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Genetic Structure of the *bphG* Gene Encoding 2-Hydroxymuconic Semialdehyde Dehydrogenase of *Achromobacter xylosoxidans* KF701

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2-Hydroxymuconic semialdehyde dehydrogenase catalyzes the conversion of 2-hydroxymuconic semialdehyde (HMS) to an enol form of 4-oxalocrotonate which is a step in the catechol *meta*-cleavage pathway. A *bphG* gene encoding HMS dehydrogenase of *A. xylosoxidans* KF701, a soil bacterium degrading biphenyl, was identified at between catechol 2,3-dioxygenase gene and HMS hydrolase gene, and its sequence was analyzed. An open reading frame (ORF) corresponding to *bphG* gene was consisted of 1461 nucleotides with ATG initiation codon and TGA termination codon. The ORF exhibited 66% of G + C content, and a putative ribosome-binding sequence, AGAGA, was identified at about 10 nucleotides upstream initiation codon of the *bphG* gene. The *bphG* gene can encode a polypeptide of molecular weight 52 kDa containing 486 amino acid residues. A deduced amino acid sequence of HMS dehydrogenase encoded in *bphG* gene from *A. xylosoxidans* KF701 exhibited the highest 94% homology with that of corresponding enzyme encoded in *xylG* from *P. putida* mt-2, 63% to 90% homology with those of other reported HMS dehydrogenases, and 29% to 42% homology with those of betaine aldehyde dehydrogenase, 5-carboxy-HMS dehydrogenase, aldehyde dehydrogenase, indole-3-acetaldehyde dehydrogenase, succinic semialdehyde dehydrogenase, methylmalonate semialdehyde dehydrogenase, and succinylglutamate 5-semialdehyde dehydrogenase. From an alignment of amino acid sequence of HMS dehydrogenase from *A. xylosoxidans* KF701 with other reported dehydrogenases, putative cofactor NAD^+-binding regions and catalytic residues were identified.

Several microorganisms which can degrade biphenyl have been identified. The upper pathway of microbial catabolism of biphenyl to form benzoate was extensively studied, but lower catabolic pathway of the resulting benzoate in biphenyl-degrading strains was not characterized in detail.

The upper pathway of biphenyl catabolism is proceeded by sequential activities of biphenyl dioxygenase, *cis*-dihydroxy-3-phenylcyclohexa-3,5-diene (*cis*-biphenyl dihydrodiol) dehydrogenase, 2,3-dihydroxybiphenyl 1,2-dioxygenase, and 2-hydroxy-6-oxo-6-phenylhexa-2,4-dienoic acid (HOPDA) hydrolase [1–7]. Biphenyl dioxygenase catalyzes the first step of biphenyl catabolism, conversion of biphenyl to *cis*-biphenyl dihydrodiol through incorporation of both atoms of molecular oxygen to 2 and 3 positions of one of the aromatic rings. The dioxygenase attack to biphenyl shares many features of other dioxygenase-catalyzing oxidations of benzene, toluene, and naphthalene to the corresponding *cis*-dihydrodiols [8–10]. Biphenyl dioxygenase is a multicomponent protein of two subunits of iron-sulfur protein, a ferredoxin, and a ferredoxin reductase where ferredoxin and ferredoxin reductase transfer electrons from NADH to iron-sulfur protein with active site for incorporation of molecular oxygen into the aromatic ring [11–15]. The resulting *cis*-biphenyl dihydrodiol is converted to 2,3-dihydroxybiphenyl by a *cis*-biphenyl dihydrodiol dehydrogenase. Subsequently 2,3-dihydroxybiphenyl undergoes aromatic ring fission at *meta* position of the dihydroxylated ring to form the yellow compound HOPDA by 2,3-dihydroxybiphenyl 1,2-dioxygenase. The *meta*-cleavage compound is then catabo-

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Abbreviations used: HMS, 2-hydroxymuconic semialdehyde; *cis*-biphenyl dihydrodiol, *cis*-dihydroxy-3-phenylcyclohexa-3,5-diene; HOPDA, 2-hydroxy-6-oxo-6-phenylhexa-2,4-dienoic acid; *bphG* gene, HMS dehydrogenase gene; ORF, open reading frame; SDS, sodium dodecylsulfate; PAGE, polyacrylamide gel electrophoresis; Ap, ampicillin; Tc, tetracycline; UV, ultraviolet.

