JOURNAL OF BACTERIOLOGY, May 2001, p. 2929–2936 Vol. 183, No. 9
0021-9193/01/$04.00+0 DOI: 10.1128/JB.183.9.2929–2936.2001

Characterization of the D-Xylulose 5-Phosphate/D-Fructose 6-Phosphate Phosphoketolase Gene (*xfp*) from *Bifidobacterium lactis*

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A D-xylulose 5-phosphate/D-fructose 6-phosphate phosphoketolase (Xfp) from the probiotic *Bifidobacterium lactis* was purified to homogeneity. The specific activity of the purified enzyme with D-fructose 6-phosphate as a substrate is 4.28 Units per mg of enzyme. K_m values for D-xylulose 5-phosphate and D-fructose 6-phosphate are 45 and 10 mM, respectively. The native enzyme has a molecular mass of 550,000 Da. The subunit size upon sodium dodecyl sulfate-polyacrylamide gel electrophoresis (90,000 Da) corresponds with the size (92,529 Da) calculated from the amino acid sequence of the isolated gene (named *xfp*) encoding 825 amino acids. The *xfp* gene was identified on the chromosome of *B. lactis* with the help of degenerated nucleotide probes deduced from the common N-terminal amino acid sequence of both the native and denatured enzyme. Comparison of the deduced amino acid sequence of the cloned gene with sequences in public databases revealed high homologies with hypothetical proteins (26 to 55% identity) in 20 microbial genomes. The amino acid sequence derived from the *xfp* gene contains typical thiamine diphosphate (ThDP) binding sites reported for other ThDP-dependent enzymes. Two truncated putative genes, *pta* and *guaA*, were localized adjacent to *xfp* on the *B. lactis* chromosome coding for a phosphotransacetylase and a guanosine monophosphate synthetase homologous to products of genes in *Mycobacterium tuberculosis*. However, *xfp* is transcribed in *B. lactis* as a monocistronic operon. It is the first reported and sequenced gene of a phosphoketolase.

Phosphoketolases (EC 4.1.2.9, EC 4.1.2.22) are thiamine diphosphate (ThDP)-dependent key enzymes of the pentose phosphate pathway of heterofermentative and facultative homofermentative lactic acid bacteria and of the D-fructose 6-phosphate (F6P) shunt of bifidobacteria (1, 2, 9, 13, 15, 29, 32). Phosphoketolases have been sporadically reported in other microorganisms (11, 14, 30). In bifidobacteria, two types of phosphoketolases have been described: a F6P specific enzyme (F6PPK) in human species like *B. dentium*, and a dual substrate xylulose 5-phosphate/fructose 6-phosphate (X5P/F6P) phosphoketolase (Xfp) in animal species like *B. globosum* (12, 31). No molecular data are available for any of the phosphoketolases defining for one active protein molecular size, subunit size, and amino acid sequence. As a preliminary to studies of the biochemistry, genetics, and regulation of these landmark enzymes in the nutritionally important bifidobacteria of the human and animal intestine, we report the purification of the dual substrate Xfp from *Bifidobacterium lactis*, a bacterium used as a probiotic supplement in fermented food (16, 22). The gene (*xfp*) was identified, cloned, and sequenced.

MATERIALS AND METHODS

Bacterial strains, plasmids, and growth conditions. The bacterial strains and plasmids used in this study are listed in Table 1. *Bifidobacterium lactis* was grown anaerobically at 37°C in Bif medium (brain heart infusion [37 g/liter; Biolife] supplemented with yeast extract [5 g/liter], L-cysteine hydrochloride [0.5 g/liter], and resazurine [2 mg/liter]) as described before (22). *Escherichia coli* was routinely grown at 37°C in Luria-Bertani medium (27). If required, the following supplements were added to the media: ampicillin, 50 μg/ml; spectinomycin, 50 μg/ml; tetracycline, 10 μg/ml; kanamycin, 30 μg/ml; chloramphenicol, 30 μg/ml; 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal), 20 μg/ml; and isopropylthio-β-D-galactopyranoside (IPTG), 240 μg/ml.

Preparation of protein extracts. All procedures were carried out at 0 to 4°C. *B. lactis* cells were harvested from liquid medium in the exponential growth phase (A_{600} between 1.2 and 1.4) by centrifugation (10,000 × *g* for 20 min), washed twice with a saline solution (0.85 M NaCl) and resuspended in 4 ml/g (wet weight) of buffer A, composed of 100 mM potassium phosphate containing 30 mM KCl, 0.1 mM EDTA, 1 mM $MgCl_2$, 0.2 mM phenylmethylsulfonyl fluoride, and 2 mM 1,4-dithio-DL-threitol at pH 7.0. Cells were disrupted by 12 passages through a chilled French pressure cell (SLM Amico; Lightening Instruments, Lausanne, Switzerland) at approximately 120 MPa. After centrifugation at 20,000 × *g* for 40 min, the clarified supernatant was extensively dialyzed against buffer A to get a so-called crude extract. Crude extracts of *E. coli* recombinant clones were obtained by the same procedure with the exception that only three passages through the French pressure cell were performed.

Purification of phosphoketolase activity. Column chromatographies were done at room temperature. All other procedures in between were performed at 4°C. The amount of protein used in a typical purification are given in Table 2.

(i) Step 1: DEAE-anion-exchange chromatography. The crude extract was loaded onto a column (15 by 2.6 cm) of DEAE Sepharose Fast Flow (Pharmacia) equilibrated with buffer A. Proteins were eluted in four subsequent steps with buffer A at potassium phosphate concentrations of 100, 150, 300, and 600 mM, respectively, and at a flow rate of 1.1 ml/min (corresponding to 12.5 cm/h). In each step, the column was eluted with 4.4 column volumes of the corresponding buffer. The fractions containing F6PPK activity which eluted at a phosphate buffer concentration of 300 mM were pooled and concentrated by membrane filtration (Centriprep YM-10, 10,000 Da; Millipore). The concentrated sample was dialyzed overnight against 20 mM Tris-HCl at pH 8.0 (buffer B).

(ii) Step 2: Mono Q-anion-exchange chromatography. The resulting sample (2.1 ml) was applied to a 1-ml Mono Q 5/5 anion-exchange fast-performance liquid chromatography column (Pharmacia) which had been equilibrated with

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TABLE 1. Bacterial strains, plasmids, and oligonucleotides used in this study

Strain, plasmid, or oligonucleotide	Relevant characteristics, construction, or nucleotide sequence (5′ → 3′)[a]	Reference or source
Strains		
B. lactis DSM 10140	Wild type	22
E. coli XL1-Blue	*recA1 lac endA1 gyrA96 thi hsdR17 supE44 relA1* (F′ *proAB lacI*^{q} *lacZ*Δ*M15* Tn*10* Tet^r)	3
E. coli BL21(DE3)/pLysS	F$^-$ *ompT hsdS*$_B$(r_B^- m_B^-) *gal dcm* (DE3)/pLysS (Cm^r)	Novagen
Plasmids		
pUC18	Amp^r LacZ′; cloning vector; 2.7 kb	35
pCL1920	Spc^r Str^r; cloning vector; 4.6 kb	19
pGEM-T Easy	Amp^r LacZ′; annealed T in both 3′ ends after linearization with *Eco*RV; 3.0 kb	Promega
pET-28a(+)	Kan^r LacI; expression vector; 5.4 kb	Novagen
pFPK1	Amp^r; 2.62-kb *Bam*HI-*Bam*HI fragment from strain DSM 10140 in pUC18; 5.3 kb	This study
pFPK2	Amp^r; 1.6-kb PCR-derived fragment from strain DSM 10140 in pGEM-T Easy; 4.6 kb	This study
pFPK3	Spc^r Str^r; 0.85-kb *Bam*HI-*Sma*I fragment from pFPK2 insertion in pCL1920; 5.4 kb	This study
pFPK4	Spc^r Str^r; 2.62-kb *Bam*HI-insertion from pFPK1 in the *Bam*HI site of pFPK3; 8.1 kb	This study
pFPK5	Kan^r; 2.5-kb PCR-derived fragment from strain DSM 10140 in pET-28a(+); 7.9 kb	This study
Oligonucleotides[b]		
pk5	5′-GGIACICCITGGCARAAR-3′ (974–991)	This study
pk6	5′-ATATATATARTAYTTRTCCATICCIATIAT-3′ (1019–1039 reverse)[c]	This study
pk7	5′-CATGGCAGAAGCTGGATCGTCCGGT-3′ (981–1005)	This study
pk9	5′-GCGAGATCCCGTGGCGT-3′ (2526–2542)	This study
pk15	5′-ATATATGAATTCATGACTAATCCTGTTATTGGTACC-3′ (956–979)[c]	This study
pk16	5′-AATTACAAGCTTTCACTCGTTGTCGCCGGCGG-3′ (3414–3433 reverse)[c]	This study

