, is widely used to stimulate the fermentation of silage, sourdough, and diverse vegetables, such as the white cabbages used in the production of sauerkraut. *L. plantarum* cannot ferment D-xylose, although this property could potentially improve the competitive position of this organism in the fermentation of plant materials and would conform to the food-grade status of recombinant strains. The use of D-xylose metabolism as a food-grade selection marker for heterofermentative lactobacilli has already been proposed by Posno et al. (24). A plasmid, pLP3537-xyl, harboring the D-xylose catabolizing genes of *L. pentosus*, was used to complement the inability of *Lactobacillus casei* ATCC 393 to metabolize this pentose. However, the growth of the *L. casei* transformants carrying pLP3537-xyl was slow compared to that of *L. pentosus*, which naturally ferments D-xylose. In that study, the authors postulated that the transport of D-xylose in *L. casei* was the limiting function for growth on this compound. Indeed, pLP3537-xyl lacked a specific transporter for D-xylose, the absence of which could limit its general use.

Consequently, we aimed at the characterization and functional analysis of a D-xylose transporter from a *Lactobacillus* species that could be used to optimize the food-grade vector based on D-xylose fermentation. So far, no evidence indicating the presence of a specific transporter for D-xylose in *L. pentosus* has been obtained, but the situation could be different with *L. brevis*.

The *xylA* gene (encoding D-xylose isomerase) of *L. brevis* has previously been cloned and sequenced (2). Sequencing of regions downstream of the *xylA* gene revealed the presence of another, albeit incomplete, gene: *xylB* (encoding D-xylose kinase). The possibility that one or more genes specifying a
D-xylene transporter of *L. brevis* could be located in the surrounding of the *xylA* and *xylB* genes was investigated.

We report here the cloning of the *xylT* gene encoding the proton motive force (PMF)-linked D-xylene transport system of *L. brevis* and its functional expression in *L. plantarum* 80 by using a *Lactobacillus* expression system suitable for developing a food-grade vector based on D-xylene fermentation. We also provide information on the arrangement of the *xyl* genes in *L. brevis*, an arrangement that is similar to that which is found in *Bacillus megaterium* (30) and Tetragnococcus halophila (33), except that a repressor gene, *xylR*, is lacking in front of *xylA* in *L. brevis*. We also show that the *L. brevis* D-xylene transporter is remarkably similar to XylE of Escherichia coli (8) and *T. halophila* and to XylT of *B. megaterium* in its primary sequence and substrate specificity.

**MATERIALS AND METHODS**

**Bacterial strains, plasmids, and growth conditions.** *E. coli* DH15a (supE44 ΔlacU169 (de3lacZAM15) hsdR17 recA1 endA1 galK1 thr-1 leuA1) was used for the propagation of the *Lactobacillus-E. coli* shuttle vectors, and *E. coli* TG1 (supE44 thi-1 hsdR17 recA1 endA1 galK1 thr-1 leuA1) was used for the subcloning of the *L. brevis* *xylABT* locus. They were maintained on Luria-Bertani broth, and ampicillin was added at a concentration of 100 μg/ml when necessary. *Lactobacillus* strains were cultured on MRS medium (Difco Laboratories, Detroit, Mich.) or in M medium (19) supplemented with 0.5% or 1% (wt/vol) concentration of the indicated sugar. Erythromycin (5 μg/ml) was used for the selection. Identification of *L. plantarum* 80(LPA9) transformants was performed on M medium agar plates containing 25 mM *L*-glutamic acid and 60 μg of 5-bromo-4-chloro-3-indolyl-β-D-glucuronic acid per ml (Sigma Chemicals Co., St. Louis, Mo.), and 100 mM potassium phosphate (pH 7.4).

