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Kinetic Property and Phylogenic Relationship of 2-Hydroxy-muconic Semialdehyde Dehydrogenase Encoded in *tomC* Gene of *Burkholderia cepacia* G4

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2-Hydroxymuconic semialdehyde (2-HMS) dehydrogenase catalyzes the conversion of 2-HMS to 4-oxalocrotonate, which is a step in the *meta* cleavage pathway of aromatic hydrocarbons in bacteria. A *tomC* gene that encodes 2-HMS dehydrogenase of *Burkholderia cepacia* G4, a soil bacterium that can grow on toluene, cresol, phenol, or benzene, was overexpressed into *E. coli* HB101, and its gene product was characterized in this study. 2-HMS dehydrogenase from *B. cepacia* G4 has a high catalytic efficiency in terms of V_{max}/K_m towards 2-hydroxy-5-methyl-muconic semialdehyde followed by 2-HMS but has a very low efficiency for 5-chloro-2-hydroxymuconic semialdehyde. However, the enzyme did not utilize 2-hydroxy-6-oxo-hepta-2,4-dienoic acid and 2-hydroxy-6-oxo-6-phenylhexa-2,4-dienoic acid as substrates. The molecular weight of 2-HMS dehydrogenase from *B. cepacia* G4 was predicted to be 52 kDa containing 485 amino acid residues from the nucleotide sequence of the *tomC* gene, and it exhibited the highest identity of 78% with the amino acid sequence of 2-HMS dehydrogenase that is encoded in the *aphC* gene of *Comamonas testosteroni* TA441. 2-HMS dehydrogenase from *B. cepacia* G4 showed a significant phylogenetic relationship not only with other 2-HMS dehydrogenases, but also with different dehydrogenases from evolutionarily distant organisms.

Key words: *Burkholderia cepacia* G4, 2-Hydroxymuconic semialdehyde dehydrogenase, Kinetic parameter, Phylogenetic relationship

INTRODUCTION

Burkholderia cepacia G4, formerly known as *Pseudomomas cepacia*, is known to grow in toluene, cresol, phenol, or benzene by utilizing these organic molecules as the sole carbon and energy source (Nelson *et al.*, 1986). There are five unique bacterial pathways that result in the oxygenase-catalyzed hydroxylation of toluene. One route, which is encoded by a catabolic TOL plasmid, involves the oxidation of toluene through benzyl alcohol to catechol (Worsey and Williams, 1975). The other routes initiate toluene oxidation through the hydroxylation of aromatic carbons by toluene dioxygenase or monooxygenase. Toluene 2,3-dioxygenase from *P. putida* F1 produces *cis*-toluene-2,3-dihydrodiol from toluene (Gibson *et al.*, 1970). Toluene monooxygenases hydroxylate the aromatic carbons at the *ortho*-, *meta*-, or *para*-position to form the corresponding cresols; toluene *ortho*-monooxygenase from *B. cepacia* G4 (Shields *et al.*, 1995), toluene *meta*-monooxygenase from *P. pikettii* PKO1 (Kukor and Olsen, 1991), and toluene *para*-monooxygenase from *P. mendocina* KR1 (Whited and Gibson, 1991).

The toluene monooxygenase from *B. cepacia* G4 has a broad range of substrate specificity. Thus, it hydroxylates toluene at the *ortho*-position, and the same enzyme oxidizes cresol, phenol, and benzene in order to form catecholic compounds (Shields *et al.*, 1995). The toluene monooxygenase from *B. cepacia* G4 is also known to play an important role in the oxidation of trichloroethylene, the most frequently encountered priority pollutant of ground waters (Folson and Chapman, 1991). In *B. cepacia* G4, catecholic compounds that were metabolized from the

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aromatic hydrocarbons are completely oxidized to TCA cycle intermediates through the *meta* cleavage pathway (Oh *et al.*, 1997).

Catabolic genes that are responsible for the degradation of aromatic hydrocarbons, including toluene, are known to be encoded in a self-transmissible TOM plasmid, which is native to *B. cepacia* G4 (Shields *et al.*, 1995). In this study, the catabolic genes encoded in the TOM plasmid were cloned as a gene cluster, and each of the genes was localized. The kinetic property and phylogenetic relationship of 2-hydroxymuconic semialdehyde (2-HMS) dehydrogenase, an enzyme catalyzing the conversion of 2-HMS to 4-oxalocrotonate in the *meta* cleavage pathway of aromatic hydrocarbon catabolism, were also determined.

