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6-Phospho-α-D-Glucosidase from *Fusobacterium mortiferum*: Cloning, Expression, and Assignment to Family 4 of the Glycosylhydrolases

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The *Fusobacterium mortiferum malH* gene, encoding 6-phospho-α-glucosidase (maltose 6-phosphate hydrolase; EC 3.2.1.122), has been isolated, characterized, and expressed in *Escherichia coli*. The relative molecular weight of the polypeptide encoded by *malH* (441 residues; M_r of 49,718) was in agreement with the estimated value (~49,000) obtained by sodium dodecyl sulfate-polyacrylamide gel electrophoresis for the enzyme purified from *F. mortiferum*. The N-terminal sequence of the MalH protein obtained by Edman degradation corresponded to the first 32 amino acids deduced from the *malH* sequence. The enzyme produced by the strain carrying the cloned *malH* gene cleaved [U-^{14}C]maltose 6-phosphate to glucose 6-phosphate (Glc6P) and glucose. The substrate analogs *p*-nitrophenyl-α-D-glucopyranoside 6-phosphate (pNPαGlc6P) and 4-methylumbelliferyl-α-D-glucopyranoside 6-phosphate (4MUαGlc6P) were hydrolyzed to yield Glc6P and the yellow *p*-nitrophenolate and fluorescent 4-methylumbelliferyl aglycons, respectively. The 6-phospho-α-glucosidase expressed in *E. coli* (like the enzyme purified from *F. mortiferum*) required Fe^{2+}, Mn^{2+}, Co^{2+}, or Ni^{2+} for activity and was inhibited in air. Synthesis of maltose 6-phosphate hydrolase from the cloned *malH* gene in *E. coli* was modulated by addition of various sugars to the growth medium. Computer-based analyses of MalH and its homologs revealed that the phospho-α-glucosidase from *F. mortiferum* belongs to the seven-member family 4 of the glycosylhydrolase superfamily. The cloned 2.2-kb *Sau*3AI DNA fragment from *F. mortiferum* contained a second partial open reading frame of 83 residues (designated *malB*) that was located immediately upstream of *malH*. The high degree of sequence identity of MalB with IIB^{Glc}-like proteins of the phosphoenol pyruvate dependent:sugar phosphotransferase system suggests participation of MalB in translocation of maltose and related α-glucosides in *F. mortiferum*.

Members of the genus *Fusobacterium* are gram-negative obligate anaerobes that colonize the oral and gastrointestinal tracts of humans and animals (5, 24, 27, 31). The frequent isolation of these species from abscesses and necrotic tissues indicates their causative or contributory roles in a variety of infections (6, 7, 15, 16, 25). The prevalence and increased numbers of certain species (e.g., *Fusobacterium nucleatum*) in patients with gingivitis and periodontitis suggest that this organism may play a role in the etiology of these oral diseases (6, 32, 50). Although the pathogenic potential and medical importance of fusobacteria are widely recognized, surprisingly little is known about the biochemistry, genetics, or regulation of energy generation in these species.

Most species of fusobacteria derive energy for growth via fermentation of amino acids such as glutamate, lysine, and serine (4, 10, 11, 17). Until recently, members of the genus were usually described as asaccharolytic or weakly fermentative (24, 31, 42). In this context, it was of considerable interest to discover that *Fusobacterium mortiferum* metabolized a wide variety of carbohydrates, including monosaccharides and both α- and β-glycosides, as energy sources (40, 41, 54, 55). These compounds are transported by the phosphoenolpyruvate-dependent sugar:phosphotransferase system (PEP:PTS) (29, 35, 43). This multicomponent group translocation system comprises both membrane-associated enzymes and cytoplasmic proteins. In concert, the PEP:PTS facilitates the simultaneous translocation, phosphorylation, and internalization of sugars by the cell. A functional PEP:PTS requires two general proteins, designated enzyme I and HPr, that are allied with sugar-specific complexes comprising enzymes IIA, IIB, and IIC (for discussions, see references 35 and 44) to effect the sequential transfer of the high-energy phosphoryl moiety from PEP to the incoming sugar.

PEP-dependent PTS have been reported for all major groups of carbohydrates in both gram-negative (29, 35) and gram-positive (19, 39, 51, 52) bacteria. However, the existence of a maltose-specific PTS has been a source of controversy, and evidence has been presented both for and against this postulate (for discussions, see references 40 and 53). In our laboratory, studies of maltose utilization by *F. mortiferum* resulted in the serendipitous isolation of maltose 6-phosphate (40). Although this finding was suggestive of a functional maltose:PEP-PTS in *F. mortiferum*, we were initially unable to demonstrate the hydrolytic cleavage of the phosphorylated disaccharide to yield the anticipated products of glucose and glucose 6-phosphate (Glc6P). The realization that maltose 6-phosphate hydrolase (MalH) (or 6-phospho-*O*-α-D-glucopyranosyl:phosphoglucohydrolase) was oxygen sensitive and required a divalent metal ion (Fe^{2+}, Co^{2+}, Ni^{2+}, or Mn^{2+}) for activation subsequently permitted the purification and characterization of the protein (53). This unique enzyme hydrolyzes a variety of

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6-phosphoryl-α-D-linked glucosides, including maltose 6-phosphate and trehalose 6-phosphate, as well as the chromogenic and fluorogenic substrates *p*-nitrophenyl-α-D-glucopyranoside 6-phosphate (pNPαGlc6P) and 4-methylumbelliferyl-α-D-glucopyranoside 6-phosphate (4MUαGlc6P), respectively.

In this report, we describe the isolation, molecular cloning, and genetic characterization of the maltose 6-phosphate hydrolase-encoding gene (*malH*) from *F. mortiferum*. The gene has been expressed in *Escherichia coli*, and to our knowledge, this phospho-α-glucosidase (maltose 6-phosphate hydrolase) is the first fusobacterial enzyme produced in active form in a heterologous organism. The high degree of sequence identity between MalH and GlvG proteins of both *E. coli* (8, 38) and *Bacillus subtilis* (58) characterizes these proteins as phospho-α-glucosidases; accordingly, they are assigned to family 4 of the glycosylhydrolase superfamily (20, 21).

MATERIALS AND METHODS

Organisms and culture maintenance. *F. mortiferum* ATCC 25557 was obtained from the American Type Culture Collection, Rockville, Md. The organism was maintained by semimonthly transfer in BBL fluid thioglycolate medium containing 0.5% beef extract and solid $CaCO_3$. *E. coli* XLOLR was obtained from Stratagene and was grown in Luria-Bertani (LB) medium. Plasmid-containing *E. coli* XLOLR(pCB 4.11) was grown in the same medium supplemented with 50 μg of kanamycin per ml.

Growth of cells. *F. mortiferum* 25557 was grown anaerobically (GasPak; BBL Microbiology Systems) at 37°C in modified Todd-Hewitt broth (41, 42) containing 0.25% (wt/vol) maltose. The prereduced medium was inoculated with ~5% (vol/vol) freshly grown culture. The cells were grown to stationary phase (~22 h), and the yield was ca. 3.5 to 4.0 g (wet weight) of cells per liter of culture. Cells were harvested by centrifugation (13,000 × *g* for 10 min at 4°C), and the cell pellets were washed by resuspension and centrifugation from 25 mM HEPES buffer (pH 7.5) containing 1 mM each dithiothreitol and $MnSO_4$.

Assay of maltose 6-phosphate hydrolase activity. Enzyme activity in cell extracts prepared from *F. mortiferum* and *E. coli* XLOLR transformants was measured with pNPαGlc6P as the substrate. The 4-ml reaction mixture contained 50 mM imidazole-HCl (pH 7.0) buffer, 1 mM pNPαGlc6P, and 1 mM the appropriate Me^{2+} ion. The mixture was warmed to 37°C in a water bath, and the requisite amount of cell extract was added to begin the reaction. At intervals of 30 s, 0.5-ml samples of reaction mixture were removed and mixed immediately with 0.5 ml of 0.5 M Na_2CO_3 solution to inactivate the enzyme and give maximum (yellow) color development. The absorbance of the 1-ml solution was measured spectrophotometrically at 400 nm, and the amount of pNPαGlc6P hydrolyzed was calculated by using a molar extinction coefficient of 18,300 M^{-1} cm^{-1} for the yellow *p*-nitrophenolate anion at pH 10.2. One unit of maltose 6-phosphate hydrolase is defined as the amount of enzyme which catalyzes the formation of 1 μmol of *p*-nitrophenolate per min at room temperature (~22°C).