**Inverse PCR.** Genomic DNA was extracted from *L. brevis* as described previously (2). DNA was restricted with *ApoI*, *BclI*, EcoRV, and *SmaI* and the restricted fragments were ligated with *T4* DNA ligase. To amplify the *xylT* gene and the remainder of the *xyl* gene, the ligatation mixtures were amplified by using Taq DNA polymerase (GIBCO BRL, Gathersburg, Md.) and two sets of primers: either Sen-2 (5′-AGCTTGACGAGCACGAAATACGT-3′), specifying codons 93 to 99 of the *xylT* gene, and Ansen-4 (5′-GGGAATACTTAAGCGTTC-3′), specifying anticodons 74 to 79 of the *xyl* gene, or Sen-3 (5′-GTTTGATTGTCGATTT-3′), specifying codons 230 to 235 of the *xyl* gene, and Ansen-5 (5′-CCATTGCTATGCGT-3′), specifying codons 207 to 212 of the *xylT* gene. The purified PCR fragments were restricted with the appropriate restriction enzymes and ligated into pUC19, yielding plasmids pYCBS0.8 (Sanal3A1), pYCBEV1.5 (EcoRV), and pYCBSBe2.3 (*BclI*) or into pUC18, yielding plasmid pYCBD2.0 (*ApoI*) (see Fig. 1A). Several randomly selected clones were sequenced.

**Construction of the *Lactobacillus* xylT expression plasmid, pLPa9.** The construction of plasmid pLPa9 was performed essentially as described previously for the construction of plasmid pTUT-MCS2. A putative RBS (italics) was also introduced eight nucleotides downstream of the original ribosome binding site (RBS [italics]). The reverse primer (5′-TTACCATCGTGGATCCTCCATTGCGATTT-3′) specifying anticodons 74 to 79 of the *xyl* gene, or Sen-3 (5′-GTTTGATTGTCGATTT-3′), specifying codons 230 to 235 of the *xyl* gene, and Ansen-5 (5′-CCATTGCTATGCGT-3′), specifying codons 207 to 212 of the *xylT* gene. The purified PCR fragments were restricted with the appropriate restriction enzymes and ligated into pUC19, yielding plasmids pYCBS0.8 (Sanal3A1), pYCBEV1.5 (EcoRV), and pYCBSBe2.3 (*BclI*) or into pUC18, yielding plasmid pYCBD2.0 (*ApoI*) (see Fig. 1A). Several randomly selected clones were sequenced.

**Enzyme assays.** The conversion of D-xylene (200 mM) to D-xylulose by D-xylulose kinase reaction (4). Enzyme activities were determined in cell extracts prepared from cells harvested during the logarithmic phase of growth as described earlier (4), except that cells (resuspended in 500 μl of 50 mM potassium phosphate buffer containing 1 mM dithiothreitol and 1 mM dithionite) were disrupted by sonication at full speed (IKA-VIBRAX-VXR, IKA-Labortechnik) for 2 h at 4°C with 100 mg of glass beads (ca. 0.1 to 0.3 mm in diameter; Pertorb Apprenticial). The protein concentration in these samples was determined by the method of Smith et al. (32).

**RESULTS**

Cloning of the *d-xylulose kinase gene and flanking regions.** The cloning strategy is summarized in Fig. 1A. Initially, a 2.5-kb *NalIII/EcoRI* region from *L. brevis* was cloned using a PCR probe generated with primers deduced from the N-terminal sequence of the *L. brevis* D-xylulose isomerase and a region conserved among virtually all bacterial D-xylulose isomerases. Its reported sequence contained a 1,347-bp open reading frame (ORF) coding for XylA (2). Within the 2.5-kb *NalIII/EcoRI* region, at 107 bp downstream of the *xylA* start codon, an ATG codon at the beginning of an ORF was found. The deduced amino acid sequence of this ORF showed considerable homology to the N-terminal amino acid sequence of the *E. coli* and *L. pentosus* D-xylulose kinases (2).

The sequence up to the *NalIII* site was also determined and revealed a putative *xylO* operator site, starting 45 bp upstream of the *xylA* start codon, and showing similarity to *xylO* operator sites found near the two *d-xylulose-inducible promoters of the L. pentosus* xylene regulon (4, 19). −35 and −10 promoter elements (TGTGCA and TATACT), spaced by 16 nucleotides, were present 81 and 59 nucleotides upstream of the *xylA* start codon, respectively (see Fig. 1B). In addition, a putative catabolite responsive element (cre), which is known to mediate CcpA-dependent catabolite repression in several gram-positive microorganisms (14, 35), was also found 90 bp upstream of the *xylA* start codon. The sequence of the 0.4-kb region upstream of the *xylA* gene did not contain any putative ORF in either orientation.