MATERIALS AND METHODS

Bacterial strains, plasmids, and culture conditions

Bacterial strains and plasmids that were used and prepared in this study are described in Table I. *Escherichia coli* HB101 as the recipient strain of the recombinant plasmid was grown in Luria Bertani (LB) medium. For the selection of an antibiotic, ampicillin at a final concentration of 50 μg/mL was supplemented. *B. cepacia* G4 was grown in either LB medium or MMO medium containing 0.1% toluene as the sole carbon and energy source. As for cloning vectors, pBR322 and pUC18 or 19 were used.

Spectrophotometric assay of 2-HMS dehydrogenase activity

E. coli HB101 that harbors pCNU374, which is a recombinant plasmid expressing 2-HMS dehydrogenase from *B. cepacia* G4, was grown in LB medium supplemented with 50 μg/mL of ampicillin to a log phase. The cells were harvested by centrifugation at 6,500×g for 10 min and were washed twice with 100 mM Tris-HCl (pH 8.5). The bacterial pellet was resuspended in the same buffer, and then, it was subjected to sonication followed by centrifugation at 12,300×g for 1 h in order to obtain a supernatant as the enzyme source. *Meta*-cleavage products (MCPs), such as 2-HMS, 2-hydroxy-6-oxo-hepta-2,4-dienoic acid (HODA), 2-hydroxy-5-methylmuconic semialdehyde (HMMS), and 5-chloro-2-hydroxymuconic semialdehyde (CHMS), were prepared from catechol, 3-methylcatechol, 4-methylcatechol, and 4-chlorocatechol, respectively, by using catechol 2,3-dioxygenase from *B. cepacia* G4 encoded in the recombinant plasmid, pCNU368. 2-Hydroxy-6-oxo-6-phenylhexa-2,4-dienoic acid (HPDA) was prepared from 2,3-dihydroxybiphenyl by using 2,3-dihydroxybiphenyl 1,2-dioxygenase from *P. putida* KF715 encoded in the recombinant plasmid, pKF6245 (Hayase *et al.*, 1990).

The 2-HMS dehydrogenase reaction was carried out at 25 °C, and the enzyme source was mixed with 100 mM Tris-HCl (pH 8.5) containing 33 μM 2-HMS or its analogs as the MCP substrate and 1 mM NAD^+ as a coenzyme (Harayama *et al.*, 1984). One unit of enzyme activity was defined as an initial rate of 1 μmole of MCP substrate disappearing per minute. The extinction coefficient (e) was taken for the respective MCP substrate and is as follows; 2-HMS (36,000 $cm^{-1}M^{-1}$ at a wavelength of 375 nm), HODA (15,000 $cm^{-1}M^{-1}$ at 388 nm), HMMS (31,500 $cm^{-1}M^{-1}$ at 382 nm), CHMS (39,600 $cm^{-1}M^{-1}$ at 379 nm), or HPDA (22,000 $cm^{-1}M^{-1}$ at 434 nm) (Oh *et al.*, 1997). The specific activity of 2-HMS dehydrogenase was defined as units per mg of protein. The protein concentration was determined by using the Bradford method.

Measurement of kinetic parameters of 2-HMS dehydrogenase

Michaelis-Menten K_m and V_{max} values of the enzyme were determined using double reciprocal plots (the inverted velocity of the reaction vs. the inverted substrate concentration) in standard assay conditions. The kinetic parameters towards each MCP of 2-HMS, HMMS, or CHMS were measured in the fixed concentration of 1 μM NAD^+ and those towards NAD^+ were measured in the fixed concentration of 33 μM each MCP.

