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Mannitol-Specific Phosphoenolpyruvate-Dependent Phosphotransferase System of *Enterococcus faecalis*: Molecular Cloning and Nucleotide Sequences of the Enzyme III^{Mtl} Gene and the Mannitol-1-Phosphate Dehydrogenase Gene, Expression in *Escherichia coli*, and Comparison of the Gene Products with Similar Enzymes

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Enzyme III^{Mtl} is part of the mannitol phosphotransferase system of *Enterococcus faecalis*. It is phosphorylated in a reaction sequence requiring enzyme I and heat-stable phosphocarrier protein (HPr). The phospho group is transferred from enzyme III^{Mtl} to enzyme II^{Mtl}, which then catalyzes the uptake and concomitant phosphorylation of mannitol. The internalized mannitol-1-phosphate is oxidized to fructose-6-phosphate by mannitol-1-phosphate dehydrogenase. In this report we describe the cloning of the *mtlF* and *mtlD* genes, encoding enzyme III^{Mtl} and mannitol-1-phosphate dehydrogenase of *E. faecalis*, by a complementation system designed for cloning of gram-positive phosphotransferase system genes. The complete nucleotide sequences of *mtlF*, *mtlD*, and flanking regions were determined. From the gene sequences, the primary translation products are deduced to consist of 145 amino acids (enzyme III^{Mtl}) and 374 amino acids (mannitol-1-phosphate dehydrogenase). Amino acid sequence comparison confirmed a 41% similarity of *E. faecalis* enzyme III^{Mtl} to the hydrophilic enzyme III^{Mtl}-like portion of enzyme II^{Mtl} of *Escherichia coli* and 45% similarity to enzyme III^{Mtl} of *Staphylococcus carnosus*. The putative N-terminal NAD$^+$ binding domain of mannitol-1-phosphate dehydrogenase of *E. faecalis* shows a high degree of similarity with the N terminus of *E. coli* mannitol-1-phosphate dehydrogenase (T. Davis, M. Yamada, M. Elgort, and M. H. Saier, Jr., Mol. Microbiol. 2:405–412, 1988) and the N-terminal part of the translation product of *S. carnosus mtlD*, which was also determined in this study. There is 40% similarity between the dehydrogenases of *E. faecalis* and *E. coli* over the whole length of the enzymes. The organization of mannitol-specific genes in *E. faecalis* seems to be similar to the organization in *S. carnosus*. The open reading frame for enzyme III^{Mtl} *E. faecalis* is followed by a stem-loop structure, analogous to a typical Rho-independent terminator. We conclude that the mannitol-specific genes are organized in an operon and that the gene order is *mtlA orfX mtlF mtlD*.

The phosphotransferase system (PTS) represents an important carbohydrate transport system in gram-negative and gram-positive bacteria (for a review, see reference 25). It consists of two general constitutive cytoplasmic phosphoproteins, enzyme I and HPr, and carbohydrate-specific components: two mannitol-specific inducible PTS enzymes exist in streptococcal cells, soluble enzyme III^{Mtl} (EIII^{Mtl}) and membrane-bound EII^{Mtl}, which catalyzes the concomitant transport and phosphorylation of mannitol. Mannitol-1-phosphate is oxidized to fructose-6-phosphate by mannitol-1-phosphate dehydrogenase.

The *mtlA* gene, encoding EII^{Mtl} of *Escherichia coli*, was cloned and sequenced by Lee and Saier (19). Recently, we cloned and sequenced the *mtlF* gene, encoding EIII^{Mtl} of *Staphylococcus carnosus* (7). There is significant similarity between the soluble EIII^{Mtl} of staphylococcal cells and the hydrophilic C terminus of EII^{Mtl} of *E. coli*. Very recently, we were able to clone and sequence *mtlA* of *S. carnosus* (8). The derived primary translation product consists of 505 amino acids (9). This protein is similar to the N-terminal part of *E. coli* EII^{Mtl}, which consists of 637 amino acids.

Little is known about the regions responsible for recognition, binding, and transport of mannitol, the phospho group transfer, or the further metabolism of mannitol-1-phosphate. No sequence of a mannitol-1-phosphate dehydrogenase of gram-positive microorganisms is yet known. This study was undertaken to determine (i) the complete primary structure of mannitol-specific proteins of a third group of microorganisms, the streptococci, (ii) their structural relationship to other sugar-specific enzymes, which could enable us to predict putative active center regions for further investigations, e.g., site-directed mutagenesis, and (iii) the organization of mannitol-specific genes in *Enterococcus faecalis*.

MATERIALS AND METHODS

Chemicals, enzymes, and isotopes. Restriction endonucleases, T4 DNA ligase, DNase, RNase, mungbean nuclease, calf intestinal alkaline phosphatase, and polymerase IK were obtained from Bethesda Research Laboratories, Boehringer Mannheim, or New England BioLabs and were used as recommended by the manufacturers. We used the T7 sequencing kit from Pharmacia with [α-^{35}S]dATP (1,000 Ci/mmol) from Amersham. 5-Bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) and isopropyl-β-D-thiogalactopyranoside (IPTG) were purchased from Biomol. Ampicillin and chloramphenicol were from Sigma Chemical Co. Bacterial medium was from Difco. Other reagents were commercial products of the highest purity grade available.