[a] Position in the nucleotide sequence according to the numbering in the GenBank database.
[b] Oligonucleotides were synthesized by Microsynth AG, Balgach, Switzerland.
[c] The underlined sequences correspond to an introduced tail for cloning purposes.

buffer B. After a 10-ml wash, the column was eluted with a 90-ml linear gradient from 0 to 500 mM KCl in buffer B. The fractions containing F6PPK activity (peak fraction at approximately 450 mM KCl) were pooled and concentrated by membrane filtration (10,000 Da).

(iii) Step 3: size exclusion chromatography. After a buffer exchange by dialysis against buffer C (Tris-HCl at pH 7.5 containing 150 mM NaCl), the sample from step 2 was subjected to gel filtration chromatography with a fast-performance liquid chromatography Superdex 200 HR 10/30 column (Pharmacia) of a volume of 24 ml. Proteins were eluted at a flow rate of 0.5 ml/min. The pooled fractions containing F6PPK activity were stored at −20°C until analytical measurements were carried out. The column had been calibrated with protein molecular weight markers (Bio-Rad Laboratories) thyroglobulin (669,000), ferritin (440,000), catalase (232,000), aldolase (158,000), and albumin (67,000). It was also used to estimate the molecular size of the purified native enzyme.

Phosphoketolase assay and activity unit definition. Phosphoketolase activity was measured spectrophotometrically as ferric acetyl hydroxamate produced from the enzymatically generated acetyl phosphate by the procedure of Racker (26). The standard reaction mixture of 0.075 ml consisted of 33.3 mM potassium phosphate (pH 6.5), L-cysteine hydrochloride (1.9 mM), sodium fluoride (23 mM), sodium iodoacetate (8 mM), either D-fructose 6-phosphate or D-xylulose 5-phosphate (Fluka) as a substrate (each at a concentration of 27 mM), and finally the protein sample to initiate the reaction. After incubating at 37°C for 30 min, 0.075 ml of hydroxylamine hydrochloride (2 M, pH 6.5) was added at room temperature. Ten minutes later, 0.05 ml of 15% (wt/vol) trichloroacetic acid, 0.05 ml of 4 M HCl, and 0.05 ml of $FeCl_3 \cdot 6\ H_2O$ (5% [wt/vol] in 0.1 M HCl) were added for the final color development of the ferric hydroxamate, which was then spectrophotometrically quantified at 505 nm by comparing to a series of acetyl phosphate (Sigma) standards. For qualitative measurements of F6PPK activity in whole cells, *E. coli* cells containing recombinant phosphoketolase or *B. lactis* cells were pretreated with hexadecyltrimethylammonium bromide (Sigma) before being assayed according to the method of Orban and Patterson (24).

TABLE 2. Purification of Xfp from *B. lactis*

Step	Vol (ml)	Total units	Total protein (mg)	Sp act (U[a]/mg)	Purification factor	Yield (%)
Crude extract	15	93.4	246.0	0.38	1.0	100
DEAE-cellulose	138	28.7	47.1	0.61	1.6	30.7
Mono Q	2.1	15.5	7.4	2.10	5.5	16.6
Superdex 200	6.0	4.1	0.96	4.28	11.1	4.4

[a] One unit is defined as the amount of extract forming 1 μmol of acetyl phosphate per min with F6P as a substrate.

One unit of phosphoketolase activity is defined as the amount of extract forming 1 μmol of acetyl phosphate per min from either F6P or X5P. Specific activity is expressed as units per milligram of protein. Protein concentrations were determined by the method of Lowry et al. (20) using bovine serum albumin as a standard.

Protein gel electrophoresis and amino acid analysis. Protein extracts were analyzed by polyacrylamide gel electrophoresis (PAGE) by established methods (6, 33) and N-terminal amino acids were determined as described elsewhere (18).

DNA isolation and manipulations. Total cellular DNA was prepared from *B. lactis* as described by Leenhouts et al. (17) with some modifications: 1.5 g (wt/vol) of frozen *B. lactis* cells (wet weight) were dissolved in 5 ml of lysis solution (0.025 M Tris-HCl [pH 8.0], 0.05 M EDTA, 0.05 M D-glucose) containing 25 mg of lysozyme (Sigma), 600 U of mutanolysin (Sigma), and 0.1 mg of RNase A (Sigma). This mixture was incubated for 30 min at 37°C and subsequently treated with proteinase K, and the DNA was precipitated as described in the original procedure (17). Electroporation of *E. coli* cells (8), small-scale plasmid DNA isolation from *E. coli*, DNA modifications, and further manipulations were carried out by standard methods (27). Transfer of DNA from 0.8% agarose gels to Nylon Plus membrane (QIAGEN) was performed according to the method of Southern (27). Synthetic oligonucleotides (approximately 5 pmol) were end-labeled by phosphorylation with T4 polynucleotide kinase and 0.1 mCi of [γ-^{32}P]ATP (5×10^3 Ci/mmol) (27) whereas plasmid DNA (approximately 0.2 μg) was nick labeled with Klenow polymerase using 0.03 mCi of [α-^{32}P]ATP (3×10^3 Ci/mmol) (27). The conditions for prehybridizations and hybridizations with oligonucleotides or DNA probes were carried out in the absence of formamide as previously described (21).

Cloning the *B. lactis* phosphoketolase gene. In order to obtain a (short) DNA fragment by PCR, degenerated oligonucleotide primers pk5 and pk6 (Table 1; Fig. 1) were deduced from the N terminus which had been determined for the *B. lactis* protein catalyzing the phosphoketolase reaction. These primers were used in a "touchdown PCR" using *B. lactis* DNA as a template. Thereby, the annealing temperature was decreased gradually from 50 to 40°C within the first 20 PCR cycles before holding it at 40°C for the next 15 cycles. Annealing time