DNA manipulations

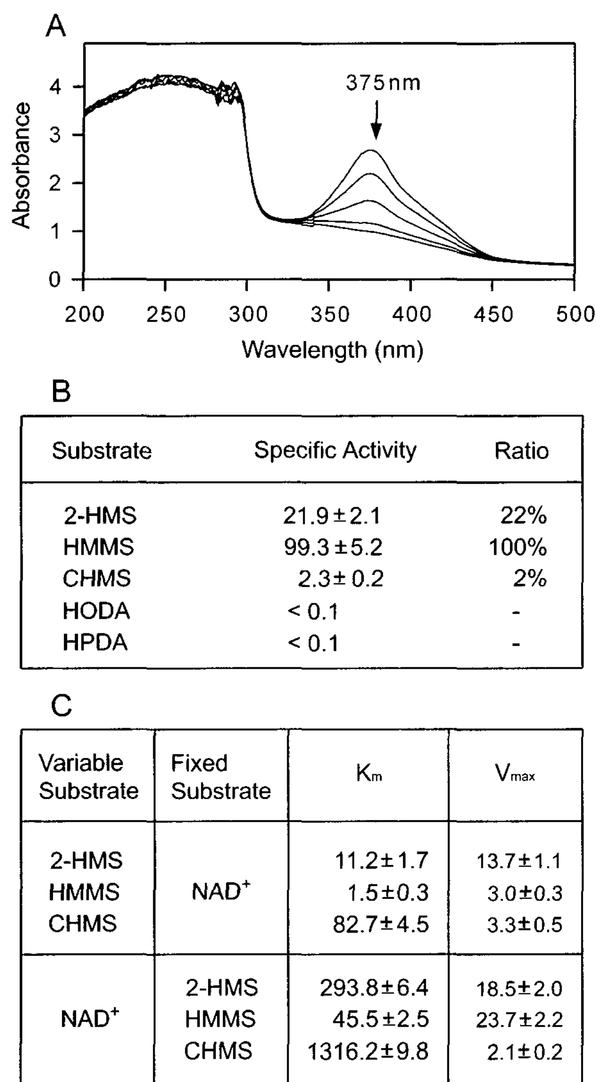
Plasmids were isolated using the alkali lysis method or by using a kit from Bioneer. DNA cleavage and ligation were accomplished under standard conditions, which were recommended by the supplier, Boehringer Mannheim. The DNA was resolved in 0.7% or 1% agarose gel by electrophoresis, and it was identified by staining with ethidium bromide followed by UV irradiation. DNA transformation was accomplished by using the calcium chloride method. The nucleotide sequences were analyzed by using softwares of DNASIS, PROSIS, and Clustal X. The nucleotide sequence corresponding to the *tomC* gene in this study was submitted to NCBI GenBank with the access no. being AY278205.

RESULTS AND DISCUSSION

Substrate specificity of 2-HMS dehydrogenase encoded in *tomC* gene

Gradual time-dependent disappearance of MCPs through the use of the enzyme has been demonstrated by scanning the absorbance change at wavelengths ranging from 200 nm to 500 nm. 2-HMS dehydrogenase from *B. cepacia* G4 decreased the absorbance at wavelength 375 nm, which was shown by 2-HMS in the presence of NAD^+ as a coenzyme (Fig. 1A). However, the enzyme did not metabolize 2-HMS in the absence of NAD^+. The specific activity of the 2-HMS dehydrogenase against 2-HMS was 21.9

units per mg of protein (Fig. 1B). The enzyme showed about a 5-fold higher specific activity against HMMS than 2-HMS as the MCP substrate but illustrated a low specific activity (2.3 units per mg of protein) against CHMS. However, 2-HMS dehydrogenase from *B. cepacia* G4 did not metabolize HODA and HPDA at all. In the chemical structure, MCPs of 2-HMS, HMMS, and CHMS contain an aldehyde group at position 6, but HODA or HPDA contains an oxo carbon substituted with a methyl or a phenyl group at the same position. Thus, it is evident that only MCPs with an aldehyde group at the terminal carbon can be served as substrates of 2-HMS dehydrogenase encoded in the *tomC* gene of *B. cepacia* G4.



