**Lactobacillus curvatus** has a glucose transport system homologous to the mannose family of phosphoenolpyruvate-dependent phosphotransferase systems

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In **Lactobacillus curvatus**, a phosphoenolpyruvate:mannose phosphotransferase system (mannose-PTS) has been characterized and it was shown to be involved in glucose and mannose transport, but no glucose-specific PTS activity could be detected. A 2.1 kb DNA fragment amplified by PCR from the *L. curvatus* genome was sequenced. Sequence analysis showed four ORFs which could encode proteins similar to PTS transporters EIIA, EIIB, EIIC and EIID of the mannose class. The expression of the *manB* gene (encoding EIIB) from *L. curvatus* in a mutant of *Lactobacillus sake* impaired in EIIMan activity restored this activity. Furthermore, this DNA fragment complemented the regulatory function of LevE (EIIB) in a *Bacillus subtilis* levE-deficient mutant, suggesting that the protein encoded by *manB* could also play a regulatory role in *L. curvatus*.

**Keywords:** *Lactobacillus curvatus*, mannose phosphotransferase system, complementation of *Bacillus subtilis* levE activity

**INTRODUCTION**

Species belonging to the genus *Lactobacillus* are found in a wide variety of fermented food products derived from milk, meat, cereals and vegetables. In particular, the species *Lactobacillus sake* and *Lactobacillus curvatus* are essential agents in dry-cured meat products (Hammes et al., 1990). In the meat mixture, added carbohydrates and certain amounts of amino acids are available to the bacterial cells. The metabolism of these compounds can generate a large amount of organic acids and flavour components. However, glucose, the most rapidly metabolized carbon source, can affect the production of enzymes, extracellular polysaccharides and secondary metabolites. This regulatory role is, to a great extent, dependent on the glucose-specific transport mechanisms present in these micro-organisms (Postma et al., 1993).

Glucose can enter the bacterial cell via two phosphoenolpyruvate (PEP)-dependent sugar phosphotransferase systems (PTS) specific for glucose or mannose (*EII\text{Gl}e* or *EII\text{Man}*, respectively). The PTS comprises two cytosolic proteins, Enzyme I and HPr, which are common to all sugar transport systems, and a family of sugar-specific EII elements, consisting of three or four domains (A, B, C or D) that can be part of a single protein or independent polypeptides (Saier & Reizer, 1992). According to the alignments of the deduced amino acid sequences of known EII proteins, Postma *et al.* (1993) grouped them into four distinct families: glucose-PTS, mannose-PTS, lactose-PTS and mannitol-PTS. The mannose-PTS class includes the mannose-specific EII of *Escherichia coli* (*EII\text{Man}*) (Erni *et al.*, 1987), the sorbose-specific EII (*EII\text{Sor}*) of *Klebsiella pneumoniae* (Wehmeier & Lengeler, 1994), the fructose-specific EII (*EII\text{Lev}*) of *Bacillus subtilis* (Martin *et al.*, 1990) and the putative N-acetylgalactosamine-specific EII (*EII\text{Aga}* of *E. coli* (Reizer *et al.*, 1996). A particular case was found in *Corynebacterium glutamicum* in which the sugar specificity of the glucose transport system corresponded to that described for the *EII\text{Man} of E. coli* (Lee *et al.*, 1993). However, the sequence alignment revealed that it belonged to the sucrose-β-glucoside subgroup of the glucose class (Lee *et al.*, 1994; Postma *et al.*, 1993). No other sequences of genes encoding EII proteins of the mannose class have been described. The mannose class EII complex is composed of two proteins (IIC and IID).
that form the transmembrane element and one or two associated cytoplasmic enzymes. In the EIIMan of E. coli, there is a single soluble protein with two domains (IIAB) (Erni et al., 1987), whilst two separate polypeptides (IIA and IIIB) are found in EIIEV (Martin et al., 1990) and EIISor (Wehmeier & Lengeler, 1994). Furthermore, mutants in different EIIEV elements of B. subtilis have been isolated (Kunst et al., 1977) in which the expression of the levanase operon was constitutive; in fact, elements IIA and IIIB act as negative regulators of the levanase operon (Martin et al., 1990).