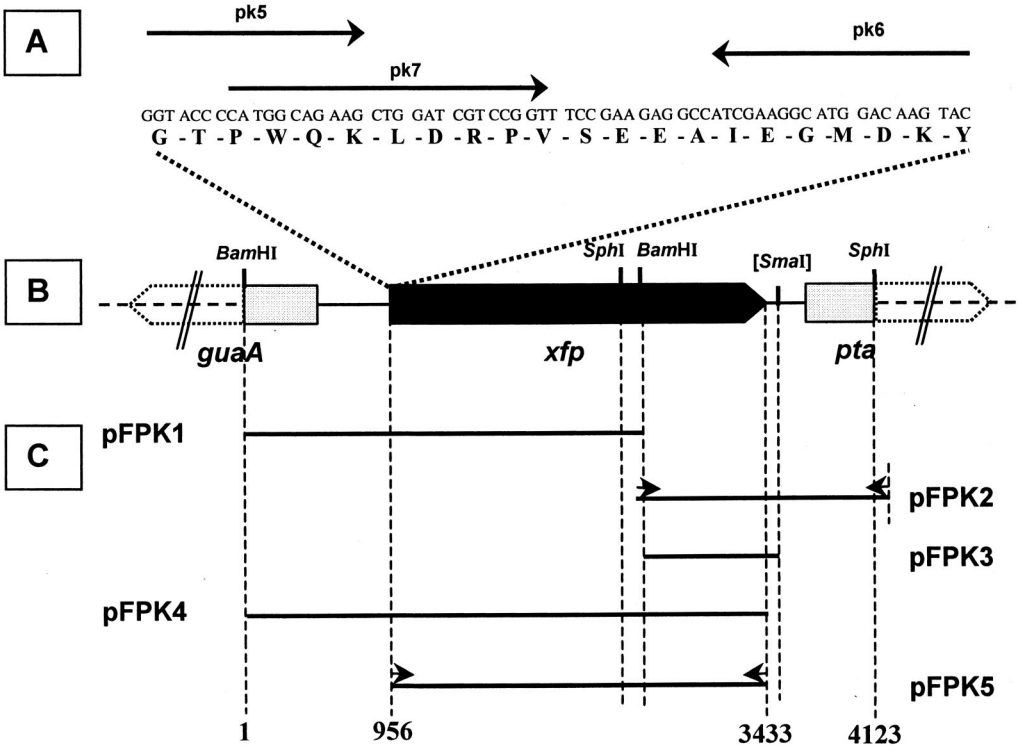

FIG. 1. Cloning strategy and restriction map of the *B. lactis xfp* gene and its adjacent region. (A) Amino acids (7 to 28) from the N terminus of the Xfp protein, position of deduced primers (arrows), and corresponding nucleotide sequence. (B) Open reading frames *guaA, xfp*, and *pta* (open and filled arrows) and restriction sites which were relevant for cloning. (C) Inserts of recombinant plasmids (solid lines) bordered by dashed vertical lines representing either restriction sites or 5′ ends of primers used for PCR; primers are indicated by short arrows and described in detail in Table 1. Numbers at the end of vertical dashed lines are assigned to nucleotide numbers as indicated in the text and in the GenBank database.

was 0.5 min. Extension occurred with *Taq* DNA polymerase (Amersham Pharmacia Biotech) at 72°C for 1 min and DNA strand separation was performed at 95°C for 0.5 min. The resulting PCR product was sequenced and the internal oligonucleotide pk7 was deduced which served as a probe in hybridizations with *B. lactis* DNA. A hybridizing 2.62-kb *Bam*HI fragment from *B. lactis* was cloned (pFPK1) (Table 1) and sequenced. An overlapping fragment from the *B. lactis* chromosome was cloned in a two-step protocol. Step 1 was a ligation of *Sph*I-digested *B. lactis* DNA (sized to 1.5 to 2.5 kb fragments) followed by transformation of *E. coli* XL-1 Blue cells and immediate growth in liquid Luria-Bertani medium. In step 2, DNA was extracted from such an overnight culture and then used for a PCR. For that, pk9 targeting the insertion of pFPK1 between the *Sph*I and *Bam*HI site (Fig. 1) and pUC18/M13(−20) (Promega) served as primers at an annealing temperature of 65°C for 0.5 min. Otherwise, the PCR conditions were the same as described above. The resulting PCR product was restricted and cloned into pGEM-T Easy to form plasmid pFPK2 (Table 1) containing a 1.62-kb recombinant fragment. In order to compose the insertions of pFPK1 and pFPK2 in the chromosomal order, an internal 0.85-kb *Bam*HI-*Sma*I fragment from the pFPK2 insertion was cloned into plasmid pCL1920 to obtain pFPK3 (Table 1 and Fig. 1). Its single *Bam*HI cleavage site was used to finally insert the 2.62-kb *Bam*HI fragment of pFPK1. The resulting plasmid, pFPK4, is supposed to contain the hypothetical phosphoketolase gene as it occurs on the *B. lactis* chromosome. After sequence analysis, the entire gene was amplified by PCR using *B. lactis* DNA, primer pair pk15-pk16 (Table 1), and *Pfu* DNA polymerase (Stratagene), and the resulting PCR product finally cloned into the expression vector pET-28a(+) (Table 1) to form plasmid pFPK5.

Nucleotide sequence determination. Nucleotide sequencing of both strands from cloned DNA was performed by the dideoxy chain termination method (28) with primer walking using the BigDye Terminator Cycle Sequencing Ready Reaction Kit and the ABI PRISM ABI 310 Genetic Analyzer apparatus (Applied Biosystems, Foster City, Calif.) for analysis. DNA sequence analysis, sequence alignments, and sequence database searching were conducted with programs contained within the Sequence Analysis Software Package (version 10.0) licensed from the Genetics Computer Group (University of Wisconsin, Madison). Sequences were compared by the algorithm of Pearson and Lipman (25) (FastA and TFastA) with sequences in the GenEMBL database copy.

Northern blot hybridization analysis. For RNA analysis experiments *B. lactis* was cultivated in 100 ml of Bif medium. RNA from both exponentially growing cells and cells from early stationary growth phase were prepared according to the hot acid-phenol extraction method of Oelmüller et al. (23). After denaturation with formamide, RNA (approximately 5 to 10 μg) was analyzed by gel electrophoresis on agarose containing 20 mM guanidine thiocyanate, followed by electroblotting onto GeneScreen Plus (NEN Life Sciences, Albany, N.Y.) membranes. Hybridization was carried out with [α-^{32}P]ATP-labeled DNA probes in a stringent sodium dodecyl sulfate (SDS) buffer (5) at 65°C.

Nucleotide sequence accession number. The nucleotide sequence reported here has been deposited in GenBank under accession number AJ293946. All nucleotide numbers used in the current study refer on the numbering of the GenBank entry dated 6 September 2000.

RESULTS

Isolation and purification of phosphoketolase activity. To approach the chemical analysis of the N-terminal amino acid sequence, the main F6P cleaving enzyme of *B. lactis* was purified to homogeneity from the initial crude extract as summarized in Table 2. The protein was purified to a specific activity of 4.28 U/mg of protein. It appeared as a single band after PAGE under nondenaturing conditions (data not shown). The protein band, when cut out from such a polyacrylamide gel, showed F6PPK activity which proved that we had purified a phosphoketolase. Its apparent molecular weight was 550,000 when estimated by gel filtration chromatography on a cali-

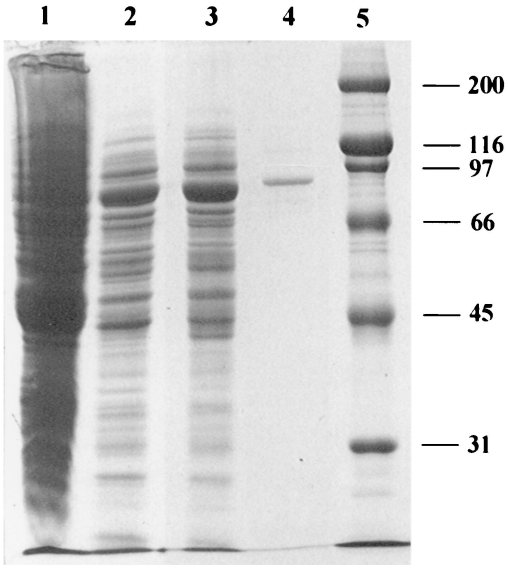

FIG. 2. SDS-PAGE of phosphoketolase preparations from *B. lactis*. Lanes: 1, crude extract; 2, the sample after DEAE-chromatography; 3, the sample after the Mono Q column; 4, the enzyme after the Superdex 200 column; 5, protein standard. Numbers indicate the molecular mass (in kilodaltons) of the standard proteins. The gel (10% polyacrylamide) was Coomassie stained.

brated Superdex 200 HR column. On SDS-PAGE, in the presence of β-mercaptoethanol, the purified enzyme displayed a single protein band with an apparent molecular mass of approximately 90,000 Da. A densitometric analysis of the stained protein (Fig. 2, lane 4) yielded more than 99% of the stain in the 90,000-Da band. When the protein which had been obtained from the single band under nondenaturing PAGE was subjected to SDS-PAGE, it migrated as a single band at the 90,000-Da position. These observations suggest that the native enzyme is a homohexamer ($6 \times 90{,}000 = 540{,}000$).