Construction and screening of an *F. mortiferum* DNA library. Genomic DNA was isolated from *F. mortiferum* ATCC 25557 by lysozyme-sodium dodecyl sulfate (SDS) treatment (47). DNA obtained by partial *Sau*3AI digestion was fractionated by sucrose density gradient centrifugation (3), and fragments of 1 to 12 kb were ligated into the lambda ZAP Express vector (Stratagene). The resulting library was plated for expression on *E. coli* XL1-Blue MRF′ as directed by the manufacturer. Proteins in plaques were transferred to nitrocellulose filters and were screened for immunoreactive material by using polyclonal antisera raised against purified maltose 6-phosphate hydrolase (53). Detection was accomplished with goat anti-rabbit horseradish peroxidase-conjugated secondary antibody (ICN). Antibody-positive plaques were isolated, amplified, and retested at least twice more. Plasmids carrying the cloned *F. mortiferum* DNA were excised from the recombinant phage clones by using the ExAssist helper phage as directed by the manufacturer (Stratagene). The plasmid (pCB 4.11) encoding *malBH* was maintained in the host strain *E. coli* XLOLR.

Isolation of *glvG* from *E. coli*. The lambda phage clone EC22-175 (which carries 20 kb of DNA from the 81.5- to 84.5-min region of the *E. coli* genome) was generously provided by G. Plunkett and F. Blattner (Laboratory of Genetics, University of Wisconsin, Madison). This phage was constructed as part of the *E. coli* Genome Sequencing Project and contains the *glvG* gene (36). Phage DNA was extracted as described by Sambrook et al. (45). The open reading frame (ORF) designated *f212* or *glvG* (8, 36) was amplified from the phage by PCR using primers *glv*-50727 (5′-GTACAAGCTTGCCACTTGCTGACTCATTAAC C-3′) and *glv*-51489 (5′-CATTCAGGTGATCATTGGTCTGC-3′), whose sequences were obtained from GenBank accession no. L10328. *Glv*-50727 contains a *Hin*dIII site (underlined) at the 5′ end, and *glv*-51489 is positioned such that a nearby *Pvu*II site in the genome will be present in the amplification product. The PCR mixture contained the following components in a final volume of 100 μl: 1× PCR buffer (Perkin-Elmer Cetus), 0.5 mM $MgSO_4$, 250 μM deoxynucleotide triphosphates, 25 pmol of each primer, 1 ng of EC22-175 DNA, and 2.5 U of Amplitaq polymerase (Perkin-Elmer Cetus). Amplification was carried out in a Perkin-Elmer 9600 Thermal Cycler for 25 cycles under the following conditions: 94°C, 30 s; 55°C, 1 min; and 72°C, 50 s. The PCR product was purified (Magic PCR Preps; Promega), digested with *Pvu*II and *Hin*dIII, and ligated into pUC18 (Life Technologies) that previously had been digested with *Sma*I and *Hin*dIII. Nucleotide sequencing confirmed that the resulting plasmid (pCB212-11) carried the desired insert.

Construction of a GlvG-MalH hybrid. A hybrid gene encoding residues 1 to 212 of GlvG and residues 207 to 441 of MalH was constructed by combinatorial PCR as described by Higuchi (23). Briefly, DNA fragments encoding residues 1 to 212 of GlvG and residues 207 to 441 of MalH were amplified separately. The PCR products were then mixed together, and the hybrid gene was constructed by another amplification using the outermost primers only. The primers for the 3′ end of *glvG* and the region of *malH* corresponding to residue 207 (the inside primers) were designed with complementary 5′ ends. For amplification of the DNA fragment encoding GlvG residues 1 to 212, the following primers were used: *glv*-51489 (sequence given above) and mal207-glv212 (5′-TAAGTCATTT CCTTGTTTATCTCTTACACCCTTTCTGAAACTGCG-3′); for amplification of the DNA fragment encoding residues 207 to 441 of MalH, the primers glv212-mal207 (5′-ATATCCCGCAGTTTCAGAAAGGTTGTAAGAGATA AACAA GGAAATGAC-3′) and malH 441-*Hin*dIII (5′-GTACAAGCTTTTATTCAAA ACTGGCCAATATTC-3′) were used. The components of the amplification mixtures (100 μl) were as described above together with the appropriate primers. Cycling parameters were the same except that an annealing temperature of 45°C was used. Both PCR products were precipitated with ethanol to remove excess primers. Subsequently, 1 μl of each product was added to an amplification reaction (components listed above) containing the outside primers *glv*-51489 and malH 441-Hind, and amplification was carried out as described above (with an annealing temperature of 45°C and an extension time of 1.5 min). The resulting PCR product was digested with *Pvu*II and *Hin*dIII and ligated into *Sma*I and *Hin*dIII-digested pBluescript SK II+. Nucleotide sequencing of the entire construct in the resulting plasmid, pH4, confirmed that no mutations had been introduced during synthesis of the hybrid gene.

Detection of the recombinant MalH, GlvG, and GlvG-MalH hybrid proteins. *E. coli* transformants carrying recombinant plasmids with either *F. mortiferum malH* (pCB4.11), *E. coli glvG* (pCB212-11), the hybrid *glvG-malH* gene (pH4), or the plasmid vector alone were grown in LB medium supplemented with ampicillin (when necessary) at 37°C to A_{600} of 0.6. Isopropylthiogalactopyranoside (IPTG) was added to a final concentration of 1 mM, and incubation was continued for an additional 1 to 2 h. The cells were harvested by centrifugation (10,000 × *g* for 10 min at 4°C), and the cell pellet was washed twice by resuspension and centrifugation from 25 mM HEPES buffer (pH 7.5) containing 1 mM dithiothreitol and 1 mM $MnSO_4$. The cells were resuspended in 2 volumes of the same buffer and were disrupted with two 1-min periods of sonication at 0°C in a Branson sonifier operated at 75% of maximum power. Proteins in the cell extracts (50 μg) were separated by SDS-polyacrylamide gel electrophoresis (PAGE) and electrophoretically transferred to nitrocellulose membranes (53). Incubation of the membranes with polyclonal antibody against MalH and immunodetection with goat anti-rabbit horseradish peroxidase-conjugated antibody were as described previously (53). An extract prepared from maltose-grown cells of *F. mortiferum* 25557 served as a control for these experiments.

Nucleotide sequencing and computer-aided analyses. Nucleotide sequencing was carried out by using the dideoxy-chain termination method (46) with [α-^{35}S]dATP (NEN-DuPont) and Sequenase 2.0 T7 DNA polymerase (U.S. Biochemicals/Amersham). Double-stranded plasmid was used in DNA sequencing reactions, which were resolved on 6 or 8% acrylamide–8 M urea gels (Sequagel; National Diagnostics). Sequence analyses were accomplished with the Genetics Computer Group suite of programs (Version 8) and the DNASYSTEM package (49). The BLASTP program (2) using the BLOSUM 62 substitution matrix was used to search the nonredundant protein sequence database at the National Center for Biotechnology Information (National Institutes of Health Bethesda, Md.).

Detection of *malH* in *Fusobacterium* spp. Genomic DNA was prepared from *F. periodonticum*, *F. nucleatum* ATCC 25586, *F. nucleatum* ATCC 10953, *F. ulcerans*, *F. varium*, *F. russii*, *F. perfoetens*, *F. mortiferum* ATCC 25557, and *F. mortiferum* ATCC 9817. Three micrograms of each preparation of genomic DNA was transferred to nylon membranes (Schleicher & Schuell) and hybridized (QuikHyb; Stratagene) with a digoxygenin-labeled oligonucleotide (Genius system; Boehringer Mannheim Biochemica) whose sequence was derived from *F. mortiferum malH*. The sequence of probe 1 corresponded to nucleotides 346 to 365, and that of probe 2 corresponded to nucleotides 1123 to 1143 of *malH*.