Bacterial strains, plasmids, and growth conditions. The bacterial strains used were the following. *E. coli* HB101 (4) is

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described as *mtl-1*; we determined the mutation as *mtlA*. *E. coli* JM109 (34) was used as a host for pT7T318U and pT7T319U (21) and p15SK(+) (10) and derivatives. Standard media and growth conditions were used for the growth of *E. coli* (20). McConkey indicator plates contained 1% (wt/vol) mannitol and 50 mg of $MgCl_2$ per liter.

Construction of genomic libraries. Standard DNA procedures were used as described by Maniatis et al. (20). *E. faecalis* chromosomal DNA was isolated as described by Götz et al. (12) for *S. carnosus* DNA.

DNA sequencing. Sequencing was done by the dideoxynucleotide chain termination method of Sanger et al. (26), with the T7 sequencing kit from Pharmacia and double-stranded DNA as a template. All fragments were propagated in pT7T318U or pT7T319U for the purposes of sequencing. We used plasmid DNA prepared by the method of Birnboim and Doly (3) after extended RNase treatment.

Computer programs. A search of the EMBL protein sequence data bank for amino acid sequence similarities was carried out on a PC-AT personal computer with PROSIS software. DNA analysis was done with the STADEN DNA software purchased from Amersham.

SDS-PAGE. Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) was carried out on slab gels containing 10% acrylamide in the presence of 0.2% SDS as described by Laemmli (18). Gels were stained with Coomassie blue and destained by a mixture of 7.5% acetic acid and 10% (vol/vol) methanol.

Construction of unidirectional deletions. Controlled unidirectional deletions in plasmid DNA were constructed by double digestion with two restriction enzymes, which produce one 3′ and one 5′ overhanging end, and subsequent exonuclease III and mungbean nuclease digestion as described by Henikoff (14).

Nucleotide sequence accession number. The sequence reported has been entered into GenBank under accession number M38386.

RESULTS

We cloned *mtlF* of *E. faecalis* by in vivo complementation of a mutant strain lacking $EIII^{Mtl}$. Because transformation of *E. faecalis* is difficult and no *E. faecalis* mannitol mutant strains are yet known, we decided to use an *E. coli* mannitol mutant strain as a host for genomic libraries of *E. faecalis* DNA. Unfortunately, the complementation of *E. coli* PTS mutants with gram-positive carbohydrate-specific PTS genes is of low efficiency, because it is known from in vitro complementation assays that the gram-negative PTS enzymes HPr and enzyme I can only poorly interact with gram-positive PTS enzymes (13a). To overcome these difficulties, we constructed a strain [HB101(p15AHI)] that combines the restriction- and recombination-negative genotype and the high transformation efficiency of *E. coli* with a mannitol-negative genotype and the ability to be complemented by $EIII^{Mtl}$ of *E. faecalis*.

Construction of p15AHI. A 2.45-kb *Sal*I-*Sst*I restriction fragment of pRF11 containing the *mtlA* gene for EII^{Mtl} of *S. carnosus* (9) was cloned into plasmid p15SK(+), and the resulting plasmid was designated p15mtlA. For the construction of p15AHI, the 3.1-kb *Xba*I restriction fragment of pHI (9) containing the *ptsH* and *ptsI* genes of *S. carnosus* was cloned into p15mtlA to yield plasmid p15AHI (Fig. 1). Transcription of the genes for HPr and enzyme I of *S. carnosus* can start from the *lac* promoter in p15AHI, while the orientation of the gene for EII^{Mtl} of *S. carnosus* is in the antisense direction and therefore must be transcribed from its own promoter, preventing harmful overexpression of the membrane enzyme. Plasmid p15AHI contains the P15A replicon (27) and a resistance gene different from the β-lactamase gene, i.e., the chloramphenicol acetyltransferase gene from Tn*9* (1). p15AHI can be used for genetic PTS complementation studies by simultaneous expression of several gram-positive PTS genes. *E. coli* HB101(p15AHI) remains mannitol negative because a gram-positive *mtlF* coding for $EIII^{Mtl}$ is absent.

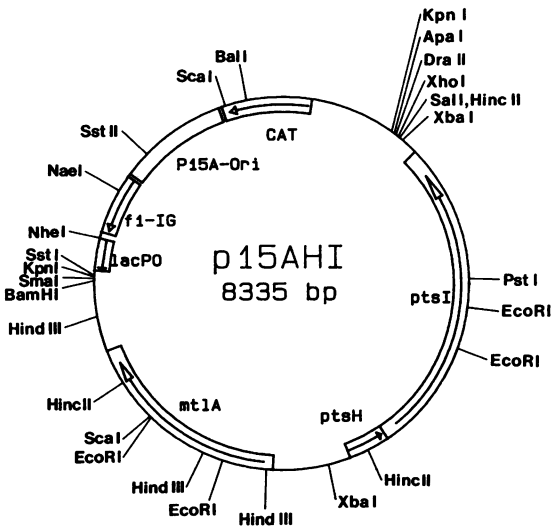

FIG. 1. Map of plasmid p15AHI, which contains the P15A replicon (P15A-Ori), the chloramphenicol acetyltransferase gene (CAT), and the *ptsH*, *ptsI*, and *mtlA* genes, encoding HPr, enzyme I, and EII^{Mtl}, respectively, from *S. carnosus*.