In lactic acid bacteria, EIIMan has been proposed to be the main phosphotransferase-mediated transport system for glucose, for which regulatory functions have been described (Chassy & Thompson, 1983; Abe & Uchida, 1989; Veyrat et al., 1994; Gauthier et al., 1990). In Lactococcus lactis and Pediococcus halophilus, EIIMan has been shown to be involved in inductor exclusion/expulsion events as well as in catabolite repression of the genes necessary for the assimilation of lactose, galactose and xylose (Thompson & Chassy, 1985; Abe & Uchida, 1989). In Lactobacillus casei, elements of EIIMan may be involved in the control of lactose and ribose metabolism (Veyrat et al., 1994), and in Streptococcus salivaruis and Streptococcus vestibularis EIIMan may control sugar uptake (Gauthier et al., 1990).

The aim of the work reported in this article was the biochemical and molecular characterization of glucose transport in L. curvatus and its involvement in the regulation of metabolic pathways leading to an efficient utilization of carbon sources. PEP-dependent PTS activity assays were carried out to test substrate specificity to determine the type of EI glucose transporter and its level of activity in bacterial cells which were grown on other sugars. The responsible genes were then amplified by PCR with the aid of degenerate primers obtained from the alignment of known sequences of members of the mannose EI family. The complete nucleotide sequence of the genes encoding EIIMan from L. curvatus was determined. The type of EIIMan-deficient mutant of B. subtilis was carried out to demonstrate the ability to complement the gene encoding EIIMan from L. curvatus to complement an EIIMan-deficient mutant of the closely related species L. sake. Finally, complementation experiments with an EIIMan-deficient mutant of B. subtilis suggest a possible regulatory function for the EIIMan protein in L. curvatus.

**METHODS**

**Bacterial strains, culture conditions and plasmids.** Lactobacillus curvatus CECT 904, Lactobacillus sake 160*1K and RV37 (Lauret et al., 1996) and Lactobacillus casei ATCC 393, cured of plZ15, were used in this work. These strains were grown at 30 °C or 37 °C under static conditions on MRS (Oxoid), Fermentation MRS medium (ADSA-MICRO) or MCD medium (Lauret et al., 1996) supplemented with 0.5% sugar, as indicated. Escherichia coli DH5α [F- endA1 hsdR17 gyrA96 thiI recA1 relA1 supE44 dcmU169 (φ80 lacZ ΔM15)] and E. coli Novablaue (Novagene) were used as hosts in cloning procedures. These cells were grown on Luria-Beranek (LB) medium (Miller, 1992) and ampicillin (100 μg ml⁻¹) was added for plasmid selection.

Bacillus subtilis QB5072 (trpC2 leuE7 sacC− lacZ ) was kindly provided by Professor G. Rapoport (Institut Pasteur, France). This strain was grown on LB or basal MMCH medium (Martin et al., 1990) at 37 °C with vigorous aeration. Bacterial growth was carried out in the presence of 1 μg erythromycin ml⁻¹ and 25 μg lincomycin ml⁻¹. For agar plates, 1.7% (w/v) agar was added to the medium.

The plasmid used in E. coli experiments was pT7Blue-T-vector (Novagen). pGAL9 (Pérez-Martinez et al., 1992) was used as a shuttle vector for cloning purposes in B. subtilis. This vector contains a secretory signal sequence (AL9) carrying transcriptional and translational initiation sequences in addition to the bacteriophage P202 promoter located upstream of the truncated α-amylase gene, the broad-host-range pWV01 replicon (Kok et al., 1984) and the erythromycin-resistance gene from pE194. B. subtilis QB5072 transformants were selected on LB plates containing 200 μg erythromycin ml⁻¹.

**DNA manipulation.** The purification of genomic DNA from Lactobacillus strains was performed according to Posnos et al. (1991) with some modifications. Cells were grown in 10 ml MRS to early stationary phase, harvested by centrifugation and washed with 10 ml 50 mM EDTA. The pellet was resuspended in 600 μl lysis buffer (10%, w/v, PEG 2000 in 15 mM Tris/HCl, pH 8; 5 mM disodium maleic acid; 5 mM MgCl₂) supplemented with lysozyme, at a final concentration of 5 mg ml⁻¹, and mutanolysin at 20 U ml⁻¹. After 1 h at 37 °C, the cells were collected by centrifugation, resuspended in 600 μl 20 mM Tris/HCl (pH 8) and lysed with 60 μl 10% (w/v) SDS in 50 mM Tris/HCl, 20 mM EDTA (pH 8). The following steps were performed as standard protocols (Sambrook et al., 1989).