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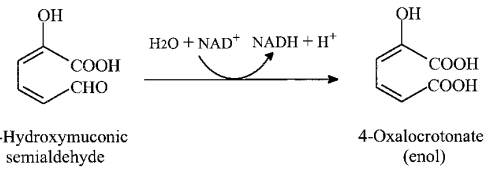

FIG. 1. The chemical reaction catalyzed by HMS dehydrogenase.

lized to form benzoate and 2-oxopent-4-enoate by HOPDA hydrolase.

The 2-oxopent-4-enoate resulting from biphenyl catabolism is degraded to acetyl-CoA by 2-hydroxypent-2,4-dienoate hydratase and acetaldehyde dehydrogenase [15]. However, catabolic pathway of resulting benzoate in biphenyl-degrading bacteria is not characterized well. Recently a gene cluster responsible for benzoate catabolism was identified in a large plasmid pWW110 encoding enzymes responsible for upper pathway of biphenyl catabolism in *Pseudomonas* sp. strain IC [16]. The gene cluster was *bph*XYZLEGFJQKIH where benzoate 1,2-dioxygenase gene (*bphXYZ*) and cis-dihydrodiol dehydrogenase gene (*bphL*) are responsible for conversion of benzoate to catechol, and catechol 2,3-dioxygenase gene (*bphE*), 2-hydroxymuconic semialdehyde (HMS) hydrolase gene (*bphF*), HMS dehydrogenase gene (*bphG*), 4-oxalocrotonate isomerase gene (*bphH*) and decarboxylase gene (*bphI*), 2-hydroxypent-2,4-dienoate hydratase gene (*bphJ*), 4-hydroxy-2-oxovalerate aldolase gene (*bphK*), and acetaldehyde dehydrogenase gene (*bphQ*) are responsible for the catechol *meta*-cleavage pathway [16].

We have characterized catechol 2,3-dioxygenase genes in biphenyl-degrading strains of *Achromobacter xylosoxidans* KF701, *Alcaligenes* sp. KF711 and *P. putida* KF715 [17–20]. The yellow *meta*-cleavage compound HMS produced from catechol by catechol 2,3-dioxygenase is converted to 4-oxalocrotonate (enol form) by HMS dehydrogenase (Fig. 1). In this study, nucleotide sequence of HMS dehydrogenase gene encoded in chromosomal DNA of *A. xylosoxidans* KF701 was determined, and compared with other reported dehydrogenases.

MATERIALS AND METHODS

Strains and plasmids. Bacterial strains and plasmids used and prepared in this study are described in Table 1. *A. xylosoxidans* KF701 was grown in LB medium or MMO medium containing 0.1% biphenyl as the sole carbon and energy source. *E. coli* HB101 and *E. coli* JM101 were used as hosts harboring each of the recombinant plasmids. *E. coli* HB101 was grown in LB medium, and *E. coli* JM101 in 2 × YT medium. For antibiotic selections, ampicillin with 50 μg/ml as a final concentration or tetracycline with 15 μg/ml was supplemented to LB or 2 × YT medium [21]. pBR322 and pUC18 or 19 were used as cloning vectors.

DNA manipulations. Plasmid was isolated by the alkali lysis method [22] or by using a kit from Qiagen. DNA cleavage and ligation were accomplished under standard conditions recommended by the supplier, Boehringer Mannheim. DNA was resolved in 0.7% or 1% agarose gel by electrophoresis, and identified by staining with ethidium bromide followed by UV irradiation [21]. Transformation was accomplished by the calcium chloride method [21]. Nucleotide sequencing was carried out by using an Applied Biosystems DNA Sequencer (Pharmacia Biotech) after dideoxy-chain termination reaction with a kit from the same company. Nucleotide sequences obtained were analyzed by using softwares of DNASIS, PROSIS, and Clustal V.