Substrate	Specific Activity	Ratio
2-HMS	21.9±2.1	22%
HMMS	99.3±5.2	100%
CHMS	2.3±0.2	2%
HODA	< 0.1	-
HPDA	< 0.1	-

Variable Substrate	Fixed Substrate	K_m	V_{max}
2-HMS	NAD^+	11.2±1.7	13.7±1.1
HMMS		1.5±0.3	3.0±0.3
CHMS		82.7±4.5	3.3±0.5
NAD^+	2-HMS	293.8±6.4	18.5±2.0
	HMMS	45.5±2.5	23.7±2.2
	CHMS	1316.2±9.8	2.1±0.2

Fig. 1. Substrate specificity and kinetic property of 2-HMS dehydrogenase. Cell lysates of *E. coli* HB01 harboring pCNU374 were used as the enzyme source. (A) The enzyme decreases the absorbance at a wavelength of 375 nm shown by the 2-HMS substrate, where absorbance changes within the wavelength range of 200 nm to 500 nm were scanned at 2-min intervals after initiation of the enzyme reaction. (B) Enzyme activity against the 2-HMS substrate or its analogs is expressed units of specific activity, which is defined by units per mg of protein. One unit of 2-HMS dehydrogenase activity was defined as the consumption of 1-mmole substrate per min. (C) Michaelis-Menten V_{max} and K_m values of the enzyme were determined using double reciprocal plot. The $K_{m\ value}$ is represented as a micromolar concentration, and the V_{max} value is represented as units per mg of protein. Values are mean±S.E. of three independent tests (n=3).

Kinetic property of 2-HMS dehydrogenase encoded in *tomC* gene

Michaelis-Menten K_m and V_{max} values of 2-HMS dehydrogenase from *B. cepacia* G4 have been determined using double reciprocal plots. K_m values for the MCP substrates of 2-HMS, HMMS, and CHMS were 11.2 μM, 1.5 μM, and 82.7 μM, respectively, and those for NAD^+ as a coenzyme were 293.8 μM, 45.5 μM, and 1316.2 μM in the presence of 2-HMS, HMMS, and CHMS, respectively (Fig. 1C). 2-HMS dehydrogenase from *B. cepacia* G4 showed a much higher affinity against MCP substrates than the NAD^+ coenzyme, where affinity potencies of MCP substrates to the enzyme were in the order of HMMS > 2-HMS > CHMS. 2-HMS dehydrogenase from *P. putida* mt-2 was also reported to show similar K_m values of 17 μM for 2-HMS and 330 μM for NAD^+ (Inoue *et al.,* 1995). 2-Aminomuconic semialdehyde dehydrogenase from *P. pseudoalcaligenes* JS45 had K_m values of 26 μM for 2-HMS and 74 μM for NAD^+ (He *et al.,* 1998).

V_{max} values for MCP substrates of 2-HMS, HMMS, and CHMS were 13.7, 3.0, and 3.3 units per mg of protein, respectively, and the V_{max} values for NAD^+ as a coenzyme were 18.5, 23.7, and 2.1 units per mg of protein in the presence of 2-HMS, HMMS, and CHMS, respectively (Fig. 1C). 2-HMS dehydrogenase from *B. cepacia* G4 exhibited approximately a 4-fold higher maximal velocity against 2-HMS than it did against HMMS and CHMS as MCP substrates. The V_{max} values for NAD^+ in the presence of 2-HMS or HMMS were similar each other, but these values were about 10 times higher than that shown in the presence of CHMS. Therefore, 2-HMS dehydrogenase from *B. cepacia* G4 exhibited high catalytic efficiency in terms of V_{max}/K_m towards HMMS followed by 2-HMS but was very low for CHMS.

Localization and sequence of *tomC* gene

To localize the *tomC* gene that encodes for the enzyme 2-HMS dehydrogenase, several subclones of pCNU301, a clone isolated from the total DNA library of *B. cepacia* G4, were constructed as described in Table I, and physical maps of the subclones are shown in Fig. 2. *E. coli* HB101 that harbored pCNU355, pCNU373, or pCNU374 exhibited 2-HMS dehydrogenase activity, but the same strain harboring pCNU357 or pCNU368 did not.