DNA fragments were recovered from agarose gels using a Sephaglas BandPrep kit (Pharmacia). The DNA probe used in Southern blot experiments and RNA dot-blotting was labelled by the non-radioactive random primed method (DIG DNA Labelling and Detection kit; Boehringer). The temperature of Southern blot hybridization was 55 °C. The RNA dot-blotting hybridization was carried out at 42 °C in the presence of 50% (v/v) formamide. The following washing and detection were carried out according to the manufacturer’s instructions. Restriction and modifying enzymes were also used according to the recommendations of the manufacturers. General cloning procedures were performed according to Sambrook et al. (1989). Preparation of competent cells and transformation of B. subtilis and L. sake were carried out as described previously by Bron & Luxen (1985) and Berthier et al. (1996).

DNA was sequenced by the dideoxy chain-termination method (Sanger et al., 1977) using a T7 Sequencing kit (Pharmacia). Synthetic DNA primers used for sequencing purposes were provided by Pharmacia. Analysis of DNA and protein sequence data was carried out with the following commercial packages: Clustal (Higgins & Sharp, 1989) and GCG (Genetics Computer Group). EMBL, GenBank, PIR and SWISS-PROT were the databases used for DNA and protein sequence similarities.

**Amplification by PCR.** In order to amplify genes encoding EIIMan from L. curvatus by PCR, various synthetic primers were designed: man01 (5'-CTCAGAAAGATACAG), man02 (5'-TGGTTCCAGATAACATCC), man11 (5'-GGAGGAGSSCCTATATAAYGC), man12 (5'-TTCAGAAACCATCTCGAGG), man21 (5'-ATYGGATGTCGTTATATTCCAGG), man22 (5'-CAARCMMACAAATGACTACANG) and man42 (5'-GGATCNCCNACNCNNGC), where B could be C.
G or T; M could be A or C; N could any nucleotide; R could be A or G; S could be C or G and Y could be C or T. The amplification reaction contained 0.1 µg of *L. curvatus* genomic DNA as template, 100 pmol of each primer, 1 U Taq DNA polymerase and the standard 10× reaction buffer provided by the manufacturer (Boehringer). The reaction was carried out in 30 cycles of 94 °C for 30 s, 55 °C for 30 s and 72 °C for 1 min, followed by a final primer extension of 72 °C for 5 min. The reactions were performed in a Techne PHC-3 thermal cycler.

**Total RNA purification and dot-blot analysis.** Buffers for RNA extraction were prepared with diethyl pyrocarbonate (DEPC)-treated water as described by Sambrook et al. (1989). *L. curvatus* was grown to early stationary phase (16 h) in 100 ml MRS fermentation medium with 0.5% glucose, mannose, fructose, ribose, maltose or galactose as carbon sources. Cells were collected by centrifugation, washed with ice-cold DEPC-treated water and resuspended in 1/10 vol. of killing solution (0.4 M ammonium acetate; 0.01 M magnesium acetate; 25%, w/v, sucrose) supplemented with 2 mg lysozyme ml⁻¹ and 10 U mutanulysin ml⁻¹. The cellular suspensions were incubated at 37 °C for 1 h and then harvested by centrifugation at 4 °C. The pellet was resuspended in 5 ml lysis buffer (0.2 M sodium acetate; 1%, w/v, SDS; 0.01 M EDTA; pH 8) containing proteinase K (100 µg ml⁻¹) and incubated for 30 min at 37 °C. Phenol/chloroform/isooamyl alcohol (10 ml) was then added. The nucleic acids, in the aqueous phase, were collected and precipitated by adding lithium chloride to a final concentration of 2 M and 2 vols ethanol and kept at −20 °C overnight. A pellet was obtained by centrifugation at 4 °C for 30 min, washed with 70% (v/v) ethanol and resuspended in DNase I buffer (20 mM Tris/HCl, pH 8; 10 mM MgCl₂) with 10 U RNase-free DNase (Boehringer). After 1 h at 37 °C, another extraction with phenol/chloroform/isooamyl alcohol was performed and RNA was precipitated with ethanol. The RNA was then resuspended in 0.1 ml DEPC-treated water. A₂₆₀ and A₂₈₀ measurements were taken to estimate the RNA concentration and purity (Hewlett Packard HP8452A spectrophotometer). Dot blotting was performed using nylon membrane (Hybond-N; Amersham) and a Bio-Dot Microfiltration Apparatus (Bio-Rad).