Materials. Electrophoresis reagents and standards for SDS-PAGE were obtained from Bio-Rad Laboratories. pNPαGlc6P was prepared in this laboratory (53). The preparative biosynthesis and purification of [U-^{14}C]maltose 6-phosphate (40) and procedures for synthesis of the fluorogenic analog 4MUαGlc6P have been described previously (55). Primers were synthesized on an Applied Biosystems model 391 DNA Synthesizer PCR-MATE. The products were deprotected as recommended by the manufacturer and were desalted on SepPak C_{18} columns (Millipore-Waters).

Nucleotide sequence accession number. The nucleotide sequences for the *malH* and *malB* genes from *F. mortiferum* ATCC 25557 have been deposited in GenBank under accession no. U81185.

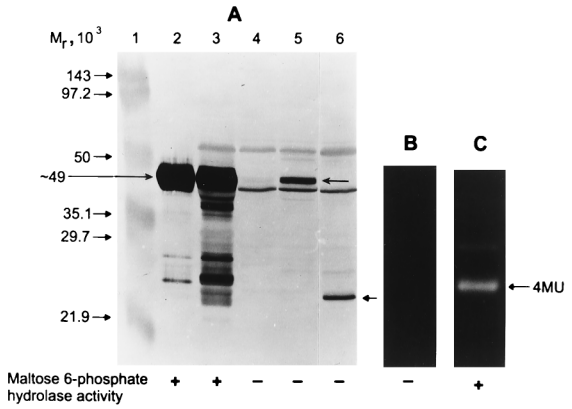

FIG. 1. Expression, immunodetection, and in situ activity staining of maltose 6-phosphate hydrolase in cell extracts. (A) Proteins were separated by electrophoresis in SDS–4 to 20% polyacrylamide gels and electrotransferred to a nitrocellulose membrane. Immunoreactive proteins to MalH antiserum were detected as described previously (53). Lanes: 1, prestained molecular weight standards; 2, ~10 μg of maltose 6-phosphate hydrolase purified from *F. mortiferum*; 3, extract (~100 μg) of *E. coli* XLOLR(pCB 4.11) (the lower-molecular-weight cross-reactive polypeptides are most probably due to proteolysis of maltose 6-phosphate hydrolase [M_r, = 49,000]); 4, extract (~100 μg) of *E. coli* XLOLR; 5, cell extract (~50 μg) prepared from *E. coli* DH5α containing plasmid pH4, which encodes the hybrid gene formed by fusion of residues 1 to 212 from *E. coli glvG* with residues 207 to 441 from *malH* of *F. mortiferum* (the arrow indicates the immunoreactive fusion protein [M_r, ~49,000]); 6, cell extract (~50 μg) of *E. coli* DH5α(pCB212-11) encoding *glvG* (the arrow indicates the expressed and immunoreactive GlvG protein [M_r, ~24,000]). (B and C) In situ activity staining showing the formation of catalytically active maltose 6-phosphate hydrolase by *E. coli* XLOLR(pCB 4.11). Proteins (~20 μg) in extracts prepared from *E. coli* XLOLR (B) and *E. coli* XLOLR(pCB4.11) (C) were separated by nondenaturing PAGE. The gel slices were then immersed for about 2 min in a solution of 50 mM HEPES buffer (pH 7.5) containing 1 mM Mn^{2+} and 0.1 mM 4MUαGlc6P. Maltose 6-phosphate hydrolase activity was revealed by the formation of the intensely fluorescent 4MU aglycon (arrow in panel C). + and − indicate the presence and absence, respectively, of maltose 6-phosphate hydrolase activity in the various extracts (with both chromogenic and fluorogenic analogs as substrates).

RESULTS

Isolation of *malH* from a genomic library *F. mortiferum*. The presence of the cloned *malH* gene, encoding maltose 6-phosphate hydrolase, was detected by immunological screening of plaques of an *F. mortiferum* DNA library (see Materials and Methods). Polyclonal antiserum raised against purified maltose 6-phosphate hydrolase (53) reacted positively with 2 plaques from a total of 2,000 screened. After amplification and retesting, one clone remained immunopositive and was retained for further study. A kanamycin resistance plasmid (pCB 4.11) was excised from this phage and was found to contain a 2.2-kb *Sau*3AI fragment of *F. mortiferum* DNA.

Expression of *malH* and activity of maltose 6-phosphate hydrolase in *E. coli*. *E. coli* XLOLR containing plasmid pCB 4.11 synthesized a polypeptide that cross-reacted with MalH antiserum. The immunoreactive recombinant protein (M_r = 49,000) (Fig. 1A, lane 3) comigrated with maltose 6-phosphate hydrolase that was purified from *F. mortiferum* (Fig. 1A, lane 2). In situ staining of nondenaturing polyacrylamide gels for maltose 6-phosphate hydrolase activity showed no cleavage of the fluorogenic substrate 4MUαGlc6P by a cell extract prepared from *E. coli* XLOLR (Fig. 1B). However, formation of the intensely fluorescent 4-methylumbelliferone (4MU) aglycon revealed expression and activity of the enzyme in an extract of *E. coli* XLOLR(pCB 4.11) that contains the plasmid-encoded *malH* gene (Fig. 1C). The recombinant enzyme exhibited properties similar to those described previously for maltose 6-phosphate hydrolase in *F. mortiferum* (53). Thus, the enzyme readily hydrolyzed the chromogenic analog pNPαGlc6P, to yield Glc6P and the yellow *p*-nitrophenolate aglycon (Table 1), and maltose 6-phosphate was cleaved to glucose and Glc6P in a 1:1 molar ratio (data not shown). Enzyme activity declined rapidly when the extract was exposed to air; maltose 6-phosphate hydrolase activity was also lost during overnight dialysis but was maintained when a divalent cation (e.g., Mn^{2+} ion) was included in the buffer; finally, the recombinant enzyme (like the native maltose 6-phosphate hydrolase from *F. mortiferum*) was reactivated after dialysis in the presence of one of four divalent cations, Ni^{2+}, Mn^{2+}, Co^{2+}, and Fe^{2+} (Table 1).

Differential expression of *malH* in *E. coli* XLOLR(pCB 4.11). Previously we showed that the amount of phospho-α-glucosidase (maltose 6-phosphate hydrolase) in cells of *F. mortiferum* was markedly dependent on the carbohydrate present in the culture medium (53). Thus, growth of the organism on α-glucosides, e.g., maltose, α-methylglucoside, palatinose, or turanose, induced high levels of enzyme activity, whereas cells grown on monosaccharides (e.g., glucose, mannose, or fructose) contained only 5 to 10% of the activities present in cells grown on α-glucosides. Paradoxically, the presence of galactose (the C-4 epimer of glucose) elicited highest expression of phospho-α-glucosidase (53). Remarkably, synthesis of phospho-α-glucosidase from the plasmid-encoded *malH* gene in *E. coli* XLOLR(pCB 4.11) was similarly regulated when these same sugars were added to the LB growth medium (Fig. 2). In situ staining for maltose 6-phosphate hydrolase activity after nondenaturing PAGE (using fluorogenic 4MUαGlc6P as the substrate) revealed a constitutive level of the enzyme in cells grown in LB broth alone (Fig. 2, lane 2). However, growth in LB medium supplemented with glucose (Fig. 2, lane 3) or fructose (Fig. 2, lane 4) resulted in significantly lower amounts of phospho-α-glucosidase. By contrast, the addition of galactose to LB broth induced high levels of enzyme activity (Fig. 2, lane 5). Extracts prepared from galactose-grown cells of the XLOLR parent strain contained no detectable phospho-α-glucosidase activity (Fig. 2, lane 1). These qualitative in situ