Assay of HMS dehydrogenase activity. Crude cell lysate was used as the enzyme source. *E. coli* HB101 harboring each of recombinant plasmids was grown in LB medium supplemented with ampicillin or tetracycline to a log phase, harvested by centrifugation at 6,500

TABLE 1

Bacterial Strains and Plasmids Used and Prepared in This Study

Strains and plasmids	Description
Strains	
E. coli HB101	*supE*44 *hsdS*58 ($r_B^- m_B^-$) *recA*13 *ara*-14 *proA*2 *lacY*1 *galK*2 *rpsL*20 *xyl*-5 *mtl*-1
E. coli JM101	*supE thi D* (*lac-proAB*) F′ (*traD*36 *proAB*$^+$*lacI*^{q} *lacZ*ΔM15)
Achromobater xylosoxidans KF701	A soil bacterium that can grow in biphenyl as the sole carbon and energy source
Plasmids	
pBR322	Cloning vector, Ap^r and Tc^r
pUC18 or 19	Cloning vector, Ap^r
pCNU202	A 10-kb *Bam*HI fragment from chromosomal DNA of the KF701 inserted into the same site of pUC18, Ap^r
pCNU203	A 3.1-kb *Kpn*I fragment of pCNU202 inserted into the same site of pUC18, Ap^r
pCNU204	A 6.9-kb *Bam*HI-*Kpn*I fragment of pCNU202 inserted into the same sites of pUC18, Ap^r
pCNU205	An 1.8-kb *Bam*HI-*Xho*I fragment of pCNU202 inserted into the *Bam*HI and *Sal*I sites of pUC18, Ap^r
pCNU209	An 1.4-kb *Xho*I-*Kpn*I fragment of pCNU202 inserted into the *Sal*I and *Kpn*I sites of pUC19, Ap^r
pCNU218	A 4.7-kb *Eco*RI fragment of pCNU202 inserted into the same site of pBR322, Ap^r and Tc^r
pCNU251	An 8.4-kb fragment of pCNU218, a deletion derivative of pCNU218 lacking a *Sma*I-*Stu*I fragment, Ap^r and Tc^r
pCNU252	A 9.0-kb *Bam*HI fragment of pCNU218, a deletion derivative of pCNU218 lacking a *Bam*HI fragment, Ap^r
pCNU253	A 2.6-kb *Stu*I-*Hin*dIII fragment of pCNU203 inserted into the same sites of pUC18, Ap^r

Note. Antibiotic resistance to ampicillin (Ap^r) or tetracycline (Tc^r).