Cloning of *mtlF* and *mtlD* of *E. faecalis*. Chromosomal DNA of *E. faecalis* was digested with *Kpn*I, and restriction fragments were eluted from preparative agarose gels and ligated into pT7T318U, which had been linearized with *Kpn*I and dephosphorylated. Competent *E. coli* HB101(p15AHI) cells were transformed with the ligation mixture to confer ampicillin resistance and mannitol utilization. The cells were plated on McConkey mannitol plates containing ampicillin and chloramphenicol. Three mannitol-metabolizing colonies could be identified by their intense red color. These colonies had identical plasmids with 4.75-kb inserts of *E. faecalis* DNA.

Transformation of these plasmids into the mannitol-1-phosphate dehydrogenase-negative strain *E. coli* 239 (30) resulted in large red colonies, while *E. coli* 239 containing pT7T318U without an insert is mannitol sensitive and only tiny white colonies are able to grow on MacConkey mannitol plates. This assay showed clearly that *mtlD* of *E. faecalis* had also been cloned. The plasmids were then transformed into HB101(pHIFD) (9), which contains the genes for HPr, enzyme I, $EIII^{Mtl}$, and mannitol-1-phosphate dehydrogenase of *S. carnosus* and lacks only a gram-positive EII^{Mtl} gene, but resulted in white colonies. These complementation studies clearly demonstrate that *mtlF* and *mtlD*, but not *mtlA* of *E. faecalis*, were cloned. The plasmids were designated pFD4,75 to indicate that they contain *mtlF* and *mtlD* on a 4.75-kb insert.

Subcloning and restriction maps. Many commonly used restriction enzymes were tested, but only *Hin*dIII and *Stu*I

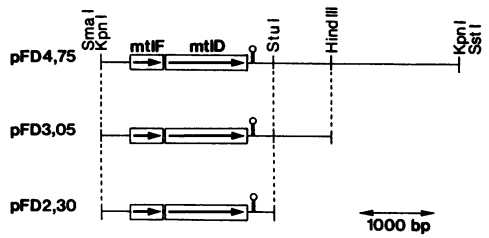

FIG. 2. Restriction maps and subclone maps. Boxes with arrows indicate the orientations and sizes of the genes. The location of the Rho-independent terminator is shown. pFD4,75 contains 4.75 kb of *E. faecalis* DNA inserted into the *Kpn*I site of pT7T318U. pFD3,05 and pFD2,30 are subclones of pFD4,75. pFD3,05 contains 3.05 kb of *E. faecalis* DNA inserted into the *Hin*dIII and *Kpn*I sites of pT7T318U; pFD2,30 contains 2.3 kb of *E. faecalis* DNA inserted into the *Sma*I site of pT7T318U. The *mtlF* and *mtlD* genes have the correct transcriptional orientation to the *lac* promoter in the vectors. Clone pFD2,30 was used as the source of DNA for sequencing.

were found to cleave the insert of pFD4,75. pFD4,75 was cleaved by *Kpn*I plus *Hin*dIII. The resulting 3.05-kb restriction fragment was isolated from a preparative agarose gel and ligated into pT7T318U, which had been linearized with *Kpn*I and *Hin*dIII. The 2.3-kb *Sma*I-*Stu*I restriction fragment of pFD4,75 was ligated into the *Sma*I site of pT7T318U. It was shown by complementation experiments that *mtlF* and *mtlD* are present on both fragments. The new plasmids (Fig. 2) were designated pFD3,05 and pFD2,3.

Sequencing strategy. Plasmid pFD2,3 was used as a source of DNA for sequencing. Unidirectional deletions were constructed by restriction with *Bam*HI plus *Pst*I and subsequent digestion with exonuclease III and mungbean nuclease (see Materials and Methods).

From the other end of the insert we obtained overlapping unidirectional deletions in the following way. The 2.3-kb insert of pFD2,3 was excised with *Sma*I and *Xba*I and ligated into pBluescript SK(+), which had been linearized with the same enzymes. The resulting plasmid, pSK2,3, was cleaved with *Apa*I and *Eco*RI, and unidirectional deletions were constructed. The complete sequencing strategy is shown in Fig. 3.

Sequences of *mtlF* and *mtlD*. The final sequence of *mtlF*, *mtlD*, and flanking regions is shown in Fig. 4. The putative ribosome binding site (RBS) is located at positions 370 to 373. This sequence is similar to the complementary sequence of 3'-terminal nucleotides of 16S rRNA from *Streptococcus lactis* and *S. aureus* (6), *Bacillus subtilis*, and *E. coli* (28, 31). The RBS is followed by an open reading frame (ORF) which starts at position 380 with ATG and runs to position 818 at the stop codon TAA. The ORF of 435 bases can potentially encode a protein of 145 amino acids with a calculated mass of 15,866 Da. The ORF for $EIII^{Mtl}$ is followed by an RBS for *mtlD*. *mtlD* starts at position 834 with ATG and runs to position 1956 at the stop codon TGA. This ORF can encode a protein of 374 amino acids with a calculated mass of 40,840 Da.