TABLE 1. Properties of 6-phospho-α-glucosidase expressed in *E. coli* XLOLR(pCB 4.11)

Determination	Sp act (μmol of pNPαGlc6P hydrolyzed/mg of protein/min)	Relative activity
Stability of enzyme in air after incubation time of:		
0 h	0.117	1
1 h	0.117	1
2 h	0.057	0.49
3 h	0.039	0.33
Effect of dialysis on enzyme activity		
Control before dialysis	0.117	1
Dialysis, no addition	0.020	0.17
Dialysis + 1 mM DTT	0.015	0.13
Dialysis + 1 mM Mn^{2+}	0.603	5.15
Restoration of enzyme activity in a dialyzed extract by divalent metal ions		
No metal addition	0.016	1
+1 mM Ni^{2+}	0.092	5.8
+1 mM Mn^{2+}	0.145	9.1
+1 mM Co^{2+}	0.181	11.1
+1 mM Fe^{2+}	0.241	15.1

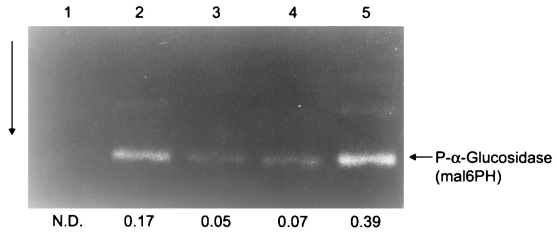

FIG. 2. Effects of different sugars on the synthesis of phospho-α-glucosidase (maltose 6-phosphate hydrolase) by cells of *E. coli* XLOLR(pCB 4.11). The organism was grown in LB medium supplemented with 0.1% (wt/vol) the appropriate sugar. Cells were disrupted by sonication, and after clarification by centrifugation, samples (20 μg) of each extract were electrophoresed in a Novex (4 to 20% polyacrylamide) nondenaturing minigel. The gel was incubated in Tris-glycine (pH 8.5) buffer containing 1 mM Mn^{2+} and 0.1 mM 4MUαGlc6P as the substrate. Enzyme activity was revealed by the formation of the intensely fluorescent 4MU aglycon; after 2 min of incubation, the gel was photographed under long-wave UV light, using a yellow filter. Lane 1 (control) contained extract from galactose-grown, plasmid-free *E. coli* XLOLR. All other lanes contained extracts prepared from *E. coli* XLOLR(pCB 4.11) grown in LB only (lane 2), LB-glucose (lane 3), LB-fructose (lane 4), and LB-galactose (lane 5). Numbers at the bottom represent specific activities of phospho-α-glucosidase in the extracts expressed as micromoles of the chromogenic substrate pNPαGlc6P hydrolyzed per minute per milligram of protein. N.D., no detectable activity.

observations were confirmed by spectrophotometric determination of the specific activities of maltose 6-phosphate hydrolase in the extracts, using the chromogenic pNPαGlc6P as the substrate (Fig. 2).

Nucleotide sequences of the *malH* and *malB* gene cluster. Figure 3 shows the nucleotide sequence of the 2.2-kb *Sau*3AI insert and the deduced amino acid sequence of *malH*. Additional upstream (*malB*) and downstream sequences are also presented. The *malH* gene comprises a coding sequence of 1,323 nucleotides beginning with an ATG codon at position 313 and terminating with a TAA (stop) codon at position 1636. This ORF encodes a polypeptide of 441 residues. As shown in Fig. 3, 29 of the first 32 deduced amino acids (aa) of *malH* were identical with those obtained by automated Edman degradation of the N-terminal portion of maltose 6-phosphate hydrolase that was purified from *F. mortiferum*. The calculated molecular mass of the protein product of *malH* (49,718 Da) is in excellent agreement with the M_r of 49,000 determined previously for the purified enzyme (53). The pI of purified maltose 6-phosphate hydrolase determined earlier (53) by analytical isoelectrofocusing (pI = 4.9) compares favorably with the pI of 5.5 predicted by computer analysis of the aa sequence. The *malH* gene is preceded by a potential ribosome-binding site (AGGAGG) centered 11 nucleotides from the initiation codon. Downstream of the stop codon one finds a GC-rich palindromic sequence (5′-GGGGCTTTAAAGCCCC-3′) and a poly(T) tract that may function as a transcriptional terminator. A partial ORF, designated *malB*, was identified upstream of *malH* (Fig. 3, nucleotides 2 to 253). The deduced amino acid sequence of *malB* contains 83 residues and is separated from the ATG start codon of *malH* by 60 bp. Overall, the G+C content of the *malH* and *malB* genes (32 to 33%) is somewhat higher than the generally accepted value of 26 to 28% for the *F. mortiferum* genome.

MalH is a member of family 4 of glycosylhydrolases. The MalH protein of *F. mortiferum* exhibits striking sequence similarity to six proteins (Table 2 and Fig. 4). that comprise family 4 of the glycosylhydrolase superfamily (20). All members of this family are of bacterial origin, and all except two are between 431 to 449 residues in size. The characterized 6-phospho-β-glucosidase from *E. coli* (designated CelF [33]) consists

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   1  GGATCTATATTCAGGAAAAGCTAAAGCATTTTTAGAAGCTTTTGGTGGTAAAGATAATAT   60
malB-> D  L  Y  S  G  K  A  K  A  F  L  E  A  F  G  G  K  D  N  I

  61  AGAACAAGTAAACAATTGTGCTACAAGACTTAGAATAAGTGTTAAAGATGAAAAAAAAGT   120
       E  Q  V  N  N  C  A  T  R  L  R  I  S  V  K  D  E  K  K  V

 121  TGGACCAGATATTCAATTTAAAGCAGCAGGAGCACATGGAGTTGTAAGAAATGGAAAAGC   180
       G  P  D  I  Q  F  K  A  A  G  A  H  G  V  V  R  N  G  K  A

                                                        -35
 181  TTTTCAAGTTATAGTAGGACTTTCAGTACCACAAGTTAGAGAGAGTTTTGAAAATTTAAT   240
       F  Q  V  I  V  G  L  S  V  P  Q  V  R  E  S  F  E  N  L  M
                  -10
 241  GGAACAAAATTAAGTATAATAAATATAAAATTAATATAACTATATTTAAATTAATATTAG   300
       E  Q  N

 301  GAGGAATAAATTATGAAACAATTTTCTATTTTAATTGCAGGTGGAGGAAGTACATTTACT   360
            malH-> M  K  Q  F  S  I  L  I  A  G  G  G  S  T  F  T

 361  CCAGGAATAATTTTAATGTTATTAGATAATCTTGATAAATTTCCGATTAGACAAATAAAA   420
       P  G  I  I  L  M  L  L  D  N  L  D  K  F  P  I  R  Q  I  K

 421  ATGTTTGATAATGATGCTGAAAGACAAGCAAAAATAGGAGAAGCATGTGCTATACTTTTA   480
       M  F  D  N  D  A  E  R  Q  A  K  I  G  E  A  C  A  I  L  L

 481  AAAGAAAAAGCACCTCAAATAAAATTTAGTTATTCAACAAATCCAGAGGAAGCATTTACA   540
       K  E  K  A  P  Q  I  K  F  S  Y  S  T  N  P  E  E  A  F  T

 541  GATATAGATTTTGTTATGGCACATATTAGAGTTGGAAAATATCCAATGAGAGAGTTAGAT   600
       D  I  D  F  V  M  A  H  I  R  V  G  K  Y  P  M  R  E  L  D

 601  GAAAAAATACCTTTAAGACATGGTGTTGTTGGTCAAGAAACTTGCGGACCAGGAGGAATA   660
       E  K  I  P  L  R  H  G  V  V  G  Q  E  T  C  G  P  G  G  I

 661  GCATATGGAATGAGAAGTATTGGAGGAGTAATTGGTCTTATAGATTATATGGAAAAATAT   720
       A  Y  G  M  R  S  I  G  G  V  I  G  L  I  D  Y  M  E  K  Y