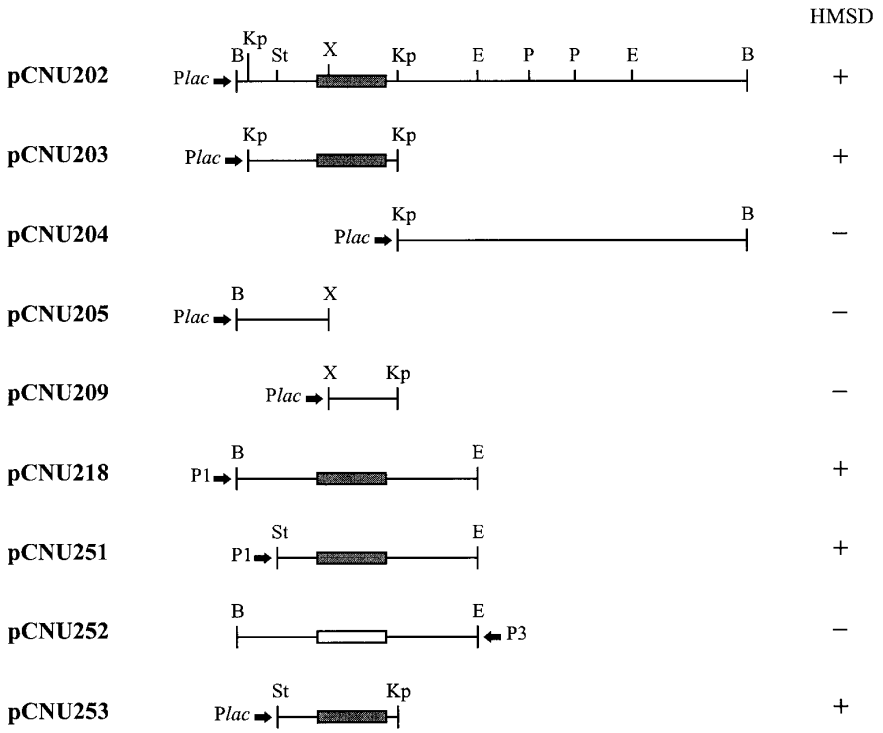

FIG. 2. Physical maps of pCNU202 and its subclones. The insert DNAs from chromosomal DNA of *A. xylosoxidans* KF701 are only shown. Restriction endonucleases are *Bam*HI (B), *Eco*RI (E), *Kpn*I (Kp), *Pst*I (P), and *Xho*I (X). Orientations of the *lac* promoter (*Plac*) in pUC18 or pUC19, and P1 promoter and P3 promoter in pBR322 are indicated by an arrow. The *bphG* gene encoding HMS dehydrogenase is located within a box. Enzyme activity of HMS dehydrogenase (HMSD) was (+) or was not (−) shown.

× g for 10 min, and washed once with 100 mM Tris-HCl (pH 8.5). The bacterial pellet was resuspended in the same buffer, disrupted by sonication, and centrifuged at 12,300 × g for 1 hr to obtain a supernatant. As the enzyme activity, disappearance of substrate HMS was monitored by measuring absorbance change at wavelength 375 nm. The enzyme reaction was carried out at 25°C in 100 mM Tris-HCl (pH 8.5) containing 33 μM HMS and 1 mM NAD^+ as described previously [23].

RESULTS AND DISCUSSION

Recombinant plasmid pCNU202 was isolated from a genomic library of *A. xylosoxidans* KF701, and nucleotide sequence of catechol 2,3-dioxygenase gene in the recombinant plasmid was analyzed in our previous works [20,24]. In this study, a *bphG* gene encoding HMS dehydrogenase was identified at downstream of the catechol 2,3-dioxygenase gene contained in pCNU202, and its nucleotide sequence was analyzed.

Localization of bphG gene. To localize the *bphG* gene encoding HMS dehydrogenase of *A. xylosoxidans* KF701, several subclones of pCNU202 were constructed as described in Table 1, and their physical maps are shown in Fig. 2. Crude lysates prepared from *E. coli* HB101 harboring pCNU202, pCNU203, pCNU218, pCNU251 or pCNU253 exhibited HMS dehydrogenase activity but those from *E. coli* HB101 harboring pCNU204, pCNU205, pCNU209 or pCNU252 did not (Fig. 2). The smallest recombinant plasmid expressing functional HMS dehydrogenase was pCNU253, and thus *bphG* gene was localized within the 2.6-kb *Stu*I-*Kpn*I fragment. pCNU209 contained an 1.4-kb *Xho*I-*Kpn*I fragment from chromosomal DNA of *A. xylosoxidans* KF701, and *E. coli* HB101 harboring the recombinant plasmid did not show HMS dehydrogenase activity. Thus, the *Xho*I site seemed to be located within the *bphG* gene, which was confirmed by sequence analysis of the gene. Both pCNU218 and pCNU252 contained the same 4.6-kb *Bam*HI-*Eco*RI fragment from *A. xylosoxidans* KF701 with P1 promoter or P3 promoter from pBR322 in opposite direction, respectively. *E. coli* HB101 harboring pCNU218 did show HMS dehydrogenase activity but that harboring pCNU252 did not. Therefore, a promoter responsible for expression of *bphG* gene in *A. xylosoxidans* KF701 would not be contained in pCNU202 or might not be recognized by *E. coli* RNA polymerase.

Nucleotide sequence of bphG gene. An open reading frame (ORF) corresponding to *bphG* gene encoding HMS dehydrogenase of *A. xylosoxidans* KF701 was identified by sequencing the 2.6-kb *Stu*I-*Kpn*I fragment of pCNU253. Nucleotide sequence and deduced amino acid sequence of the ORF were analyzed as shown in Fig. 3. The ORF corresponding to *bphG* gene was consisted of 1461 nucleotides with ATG initiation codon and TGA