**PEP-dependent PTS activity.** PTS activity, estimated by consumption of PEP in the presence of mannose, 2-deoxy-D-glucose (2-DG), glucose, fructose, ribose and methyl α-D-glucopyranoside (α-MG), was quantified according to Chassy et al. (1994). The sugar phosphate was phosphorylated yielding fructose 1-phosphate in EIIs of *E. coli* as described by Abe et al. (1994). L. *curvatus* was normally found on glucose) and, since cells could normally grow on ribose, a non-PTS transport system must be present in *L. curvatus*. The pattern of sugar specificity of this transport system was similar to that found in the EIIMan of *E. coli* and also resembled the pattern found by Veyrat et al. (1994) in *L. casei*.

**Fructose phosphorylation**

Table 1 shows that fructose phosphorylation by glucose- or mannose-grown cells was very low, but that by fructose-grown cells was slightly higher. In order to confirm the inability of *L. curvatus* EIIMan to phosphorylate fructose, two phosphorylation products were quantified, fructose 6-phosphate and fructose 1-phosphate. Fructose 6-phosphate is normally formed by EIIMan of *E. coli* and EIILev of *B. subtilis* (Erni et al., 1987; Martin et al., 1990) but the C-1 of fructose is phosphorylated yielding fructose 1-phosphate in EIISor of *K. pneumoniae* (Wöhr & Lengeler, 1990). No fructose 6-phosphate or fructose 1-phosphate was detected in cells preadapted with fructose, glucose or mannose. According to these data, *L. curvatus* EIIMan could be an unusual member of the group, as it has a low affinity for fructose.

Distinct fructose transport systems may be present in *L. curvatus*, since this micro-organism can efficiently grow on fructose and we could show that a higher EIIPru activity was found in fructose-grown cells.

**Cloning of genes encoding EIIMan, and sequence analysis**

From the sequence alignment of known EIIF proteins (Postma et al., 1993), different synthetic oligonucleotides (man11, man12, man21, man22 and man42) were designed from conserved regions of the mannose-EII family. Initially, three DNA fragments of 340, 600 and 860 bp...
**Table 1.** PTS activity in permeabilized cells of *L. curvatus*

PTS activity is expressed as nmol PEP consumed min⁻¹ (mg dry wt)⁻¹.

<table>
<thead>
<tr>
<th>Growth on:</th>
<th>Glucose</th>
<th>Mannose</th>
<th>Fructose</th>
<th>Ribose</th>
<th>α-MG</th>
<th>2-DG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>142.9</td>
<td>161.9</td>
<td>12.7</td>
<td>15.9</td>
<td>12.7</td>
<td>66.7</td>
</tr>
<tr>
<td>Mannose</td>
<td>189.8</td>
<td>206.8</td>
<td>5.6</td>
<td>8.5</td>
<td>5.6</td>
<td>82.1</td>
</tr>
<tr>
<td>Fructose</td>
<td>149.5</td>
<td>155.8</td>
<td>31.2</td>
<td>9.3</td>
<td>9.3</td>
<td>68.5</td>
</tr>
<tr>
<td>Ribose</td>
<td>326.6</td>
<td>361.3</td>
<td>17.3</td>
<td>17.3</td>
<td>11.6</td>
<td>130.1</td>
</tr>
</tbody>
</table>

Fig. 1. Strategy of cloning of the man operon from *L. curvatus*. The synthetic oligonucleotides used for PCR amplification are indicated by arrows. pCU1, pCU2 and pCU3 plasmids were obtained by cloning the indicated amplified fragment in pT7Blue T-vector. IIA, IIB, IIC and IID elements correspond to the gene order of the levanase operon in *B. subtilis*. A putative structure of the EllMan operon from *L. curvatus* is shown.