Dual substrate specificity. To find out whether we had purified the F6P specific phosphoketolase or the X5P/F6P enzyme reported in animal bifidobacteria (31), the apparent Michaelis constants (K_m values) for both substrates were analyzed separately. A Michaelis-Menten saturation kinetic was observed for F6P, whereas the kinetics for X5P presented a substrate inhibition profile (7) at X5P concentrations above 20 mM. From double-reciprocal plots apparent K_m values of 10 ± 1 mM and 45 ± 3 mM were obtained for F6P and X5P, respectively. Therefore, the purified protein belongs to the class of nonspecific phosphoketolases combining substrate specificity of both phosphoketolase-1 and phosphoketolase-2 (2). The abbreviation Xfp was chosen. The activity of the purified enzyme was not significantly changed by addition of 1 mM ThDP or 2 mM $MgCl_2$ (or both together) to the assay mixture, nor was it changed by omitting sodium fluoride or sodium iodoacetate from the assay. From the initial slopes of the reaction velocities, V_{max} values of 5.2 and 27 μmol per min per mg protein were estimated for F6P and X5P, respectively.

N-terminal amino acid sequence of the purified phosphoketolase. The N-terminal amino acid sequence of the purified enzyme was determined in parallel for the protein after both SDS-PAGE and PAGE under nondenaturing conditions. The first 30 residues were recorded by automatic Edman degradation and were found to be identical in both sources: NH_2-XXNPVIGTPWQKLDRPVVEEAIIGMDKYXRV (see also Fig. 1A). This finding supports the homohexamer structure of the native enzyme (see above).

Identification and cloning of the phosphoketolase gene (*xfp*). The N-terminal amino acid sequence obtained was used for the identification and cloning of the *xfp* gene. We applied touchdown PCR with two synthetic oligonucleotide primers (pk5 and pk6). They contain several degeneracies and were designed to target a gene segment for 22 amino acids (Fig. 1). In addition, pk6 has a tail of 9 nucleotides (nt) to prolong the desired PCR product to a length of 75 (Fig. 1). Its DNA sequence confirmed that part of the N terminus encoding DNA had been generated. A Southern blot of *B. lactis* chromosomal DNA cleaved with *Bam*HI and hybridized with ^{32}P-labeled pk7 (Fig. 1; Table 1) gave a signal of 2.6 kb (data not shown). The proper *Bam*HI fragment and an adjacent *Bam*HI-*Sma*I fragment (Fig. 1) were finally cloned as a contiguous 3.4-kb fragment in pFPK4 (Table 1) as outlined in Materials and Methods. For this purpose, the low-copy-number plasmid pCL1920 (Table 1) was used as attempts to clone the fragment into the high-copy-number plasmid pUC18 failed. However, no F6PPK activity could be detected in extracts of *E. coli*

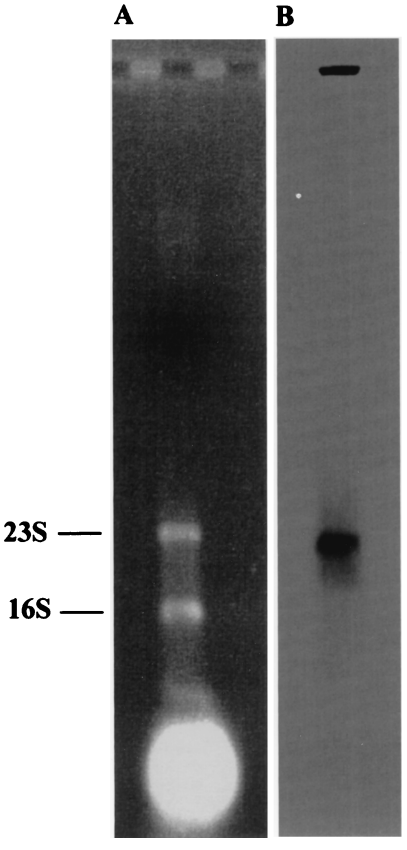

FIG. 3. Northern blot analysis of *B. lactis xfp* gene transcript. Ethidium bromide staining of RNA from *B. lactis* after agarose gel electrophoresis (A) and X-ray signal after Northern blot analysis using primer pk9 as a probe (B). 16S and 23S indicate the migrating positions of 16S and 23S rRNA, respectively.

containing pFPK4. Therefore, the *xfp* gene (nt 956 to 3433) was cloned into the expression vector pET-28a(+) by a PCR approach to construct pFPK5 (Table 1). But no F6PPK activity could be measured in extracts of IPTG-induced cultures of the *E. coli* host BL21(DE3)/pLysS, not even when 1 mM ThDP and 2 mM $MgCl_2$ were added. However, inspection of extracts from such cells revealed a band at a mass of approximately 90,000 Da after SDS-PAGE (see Fig. 5) and indicated the overproduction of the Xfp subunit at the expected size.

DNA sequence of the *xfp* gene and its adjacent region. The nucleotide sequence for a 4.1-kb segment of chromosomal *B. lactis* DNA was derived from sequencing the insertions of both plasmids pFPK1 and pFPK2. In addition, the original arrangement of both insertions on the chromosome was verified by sequence analysis of an overlapping PCR fragment. It had been generated in step 2 of the cloning protocol using pk9 as one of the PCR primers (Fig. 1). The nucleotide sequence of 4,123 bp contained an open reading frame (nt 956 to 3,430) of 825 amino acids. Its deduced N-terminal sequence matches that of the N terminus of the purified phosphoketolase (except for serine-18 [Fig. 1]). The molecular mass of the acidic Xfp polypeptide (pI 4.9) was calculated to be 92,529 Da, a value coincident with that estimated by comparative SDS-PAGE (about 90,000 Da [Fig. 2]). The G+C content of the DNA (61.9%) corresponds exactly to that of the entire *B. lactis* genome (22). A putative ribosome binding site (AGGAGC) is present 10 to 5 nt upstream of the translational start of the Xfp polypeptide. Hybridization analysis indicated that one single copy of *xfp* is present on the *B. lactis* chromosome.

The *xfp* gene is flanked by two truncated open reading frames (Fig. 1). After aligning the translated regions with those from other microorganisms, the corresponding truncated genes were named *guaA* and *pta*, standing for guanosine monophosphate synthetase (EC 6.3.5.2) and phosphotransacetylase (EC 2.3.1.8), respectively. The predicted partial *guaA* and *pta* gene products show a 61% identity with the hypothetical guanosine monophosphate synthetase and a 32.2% identity with the phosphotransacetylase reported in the *Mycobacterium tuberculosis* genome (accession no. Q50729).

Transcriptional analysis of the *xfp* gene. The probe for Northern blot analysis consisted of an internal fragment of the *xfp* gene (nt 1,385 to 2,119). Hybridizations of total RNA extracted from exponentially growing *B. lactis* cells with this probe revealed a single distinct signal indicating an mRNA transcript of approximately 2,500 nt (Fig. 3). Thus, the *xfp* gene is not cotranscribed with other adjacent genes. Two perfect inverted repeats (each 13 nt in length) were detected 25 nt downstream of the translational end of *xfp* (nt 3,459 to 3,471 and 3,476 to 3,488, respectively). They could build up transcriptional terminator structures in *B. lactis*. Potential −35 (AGGTCA, nt 729 to 734) and −10 (CATAAT, nt 849 to 854) promoter motifs are located upstream of a GA dinucleotide (nt 859 to 860). Either of these particular G or A positions represents a transcriptional start point of the *xfp* gene (starting at position 956) as we found in preliminary primer extension experiments (data not shown).