Downstream of *mtlD* a putative stem-loop structure at positions 2066 to 2103 is found, followed by seven T residues, which could form the typical Rho-independent transcriptional terminator (Fig. 5). Upstream of *mtlF* and *mtlD* is the end of another ORF. This ORF is 104 amino acids long and stops with TAA at position 315.

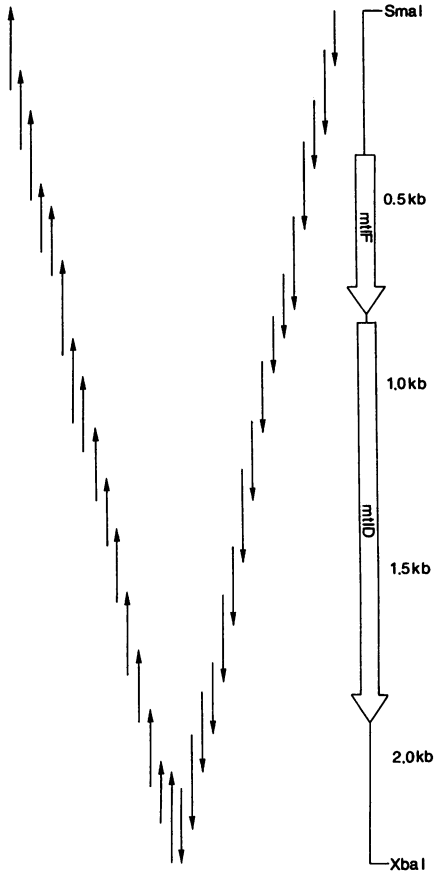

FIG. 3. DNA sequencing scheme for the 2.3-kb insert of pFD2,3. The single line represents *E. faecalis* DNA. Large open arrows indicate the coding regions of *mtlF* and *mtlD*. Small arrows show the sequencing start point and extension of subclones constructed by the unidirectional deletion method. Double-stranded templates were sequenced by the dideoxy-chain termination method with the universal sequencing primer.

In addition, we sequenced a region downstream of *mtlF* of *S. carnosus* (8) and found the start of an ORF very similar to *mtlD* of *E. faecalis* (Fig. 6 and Discussion).

Overexpression in *E. coli*. The high level expression of *E. faecalis* mannitol-1-phosphate dehydrogenase in *E. coli* is demonstrated in Fig. 7. HB101 cells containing either pFD3,05, pFD2,3, or pT7T318U without an insert were grown overnight and then broken by sonication. One can find an intense protein band migrating at the same position as the marker protein aldolase (40,000 Da).

DISCUSSION

$EIII^{Mtl}$ of *E. faecalis* is similar to the C terminus of EII^{Mtl} of *E. coli*. EII^{Mtl} of *E. coli* belongs to a PTS which lacks soluble EIII (19). As shown in Fig. 8, $EIII^{Mtl}$ of *E. faecalis* exhibits 41% similarity to the C-terminal part of this enzyme. The region of EII^{Mtl} of *E. coli* that is similar to $EIII^{Mtl}$ of *E. faecalis* starts within the hydrophilic C-terminal part at amino acid 493. Similar observations were made with $EIII^{Mtl}$ of *S. carnosus* (7), which exhibits 38% similarity to the C-terminal part of EII^{Mtl} of *E. coli*. It is also remarkable that the N-terminal part of *Salmonella typhimurium* FPr (11)