To map the regions surrounding the *xylA* gene, a Southern hybridization analysis was performed on total genomic DNA by using a fragment from the *L. brevis* xylB gene as a probe. We found that a 4.2-kb *HindIII* fragment and a 5.8-kb *EcoRI/SalI* fragment located downstream of the *xylA* gene hybridized with...
the *L. brevis* xylB probe. In *Bacillus* spp. and other gram-positive bacteria, the xylAB operon was shown to be negatively regulated at the level of transcription by a repressor protein, designated XylR (16, 18, 27, 29, 31). Since a putative xylO operator site (XylR binding site) was found in the promoter region of the *L. brevis* xylA gene, another Southern hybridization analysis to locate a xylR homologue was performed with the *L. pentosus* xylR gene as a probe. A 2.9-kb *EcoRI/SalI* fragment hybridized to this latter probe. Based upon a restriction map of the *L. brevis* xyl locus and the nearest possible 2.9-kb *EcoRI/SalI* fragment, the xylR gene must be located more than 2 kb upstream or 5 kb downstream of the xylA gene. To clone

**FIG. 1.** (A) Physical map and organization of the *L. brevis* xylABT locus. The upper part shows the xylB and xylT cloning strategy. The stem-loop structures indicate the putative transcriptional terminators. The nucleotide (nt) sequences of upstream regions of xylA and xylT are depicted in panels B and C, respectively. The open boxes denote putative −10 and −35 consensus sequences of the promoters. The putative regulatory elements (cre and xylO) are in boldface italic letters. The potential RBSs are indicated by asterisks. The beginning of the deduced amino acid sequence of the xylA and xylT genes is depicted below the nucleotide sequence. The putative transcriptional terminator located downstream of the xylB gene is underlined by thin arrows (panel C). For clarity, only the *NlaIII* and *Sau3AI* sites used for cloning are depicted.
the entire xylB gene, a minilibrary was constructed in *E. coli* TGI by using size-selected HindIII fragments. Although colony hybridization revealed several positively hybridizing clones, restriction analyses revealed that these recombinants carried inserts which were much smaller than the expected 4.2 kb and appeared to have lost most of the sequences downstream of the xylB gene. The deletions were confirmed by DNA sequencing. The cause of these deletions is unknown, however, and this dictated the use of another approach to clone xylB.

Since inverse PCR was successfully applied to clone the 432-bp xylA 5′ region (2), it was also employed to amplify the flanking region of xylB. Results of Southern hybridization were used to select restriction enzymes which generated fragments in the 0.8- to 2.5-kb range. Size-selected restriction fragments, including 2.3-kb *BciI*, 1.5-kb *EcoRV*, and 0.8-kb *Sau3AI* fragments, were self-ligated under conditions favoring the formation of monomeric circles and amplified by using primers Sen-2 and Ansen-4 to yield the xylB gene and the flanking region. The sequence of the fragments obtained was determined. Based upon this new sequence information, two additional inverse PCR primers (Sen-3 and Ansen-5) from the region downstream of xylB were synthesized and then used to amplify a 2-kb fragment from a religated *ApoI* size-selected population. This fragment was cloned into pUC18, and its sequence was determined.

**Nucleotide sequence of the xylB and xylT genes.** The xylB ORF (1,506 nucleotides long) starts with an ATG codon (located 96 nucleotides after the xylA stop codon) and terminates with a TAG codon. The xylB gene codes for a protein of 502 amino acids and showed a high degree of identical amino acids (66%) when compared to the product of the *L. pentosus* xylA gene (data not shown). Another ORF (1,371 nucleotides long), xylT, begins 222 bp downstream of the xylB stop codon with an ATG codon and terminates with a TAA codon. Both the xylB and the xylT ORFs are preceded by putative Shine-Dalgarno sequences. No obvious promoter motifs were found immediately upstream of xylB. However, starting 110 bp upstream of the xylT start codon, a xylO sequence was observed, which showed similarity to xylO found in the upstream region of the *L. brevis* and *L. pentosus* xylA genes and in the upstream region of the *L. pentosus* xylP gene (Fig. 1C). −35 and −10 promoter elements (TTTCAAT and TATGAT), spaced by 17 nucleotides, were found 145 and 128 bp upstream of the xylT start codon. Moreover, a putative cre site overlapping the −35 element was also identified. Potential Rho-independent transcriptional terminator sequences were found within the noncoding regions after xylA (ΔG° = −17.1 kcal mol⁻¹), xylB (ΔG° = −14.6 kcal mol⁻¹), and xylT (ΔG° = −18.4 kcal mol⁻¹).