Fig. 2 shows the longest nucleotide sequence obtained from *L. curvatus*. Primary structure analysis revealed four ORFs (ORF1–4), although ORF4 was incomplete. The first two ORFs could encode proteins of 143 and 163 aa, respectively, and ORF3 could encode a protein of 271 aa. Detailed analysis showed Shine–Dalgarno sequences 7–10 nucleotides upstream of the putative start codons, ATG, in all four ORFs. The first codon of ORF2 overlapped with the stop codon (TGA) of ORF1, which could indicate translational coupling of ORF1 and ORF2. The intercistronic distance between ORF2 and ORF3 was 19 bp and that between ORF3 and ORF4 was 12 bp.

The deduced amino acid sequences were tested for similarities with sequences in the databases. Higher sequence similarities were found to EII belonging to the mannose class. Alignments with the deduced amino acid sequences of *E. coli* EII₅₄₅ (Erni et al., 1987), *B. subtilis* EII₅₄₅ (Martin et al., 1990), K. pneumoniae EII₅₄₅ (Wehmeier & Lengeler, 1994) and *E. coli* EII₅₄₅ (Reizer et al., 1996) are shown in Fig. 3. The calculated percentages of similarity and identity are shown in Table 2. The sequence of EII₅₄₅ from *E. coli* was not included in the comparison, as it is not included in the operon. In general terms, the degree of similarity between the EII₅₄₅ proteins of *L. curvatus* and the EII₅₄₅ proteins of *B. subtilis* was higher than to any other EII complex. The trend of sequence divergence between the different EIIA proteins, also found between EII₅₄₅ of *L. curvatus* and the other EIIA compared, as also shown by Reizer et al. (1996), was also noticeable. Therefore, it can be considered that the sequenced fragment of *L. curvatus* DNA encodes an operon with a structure similar to the levanase operon of *B. subtilis* and the sorbose operon of *K. pneumoniae* and, considering their structure, they could also have translational coupling (Martin et al., 1990; Wehmeier & Lengeler, 1994). However, a difference between these two organisms is that the expression of these genes in *L. curvatus* seems to be constitutive (see below) as is the expression of the genes encoding EII₅₄₅ of *E. coli*. Sequence analysis also showed that proteins IIA₅₄₅ and IIB₅₄₅ of *L. curvatus* have a histidine residue (His-9 and His-14, respectively) that could be involved in the transfer of the phosphate group to the carbohydrate, as occurs in the other members of the family. There are also highly...
**Fig. 2.** Nucleotide sequence of the *L. curvatus* DNA fragment containing four ORFs. The deduced amino acid sequences are written below the corresponding nucleotide codons. Sequences resembling ribosome-binding sites are underlined. Putative conserved regions in the EIIC proteins of all four micro-organisms.

The existence of a chromosomal copy of the cloned fragments was demonstrated by Southern blotting in which the genomic DNA of *L. curvatus* was digested with EcoRI (Fig. 4). In the same experiment, the presence of a similar complex in *L. casei* was tested and its genomic DNA was digested with the endonucleases EcoRI and BamHI. The probe used was the 600 bp fragment cloned in pCU2. It hybridized with a band of 2.1 kb from *L. curvatus* DNA. No hybridizing bands were found with *L. casei* DNA (Fig. 4).
Table 2. Similarity between the A, B, C and D domains of the EII members of the mannose class

Percentage identities are in parentheses. The entire proteins (domains in the case of the EIIABman of E. coli) were used for the comparison, except for the comparison of the EIIDman of L. curvatus with the other EIID, where only the first 90 aa were used. Eco, E. coli; Bsu, B. subtilis; Kpn, K. pneumoniae; Lcu, L. curvatus.