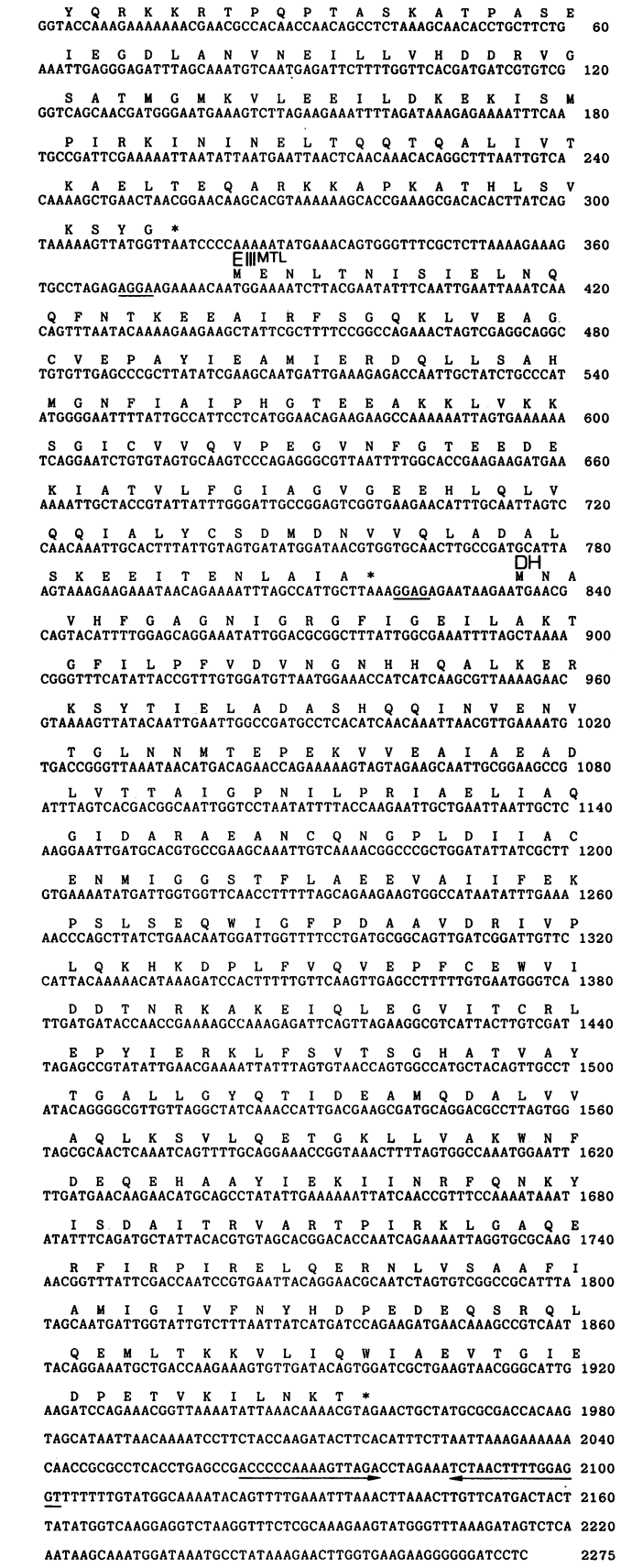

FIG. 4. DNA sequence of *mtlF*, *mtlD*, and flanking regions. Deduced amino acids encoded by *mtlF*, *mtlD*, and *orfX* are given above the nucleotide sequence. The RBS and Rho-independent terminator are underlined (see Fig. 5). DH, dehydrogenase.

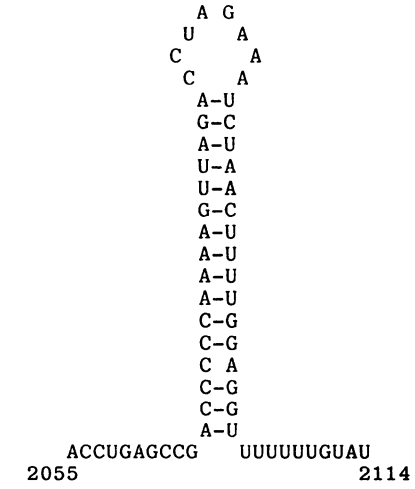

FIG. 5. Structure of the putative Rho-independent terminator of the mannitol operon (see Fig. 4).

shows 38% similarity to $EIII^{Mtl}$ of *E. faecalis*, while the C-terminal part of FPr is similar to HPr.

The highest degree of conservation is around His-554 of EII^{Mtl} of *E. coli* (Fig. 9). It could indeed be demonstrated that the motif PHGT is the active center in $EIII^{Mtl}$ of *S. carnosus* and EII^{Mtl} of *E. coli* (22, 24). Therefore, we assume that His-62 is phosphorylated in $EIII^{Mtl}$ of *E. faecalis* also.

Recently, van Weeghel et al. have subcloned the extreme C terminus (Ser-490 to Lys-637) of *E. coli* EII^{Mtl} and purified the overexpressed 16-kDa domain. This domain is enzymatically active and can restore the phosphoenolpyruvate-dependent phosphorylation activity of an EII^{Mtl}–His-554 mutant to 30% of wild-type levels. Furthermore, $EIII^{Mtl}$ of *S. carnosus* is able to substitute for the C-terminal domain in the same mannitol phosphorylation assay, and vice versa, the C-terminal domain can substitute for $EIII^{Mtl}$ in the assay of *S. carnosus* EII^{Mtl} phosphorylation. These facts lead to the conclusions that the active sites of EII^{Mtl} of *E. coli* are positioned on different functional domains of the same protein, while these domains are present on different proteins in gram-positive organisms.

Similarity of mannitol-1-phosphate dehydrogenases. The amino acid sequence of mannitol-1-phosphate dehydrogenase of *E. faecalis* showed only a little similarity with mannitol-1-phosphate dehydrogenase of *E. coli* (5). During the preparation of this report, a corrected sequence of *mtlD* of *E. coli* was published (35). Now one can find 40% identity.

The N-terminal amino acids are particularly well conserved in mannitol-1-phosphate dehydrogenases of *E. faecalis*, *S. carnosus*, and *E. coli*. One can find a motif of 13

```
 E  I  K  A  V  F  E  E  A  D  V  *
                                    M  K  A  V  H  F  G  A  G
*        *        *        *        *        *
1        11       21       31       41       51
GAAATCAAAGCAGTTTTTGAGGAGGCTGACGTATAATGAAAGCAGTTCATTTTGGAGCCG

   N  I  G  R  G  F  N  G  Q  I  L  S  D  N  K  V  E  V  T
*        *        *        *        *        *
61       71       81       91       101      111
GGAATATAGGCCGAGGTTTCAACGGCCAAATTCTTTCAGATAATAAAGTAGAAGTTACCT
```

FIG. 6. DNA sequence and deduced amino acids encoded by the end of *mtlF* and the start of *mtlD* from *S. carnosus*. The start codon of *mtlD* begins with the last A of the stop codon from *mtlF*. The RBS of *mtlD* is underlined.