 721  TCTCCAAATGCTTGGATGTTAAACTATTCAAACCCAGCTGCAATAGTTGCAGAAGCAACA   780
       S  P  N  A  W  M  L  N  Y  S  N  P  A  A  I  V  A  E  A  T

 781  AGAAGATTAAGACCAAATTCGAAAGTTTTAAATATTTGTGATATGCCAATAGGAATAGAA   840
       R  R  L  R  P  N  S  K  V  L  N  I  C  D  M  P  I  G  I  E

 841  GTTAGAATGGCTGAAATTTTAGGACTTGAATCAAGAAAAGATATGGATATTATGTACTAT   900
       V  R  M  A  E  I  L  G  L  E  S  R  K  D  M  D  I  M  Y  Y

 901  GGATTGAATCATTTTGGATGGTGGAAATCAGTAAGAGATAAACAAGGAAATGACTTAATG   960
       G  L  N  H  F  G  W  W  K  S  V  R  D  K  Q  G  N  D  L  M

 961  CCTAAATTAAGAGAACACGTATCTCAATATGGATATGTAGTACCTAAAGGAGATAATCAA  1020
       P  K  L  R  E  H  V  S  Q  Y  G  Y  V  V  P  K  G  D  N  Q

1021  CATACAGAAGCTAGCTGGAATGATACATTTGCTAAGGCTAAAGATGTTCTTGCATTAGAC  1080
       H  T  E  A  S  W  N  D  T  F  A  K  A  K  D  V  L  A  L  D

1081  CCTACAACTTTACCAAATACATATTTAAAATACTACTTATTCCCAGACTATGTAGTTGAA  1140
       P  T  T  L  P  N  T  Y  L  K  Y  Y  L  F  P  D  Y  V  V  E

1141  CATTCAAACAAAGAATACACAAGAGCTAATGAAGTAATGGACGGAAGAGAAAAGTTTGTA  1200
       H  S  N  K  E  Y  T  R  A  N  E  V  M  D  G  R  E  K  F  V

1201  TTTGGAGAATGTGAAAAAGTTGTAAAAAATCAATCTTCAGAAGGATGCGCTTTACATATA  1260
       F  G  E  C  E  K  V  V  K  N  Q  S  S  E  G  C  A  L  H  I

1261  GATGAGCATGCTTCATATATAGTTGACTTAGCTAGAGCAATAGCTTTTAATACTAAAGAA  1320
       D  E  H  A  S  Y  I  V  D  L  A  R  A  I  A  F  N  T  K  E

1321  AAAATGCTATTAATAGTAGAGAATAATGGAGCGATTGTAAACTTTGATTCAACAGCAATG  1380
       K  M  L  L  I  V  E  N  N  G  A  I  V  N  F  D  S  T  A  M

1381  GTAGAAATACCTTGTATTGTAGGAAGTAATGGACCAGAGCCATTAGTAGTTGGAAGAATT  1440
       V  E  I  P  C  I  V  G  S  N  G  P  E  P  L  V  V  G  R  I

1441  CCTCAATTCCAAAAAGGAATGATGGAGCAACAAGTAACAGTTGAAAAGTTAACAGTTGAG  1500
       P  Q  F  Q  K  G  M  M  E  Q  Q  V  T  V  E  K  L  T  V  E

1501  GCATGGATAGAGGGATCTTATCAAAAATTATGGCAAGCAATAACAATGTCTAAAACAGTA  1560
       A  W  I  E  G  S  Y  Q  K  L  W  Q  A  I  T  M  S  K  T  V

1561  CCAAGTGCAAAAGTTGCAAAAGATATTTTAGATGATTTAATTGAAGCTAATAAAGAATAT  1620
       P  S  A  K  V  A  K  D  I  L  D  D  L  I  E  A  N  K  E  Y

1621  TGGCCAGTTTTGAAATAAAAATTATTAAAAAGGGGCTTTAAAGCCCCCTTTTTAATAAAA  1680
       W  P  V  L  K

1681  TATAGGAGAACAGAATGAAAATAGAAATAATTGGATTAATAGCAGCATGTTTAACAACAA  1740

1741  GTTCTTTCTTACCACAAGTTTTAATGGTTATAAAAACGAAAGATACTAAAAGTATTTCTT  1800

1801  TAAAAATGTATAGTATTTTTGTCATAGGAACAGCTCTTTGGATATTTTATGGATTAAGTA  1860

1861  CTAATAATATGCCAATAGTATTAGCTAATAGTATTACAGAAATGTTAGCCTTAATAATAC  1920

1921  TTTATTACAAAGTAAAAGAGAAAAAAATTTAATTATTATTTTTAGTTACTGATAAAAGAT  1980

1981  ATTCAAAAAAAAGAATTGTATTTCCAAAAAAATCATTTTCCTTAAGATTTCTATCATCAA  2040

2041  TGGGATTAGAATCTTTAATTATAAAAGTAAGGTCAGCTAAGTTTTCAAGTGTAGAATTAG  2100

2101  ATGTACCTGTAAATAAGACAATTGGAACATTTCTCAAATTAGCTTTTTCACATAACATGA  2160

2161  TAATTTGAGAAGTTTCTCCAGATTTAGAGACAACTAATAATATAGCTTTTGATGGTATTT  2220

2221  AGGTTCCATAGTTTCCATATATTCAGATCC  2250
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FIG. 3. Nucleotide sequence of *malBH*. The nucleotide sequence of a 2,250-bp *Sau*3AI fragment of *F. mortiferum* DNA is presented. A partial ORF designated *malB* extends from nucleotides 1 to 253 and encodes most of the enzyme IIB component of the PEP-dependent maltose (α-glucoside):PTS. The gene (*malH*) that encodes maltose 6-phosphate hydrolase (6-phospho-α-glucosidase) extends from nucleotides 313 to 1638. Residues deduced by translation of the sequence encoded by *malH* that match those obtained by automated Edman degradation of the purified protein (53) are underlined. A potential ribosomal-binding site preceding *malH* is indicated by double underlining, and a putative ρ-independent terminator following *malH* is shown by arrows above the sequence. The −10 and −35 regions of the putative promoter of *malH* are shaded and boldfaced.

TABLE 2. Binary comparisons of the proteins comprising family 4 of glycosyl hydrolases[a]

Protein	% Identity for segment having the no. of compared residues indicated in parentheses					
	LpID(Bsu) (446)	AgaL(Eco) (451)	CelF(Eco) (372)	CelF(Bsu) (442)	GlvG(Eco) (212)	GlvG(Bsu) (449)
MalH(Fmo) (441)	22(349) [20]	22(443) [27]	26(375) [53]	31(442) [66]	77(207) [137]	74(439) [216]
LpID(Bsu) (446)		25(429) [40]	26(188) [19]	25(370) [27]	26(156) [18]	28(196) [20]
AgaL(Eco) (451)			24(377) [35]	24(459) [41]	25(196) [16]	22(442) [27]
CelF(Eco) (372)				57(368) [129]	35(200) [43]	25(376) [43]
CelF(Bsu) (442)					37(203) [53]	31(446) [64]
GlvG(Eco) (212)						78(204) [95]

[a] Values in parentheses refer to the numbers of residues in the proteins. The FASTA program using the dipeptide identities mode (34) was used to assess similarities of the indicated proteins. Comparison scores in standard deviations using the RDF2 program (34) and 150 shuffles are given in brackets. The abbreviations used, references to the published sequences, and data bank accession numbers are as follows: MalH, maltose 6-phosphate hydrolase of *F. mortiferum* [MalH(Fmo); GenBank accession no. U81185]; LpID, uncharacterized glycosyl hydrolase of *B. subtilis* [LpID(Bsu); SwissProt identifier P39130]; AgaL, α-galactosidase of *E. coli* [AgaL(Eco) (28); SwissProt identifier P06720]; CelF, 6-phospho-β-glucosidase of *E. coli* [CelF(Eco) (33); SwissProt identifier P17411] and *B. subtilis* [CelF(Bsu); SwissProt identifier P46320]; GlvG, a putative 6-phospho-α-glucosidase of *E. coli* [GlvG(Eco) (36); SwissProt identifier P31450] and *B. subtilis* [GlvG(Bsu) (58); GenBank accession no. D50543].