Table I. Bacterial strains and plasmids that were used and prepared in this study

Strain or plasmid	Description
Strains	
E. coli HB101	*SupE44 hsdS20* ($r_B^- m_B^-$) *recA13 ara-14 proA2 lacY1 galK2 rpsL20 xyl-5 mtl-1*, Str^r
B. cepacia G4	A soil bacterium can grow in toluene, cresol or phenol as the sole carbon and energy source
Plasmids	
pBR322	Cloning vector, Ap^r and Tet^r
pUC18 or 19	Cloning vector, Ap^r
pBluescript SK(+)	Cloning vector, Ap^r
pCNU301	A plasmid clone selected from total DNA library of *B. cepacia* G4, a 23.0 kb *Bam*HI fragment of the G4 inserted into the *Bam*HI site of pBR322, Ap^r & Tet^r
pCNU341	A 23.9 kb *Not*I fragment of pCNU301, deletion derivative of pCNU301 lacking a *Not*I fragment, Ap^r
pCNU342	A 19.9 kb *Sma*I fragment of pCNU301, deletion derivative of pCNU301 lacking a *Sma*I fragment, Ap^r
pCNU347	A 12.0 kb *Sma*I-*Bam*HI fragment of pCNU342 inserted into the same sites of the pUC19, Ap^r
pCNU355	An 8.0 kb *Sac*I fragment of pCNU341 inserted into the same site of pUC18, Ap^r
pCNU357	A 4.6 kb *Eco*RI fragment of pCNU355, deletion derivative of pCNU355 lacking an *Eco*RI fragment, Ap^r
pCNU368	A 3.2 kb *Apa*I-*Sac*I fragment of pCNU355 inserted into the same sites of pBluescript SK(+), Ap^r
pCNU373	An 8.7 kb *Pst*I fragment of pCNU347, deletion derivative of pCNU347 lacking *Pst*I fragment, Ap^r
pCNU374	A 6.3 kb *Sac*I fragment of 373, deletion derivative of pCNU373 lacking a *Sac*I fragment, Ap^r

Note: Abbreviations are resistance to ampicillin (Ap^r), streptomycin (Str^r), and tetracycline (Tet^r).