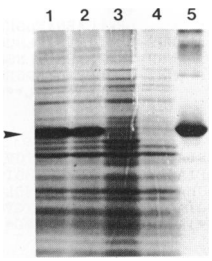

FIG. 7. PAGE with crude extract. Lanes: 1, HB101(pFD3,05); 2, HB101(pFD2,30); 3, HB101(pT7T318U); 4, HB101; 5, 5 μg of aldolase. The position of aldolase and mannitol-1-phosphate dehydrogenase is marked by an arrow.

identical residues between *E. faecalis*, *S. carnosus*, and *E. coli* N termini of mannitol-1-phosphate dehydrogenases (Fig. 8).

Prediction of the coenzyme binding domain by sequence comparison with other $NAD(P)^+$-dependent dehydrogenases. Although a computer-aided search of the EMBL protein sequence data bank revealed rather low overall sequence similarities between mannitol-1-phosphate dehydrogenases and other pyridine nucleotide-dependent dehydrogenases, the N-terminal 28 residues were found to contain several residues identical to those in the nicotinamide coenzyme binding region of various dehydrogenases (Fig. 8). Two possible overlapping NAD^+ binding sites in the N-terminal regions of the mannitol-1-phosphate dehydrogenases were identified. The first potential NAD^+ binding site starts within the mannitol-1-phosphate dehydrogenases at glycine residue 7 and exhibits significant correlations with the nucleotide binding site of the following enzymes: *Bacillus sphaericus* and *B. stearothermophilus* alanine dehydrogenases and *B. sphaericus* phenylalanine dehydrogenase (17), *Drosophila melanogaster* alcohol dehydrogenase (16), *Lactobacillus casei* lactate dehydrogenase (15), *B. stearothermophilus* lactate dehydrogenase (33), dogfish lactate dehydrogenase (32), pig glutaraldehyde-3-phosphate (GAP) dehydrogenase (13), *B. stearothermophilus* GAP dehydrogenase (2), and *Kluyveromyces lactis* GAP dehydrogenase (29). The correlation with the second putative NAD^+ binding site starting at glycine residue 12 is smaller.

Consequently, the first potential NAD^+ binding site is favored. The significance of two overlapping sequences possibly capable of serving as NAD^+ binding sites is not known. Other proteins exhibiting significant homology with

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                                  *  *  *                    *
Mtl-1-P-Dh E.coli          KALHFGAGNIGRGFIG-KLLADAGIQLTFAD
Mtl-1-P-Dh S.faec.         NAVHFGAGNIGRGFIG-EILAKTGFILPFVD
Mtl-1-P-Dh S.carn.         KAVHFGAGNIGRGFNG-QILSDNKVEVTLID
AlaDh B.sphaer.            KVTVIGGGIAGTNAA--KIAVGMGADVTVID
AlaDh B.stearo.            KVTIIGGGTAGTNAA--KIGVGLGADVTILD
AlcDh Dros.melano.         VIFVAGLGGIGLDTSL-ELLKRDLKNLVILD
PheDh B.sphaer.            TYAIQGLGKVGYKVAE-QLLK-AGADLFVYD
LDh B.stearo.              RVVVIGAGFVGASYVFALMNQGIADEIVLID
LDh L.casei                KVILVGDGAVGSSYAFAMVLQGIAQEIGIVD
LDh dogfish                KITVVGVGAVGMACAISILMKDLADEVALVD
GAPDh pig                  KVGVNGFGRIGRLVLRAALSCGKVDIVAIND
GAPDh B.stearo.            KVGINGFGRIGRNVFRAALKNPDIEVVAVND
GAPDh Kluyv.lactis         KVAINGFGRIGRLVLRIALQRKALEVVAVND
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FIG. 8. Comparison of the amino acid sequences of putative nucleotide binding sites of different dehydrogenases (Dh). Conserved residues which are thought to be essential for nucleotide binding are marked by asterisks. Mtl-1-P, mannitol-1-phosphate; Ala, alanine; Alc, alcohol; Phe, phenylalanine; L, lactate.

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                                    495
EIImtl   E.coli      :  ....LFKLGAENIFLGRKAATKEEAIRFAGEQLVKGGYV
EIIImtl  S.faecalis  :  NH2-MENLTNISIELNQQFNTKEEAIRFSGQKLVEAGCV
EIIImtl  S.carnosus  :  NH2-TELFSNENIFLNQSFEDQNEAIEKAGQALVDAGAV
FPr   S.typhimurium  :  NH2-MFQLSVQDIHPGEQAGNKEEAIRGIAAALAGAGNV
Consensussequence:          **:L****I*L****:*KEEAIRF*G**LV:AG:V