**Sequence homology of XylT with sugar transporters.** The Blast computer program (1) was used to search entries in the Swiss-Prot protein database showing similarity to the deduced amino acid sequence of xylT from *L. brevis*. XylT demonstrated strong similarity throughout the entire sequence to other bacterial monosaccharide transporters of the “major facilitator superfamily” (MFS) (13, 20, 22), especially to the l-arabinose–H⁺ symporter of *E. coli*, AraE (60%); the d-galactose–H⁺ symporter of *E. coli*, GalP (59.5%); the glucosyl transferase of *Zymomonas mobilis*, Glz (57%); the d-xylose–H⁺ symporters of *B. megaterium*, XylT (58%) and of *E. coli*, XylE (57%); and the d-xylose–Na⁺ symporter of *T. halophila*, Xyle (57%). An alignment is presented in Fig. 2. Surprisingly, the similarity score shared between XylT of *L. brevis* and the three bacterial d-xylose-cation symporters was significantly lower than the score shared between Xyle of *E. coli* and *T. halophila* and XylT of *B. megaterium* (73% to 81%). Moreover, XylT of *L. brevis* shared the highest similarity score with the l-arabinose–H⁺ symporter, AraE, of *E. coli*.

**Regulation of d-xylose uptake and d-xylose catabolism in *L. brevis***. It is not known whether d-xylose is required for the expression of the *L. brevis* xyl genes. We could, however, identify putative xylO sequences in the regions upstream of the xylA and xylT genes, suggesting that their expression would be induced by d-xylose. Moreover, the presence of putative cre sites upstream of the xylA and of the xylT genes suggested a possible negative regulation by glucose and other carbohydrates. Therefore, the activity of d-xylose isomerase and d-xyulose kinase were measured in cell extracts of *L. brevis* grown in the presence of d-xylose, d-ribose, l-arabinose, d-glucose, maltose, and a mixture of d-glucose and d-xylose. Active d-xylose transport was also measured in *L. brevis* cells in the presence of an exogenous energy source, l-arginine or d-glucose (Table 1). D-xylose isomerase and d-xyulose kinase activity and the transport of d-xylose were only detected when cells were grown on d-xylose. Moreover, the addition of d-glucose to cells growing on d-xylose decreased the total activity of d-xylose isomerase and d-xyulose kinase by fourfold, and the d-xylose transport activity was decreased by sixfold. In addition, it has been demonstrated that the repressor gene, *xylR*, of the *Bacillus subtilis* and *L. pentosus* *xyl* regulons is involved in the negative control of a β-xylosidase and of an α-xylosidase encoding gene, respectively (4, 12, 17). In *L. brevis*, we could identify a xylR repressor gene homologue, although it was not found within the xyl/αβ locus. This finding prompted us to investigate the presence of α- or β-xylosidase activities in *L. brevis*. No α- and β-xylosidase activity could be detected, but a low level of β-xylosidase activity was detected in cell extracts with most of the growth substrates (see Table 1). However, this activity was increased about 10-fold in the presence of d-xylose, suggesting a mechanism of induction. The β-xylosidase activity was reduced about twofold when both d-glucose and d-xylose were added to the growth medium compared to that of cells grown on d-xylose only.