DISCUSSION

The purified phosphoketolase Xfp from *B. lactis* has K_m values of 10 mM (for F6P) and 45 mM (for X5P). The calculated V_{max} values for F6P and X5P are 5.2 and 27 μmol per min per mg of protein, yielding a ratio of 1:5. Xfp of *B. lactis* is similar to a phosphoketolase partially purified from *Bifidobacterium globosum*, whose habitat is the bovine rumen (31). The typical feature of the *B. globosum* enzyme was reported to be a high reaction rate with X5P compared to F6P. In this respect, the enzyme resembles also the phosphoketolase from *Acetobacter xylinum* (30). Also, the crystallized phosphoketolase from *Leuconostoc mesenteroides* accepts both substrates with K_m values of 4.7 mM (X5P) and 29 mM (F6P), respectively (10).

The molecular weight of the native enzyme from *B. lactis* is 550,000, compared to 290,000 (31) or 300,000 (12) estimated by gel filtration for the *B. globosum* enzyme. The X5P specific enzyme from *Lactobacillus plantarum* had a molecular weight of 550,000 as determined by ultracentrifugation and the resulting Svedberg constant (13). Our homohexamer hypothesis for the *B. lactis* enzyme was derived from the genetically or electrophoretically determined subunit size of 92,529 Da and the calibrated gel filtration experiment. It will have to be substantiated by additional biochemical evidence such as controlled chemical cross-linking followed by chromatographic molecular weight estimations. Furthermore, the size and subunit composition of the Xfp protein is totally different from the αβ subunit structure of a recently described F6PPK activity (molecular weight, 110,000 to 115,000) purified from *B. asteroides* extracts (K. G. Fandi, H. M. Ghazali, and A. M. Yazid, Abstr. 100th Gen. Meet. Am. Soc. Microbiol., p. 436, 2000). Our data do not allow yet conclusions as to whether the enzyme is involved in both steps (phosphoketolase-1 and -2) of the so-called *Bifidobacterium bifidum* pathway (2). It should be mentioned that the crude extract of *B. lactis* contains a second phosphoketolase activity which can be separated by gel filtration from Xfp. It seems to be specific for F6P (L. Rohr, unpublished observation).

Although the phosphoketolase pathway has been proposed in the past to be specific for a small group of bacteria and yeasts, sequences highly homologous to *xfp* of *B. lactis* were detected in the genomes from a variety of eubacteria and eucarya, but not from archaea. On the protein level, the calculated amino acid sequence identities vary between 26 and 55%; similarities range up to 63%. The taxonomic diversity is represented in the alignment shown in Fig. 4.

In *Clostridium acetobutylicum* ATCC 824, the *xfp*-homologous gene (coding for open reading frame CAC1622) is situated in a cluster of genes for the pentose phosphate pathway and some anaplerotic reactions: *araE, araR*, L-ribulose phosphate 4-epimerase, L-arabinose isomerase, *xfp*, xylulose kinase, *ywtG* (metabolite transport), L-arabinose isomerase, transaldolase, transketolase, and an aldose 1-epimerase (nt 1,477,589 to 1,494,444 of the chromosome) (data from Genomic Therapeutics Corp.).

The amino acid sequence contains a ThDP-dependent enzyme signature sequence Y-G-X_5-P-X_3-V-X_2-I-X-G-D-G-E (amino acids 165 to 184 in Xfp) matching closely the consensus pattern [LIVMF]-G-X_5-P-X_4-V-X-I-X-G-D-G-[GSAC] (PRO-