530                                 560
EPEYVQAMLDREKLTPTYLGESIAVPHGTVEAKDRVLKTGVVFCQYPEGVRFGEEEDD-IA
EPAYIEAMIERDQLLSAHMGNFIAIPHGTEEAKKLVKKSGICVVQVPEGVNFGTEEDEKIA
TEDYIQAMKDREAVVSTFMGNGLAIPHGTDEAKSAVLQSGLTLLQIPEGVQWG---DD-VA
AGGYVDGMLAREQQTSTFLGNGIAIPHGTTDTRDQVLKTGVQVFQFPQGVTWGE---GQ-VA
**:Y**AM**RE***ST**GN*IAIPHGT:EAK*:VLK*G*:*:Q:PEGV:*G***D*:*A

590                                 620             637
RLVIGIAARNNEHIQVITSLTNALDDESVIERLAHTTSVDEVLELLAGRK*
TVLFGIAGVGEEHLQLVQQIALYCSDMDNVVQLADALSKEEITENLAIA*
KVVVGIAGKDGEHLDLLSKIAITFSEEENVDRIVNTKSPEEIKAVFEEADV*
YVAIGIAASSDEHLGLLRQLTHVLSDDSVAEQLKSATTAEELRALLMGEKQ.....
:V**GIA*:::EHL*L*:***::*SD:*****L*:**S:EE*:**L****:
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FIG. 9. Comparison of the amino acid sequences of $EIII^{Mtl}$ of *E. faecalis* with EII^{Mtl} of *E. coli*, $EIII^{Mtl}$ of *S. carnosus*, and FPr of *Salmonella typhimurium*. The consensus sequence is presented. Notation: *, two identical residues; :, no identical residues; letter, three identical residues; underlined letter, all residues identical. The last digit of each number is aligned with the corresponding amino acid of EII^{Mtl} *E. coli*.

the mannitol-1-phosphate dehydrogenase of *E. faecalis* were not found.

Putative order of mannitol-specific genes in gram-positive microorganisms. The mannitol-specific genes of *S. carnosus* are organized in the following way. The first gene is *mtlA*, followed by *mtlF* and *mtlD*. Between *mtlA* and *mtlF* is a space of about 2,000 bp (9). No space is found between *mtlF* and *mtlD*; the start codon ATG of *mtlD* begins with the last A of the *mtlF* stop codon.

In *E. faecalis*, we find a very similar organization of *mtlF* and *mtlD*: the gene order is identical, and the space is only 14 bp. Upstream of *mtlF* one can find the end of an ORF. This ORF exhibits some similarity to the C-terminal part of EII^{Mtl} of *E. coli*, but the similarity stops just before the active center cysteine (22, 23) of EII^{Mtl}, so we think that this ORF is not identical to *mtlA* of *E. faecalis*. We conclude that the genes in both staphylococci and streptococci are organized in an operon and have the order *mtlA*→*orfX*→*mtlF*→*mtlD*. *orfX* might encode a regulator protein. These speculations must be confirmed by further sequence analysis and transcriptional experiments.

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REFERENCES

1. **Alton, N. K., and D. Vapnek.** 1979. Nucleotide sequence analysis of the chloramphenicol resistance transposon Tn9. Nature (London) **282**:864–869.
2. **Biesecker, G., J. Ieuan Harris, J. C. Thierry, J. E. Walker, and A. J. Wonacott.** 1977. Sequence and structure of D-glyceraldehyde-3-phosphate dehydrogenase from *Bacillus stearothermophilus*. Nature (London) **266**:328–332.
3. **Birnboim, H. C., and J. Doly.** 1979. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Res. **7**:1513–1523.
4. **Boyer, H. W., and D. Rouland-Dussoix.** 1969. A complementation analysis of the restriction and modification of DNA in *Escherichia coli*. J. Mol. Biol. **41**:459–472.
5. **Davis, T., M. Yamada, M. Elgort, and M. H. Saier, Jr.** 1988. Nucleotide sequence of the mannitol (mtl) operon in *Escherichia coli*. Mol. Microbiol. **2**:405–412.
6. **De Vos, W. M.** 1987. Gene cloning and expression in lactic streptococci. FEMS Microbiol. Rev. **46**:281–295.
7. **Fischer, R., R. Eisermann, B. Reiche, and W. Hengstenberg.** 1989. Cloning, sequencing and overexpression of the mannitol-specific enzymeIII-encoding gene of *Staphylococcus carnosus*. Gene **82**:249–257.
8. **Fischer, R., and W. Hengstenberg.** 1989. The mannitol operon of *Staphylococcus carnosus*. Biol. Chem. Hoppe-Seyler **370**:896–897.
9. **Fischer, R., and W. Hengstenberg.** Eur. J. Biochem., submitted for publication.
10. **Fischer, R., and W. Hengstenberg.** Plasmid, submitted for publication.
11. **Geerse, R. H., and P. W. Postma.** 1989. The PEP:fructose phosphotransferase system in *Salmonella typhimurium*. FPr combines enzymeIII^{Fru} and pseudo-HPr activities. Mol. Gen. Genet. **216**:517–525.
12. **Götz, F., J. Zabielsky, L. Philipson, and M. Lindberg.** 1983. DNA homology between the arsenate resistance plasmid pSX267 from *Staphylococcus xylosus* and the penicillinase plasmid pI258 from *Staphylococcus aureus*. Plasmid **9**:126–137.
13. **Harris, J. I., and R. N. Perham.** 1968. Glyceraldehyde-3-phosphate dehydrogenase from pig muscle. Nature (London) **219**:1025–1028.