**Functional expression of the d-xylose transport gene in *L. plantarum***. The *L. brevis* strain studied here could not be transformed with plasmid DNA when standard *Lactobacillus* electrotransformation procedures were used (unpublished observations). Therefore, the xylT gene could not be inactivated by plasmid integration to determine its role in d-xylose uptake in *L. brevis*. However, we have recently developed a *Lactobacillus* expression system which was used to characterize the α-xylosidase transporter of *L. pentosus*, XylP (5). Consequently, an xylT expression vector was constructed, pLP9 (Fig. 3) and was used to transform *L. plantarum* 80, a strain lacking PMF-linked d-xylose transport activity. To demonstrate that the d-xylose transport gene of *L. brevis* was functionally expressed in *L. plantarum* 80, d-xylose transport activity with [1-UT-14C]dxylose was assayed under conditions in which a PMF was generated from either malolactic fermentation (21) or glucose fermentation. Under both conditions, *L. plantarum* 80 harboring plasmid pLP9 could transport and efficiently accumulate significant amounts of d-xylose, whereas the parental wild-type strain could not (Fig. 4). The initial rates of uptake in *L. plantarum* 80(pLP9) were approximately 9 and 12 nmol/min per mg (dry weight) when l-malate and d-glucose were the source of the PMF, respectively. The accumulation level was 30-fold when 5 mM glucose was the source of metabolic energy. In addition, increasing the glucose concentration to 20 mM resulted in a slower rate of uptake (ca. 3 nmol/min per mg [dry weight]), and in a fourfold decrease of...
FIG. 2. Comparison of the primary sequences of XylT of L. brevis (XylT-Lb); GlfZ of Z. mobilis (GlfZ-Zm, Swiss-Prot accession number P21906); XylT of B. megaterium (XylT-Bm, EMBL gene bank accession number Z71474); GalP, AraE, and XylE of E. coli (GalP-Ec, Swiss-Prot accession number P37021; AraE-Ec, Swiss-Prot accession number P09830; XylE-Ec, Swiss-Prot accession number P09098); and XylE of T. halophila (XylE-Th, EMBL gene bank accession number AB009593). The alignment was done by using the Pileup program (9), and some gaps were introduced to maximize the alignment. The identical amino acids are shown in white letters on a solid background. The 12 putative transmembrane segments are indicated by arrows above the alignment. The numbers on the left of the alignment correspond to the amino acid positions for each protein.
the D-xylose level of accumulation. These findings suggest that the metabolism of glucose can negatively affect the activity of XylT and/or the maintenance of D-xylose inside the cell. Without L-malate or glucose as source of the PMF, D-xylose could not be transported or accumulated in *L. plantarum* 80(pLP9). These results clearly indicate that the *xylT* gene of *L. brevis* encodes a D-xylose transporter.

**Substrate specificity and kinetic parameters of XylT.** The kinetic parameters of XylT in *L. plantarum* 80(pLP9) were determined with an L-malate-generated PMF. The *K*ₘ and *V*ₘₐₓ for D-xylose transport were 215 ± 15 μM and 35 ± 2 nmol/min per mg (dry weight), respectively. The effects of various sugars or sugar alcohols on the initial rate of uptake of D-xylose were also tested (Table 2). An excess of the test substrate (50-fold) was added 5 s before the addition of D-[U-¹⁴C]xylose, and the initial velocity of D-xylose transport was measured (20 s). D-XYLOSE transport by XylT was poorly inhibited (20%) by a 50-fold excess of L-arabinose and methyl-β-D-xylose, but no inhibition could be detected with methyl-D-glucose and D-glucose. Six-Deoxy-D-glucose and D-glucose inhibited the transport of D-xylose. The inhibition of D-xylose transport by 6-deoxy-D-glucose was found to be competitive (Fig. 5), with a *K*ₐ of 220 ± 3 μM. As described above, the metabolism of D-glucose in *L. plantarum* 80 affected both the initial rate of uptake and the accumulation level of D-xylose. Since the metabolic inhibition of D-xylose was different with two different concentrations of D-glucose, the role of D-glucose as a potential competitive inhibitor of D-xylose uptake by XylT could not be assessed in our assay conditions.