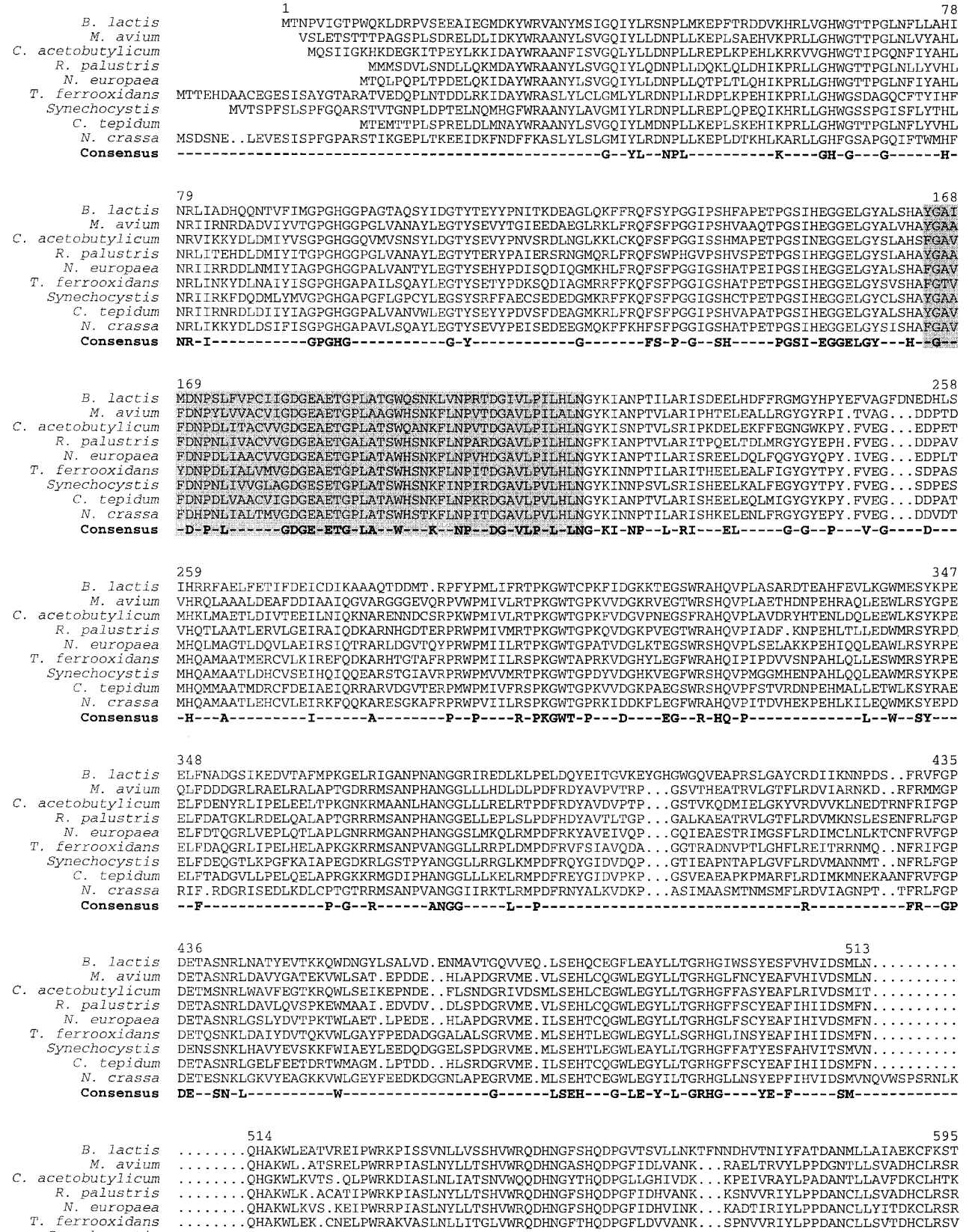
1 78
B. lactis MTNPVIGTPWQKLDRPVSEEAIEGMDKYWRVANYMSIGQIYLRSNPLMKEPFTRDDVKHRLVGHWGTTPGLNFLLAHI
M. avium VSLETSTTTPAGSPLSDRELDLIDKYWRAANYLSVGQIYLLDNPLLKEPLSAEHVKPRLLGHWGTTPGLNLVYAHL
C. acetobutylicum MQSIIGKHKDEGKITPEYLKKIDAYWRAANFISVGQLYLLDNPLLREPLKPEHLKRKVVGHWGTIPGQNFIYAHL
R. palustris MMMSDVLSNDLLQKMDAYWRAANYLSVGQIYLQDNPLLDQKLQLDHIKPRLLGHWGTTPGLNLLYVHL
N. europaea MTQLPQPLTPDELQKIDAYWRAANYLSVGQIYLLDNPLLQTPLTLQHIKPRLLGHWGTTPGLNFIYAHL
T. ferrooxidans MTTEHDAACEGESISAYGTARATVEDQPLNTDDLRKIDAYWRASLYLCLGMLYLRDNPLLRDPLKPEHIKPRLLGHWGSDAGQCFTYIHF
Synechocystis MVTSPFSLSPFGQARSTVTGNPLDPTELNQMHGFWRAANYLAVGMIYLRDNPLLREPLQPEQIKHRLLGHWGSSPGISFLYTHL
C. tepidum MTEMTTPLSPRELDLMNAYWRAANYLSVGQIYLMDNPLLKEPLSKEHIKPRLLGHWGTTPGLNFLYVHL
N. crassa MSDSNE..LEVESISPFGPARSTIKGEPLTKEEIDKFNDFFKASLYLSLGMIYLRDNPLLKEPLDTKHLKARLLGHFGSAPGQIFTWMHF
Consensus ----------G--YL--NPL----------K----GH-G---G------H-
79 168
B. lactis NRLIADHQQNTVFIMGPGHGGPAGTAQSYIDGTYTEYYPNITKDEAGLQKFFRQFSYPGGIPSHFAPETPGSIHEGGELGYALSHAYGAI
M. avium NRIIRNRDADVIYVTGPGHGGPGLVANAYLEGTYSEVYTGIEEDAEGLRKLFRQFSFPGGIPSHVAAQTPGSIHEGGELGYALVHAYGAA
C. acetobutylicum NRVIKKYDLDMIYVSGPGHGGQVMVSNSYLDGTYSEVYPNVSRDLNGLKKLCKQFSFPGGISSHMAPETPGSINEGGELGYSLAHSFGAV
R. palustris NRLITEHDLDMIYITGPGHGGPGLVANAYLEGTYTERYPAIERSRNGMQRLFRQFSWPHGVPSHVSPETPGSIHEGGELGYSLAHAYGAA
N. europaea NRIIRRDDLNMIYIAGPGHGGPALVANTYLEGTYSEHYPDISQDIQGMKHLFRQFSFPGGIGSHATPEIPGSIHEGGELGYALSHAFGAV
T. ferrooxidans NRLINKYDLNAIYISGPGHGAPAILSQAYLEGTYSETYPDKSQDIAGMRRFFKQFSFPGGIGSHATPETPGSIHEGGELGYSVSHAFGTV
Synechocystis NRIIRKFDQDMLYMVGPGHGAPGFLGPCYLEGSYSRFFAECSEDEDGMKRFFKQFSFPGGIGSHCTPETPGSIHEGGELGYCLSHAYGAA
C. tepidum NRIIRNRDLDIIYIAGPGHGGPALVANVWLEGTYSEYYPDVSFDEAGMKRLFRQFSFPGGIPSHVAPATPGSIHEGGELGYALSHAYGAV
N. crassa NRLIKKYDLDSIFISGPGHGAPAVLSQAYLEGTYSEVYPEISEDEEGMQKFFKHFSFPGGIGSHATPETPGSIHEGGELGYSISHAFGAV
Consensus NR-I----------GPGHG----------G-Y----------G-------FS-P-G--SH-----PGSI-EGGELGY---H--G--
169 258
B. lactis MDNPSLFVPCIIGDGEAETGPLATGWQSNKLVNPRTDGIVLPILHLNGYKIANPTILARISDEELHDFFRGMGYHPYEFVAGFDNEDHLS
M. avium FDNPYLVVACVIGDGEAETGPLAAGWHSNKFLNPVTDGAVLPILALNGYKIANPTVLARIPHTELEALLRGYGYRPI.TVAG...DDPTD
C. acetobutylicum FDNPDLITACVVGDGEAETGPLATSWQANKFLNPVTDGAVLPILHLNGYKISNPTVLSRIPKDELEKFFEGNGWKPY.FVEG...EDPET
R. palustris FDNPNLIVACVVGDGEAETGALATSWHSNKFLNPARDGAVLPILHLNGFKIANPTVLARITPQELTDLMRGYGYEPH.FVEG...DDPAV
N. europaea FDNPDLIAACVVGDGEAETGPLATAWHSNKFLNPVHDGAVLPILHLNGYKIANPTILARISREELDQLFQGYGYQPY.IVEG...EDPLT
T. ferrooxidans YDNPDLIALVMVGDGEAETGPLATSWHSNKFLNPITDGAVLPVLHLNGYKINNPTILARITHEELEALFIGYGYTPY.FVEG...SDPAS
Synechocystis FDNPNLIVVGLAGDGESETGPLATSWHSNKFINPIRDGAVLPVLHLNGYKINNPSVLSRISHEELKALFEGYGYTPY.FVEG...SDPES
C. tepidum FDNPDLVAACVIGDGEAETGPLATAWHSNKFLNPKRDGAVLPVLHLNGYKIANPTVLARISHEELEQLMIGYGYKPY.FVEG...DDPAT
N. crassa FDHPNLIALTMVGDGEAETGPLATSWHSTKFLNPITDGAVLPVLHLNGYKINNPTILARISHKELENLFRGYGYEPY.FVEG...DDVDT
Consensus -D-P-L------GDGE-ETG-LA--W---K--NP--DG-VLP-L-LNG-KI-NP--L-RI---EL-----G-G--P---V-G----D---
259 347
B. lactis IHRRFAELFETIFDEICDIKAAAQTDDMT.RPFYPMLIFRTPKGWTCPKFIDGKKTEGSWRAHQVPLASARDTEAHFEVLKGWMESYKPE
M. avium VHRQLAAALDEAFDDIAAIQGVARGGGEVQRPVWPMIVLRTPKGWTGPKVVDGKRVEGTWRSHQVPLAETHDNPEHRAQLEEWLRSYGPE
C. acetobutylicum MHKLMAETLDIVTEEILNIQKNARENNDCSRPKWPMIVLRTPKGWTGPKFVDGVPNEGSFRAHQVPLAVDRYHTENLDQLEEWLKSYKPE
R. palustris VHQTLAATLERVLGEIRAIQDKARNHGDTERPRWPMIVMRTPKGWTGPKQVDGKPVEGTWRAHQVPIADF.KNPEHLTLLEDWMRSYRPD
N. europaea MHQLMAGTLDQVLAEIRSIQTRARLDGVTQYPRWPMIILRTPKGWTGPATVDGLKTEGSWRSHQVPLSELAKKPEHIQQLEAWLRSYRPE
T. ferrooxidans MHQAMAATMERCVLKIREFQDKARHTGTAFRPRWPMIILRSPKGWTAPRKVDGHYLEGFWRAHQIPIPDVVSNPAHLQLLESWMRSYRPE
Synechocystis MHQAMAATLDHCVSEIHQIQQEARSTGIAVRPRWPMVVMRTPKGWTGPDYVDGHKVEGFWRSHQVPMGGMHENPAHLQQLEAWMRSYKPE
C. tepidum MHQMMAATMDRCFDEIAEIQRRARVDGVTERPMWPMIVFRSPKGWTGPKVVDGKPAEGSWRSHQVPFSTVRDNPEHMALLETWLKSYRAE
N. crassa MHQAMAATLEHCVLEIRKFQQKARESGKAFRPRWPVIILRSPKGWTGPRKIDDKFLEGFWRAHQVPITDVHEKPEHLKILEQWMKSYEPD
Consensus -H---A---------I------A--------P--P----R-PKGWT-P---D----EG--R-HQ-P-------------L--W--SY---
348 435
B. lactis ELFNADGSIKEDVTAFMPKGELRIGANPNANGGRIREDLKLPELDQYEITGVKEYGHGWGQVEAPRSLGAYCRDIIKNNPDS..FRVFGP
M. avium QLFDDDGRLRAELRALAPTGDRRMSANPHANGGLLLHDLDLPDFRDYAVPVTRP...GSVTHEATRVLGTFLRDVIARNKD..RFRMMGP
C. acetobutylicum ELFDENYRLIPELEELTPKGNKRMAANLHANGGLLLRELRTPDFRDYAVDVPTP...GSTVKQDMIELGKYVRDVVKLNEDTRNFRIFGP
R. palustris ELFDATGKLRDELQALAPTGRRRMSANPHANGGELLEPLSLPDFHDYAVTLTGP...GALKAEATRVLGTFLRDVMKNSLESENFRLFGP
N. europaea ELFDTQGRLVEPLQTLAPLGNRRMGANPHANGGSLMKQLRMPDFRKYAVEIVQP...GQIEAESTRIMGSFLRDIMCLNLKTCNFRVFGP
T. ferrooxidans ELFDAQGRLIPELHELAPKGKRRMSANPVANGGLLRRPLDMPDFRVFSIAVQDA...GGTRADNVPTLGHFLREITRRNMQ..NFRIFGP
Synechocystis ELFDEQGTLKPGFKAIAPEGDKRLGSTPYANGGLLRRGLKMPDFRQYGIDVDQP...GTIEAPNTAPLGVFLRDVMANNMT..NFRLFGP
C. tepidum ELFTADGVLLPELQELAPRGKKRMGDIPHANGGLLLKELRMPDFREYGIDVPKP...GSVEAEAPKPMARFLRDIMKMNEKAANFRVFGP
N. crassa RIF.RDGRISEDLKDLCPTGTRRMSANPVANGGIIRKTLRMPDFRNYALKVDKP...ASIMAASMTNMSMFLRDVIAGNPT..TFRLFGP
Consensus --F-------------P-G--R------ANGG-----L--P----------------------------R----------FR--GP
436 513
B. lactis DETASNRLNATYEVTKKQWDNGYLSALVD.ENMAVTGQVVEQ.LSEHQCEGFLEAYLLTGRHGIWSSYESFVHVIDSMLN.....
M. avium DETASNRLDAVYGATEKVWLSAT.EPDDE..HLAPDGRVME.VLSEHLCQGWLEGYLLTGRHGLFNCYEAFVHIVDSMLN.....
C. acetobutylicum DETMSNRLWAVFEGTKRQWLSEIKEPNDE..FLSNDGRIVDSMLSEHLCEGWLEGYLLTGRHGFFASYEAFLRIVDSMIT.....
R. palustris DETASNRLDAVLQVSPKEWMAAI.EDVDV..DLSPDGRVME.VLSEHLCQGWLEGYLLTGRHGFFSCYEAFIHIIDSMFN.....
N. europaea DETASNRLGSLYDVTPKTWLAET.LPEDE..HLAPDGRVME.ILSEHTCQGWLEGYLLTGRHGLFSCYEAFIHIVDSMFN.....
T. ferrooxidans DETQSNKLDAIYDVTQKVWLGAYFPEDADGGALALSGRVME.MLSEHTLEGWLEGYLLSGRHGLINSYEAFIHIIDSMFN.....
Synechocystis DENSSNKLHAVYEVSKKFWIAEYLEEDQDGGELSPDGRVME.MLSEHTLEGWLEAYLLTGRHGFFATYESFAHVITSMVN.....
C. tepidum DETASNRLGELFEETDRTWMAGM.LPTDD..HLSRDGRVME.ILSEHTCQGWLEGYLLTGRHGFFSCYEAFIHIIDSMFN.....
N. crassa DETESNKLGKVYEAGKKVWLGEYFEEDKDGGNLAPEGRVME.MLSEHTCEGWLEGYILTGRHGLLNSYEPFIHVIDSMVNQVWSPSRNLK
Consensus DE--SN-L----------W----------------G------LSEH---G-LE-Y-L-GRHG----YE-F-----SM----------
514 595
B. lactis .....QHAKWLEATVREIPWRKPISSVNLLVSSHVWRQDHNGFSHQDPGVTSVLLNKTFNNDHVTNIYFATDANMLLAIAEKCFKST
M. avium .....QHAKWL.ATSRELPWRRPIASLNYLLTSHVWRQDHNGASHQDPGFIDLVANK...RAELTRVYLPPDGNTLLSVADHCLRSR
C. acetobutylicum .....QHGKWLKVTS.QLPWRKDIASLNLIATSNVWQQDHNGYTHQDPGLLGHIVDK...KPEIVRAYLPADANTLLAVFDKCLHTK
R. palustris .....QHAKWLK.ACATIPWRKPIASLNYLLTSHVWRQDHNGFSHQDPGFIDHVANK...KSNVVRIYLPPDANCLLSVADHCLRSR
N. europaea .....QHAKWLKVS.KEIPWRRPIASLNYLLTSHVWRQDHNGFSHQDPGFIDHVINK...KADTIRIYLPPDANCLLYITDKCLRSR
T. ferrooxidans .....QHAKWLEK.CNELPWRAKVASLNLLITGLVWRQDHNGFTHQDPGFLDVVANK...SPNVVRIYLPPDANCLLSVTDHCLRSV
Synechocystis .....QHAKWLDI.CRHLNWRADISSLNILMTSTVWRQDHNGFTHQDPGFLDVILNK...SPDVVRIYLPPDVNSLLSVADHCLQSK
C. tepidum .....QHAKWLKVTGAXIPWRRPIASLNYFLTSHVWRQDHNGFSHQDPGFIDHVVNK...KSSVIRVYLPPDANSLLSVTNHCLRSR
N. crassa *LSLTIGFQHCKWLEK.CLEVEWRHKVASLNILLTAVVWRQDHNGFTHQDPGFLDVVANK...SPEIVRIYLPPDGNCLLSVTDHCLRSS
Consensus --------QH-KWL--------WR----S-N------VW-QDHNG--HQDPG-------K----------Y---D-N-LL-----C----