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-150 gag att  aca act  acc cgg acc aca aac cgg tga cct gga cca ctg acc agc tgg gca agg cga tct  ttt  acc acg
-75  acc gca ttc  tca  acg aac gat tca tga ccg tgc tga cct gaA GGC CCG GTT CGA CTT ATT GCA GAG ATT GCG CAG
                                                                                    RBS
1    ATG AAA GAA ATC AAG CAT TTC ATT AAC GGT GCC TTC GTC GGT TCG GCC AGC GGC AAG CTG TTC GAC AAT GTC AGC
1    Met Lys Glu Ile  Lys His Phe Ile  Asn Gly Ala Phe Val Gly Ser Ala Ser Gly Lys Leu Phe Asp Asn Val Ser
76   CCG GCC AAC GGC CAG GTG ATC GGC CGC GTC CAC GAG GCA GGC CGC GCC GAG GTC GAC GCT GCG GTC AAA GCT GCC
26   Pro Ala Asn Gly Gln Val Ile  Gly Arg Val His Glu Ala Gly Arg Ala Glu Val Asp Ala Ala Val Lys Ala Ala
151  CGT GCC GCG CTC AAG GGA CCC TGG GGG AAG ATG ACG GTG GCC GAG CGC GCT GAG ATT CTG CAT CGC GTG GCC GAT
51   Arg Ala Ala Leu Lys Gly Pro Trp Gly Lys Met Thr Val Ala Glu Arg Ala Glu Ile  Leu His Arg Val Ala Asp
226  GGC ATC ACG GCG CGC TTC GAC GAG TTT CTC GAG GCC GAA TGC CTC GAC ACC GGC AAG CCC AAA TCT CTG GCC AGC
76   Gly Ile  Thr Ala Arg Phe Asp Glu Phe Leu Glu Ala Glu Cys Leu Asp Thr Gly Lys Pro Lys Ser Leu Ala Ser
301  CAC ATC GAC ATT CCG CGC GGC GCG GCC AAT TTC AAG GTG TTC GCC GAC CTG CTC AAG AAT GTT GCC AAT GAA ACC
101  His Ile  Asp Ile  Pro Arg Gly Ala Ala Asn Phe Lys Val Phe Ala Asp Leu Leu Lys Asn Val Ala Asn Glu Thr
376  TTC GAG ATG GCC ACC CCG GAC GGC GCC GGT GCA CTC AAC TAC GGA GTG CGC CGG CCC AAG GGG GTG ATC GGG GTG
126  Phe Glu Met Ala Thr Pro Asp Gly Ala Gly Ala Leu Asn Tyr Gly Val Arg Arg Pro Lys Gly Val Ile  Gly Val
451  ATC AGC CCG TGG AAC CTG CCG CTG CTG CTG ATG ACC TGG AAA GTC GGC CCG GCC CTG GCC TGC GGC AAC TGC GTG
151  Ile  Ser Pro Trp Asn Leu Pro Leu Leu Leu Met Thr Trp Lys Val Gly Pro Ala Leu Ala Cys Gly Asn Cys Val
526  GTG GTC AAA CCA TCC GAG GAA ACC CCG CTG ACC GCC ACC CTG CTC GGC GAG GTG ATG CAG GCC GCC GGT GTG CCG
176  Val Val Lys Pro Ser Glu Glu Thr Pro Leu Thr Ala Thr Leu Leu Gly Glu Val Met Gln Ala Ala Gly Val Pro
601  GCC GGC GTG TAC AAC GTG GTG CAC GGT TTC GGC GGC GAT TCG GCC GGG GCC TTC CTC ACC GAG CAC CCG GAC GTC
201  Ala Gly Val Tyr Asn Val Val His Gly Phe Gly Gly Asp Ser Ala Gly Ala Phe Leu Thr Glu His Pro Asp Val
676  GAC GCC TAC ACC TTC ACC GGC GAG ACC GGC ACC GGC GAA ACC ATC ATG CGC GCC GCG GCC AAG GGC GTG CGC CAG
226  Asp Ala Tyr Thr Phe Thr Gly Glu Thr Gly Thr Gly Glu Thr Ile  Met Arg Ala Ala Ala Lys Gly Val Arg Gln
751  GTG TCG CTG GAG CTG GGC GGC AAG AAC GCC GGC ATC GTC TTC GCC GAC TGC GAT ATG GAC AAG GCC ATC GAG GGC