of 372 aa, and a second putative 6-phospho-α-glucosidase in this organism (designated GlvG [36]) comprises 212 residues (Fig. 4). The binary sequence similarities shown in Table 2 and their statistical significance (comparison scores of 16 to 216 standard deviations) establish that the seven proteins are indeed homologous and comprise a single family. Not included in the MalH family of proteins in Table 2 are the following partially sequenced homologs: (i) AgaL of *Salmonella typhimurium* (GenBank accession no. P30877; 43 aa; 93% identity with AgaL of *E. coli*); (ii) α-galactosidase of *Klebsiella pneumoniae* (A44166; 34 aa; 91% identity with AgaL of *E. coli*); and (iii) hypothetical protein of *Clostridium absonum* (A61476; 34 aa; 71% identity with CelF of *B. subtilis*). Previously published in silico analyses suggested that the two GlvG proteins of *E. coli* (36) and *B. subtilis* (58) functioned in the metabolism of α- or β-glucosides. However, the higher sequence similarity of these proteins (74 to 77% identity) with the biochemically characterized maltose 6-phosphate hydrolase of *F. mortiferum* than with the 6-phospho-β-glucosidase (CelF) of *E. coli* or *B. subtilis* (25 to 37% identity) strongly suggests that the two GlvG proteins represent intact or truncated 6-phospho-α-glucosidase(s).

The average similarity plot of MalH and its homologs (not shown) and the multiple alignment presented in Fig. 4 reveal that the N-terminal halves of these proteins exhibit a higher degree of sequence similarity than the C-terminal regions. The N-terminal moiety may therefore be of greatest structural or functional significance, whereas the poorly conserved C-terminal portion may play a regulatory role. The phylogenetic tree for the members of this family is illustrated in Fig. 5. Three clusters that correlate with the binary sequence similarities of these proteins (Table 2) and with their physiological and catalytic functions are discernible: the biochemically characterized phospho-α-glucosidase (MalH) from *F. mortiferum* and the two proposed phospho-α-glucosidases (GlvG) of *E. coli* and *B. subtilis* cluster together; the two phospho-β-glucosidases (CelF; EC 3.2.1.86) of *B. subtilis* and *E. coli* comprise a second cluster; and the third (albeit diffuse) cluster includes the uncharacterized glycosylhydrolase (LpID) of *B. subtilis* and the *melA* gene product (α-galactosidase, AgaL; EC 3.2.1.22) from *E. coli* (28).

GlvG proteins as potential 6-phospho-α-glucosidases. To date, no biochemical studies have been carried out with GlvG from either *E. coli* or *B. subtilis*. Indeed, it is not known whether these genes are actually expressed by these organisms. Translation of the *glvG* gene of *E. coli* predicts a protein of 212 residues which shows 77% identity to the N-terminal half of MalH of *F. mortiferum*. By contrast, GlvG from *B. subtilis* exhibits 74% residue identity with MalH throughout its entire length (Table 2). It occurred to us that GlvG of *E. coli* might represent a truncated phospho-α-glucosidase. To test this hypothesis, plasmids containing *glvG* and a *glvG-malH* hybrid gene were constructed, and the respective gene products were expressed in *E. coli* (Fig. 1). The M_r of the GlvG polypeptide, 24,000, was in excellent agreement with the value of 23,723 calculated from the translated *glvG* sequence (8), and the polypeptide cross-reacted with MalH antiserum (Fig. 1A, lane 6). The hybrid protein comprised the *E. coli* GlvG (residues 1 to 212) linked to the carboxy-terminal portion of MalH (residues 207 to 441). This construct (designated pH4) also yielded an immunologically reactive protein with the expected M_r of ~49,000 (Fig. 1A, lane 5). Cell-free preparations from the two *E. coli* clones were assayed for both phospho-α- and phospho-β-glucosidase activities. However, neither the chromogenic pNPαGlc6P nor pNPβGlc6P analogs were hydrolyzed by the extracts. These data suggest that the GlvG protein is truncated and consequently inactive, whereas the full-length hybrid protein may be unable to fold to yield the secondary or tertiary structures necessary for catalytic activity.

Distribution of *malH* among *Fusobacterium* spp. Genomic DNA was prepared from eight species of fusobacteria. Two *malH*-specific probes (see Materials and Methods) hybridized only to *F. mortiferum* DNA (data not shown). Another oligonucleotide probe derived from the partial sequence of *malB* also hybridized only to DNA from *F. mortiferum* (data not shown). While these results indicate that the genes encoding maltose utilization may be unique to *F. mortiferum*, it is possible that different hybridization stringencies may reveal the presence of homologs in other *Fusobacterium* species.

***malB* encodes a maltose-specific enzyme IIB of the PTS.** The incomplete ORF *malB* (Fig. 3) encodes 83 residues which exhibit 30 to 71% identity (in a 56- to 79-residue overlap) to