FIG. 4

```
                 596                                                                                       682
        B. lactis  NKINAIFAGKQPAATWITLDEVRAELEAGAAEWKWASN.AKSNDEVQVVLAAAGDVPTQEIMAASDALNKM..GIKFKVVNVVDLIKLQS
         M. avium  DYINVIVAGKQPALAYLDMDAAIAHCTRGLGIWDWASTARSIGAEPDVVLACAGDIPTLETLAAADILRRELPDLAVRVVNVVDLMRLQP
C. acetobutylicum  HKINLLVTSKHPRQQWLTMDQAVKHVEQGISIWDWASNDK..GQEPDVVIASCGDTPTLEALAAVTILHEHLPELKVRFVNVVDMMKLLP
      R. palustris  NYVNLIVAGKQPEWQWLDIDAAVRHCTTGAGIWHWASD...EG.EPDVVMACAGDVPTVETLAAVKLLREYVPDIKIRVVNVVDLMVLQP
       N. europaea  NFVNVIVAGKQPQLQWLDMDAAIKHCTAGIGIWGWASND.QAG.EPDVVIACAGDVPTIEVLAAVSILREHLPDLRIRVINVVDLMTLQH
   T. ferrooxidans  NYINVIVADKQTHLQYLDMDAAMAHCAKGAGIWEWASNDM..GEEPDVVMASCGDVPTMESLAATALLRQHLPDIKIRFVNVVDLFKLVP
     Synechocystis  NYINIIVCDKQAHLQYQDMTSAIRNCTKGVDIWEWASNDA..GTEPDVVMAAAGDIPTKEALAATAMLRQFFPNLRIRFVSVIDLLKLQP
        C. tepidum  NYINVIVAGKQPAWQWLDMESAVRHCTSGIGIWEWASNDANEG.EPDVVMACAGDVPTLETLAAVKILRKLAPELKIRVVNVVDLMTLQP
         N. crassa  NYVNVIVADKQEHLQYLSMDDATIHCTKGIGIWPQFSSDR..GEEPDLVMASCGDISTHESLAAIDLLLQHFPELKIRCVNCVDLFKLIS
         Consensus  ---N-----K------------------G---W---S-------E---V-A--GD--T-E--AA---L---------------D---L--

                 683                                                                                       769
        B. lactis  SKENDEAMSDEDFADLFTADKPVLFAYHSYAQDVRGLIYDRPNHDNFTVVGYKEQGSTTTPFDMVRVNDMDRYALQAKALELID...ADK
         M. avium  DSEHPHGLPDREFDALFTRDRPVIFAYHGYPWLIHRLTYRRANHAQLHVRGFKERGTTTTPFDMVMLNDLDRFHLVIDVLDRVEG.LASR
C. acetobutylicum  ENEHPHGLSDKDYNALFTTDKPVIFAFHGFAHLINQLTYHRENR.NLHVHGYMEEGTITTPFDMRVQNKLDRFNLVKDVVENLPQ.LGNR
      R. palustris  SSEHPHGLDDRRFDELFTTDKPVIFAFHGYPWLIHRLTYRRRNHVNIHVRGYKEEGTTTTPFDMVVLNDLDRYRLALDAILRIPR.LADQ
       N. europaea  DREHPQGLSDREFDTLFTTDKPIIFAYHGYPWLIHRLTYRRTNHANLHVRGYKEEGTTTTPFDMTVLNDMDRFHLVDDVIDRVPH.LGYK
   T. ferrooxidans  HTEHPHGMTDREFEALFTSSKPVIFNFHSYPWLIHRLTYRRPAQHHIHVRGYKEKGNIDTPLELAIRNQTDRFSLAIDAIDRIPRFC.DT
     Synechocystis  ESEHPHGLSDRDFDSLFTTDKPIIFNFHAYPWLIHRLTYRRTNHGNLHVRGYKEKGNINTPMDLAIQNQIDRFSLAIDVIDRLPQ.LRVA
        C. tepidum  KEEHPHGLADRDFDDMFTTDKPIIFAYHGYPWLIHRLTYRRTNHHNLHVRGYKEEGTTTTPFDMVVMNELDRFHLVADVANRVES.LRPQ
         N. crassa  HDEHPHGLTDAEWISLFTDNRPIIFNFHSYPWLVHRLTYKRPGSHNVHVRGYREKGNIDTPLELAIRNGTDRFSLAMAAIDHMTGILGNR
         Consensus  --E------D------FT---P--F--H--------L-Y-R-------V-G--E-G---TP------N--DR--L---------------

                 770                                                     825
        B. lactis  YADKINELNEFRKTAFQFAVDNGYDIPEFTDWVYPDVKVDETSMLSATAATAGDNE
         M. avium  AAMLRQRMVDARLAARMYTREHGEDDPAIANWTWEPSERNSRSE
C. acetobutylicum  GAHLVQLMNDKLVEHNQYIREVGEDLPEITNWQWHV
      R. palustris  RDAATSRYWATMQRHKLYIGEHGDDLPEVRDWRWSA
       N. europaea  AAYLRQIMRDKLVEHREYINRHGEDMPEIRDWKWTAP
   T. ferrooxidans  GSGVREILLNLQFACKNHAYEYGVDPQEITDWQWPFRDTP
     Synechocystis  GAHIKEMLKDMQIDCTNYAYEHGIDMPEIVNWRWPL
        C. tepidum  AAYIKQYVRDRLIEHKEYITKYGEDMPEVRDWRWED
         N. crassa  GAAAREQLRNEQLKAKAMAFEVGMDPPFLKNWTWPHTGLWSKGEARVASEQHLH
         Consensus  ----------------------G-D------W------------------------
```