**Role of the PMF components on D-xylose uptake.** At pH 4.5, the PMF generated by L-malate transport and metabolism (∼160 mV) is composed of an electrochemical membrane potential, *Δψ* (∼70 mV) and of an electrochemical proton gradient, *ZΔpH* (∼90 mV), where *Z* equals 2.3 (*RT/F*) and *R*, *T*, and *F* have their usual meanings (21). To determine the role of these components in the transport of D-xylose by XylT, the effects of uncoupling agents on the initial rate of uptake and on the accumulation level of D-xylose were studied (Table 3). The ionophore nigericin (H⁺/K⁺ antiporter), which dissipates the *ΔH*, decreased the initial rate of uptake and lowered the accumulation level about 80% when used at a concentration of 0.5 μM or higher. A similar but stronger effect was obtained with the protonophore carbonyl cyanide *m*-chlorophenylhydrazone (CCCP), which collapses the total PMF.

**DISCUSSION**

The aim of this study was twofold. First, we wanted to characterize a D-xylose transporter from a *Lactobacillus* species and, second, we wanted to construct a system enabling efficient expression of the transporter. The expression system should be of value in the development of food-grade vectors for heterofermentative lactobacilli based on D-xylose fermentation.

**Similarity between the *L. brevis* xylABT locus, the xylABT operon of *B. megaterium*, and the xylABE operon of *T. halophila*.** The cloning and sequencing of the region downstream from the *xylA* and *xylB* genes of *L. brevis* revealed the presence of a potential xyl gene, *xylT*, encoding a putative membrane-embedded protein. On the basis of homology between the primary structure of XylT from *L. brevis* (further referred to as XylTⁿ) and some members of the MFS, including the low-affinity D-xylose–cation symporters XylE of *E. coli* (further

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**TABLE 1. D-Xylose transport and D-xylose catabolic enzyme activities in *L. brevis* grown on different carbohydrates**

<table>
<thead>
<tr>
<th>Energy source</th>
<th>Initial D-xylose transport ratea (nmol/min/mg [dry wt]) with:</th>
<th>Enzyme activity (nmol/min/mg of total protein) with:</th>
</tr>
</thead>
<tbody>
<tr>
<td>No addition</td>
<td>20 mM of L-arginine</td>
<td>5 mM of D-glucose</td>
</tr>
<tr>
<td>Maltose</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Fructose</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Glucose</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Xylose</td>
<td>&lt;0.1</td>
<td>9.1 ± 0.4</td>
</tr>
<tr>
<td>Glucose-xylose</td>
<td>&lt;0.1</td>
<td>1.9 ± 0.1</td>
</tr>
<tr>
<td>Arabinose</td>
<td>&lt;0.1</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>Ribose</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
</tr>
</tbody>
</table>

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*a* Cells were grown in M medium supplemented with 0.5% (wt/vol) of the corresponding energy source. In all cases, inoculations were performed by diluting an overnight M medium culture (OD₆₀₀ = ∼0.9) 1/100 into fresh medium. Pregrowth was conducted in the presence of the same energy source used for the assay, except for the mixture of D-glucose plus D-xylose, for which cells were pregrown on D-xylose. Each assay was conducted in triplicate with individual *L. brevis* cultures.

*b* Initial rates of D-xylose transport were measured after 20 s. Transport was carried out at 30°C and at pH 6.5 in the presence of 0.5 mM of D-[U-¹⁴C]xylose. *L. brevis* cells were energized with either 20 mM of L-arginine 2 min prior to start the transport reaction or with 5 mM of D-glucose 5 min prior to start the transport reaction.
referred to as \(Xyle\), \(Xyle\) of \(T.\) halophila (further referred to as \(Xyle\)), \(Xyle\) of \(T.\) halophila (further referred to as \(Xyle\)), \(Xyle\) of \(B.\) megaterium (further referred to as \(Xyle\)), a \(\alpha\)-xylose–\(H^{+}\) symporter activity has been assigned to \(Xyle\). The organization of the \(L.\) brevis \(xyl\) locus showed similarity to that of the \(B.\) megaterium \(xyl\) operon (30) and the \(T.\) halophila \(xyl\) operon. No tightly linked \(xylR\) gene could be found upstream of \(xylA\) in \(L.\) brevis. This finding does not exclude, however, that the \(xylR\) gene in \(L.\) brevis may be located in another operon, perhaps serving to regulate a distinct set of genes. Preliminary results have indicated that the expression of the \(xyl\) genes in \(L.\) brevis is inducible by \(\alpha\)-xylose and is repressed by \(\gamma\)-glucose. Putative \(xylO\) sequences (\(XylR\) binding sites) and cre-like elements (\(CcpA\) binding sites) upstream of \(xylAB\) and \(xylT\) genes strongly suggest a negative regulation by \(XylR\) and \(CcpA\) homologues. However, the transcriptional regulation of the \(L.\) brevis \(xyl\) locus is not yet known, and the role of these transcriptional regulators remains to be demonstrated.