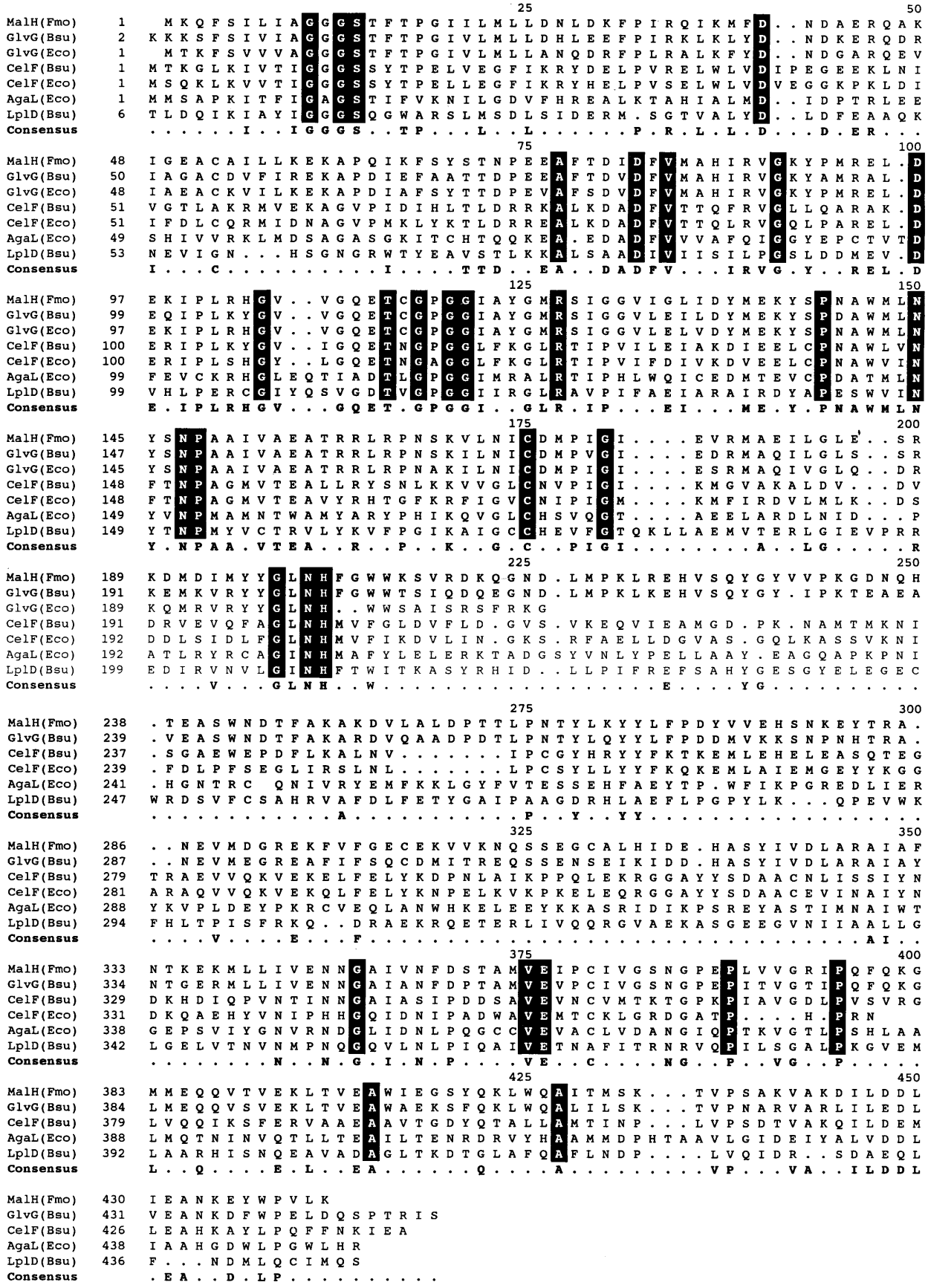

FIG. 4. Multiple alignment of the proteins comprising family 4 of the glycosyl hydrolases. Fully conserved residues are highlighted (black background). The consensus sequence is shown at the bottom of the alignment. Numbers to the left denote residue positions; numbers above the sequences refer to alignment positions and not to any one of the aligned proteins. The abbreviations used and references to the published sequences are given in the footnote to Table 2.

proteins or domains that comprise the IIB^{Glc}-like family of PTS permeases (37). Significantly, MalB exhibits greater sequence similarity with IIB^{Glv} of *B. subtilis* (71% identity in a 73-residue overlap), IIB^{Glv} of *E. coli* (48% identity in a 79-residue overlap), and IIB^{Mal} of *E. coli* (45% identity in a 74-residue overlap) than to all other IIB members of this family. Multiple alignment of the partially sequenced MalB protein (designated IIB^{Mal}) with all 37 currently identified homologs that constitute the IIB^{Glc}-like family is presented in Fig. 6. Except for the IIB^{Glc1} protein of *Mycoplasma genitalium*, the region around the active-site cysteyl residue (alignment position 17 [30]) is strongly conserved in all members of this family, including IIB^{Mal} of *F. mortiferum*. This region was used to derive a signature sequence for this family of IIB^{Glc}-like proteins or domains. The sequence N[LIVMF]X(5)[CT]XT[RQK]L[RI][LIVMF]X(3)[DNQ] proved to be specific to the 38 members of the family when the SwissProt database (version 33) was screened. A phylogenetic tree of this family of IIB proteins or domains was recently published (37). An updated version of this tree including IIB^{Mal} of *F. mortiferum* as well as additional recently sequenced IIB^{Glc}-like domains revealed that IIB^{Mal} of *F. mortiferum* clusters together with the orthologous IIB^{Glv} of *E. coli* and *B. subtilis* (data not shown). It would therefore be reasonable to propose that the proteins encoded in the *glv* operons of these organisms function primarily in the PTS-dependent transport and metabolism of α-glucosides. It remains to be determined whether *malB* encodes merely a IIB protein (as is the case with the orthologous GlvB of *E. coli* [34]) or whether it comprises a polypeptide chain that includes a IIB domain fused to the IIC and/or IIA domains of the PTS. Regardless, the presence of a structural gene (*malB*) encoding a IIB protein (or domain) that lies adjacent to the maltose 6-phosphate hydrolase (*malH*) gene suggests that MalB functions in PTS-catalyzed transport of maltose in *F. mortiferum.*

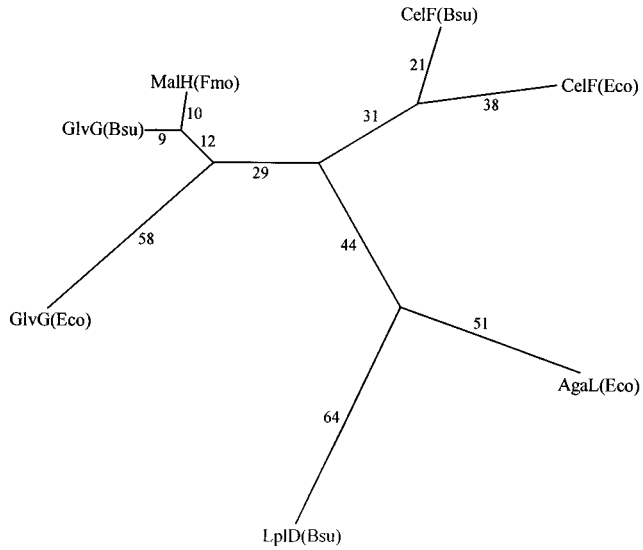

FIG. 5. Phylogenetic tree of the proteins comprising family 4 of glycosyl hydrolases. The tree was constructed as described by Reizer and Reizer (38), using the progressive multiple alignment method of Feng and Doolittle (14). Relative branch lengths (in arbitrary units) are provided adjacent to the branches. The abbreviations used and references to the published sequences are given in the footnote to Table 2. The long branch length of GlvG(Eco) is most likely due to the smaller size of this protein (212 residues) compared to the *B. subtilis* GlvG and the *F. mortiferum* MalH (449 and 441 residues, respectively).

DISCUSSION

In previous studies with *F. mortiferum*, evidence for PEP-dependent:maltose PTS activity was indicated by the finding of maltose 6-phosphate in this anaerobic pathogen (40). Subsequently, an oxygen-sensitive and Me^{2+}-dependent maltose 6-phosphate hydrolase was purified to electrophoretic homogeneity, and the physicochemical properties and substrate specificity of the enzyme were reported (53). We now describe the isolation, cloning, and complete nucleotide sequence of the gene (*malH*) that encodes maltose 6-phosphate hydrolase in *F. mortiferum*. This unique enzyme catalyzes the intracellular hydrolytic cleavage of maltose 6-phosphate and related 6-phospho-α-D-glucosides. By virtue of its broad substrate specificity, maltose 6-phosphate hydrolase may be systematically classified as a 6-phosphoryl-*O*-α-D-glucopyranosyl:6-phospho-glucosylhydrolase (13). It should be noted that Würsch and Koellreutter (56) have previously described maltose 6-phosphate hydrolase activity in *Streptococcus mutans* OMZ 176. However the enzyme was not purified, and we failed to detect maltose 6-phosphate hydrolase activity in maltose-grown cells of this strain. Surprisingly, the putative phospho-α-glucosidase reported by Würsch and Koellreutter has been assigned Enzyme Commission number EC 3.2.1.122 (13).

The orientation of *malH* in pCB 4.11 is directionally opposite that of the *lac* promoter of the vector, and with or without addition of IPTG, catalytically active phospho-α-glucosidase was expressed by *E. coli* XLOLR(pCB 4.11). Presumably an associated promoter is present within the cloned *malBH* fragment from *F. mortiferum*. Nucleotides that may represent the −10 and −35 regions of the putative *malH* promoter are shown in Fig. 3. Two important findings emerged from our growth studies with *E. coli* XLOLR(pCB 4.11). First was the observation that phospho-α-glucosidase was expressed constitutively during growth of the organism in LB medium. However, it has been reported that 1 g of yeast extract contains ~113 mg of trehalose (57), which in LB medium approximates to a concentration of 1.65 mM. Trehalose (1-*O*-α-D-glucopyranosyl-α-D-glucopyranoside) elicits synthesis of maltose 6-phosphate hydrolase in *F. mortiferum* (53), and this α,α-linked diglucoside may serve as the inducer for phospho-α-glucosidase in LB-grown cells of *E. coli* XLOLR(pCB 4.11). Second, and surprisingly, the addition of different sugars to LB broth influenced the amount of phospho-α-glucosidase produced in *E. coli* as was found with *F. mortiferum* (Fig. 2 and reference 53). The mechanism by which these various sugars modulate synthesis of MalH both in the parental *F. mortiferum* strain (53) and in *E. coli* XLOLR(pCB 4.11) is presently unknown.