<table>
<thead>
<tr>
<th>Man (Eco)</th>
<th>Fru (Bsu)</th>
<th>Sor (Kpn)</th>
<th>Aga (Eco)</th>
<th>Aga' (Eco)</th>
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</thead>
<tbody>
<tr>
<td>Man (Lcu)</td>
<td>A 44.2 (25.8)</td>
<td>61.6 (42.5)</td>
<td>54.1 (30.4)</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>B 72.0 (54.0)</td>
<td>80.0 (61.2)</td>
<td>67.9 (46.3)</td>
<td>55.8 (27.9)</td>
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<tr>
<td></td>
<td>C 81.0 (60.5)</td>
<td>88.8 (77.3)</td>
<td>77.6 (56.3)</td>
<td>56.5 (24.8)</td>
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<td></td>
<td>D 82.8 (58.6)</td>
<td>83.9 (74.7)</td>
<td>72.4 (55.2)</td>
<td>65.1 (38.4)</td>
</tr>
<tr>
<td>Man (Eco)</td>
<td>A 54.6 (31.2)</td>
<td>48.5 (29.8)</td>
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<td></td>
<td>B 63.2 (47.2)</td>
<td>66.0 (50.6)</td>
<td>53.5 (33.6)</td>
<td>54.8 (38.1)</td>
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<td></td>
<td>C 79.5 (59.3)</td>
<td>80.1 (60.9)</td>
<td>57.6 (25.8)</td>
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<td>D 81.8 (60.4)</td>
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<td>Fru (Bsu)</td>
<td>A 54.8 (29.6)</td>
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<td></td>
<td>B 65.4 (43.8)</td>
<td>57.1 (27.3)</td>
<td>56.6 (32.5)</td>
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<tr>
<td></td>
<td>C 77.2 (54.4)</td>
<td>56.9 (25.0)</td>
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<td></td>
<td>D 78.1 (57.7)</td>
<td>62.8 (35.6)</td>
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<tr>
<td>Sor (Kpn)</td>
<td>A –</td>
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<tr>
<td></td>
<td>B 51.9 (29.9)</td>
<td>55.8 (34.0)</td>
<td>–</td>
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<td></td>
<td>C 53.8 (25.8)</td>
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<tr>
<td></td>
<td>D 61.3 (36.4)</td>
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<tr>
<td>Aga (Eco)</td>
<td>A –</td>
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As discussed above, the sugar specificity pattern of L. curvatus was very similar to that previously described in L. casei (Veyrat et al., 1994). This hybridization experiment indicates that the sequence divergence between the genes encoding EII within this genus could be high. A second possibility would be that the mannose-glucose-specific EII found in L. casei could have a different structure, as described for oral streptococci, in which the type and number of glucose transport systems is variable, even among strains of the same species (Pelletier et al., 1995).

Transcription of the genes encoding EIIMan

In an attempt to determine whether the genes encoding EIIMan were subject to some type of regulation, total RNA extracted from L. curvatus grown on glucose, mannose, fructose, ribose, galactose or maltose was blotted and hybridized with an internal DNA fragment of the mannose complex (pCU2 insert) (Fig. 5). Clear hybridization signals found under all the culture conditions used suggested that these genes are not under tight repression or induction.

Complementation of an EIIMan mutant of L. sake

The amplified fragments from L. curvatus DNA were shown to have a similar sequence and structure to other genes encoding EIIMan, but the real function had to be proved. A BamHI–HindIII fragment from pCU3, containing the manB gene from L. curvatus, was subcloned in pGAL9. Therefore, manB could be expressed in other Gram-positive bacteria from Spo2 and Al9 promoters. A mutant of L. sake 160*K which had been shown to lack a functional EIIMan, strain RV37 (Lauret et al., 1996), was transformed with pMJ18. Complemented colonies were selected on MCD medium containing mannose as the only carbon source. The restriction map demonstrated the presence of pMJ18 in the transformants.