251  Val Ser Leu Glu Leu Gly Gly Lys Asn Ala Gly Ile  Val Phe Ala Asp Cys Asp Met Asp Lys Ala Ile  Glu Gly
826  ACC CTG CGC TCG GCC TTC GCC AAC TGC GGC CAG GTC TGC CTG GGC ACC GAG CGG GTG TAT GTC GAG CGG CCG ATC
276  Thr Leu Arg Ser Ala Phe Ala Asn Cys Gly Gln Val Cys Leu Gly Thr Glu Arg Val Tyr Val Glu Arg Pro Ile
901  TTC GAC GCG TTC GTC GCC CGC CTG AAG GCC GGC GCC GAA GCG TTG AAG ATC GGC GAA CCG AAC GAT CCA GAG GCC
301  Phe Asp Ala Phe Val Ala Arg Leu Lys Ala Gly Ala Glu Ala Leu Lys Ile  Gly Glu Pro Asn Asp Pro Glu Ala
976  AAT TTC GGC CCG CTG ATC AGC CAT AAG CAC CGT GAA AAA GTC CTC AGT TAC TAC CAG CAG GCA GTC GAC GAC GGC
326  Asn Phe Gly Pro Leu Ile  Ser His Lys His Arg Glu Lys Val Leu Ser Tyr Tyr Gln Gln Ala Val Asp Asp Gly
1051 GCC ACC GTT GTC ACC GGC GGC GGC GTG CCG GAG ATG CCG GCG CAC CTG GCC GGC GGC GCC TGG GTG CAG CCG ACC
351  Ala Thr Val Val Thr Gly Gly Gly Val Pro Glu Met Pro Ala His Leu Ala Gly Gly Ala Trp Val Gln Pro Thr
1126 ATC TGG ACC GGC TTG GCC GAC GAT TCG GCG GTG GTC ACC GAG GAA ATC TTC GGC CCC TGC TGC CAT ATC CGC CCG
376  Ile  Trp Thr Gly Leu Ala Asp Asp Ser Ala Val Val Thr Glu Glu Ile  Phe Gly Pro Cys Cys His Ile  Arg Pro
1201 TTC GAC AGC GAG GAG GAA GCC ATT GAA CTG GCC AAC AGC CTG CCT TAC GGC CTG GCC TCG GCG ATC TGG ACC GAG
401  Phe Asp Ser Glu Glu Glu Ala Ile  Glu Leu Ala Asn Ser Leu Pro Tyr Gly Leu Ala Ser Ala Ile  Trp Thr Glu
1276 AAC GCT TCG CGC GCC CAC CGC GTC GCC GGG CAG ATT GAG GCC GGC ATC GTC TGG GTC AAC AGC TGG TTC CTG CGC
426  Asn Ala Ser Arg Ala His Arg Val Ala Gly Gln Ile  Glu Ala Gly Ile  Val Trp Val Asn Ser Trp Phe Leu Arg
1351 GAC CTG CGC ACT GCC TTC GGC GGC AGC AAG CAG TCG GGC ATC GGG CGC GAA GGG GGT GTG CAC TCG CTG GAG TTC
451  Asp Leu Arg Thr Ala Phe Gly Gly Ser Lys Gln Ser Gly Ile  Gly Arg Glu Gly Gly Val His Ser Leu Glu Phe
1426 TAC ACC GAG CTG AAA AAC ATC TGT GTG AAA CTC TGA GGA CCT GGT Cat gaa cgc acc gca gca aag ccc tga aat
476  Tyr Thr Glu Leu Lys Asn Ile  Cys Val Lys Leu ***
1501 cgg tcg cga aat cct cgc cgc cgg cta ccg cac caa cct gca tga tca ggg cga agg ctt ccc agt cct gct gat
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FIG. 3. Nucleotide sequence and deduced amino acid sequence of HMS dehydrogenase from *A. xylosoxidans* KF701. An ORF corresponding to *bphG* gene encoding HMS dehydrogenase is shown at position 1 to 1461, where a termination codon is indicated by ***. A 3′-terminal sequence of catechol 2,3-dioxygenase gene at upstream the *bphG* gene and a 5′-terminal sequence of HMS hydrolase gene at downstream the *bphG* gene are shown by lower case letters. A putative ribosome-binding sequence (RBS) is underlined.