FIG. 4. Alignment of the *B. lactis* Xfp amino acid sequence with homologous hypothetical proteins identified in the chromosomes of *Chlorobium tepidum* (The Institute for Genomic Research [TIGR]), *C. acetobutylicum* (Genome Therapeutics), *Mycobacterium avium* (TIGR), *Neurospora crassa* (Heinrich Heine Universität Düsseldorf), *Nitrosomonas europaea* (Doe Joint Genome Institute), *Rhodopseudomonas palustris* (Doe Joint Genome Institute), *Synechocystis* sp. strain PCC6803 (P74690), and *Thiobacillus ferrooxidans* (TIGR) (sequence sources are given in parenthesis). Amino acid residues that occur in all of the 9 sequences are included in a consensus sequence. The thiamine diphosphate binding motif is shadowed. Amino acid numbering refers to the *B. lactis* Xfp protein. Further homologies were found in the chromosomes of *Anabaena* sp. strain PCC7120 (two different copies) (Kazusa DNA Research Institute), *Brucella suis* (TIGR), *Lactococcus lactis* IL1403 (Genoscope), *Mesorhizobium loti* (AP003009), *Mycobacterium smegmatis* (TIGR), *Nostoc punctiforme* (Doe Joint Genome Institute), *Pseudomonas aeruginosa* (*Pseudomonas* Genome Project), *Pseudomonas syringae* pv. *tomato* (TIGR), *Schizosaccharomyces pombe* (SPBC24C6), *Shewanella putrefaciens* (TIGR), *Sinorhizobium meliloti* (Stanford University), and *Streptomyces coelicolor* (SCF55/SCF56).

SITE PS00187 [Fig. 4]). Another motif, G-D-G-X_{24-27}-N-N, of ThDP-binding enzymes like acethydroxyacid synthases, transketolases, E1 components of 2-ketoacid and acetoin dehydrogenases, and others (4) is also present in Xfp in a slightly modified form (G-D-G-E-X_{30}-N) (amino acids 181 to 215 [Fig. 4]).

In the course of cloning the *B. lactis xfp* gene in *E. coli*, we failed to clone the entire gene in the high-copy-number vector pUC18. It might point to the effect that the basic carbohydrate pathways of *E. coli* might be severely disturbed by *xfp* and its expression, leading to a lethal imbalance of metabolites (34). We failed to detect F6PPK activity in *E. coli* harboring the recombinant *xfp* gene in both the low-copy-number vector pCL1920 and in the expression vector pET-28a(+), respectively (Table 1). However, the Xfp subunit seemed to be synthesized in the *E. coli* host carrying pFPK5 (Fig. 5). Due to the additional His tag arrangement (36 amino acids encoded by the expression vector) at the N terminus of the recombinant Xfp, the functionality was probably hampered by an inappropriate subunit assembling to the hexamer. Although expression of an active enzyme in *E. coli* is still pending, the biochemical evidence that the characterized gene *xfp* codes for the purified Xfp is sufficiently substantiated.

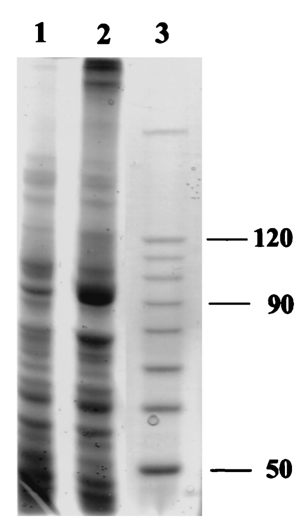

FIG. 5. Expression of the *xfp* gene from *B. lactis* in *E. coli*. Protein extracts of uninduced (lane 1) and IPTG-induced (lane 2) cells from *E. coli* BL21(DE3)/pLysS harboring plasmid pFPK5 were subjected to SDS-PAGE. The masses (in kilodaltons) of the corresponding protein standards run in lane 3 are indicated at right. The gel (7.5 % polyacrylamide) was Coomassie stained.

ACKNOWLEDGMENTS

We thank Alexandra Kulangara for help during the purification of the protein.

This work was supported by grant 01016/41-2705.5 from ETH Zurich.

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