**Characteristics of \(Xyle\)**. The \(L.\) brevis \(xylT\) gene was functionally expressed in \(L.\) plantarum 80 by using a recently described \(Lactobacillus\) expression system, which confirmed \(Xyle\) to be a \(\alpha\)-xylose–\(H^{+}\) symporter and enabled the determination of some properties of this transporter. It is interesting to note that throughout the whole sequence, \(Xyle\) appeared to be more closely related to the \(E.\) coli \(\alpha\)-arabinose–\(H^{+}\) transport protein, \(AraE\), than to the bacterial \(\alpha\)-xylose–\(cation\) symporters, \(Xyle\), \(Xyle\), and \(Xyle\). This difference extended to the large gap found between the third and the fourth putative transmembrane region in the sequence of \(Xyle\). \(AraE\), and \(GalP\) introduced to optimize the alignment with

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**TABLE 2. Substrate specificity of the \(L.\) brevis \(XylT\) transporter**

<table>
<thead>
<tr>
<th>Addition of sugars or sugar analogs (5 mM, final conc.)</th>
<th>Relative uptake of (\alpha)-(\gamma-H^{+})xylose (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None .................................................................................</td>
<td>100</td>
</tr>
<tr>
<td>(\alpha)-(\gamma)-xylose .......................................</td>
<td>15</td>
</tr>
<tr>
<td>6-Deoxy-(\gamma)-glucose ........................................</td>
<td>10</td>
</tr>
<tr>
<td>(\gamma)-glucose ..................................................</td>
<td>60</td>
</tr>
<tr>
<td>methyl-(\alpha)-(\gamma)-xylose ................................</td>
<td>80</td>
</tr>
<tr>
<td>(\alpha)-(\gamma)-arabinose ......................................</td>
<td>80</td>
</tr>
<tr>
<td>(\gamma)-galactose ................................................</td>
<td>90</td>
</tr>
<tr>
<td>methyl-(\beta)-(\gamma)-xylose ..................................</td>
<td>100</td>
</tr>
<tr>
<td>(\beta)-ribose .....................................................</td>
<td>100</td>
</tr>
<tr>
<td>(\gamma)-fucose .....................................................</td>
<td>100</td>
</tr>
</tbody>
</table>

* a Initial rates of \(\alpha\)-xylose transport in \(L.\) plantarum 80(pLPA9) were measured after 20 s at pH 4.5 in the presence of 50 mM of \(\gamma\)-malate as a PMF-generating system and with 100 \(\mu\)M of \(\gamma\)-\(\gamma\-H^{+}\)xylose. The control rate (100%) was 9 nmol/min per mg (dry weight). The potential competitors were added 5 s before the initiation of the transport reaction. All values are the average of two separate experiments.

**FIG. 5. Eadie-Hofstee plot of the \(\alpha\)-\(\gamma\-H^{+}\)xylose uptake rate by cells of \(L.\) plantarum 80(pLPA9) as a function of the \(\gamma\)-xylose concentration, without inhibitor (C) or with 0.5 mM (○) or 1 mM (●) 6-deoxy-\(\gamma\)-glucose. Cells were preenergized at 30°C by incubation with 50 mM \(\gamma\)-malate for 2 min at an extracellular pH of 4.5. Rates were calculated after an uptake of 20 s.**
TABLE 3. Effect of ionophores and of the F,R,F,-ATPase inhibitor CCCP on d-xylene uptake by the L. brevis XylIT transporter∗