The fermentation abilities of L. sake 160*K (wild-type), L. sake RV37 and L. sake RV37(pMJ18) were tested on Fermentation MRS medium containing glucose, mannose, fructose, ribose and N-acetylglucosamine. Acid production of L. sake RV37(pMJ18) on these sugars was identical to the wild-type L. sake 160*K. PEP consumption of both strains in the presence of mannose was very similar [24.3 nmol min⁻¹ (mg dry wt)⁻¹] in the wild-type versus 21.8 nmol min⁻¹ (mg dry wt)⁻¹ in RV37(pMJ18)], while strain RV37 showed lower activity [2.9 nmol min⁻¹ (mg dry wt)⁻¹]. Therefore, the gene from L. curvatus encoding EIIMan could complement the impaired EIIMan of L. sake RV37. This experiment showed that (i) L. sake RV37 mutation must be affecting element EIIMan and that (ii) the sequences described in this work corresponded to the proteins of a true EIIMan system belonging to the mannose class.
the operon has been proposed (Martin et al., 1990). The product of the \( levR \) gene would undergo a phosphorylation by EII\( ^{Lev} \), being inactivated in this form when fructose in the medium is limited. In fact, proteins LevD and LevE (EII\( ^{Lev} \) and EIIB\( ^{Lev} \)) would be involved in this regulation, since the expression of the levanae operon becomes constitutive in mutants deficient in these enzymes. \( B. \ subtilis \) QB5072 carries a point mutation in the \( levE \) gene (G to A) producing the codon change Trp-22 to stop-22. The impaired EII\( ^{Lev} \) is unable to transport fructose, but the expression of the operon becomes constitutive. Also in this strain, the levanae gene (\( sacC \)) has been fused to the lacZ gene of \( E. \ coli \) to facilitate the study of the regulation of this operon.

In order to determine if EIIB\( ^{Man} \) from \( L. \ curvatus \) could have a similar regulatory function to the LevE protein of \( B. \ subtilis \), mutant \( B. \ subtilis \) QB5072 was transformed with pMJ18 and pGAL9. When \( \beta \)-galactosidase activity of these transformants was assayed on LB plates containing erythromycin and X-Gal, strains carrying pGAL9 gave dark-blue colonies due to the constitutive expression of the fused operon, while colonies bearing pMJ18 remained white. In order to quantify this activity, both strains were grown on basal medium with glycerol or fructose as carbon sources. \( \beta \)-Galactosidase expression became inducible by fructose in the strain transformed with pMJ18, the activity increasing from 34-8 (with glycerol) to 118-8 (with fructose) U min\(^{-1} \) (mg protein\(^{-1} \)). The values for QB5072(pGAL9) were 264-3 and 262-8 U min\(^{-1} \) (mg protein\(^{-1} \)), respectively.

This experiment shows that the constitutive expression of the levanae operon was abolished when the \( B. \ subtilis \) levE mutant was transformed with the \( manB \) gene from \( L. \ curvatus \). This complementation of this regulatory effect suggests that EIIB\( ^{Man} \) of \( L. \ curvatus \) could phosphorylate LevR in \( B. \ subtilis \), as suggested by Strülke et al. (1995).

Whether EIIB\( ^{Man} \) is involved in a similar process in \( L. \ curvatus \) has yet to be proved. Genetic inactivation techniques which would be required to establish the real role of EIIB\( ^{Man} \) in this micro-organism are currently being developed.

Concluding remarks

We have physiologically characterized the glucose transport system and sequenced a DNA fragment of \( L. \ curvatus \), showing that: (i) its sugar specificity pattern corresponds to an EII\( ^{Man} \)-type of transporter; (ii) fructose is not transported by this system; (iii) ribose is not transported by PTS in this micro-organism; (iv) the amino acid sequence deduced from the fragment amplified by PCR has homology to the other members of the mannose-EII family; (v) sugar phosphorylation as well as dot-blot assays showed that EII\( ^{Man} \) is constitutive in \( L. \ curvatus \); (vi) EIIB\( ^{Man} \) from \( L. \ curvatus \) can complement a mutant of \( L. \ sake \) impaired in EII\( ^{Man} \), therefore the cloned operon could encode the PTS transporter of mannose and glucose; (vii) EIIB\( ^{Man} \) from \( L. \ curvatus \) can complement the regulatory functions of LevE from \( B. \ subtilis \), therefore it could play a similar role in \( L. \ curvatus \).
The PTS is known to play a central role, in both the uptake of a number of sugars and regulatory processes, specially by the common elements, HPr and EI. However, the glucose-specific EI elements are also known to mediate in a number of regulatory events (Abe & Uchida, 1989; Veyrat et al., 1994; Martin et al., 1990). Additionally, it has been shown in this work that in L. curvatus EI²Man could possibly interact with other proteins, such as B. subtilis LeVE.

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