termination codon. The *bphG* gene exhibited 66% of G + C content, and its codon usage preferred G or C in the wobble positions. The G + C content of *bphG* gene is significantly lower than 58% of G + C content of catechol 2,3-dioxygenase gene from the same bacterial strain [20]. A putative ribosome-binding sequence, AGAGA, was identified at about 10 nucleotides upstream initiation codon of the *bphG* gene. The ORF corresponding to *bphG* gene can encode a polypeptide of molecular weight 52 kDa containing 486 amino acid residues. The predicted HMS dehydrogenase of *A. xylosoxidans* KF701 is similar in size with corresponding enzyme encoded in *xylG* gene of *P. putida* mt-2 whose size was identified as 57 kDa by SDS-PAGE [23,25]. The ORF corresponding to *bphG* gene of *A. xylosoxidans* KF701 was located at between catechol 2,3-dioxygenase gene and HMS hydrolase gene, where catechol 2,3-dioxygenase gene was located at upstream the *bphG* gene and HMS hydrolase gene was at downstream.

Sequence comparison of HMS dehydrogenase with other corresponding enzymes. Nucleotide and amino acid sequences of HMS dehydrogenase from *A. xylosoxidans* KF701 exhibited the highest homology, 98% identity at nucleotide level and 94% identity at amino acid

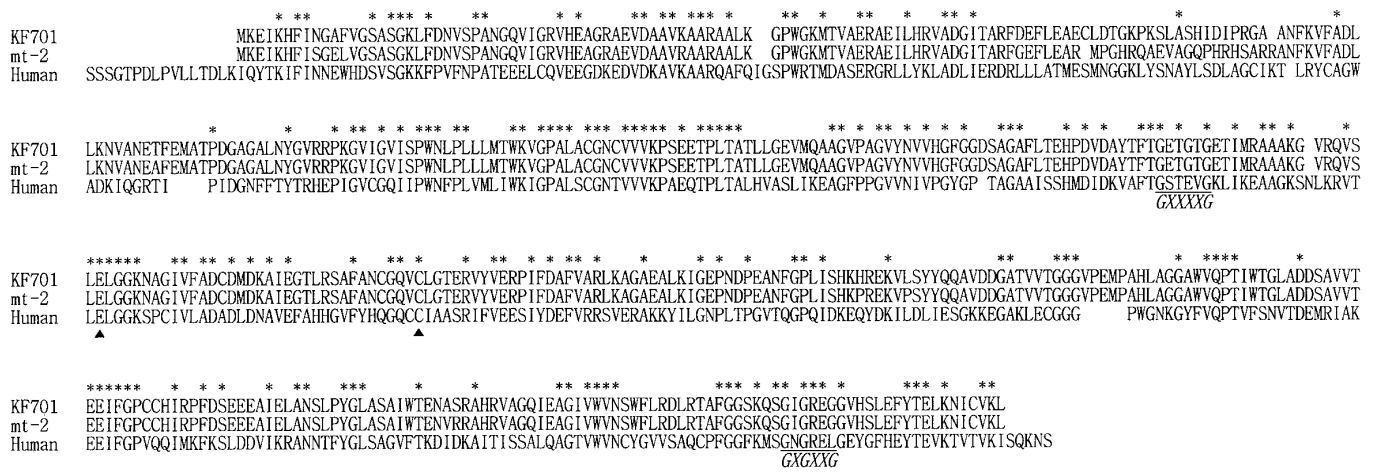

FIG. 4. Amino acid alignment of HMS dehydrogenases and aldehyde dehydrogenase. Enzymes are HMS dehydrogenases from *A. xylosoxidans* KF701 (KF701) and *P. putida* mt-2 (mt-2), and aldehyde dehydrogenase from human mitochondria (Human). Identical residues among the dehydrogenases are indicated by an asterisk, and catalytically important residues in aldehyde dehydrogenase by an solid triangle. Cofactor NAD^+-binding regions in the dehydrogenases are underlined.

level with those of corresponding enzyme encoded in *xylG* gene of *P. putida* mt-2 (Table 2) [26]. The amino acid sequence of HMS dehydrogenase from *A. xylosoxidans* KF701 exhibited 63% to 90% identity with those of other reported HMS dehydrogenases, and significant homology, 29% to 42% identity with those of betaine aldehyde dehydrogenase, 5-carboxy-HMS dehydrogenase, aldehyde dehydrogenase, indole-3-acetaldehyde dehydrogenase, succinic semialdehyde dehydrogenase, methylmalonate semialdehyde dehydrogenase, and succinylglutamate semialdehyde dehydrogenase (Table 2).