Multiple alignment of the family 4 glycosylhydrolases (Fig. 4) revealed many conserved amino acids, with prolyl, aspartyl, and glycyl residues being particularly prominent. Members of glucosylhydrolase family 1 (β-glycosidases and phospho-β-glycosidases) contain two conserved glutamyl residues that participate in the hydrolytic cleavage of substrates (for discussion and additional references, see reference 55). By contrast, little is known concerning the active-site residues in proteins that comprise family 4. The sequences GGGS (alignment positions 11 to 14), DADFV (80 to 84), GQET(X)GPGGI (113 to 122), PNAWMLN (144 to 150), and GLNH (209 to 212) are positionally conserved in all members of family 4, and it is perhaps significant that cysteyl and glutamyl residues are present in all family members at positions 175 and 376, respectively.

We believe that the partially sequenced gene (83 residues) encodes most of the IIB protein (domain) that participates in PEP-dependent phosphorylation and translocation of maltose

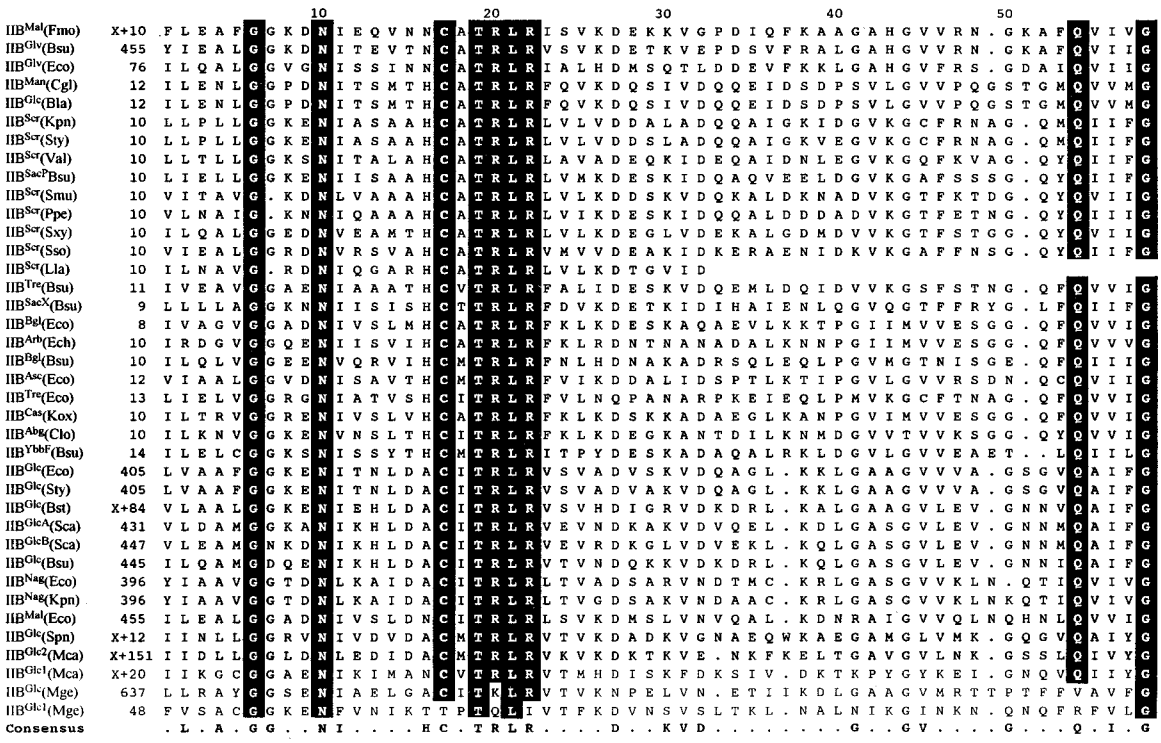

FIG. 6. Multiple alignment of a short region surrounding the catalytic cysteyl residue of the IIB^{Glc}-like proteins (or domains). Fully conserved residues or residues conserved in all members of the IIB^{Glc}-like family except IIB^{Glc1} and IIB^{Glc} of *M. genitalium* are highlighted (black background). Numbers to the left denote residue numbers. The sequences of proteins IIB^{Mal}(Fmo), IIB^{Glc}(Bst), IIB^{Glc}(Spn), IIB^{Glc1}(Mca), and IIB^{Glc2}(Mca) have yet to be completed; for these polypeptides, the first sequenced residue is designated X. Numbers above the sequences refer to alignment positions and not to any one of the aligned proteins. The consensus sequence is shown at the bottom of the alignment. Sugar abbreviations: Abg, aryl-β-glucoside; Arb, arbutin; Asc, arbutin, salicin, and cellobiose; Bgl, β-glucoside; Cas, cellobiose, arbutin, and salicin; Glc, glucose; Glc1, Glc2, and Glv, glucose-like; Nag, *N*-acetylglucosamine; Mal, maltose; Man, mannose; Scr, SacP, and SacX, sucrose; Tre, trehalose; YbbF, IIB^{Scr}-like protein domain of as yet uncharacterized sugar-specific enzyme II. Organism abbreviations: Bsu, *B. subtilis*; Bst, *Bacillus stearothermophilus*; Bla, *Brevibacterium lactofermentum*; Cgl, *Corynebacterium glutamicum*; Clo, *Clostridium longisporum*; Eco, *E. coli*; Ech, *Erwinia chrysanthemi*; Fmo, *Fusobacterium mortiferum*; Kox, *Klebsiella oxytoca*; Kpn, *K. pneumoniae*; Lla, *Lactococcus lactis*; Mca, *Mycoplasma capricolum*; Mge, *M. genitalium*; Ppe, *Pediococcus pentosaceus*; Sca, *Staphylococcus carnosus*; Smu, *Streptococcus mutans*; Spn, *Streptococcus pneumoniae*; Sty, *Salmonella typhimurium*; Sso, *Streptococcus sobrinus*; Sxy, *Staphylococcus xylosus*; Val, *Vibrio alginolyticus*. Accession numbers (the prefixes P or Q, MG, and S, Z, D, U, or L denote accession numbers from SwissProt, the *M. genitalium* databank at The Institute for Genomic Research, and GenBank/EMBL/DDBJ, respectively) are shown at the right.

and other α-glucosides by *F. mortiferum*. These findings provide the first evidence in any bacterium for a genetic linkage between a 6-phospho-α-glucosylhydrolase and maltose-specific PEP-PTS components. Confirmation of the existence of a *mal* operon will require the isolation, transfer, and expression of the *mal* genes in an α-glucoside-negative *Fusobacterium* strain (e.g., *F. nucleatum*). It is our contention that acquisition of the putative *mal* operon will confer upon the recipient the novel capacity to utilize maltose and related α-glucosides as energy sources for growth. Presently, we are attempting to construct the requisite delivery systems for the *mal* genes, using either plasmids or transposons as vectors for interspecies transfer.

Our studies of carbohydrate metabolism by fusobacteria (41, 42, 54), and specifically the discovery of PEP-dependent sugar: PTS activities in *F. mortiferum* (40, 53), raise other important questions. For example, of the ~16 species comprising the genus *Fusobacterium* (1, 5, 6, 9, 32, 48), only *F. mortiferum* has the capacity to utilize such an extraordinarily wide variety of carbohydrates (monosaccharides, disaccharides, and both α- and β-glycosides) as energy sources. One wonders whether *F. mortiferum* has acquired the necessary genetic information for the transport and dissimilation of these carbohydrates from other bacterial species. Alternatively, the genes encoding these metabolic traits may have been deleted from the genomes of other species of fusobacteria, perhaps due to environmental pressures or simply because of the greater availability of amino acids as potential sources of energy. Answers to these queries can provide a rationale for the unique ecological niches occupied by the different species of fusobacteria. Importantly, we may also obtain a new perspective for microbe-host interactions (5, 7, 16) that must contribute to the etiology of periodontitis (6, 50), gingivitis (32), abscess formation (12, 15, 18), ulceration (1), and bacteremia (22, 26) caused by these human pathogens.

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