<table>
<thead>
<tr>
<th>Addition</th>
<th>Conc (nM)</th>
<th>Initial rate of uptakea (nmol/min/mg [dry wt])</th>
<th>Level of accumulation [xylosein]/[xyloseout]</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>9</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>CCCP</td>
<td>50</td>
<td>6</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>0.5</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>1,000</td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>Nigericin</td>
<td>50</td>
<td>9</td>
<td>52</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>5.5</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>1,000</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

∗ The transport assays were carried out with L. plantarum 80(pLP9) cells at pH 4.5 and in the presence of 100 μM d-(U-14C)xylene and 50 mM L-malate as the PMF-generating system. Initial rates were measured after 20 s, and the accumulation levels were calculated after an uptake of 2 min. The potential inhibitors CCCP and nigericin were added 5 s before the initiation of the transport reaction. All values are the average of two separate experiments.

XylIEEc, XylITLb, GfzL, and XytITBm. Even though such a homology would suggest a higher affinity of XytITLb for L-arabinose than for d-xylose, L-arabinose proved to be a very poor competitor of d-xylose uptake by XytITLb. This finding suggests that L-arabinose is presumably not a physiological substrate for the XytITLb protein. In fact, the pattern of inhibition of d-xylose transport by several sugars or sugar analogs indicated that the properties of the XytIEEc and XytITLb transport proteins are very similar. In contrast to the AraE protein of E. coli, for which L-arabinose, d-fucose, and d-xylose are substrates (13), transport of d-xylose by XytITLb is only inhibited by 6-deoxy-d-glucose (6-methyl-d-xylose) and possibly d-glucose. XytITLb can also discriminate between d-xylose and d-xylose analogs with a methyl substitution on the C1 of the pyranoside ring, since neither methyl-α-d-xylose nor methyl-β-d-xylose were efficient competitors of d-xylose transport. A similar specificity was previously found for XylIEEc (13). These results indicate that the primary sequence homology between members of the MFS is clearly not sufficient to predict their substrate specificity. Substrate recognition by these proteins may reside in the positions of specific charged residues (mostly histidine, glutamic acid, or aspartic acid) located in the hydrophilic regions, which may help to bind the substrates (for a review, see reference 23). The alignment shown in Fig. 3, however, did not reveal such conserved residues in XytITLb, XytITBm, XytIEEc, and XylITLb, which could discriminate the d-xylose–cation symporters from the other monocarhide-cation symporters. Nonetheless, the apparent affinity constant of the XytITLb protein for d-xylose (215 μM) is substantially higher than the apparent affinity constant of XytITBm (100 μM) and of XylIEEc (60 μM) for d-xylose (36). Finally, the susceptibility of d-xylose transport to the protonophore CCCP, which collapses the PMF, and to the ionophore nigericin, which discharges the ΔpH, indicates that d-xylose transport by XytITLb proceeds most likely in symport with a proton.

The metabolism of d-glucose in L. plantarum 80, resulted in inhibition of d-xylose transport via XytITLb. A similar inhibition of d-xylose transport could be observed in L. brevis when a high concentration of d-glucose (>20 mM) was used to energize the cells for transport (data not shown). Addition of d-glucose decreased the initial rate of uptake and the overall accumulation level, suggesting a mechanism which may inactivate the activity of XytITLb. A similar mechanism of inhibition, called inducer exclusion, has already been suggested to regulate methyl-d-β-thiogalactopyranoside accumulation in L. brevis (36). Whether a similar mechanism is active in L. plantarum 80 and whether it is responsible for the inhibition of d-xylose uptake by a high concentration of d-glucose in L. brevis remains to be demonstrated.

Development of a d-xylose food-grade vector. In this study, we have shown that the Lactobacillus expression system, pLP9, allowed the functional expression of a d-xylose transporter in L. plantarum 80. This plasmid could serve as the basis for the construction of a food-grade vector based on the metabolism of d-xylose by cloning of the xylA and xylB genes downstream of the xylT gene. Such a vector, which makes use of a strong and constitutive Lactobacillus promoter (pLPA9 from L. casei ATCC 393) may represent a useful tool for improving the catalytic capacity of heterofermentative lactic acid bacteria that are important in the fermentation of plant materials and in the production of fermented food.

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REFERENCES