Both HMS dehydrogenase and aldehyde dehydrogenase require NAD^+ as a cofactor, and catalyze similar chemical reactions. Catalytically important residues in aldehyde dehydrogenase from human mitochondria have been studied extensively [27–29]. Amino acid sequence of HMS dehydrogenase from *A. xylosoxidans* KF701 exhibited 37% identity with that of aldehyde dehydrogenase from human mitochondria. Even though low but significant sequence homology between HMS dehydrogenase and aldehyde dehydrogenase was shown, amino acid sequence of HMS dehydrogenase from *A. xylosoxidans* KF701 was well aligned with those of HMS dehydrogenases from *P. putida* mt-2 and of aldehyde dehydrogenase from human mitochondria (Fig. 4). Putative NAD^+-binding $\beta\alpha\beta$-fold fingerprint regions in HMS dehydrogenase from *A. xylosoxidans* KF701 were identified as $G^{232}XXXXG^{237}$ and $G^{463}XGXXG^{469}$ from the sequence alignment. In aldehyde dehydrogenase from human mitochondria, Cys^{302} is known to function in catalysis as a nucleophile and Glu^{268} as a general base necessary to activate Cys^{302} [27,28]. The corresponding Cys and Glu residues are well conserved in HMS dehydrogenases from *A. xylosoxidans* KF701 and *P. putida* mt-2. Importance of the conserved amino acid residues identified as putative catalytic residues and cofactor-binding regions in HMS dehydrogenase from *A. xylosoxidans* KF701 will be elucidated by site-directed mutagenesis in a future study.

TABLE 2

Sequence Homology of HMS Dehydrogenase from *A. xylosoxidans* KF701 with Other Reported Dehydrogenases

Strain (*gene*)	Identity (%) aa	Identity (%) nt	GenBank #
P. putida mt-2 (*xylG*)	94	98	PWWXYL
P. putida CF600 (*dmpC*)	90	85	PPDMPCD
Acinetobacter sp. YAA (*atdC*)	83	77	AB008831
Sphingomonas sp. HV3 (*cmpC*)	67	69	SSPZ84817
Pseudomonas sp. DJ77 (*phnG*)	63	67	AFD23839
E. coli K-12 (*betB*)	42	55	ECOBET
E. coli ATCC 11105 (*hpaE*)	41	54	EC4HPADNA
Emericella nidulans (*aldA*)	41	52	EMEALDA
Xantobacter autotrophicus GJ10 (*aldB*)	40	57	AF029734
Sinorhizobium meliloti 102F34 (*betB*)	40	56	RMU39940
Ustilago maydis FB1 (*iad1*)	40	53	UMU74468
Alcaligenes eutrophus H16 (*acoD*)	39	58	AFAACOD
Pichia angusta (*ALD*)	39	53	PAU40996
E. coli K-12 (*gabD*)	38	52	ECOSUSED
Streptomyces coelicolor A3 (*msdA*)	33	56	STMMSDA
P. aeruginosa PAO1 (*aruD*)	29	54	AF011922

Note. Homology of deduced amino acid sequence (aa) and nucleotide sequence (nt) of HMS dehydrogenase from *A. xylosoxidans* KF701 with those of other reported dehydrogenases is expressed as % of identity. Enzymes are HMS dehydrogenase encoded in *xylG, dmpC, atdC, cmpC* or *phnG,* betaine aldehyde dehydrogenase encoded in *betB,* 5-carboxy-HMS dehydrogenase encoded in *hpaE,* aldehyde dehydrogenase encoded in *aldA, aldB, ALD* or *acoD,* indole-3-acetaldehyde dehydrogenase encoded in *iad1,* succinic semialdehyde dehydrogenase encoded in *gabD,* methylmalonate semialdehyde dehydrogenase encoded in *msdA,* and succinylglutamate semialdehyde dehydrogenase encoded in *aruD.*

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