13a. **Hengstenberg, W.** Unpublished data.

14. **Henikoff, S.** 1984. Unidirectional digestion with exonuclease III creates targeted breakpoints for DNA sequencing. Gene **28**:351–359.
15. **Hensel, R., U. Mayr, and Y. Chao-yuh.** 1983. The complete primary structure of the allosteric L-lactate dehydrogenase from *Lactobacillus lactis*. Eur. J. Biochem. **134**:503–511.
16. **Kreitman, M.** 1983. Nucleotide polymorphism at the alcohol-dehydrogenase locus of *Drosophila melanogaster*. Nature (London) **304**:412–417.
17. **Kuroda, S., K. Tanizawa, Y. Sakamoto, H. Tanaka, and K. Soda.** 1990. Alanine dehydrogenases from two *Bacillus* species with distinct thermostabilities: molecular cloning, DNA and protein sequence determination, and structural comparison with other NAD(P)$^+$-dependent dehydrogenases. Biochemistry **29**: 1009–1015.
18. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
19. **Lee, C. A., and M. H. Saier, Jr.** 1983. Mannitol-specific enzymeII of the bacterial phosphotransferase system. J. Biol. Chem. **258**:10761–10767.
20. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
21. **Mead, A. D., E. Szczesna-Skorupa, and B. Kemper.** 1986. Single-stranded DNA 'blue' T7 promoter plasmids: a versatile tandem promoter system for cloning and protein engineering. Protein Eng. **1**:67–74.
22. **Pas, H. H., and G. T. Robillard.** 1988. S-phosphocysteine and phosphohistidine are intermediates in the phosphoenolpyruvate dependent mannitol transport catalyzed by *Escherichia coli* EII^{Mtl}. Biochemistry **27**:5835–5839.
23. **Pas, H. H., R. H. Ten Hoeve-Duurkens, and G. T. Robillard.** 1988. Bacterial phosphoenolpyruvate dependent phosphotransferase system: mannitol-specific EII contains two phosphoryl binding sites per monomer and one high-affinity mannitol binding site per dimer. Biochemistry **27**:5520–5525.
24. **Reiche, B., R. Frank, J. Deutscher, N. Meyer, and W. Hengstenberg.** 1988. Staphylococcal phosphoenolpyruvate-dependent phosphotransferase system: purification and characterisation of the mannitol-specific enzymeIII^{mtl} of *Staphylococcus aureus* and *Staphylococcus carnosus* and homology with the enzyme II^{mtl} of *Escherichia coli*. Biochemistry **27**:6512–6516.
25. **Reizer, J., M. H. Saier, Jr., J. Deutscher, F. Grenier, J. Thompson, and W. Hengstenberg.** 1988. The phosphoenolpyruvate:sugar phosphotransferase system in gram-positive bacteria: properties, mechanism and regulation. Crit. Rev. Microbiol.

15:297–338.

26. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
27. **Selzer, G., T. Som, T. Itoh, and J.-I. Tomizawa.** 1983. The origin of replication of plasmid p15A and comparative studies on the nucleotide sequences around the origin in related plasmids. Cell **32**:119–129.
28. **Shine, J., and L. Dalgarno.** 1974. The 3′-terminal sequence of *Escherichia coli* 16S ribosomal RNA: complementarity to nonsense triplets and ribosome binding sites. Proc. Natl. Acad. Sci. USA **4**:1342–1346.
29. **Shuster, J. R.** 1990. *Kluyveromyces lactis* glyceraldehyde-3-phosphate dehydrogenase and alcohol dehydrogenase-1 genes are linked and divergently transcribed. Nucleic Acids Res. **18**:4271.
30. **Solomon, E., and E. C. C. Lin.** 1972. Mutations affecting the dissimilation of mannitol by *Escherichia coli* K-12. J. Bacteriol. **111**:566–574.
31. **Van Charldorp, R., and P. H. Van Knippenberg.** 1982. Sequence, modified nucleotides and secondary structure at the 3′-end of small ribosomal subunit RNA. Nucleic Acids Res. **4**:1149–1158.
32. **Wierenga, R. K., P. Terpstra, and W. G. J. Hol.** 1986. Prediction of the occurrence of the ADP-binding βαβ-fold in proteins, using an amino acid sequence fingerprint. J. Mol. Biol. **187**:101–107.
33. **Wilks, H. M., K. W. Hart, R. Feeney, C. R. Dunn, H. Muirhead, W. N. Chia, D. A. Barstow, T. Atkinson, A. N. Clarke, and J. J. Holbrook.** 1988. A specific, highly active malate dehydrogenase by redesign of a lactate dehydrogenase framework. Science **242**:1541–1544.
34. **Yanisch-Perron, C., J. Vieira, and J. Messing.** 1985. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. Gene **33**:103–119.
35. **Yiang, W., L. F. Wu, J. Tomich, M. H. Saier, Jr., and W. G. Niehaus.** Mol. Microbiol., in press.