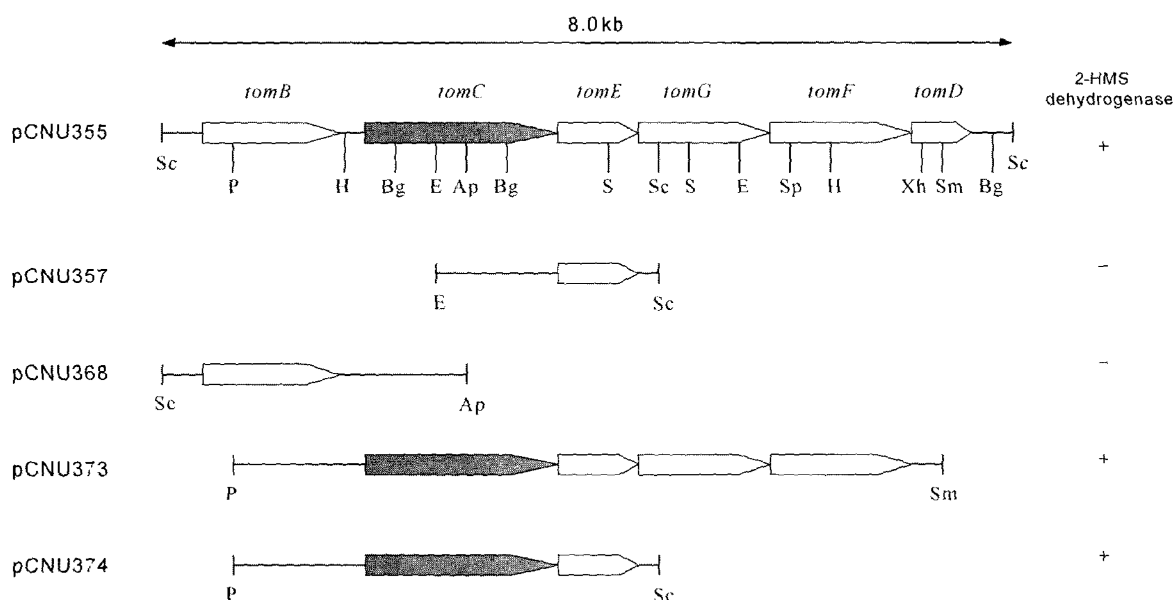


Fig. 2. Physical and genetic maps of pCNU355 and its subclones. Restriction endonucleases are *Apa*I (AP), *Bgl*II (Bg), *Eco*RI (E), *Hin*dIII (H), *Pst*I (P), *Sac*I (Sc), *Sma*I (Sm), *Sph*I (Sp), and *Xho*I (Xh). The catabolic genes are catechol 2,3-dioxygenase gene (*tomB*), 2-HMS dehydrogenase gene (*tomC*), 2-hydroxypenta-2,4-dienoate hydratase gene (*tomE*), acetaldehyde dehydrogenase gene (*tomG*), 4-hydroxy-2-oxovalerate aldolase gene (*tomF*), and 4-oxalocrotonate decarboxylase gene (*tomD*). *E. coli* HB101 that harbored pCNU355, pCNU373, or pCNU374 exhibited 2-HMS dehydrogenase activity. (+) and *E. coli* HB101 harboring pCNU357 or pCNU368 did not (-).

The *tomC* gene was located 0.6 kb downstream of the catechol 2,3-dioxygenase gene and just upstream of the 2-hydroxypent 2,4-dienoate hydratase gene (Fig. 2). No open reading frame (ORF) was found in the intergenic sequence between the catechol 2,3-dioxygenase gene and *tomC* gene. In the case of *P. putida* CF600, 2-HMS dehydrogenase gene (*dmpC*) is located just downstream of the catechol 2,3-dioxygenase gene (*dmpB*). However,

2-HMS dehydrogenase gene (*aphC*) from *Comamonas testosteroni* TA441 is located 2.7 kb downstream of the catechol 2,3-dioxygenase gene (*aphB*), and two unknown ORFs and a putative regulatory gene were identified in the intergenic sequence between the *aphB* and *aphC* genes (Arai *et al.*, 2000; Shingler *et al.*, 1992). The other catabolic genes that are responsible for the aromatic hydrocarbon degradation of *B. cepacia* G4 were also identified as a cluster in sequential order of acetaldehyde dehydrogenase gene (*tomG*), 4-hydroxy-2-oxovalerate aldolase gene (*tomF*), and 4-oxalocrotonate decarboxylase gene (*tomD*), downstream 2-hydroxypent 2,4-dienoate hydratase gene (*tomE*) (Fig. 2). A monooxygenase gene, which is responsible for the initial catabolism of toluene, cresol, phenol, and benzene, was located just upstream of the catechol 2,3-dioxygenase gene from *B. cepacia* G4 (data not shown).

The smallest subclone that expresses 2-HMS dehydrogenase from *B. cepacia* G4 was pCNU374, and thus, the *tomC* gene within the recombinant plasmid was sequenced. As it is registered in GenBank with the accession no. AY278205, the *tomC* gene consists of 1,458 nucleotides with the ATG initiation codon and TGA termination codon. The G+C content of the *tomC* gene is 63%, whereas it was only 55% in the catechol 2,3-dioxygenase gene from the identical *B. cepacia* G4 that was reported in our previous study (Oh *et al.*, 1997). The ORF corresponding to the *tomC* gene can encode a polypeptide of a molecular weight of 52 kDa containing 485 amino acid residues. The predicted 2-HMS dehydrogenase from *B. cepacia* G4 is similar in size with the corresponding enzymes from other bacterial strains of *P. putida* mt-2, *Pseudomonas* sp. DJ77, and *Achromobacter xylosoxidans* KF701 (Inoue *et al.*, 1995; Kang *et al.* 1998; Kim *et al.*, 1997).

Sequence comparison of 2-HMS dehydrogenase from *B. cepacia* G4 with other dehydrogenases

The amino acid sequence of 2-HMS dehydrogenase from *B. cepacia* G4 exhibited the highest identity of 78% with the amino acid sequence of 2-HMS dehydrogenase encoded in the *aphC* gene of *C. testosteroni* TA441 (Arai *et al.*, 2000). As shown in Fig. 3, 2-HMS dehydrogenases from *B. cepacia* G4, *C. testosteroni* TA441, and *C. oligotrophus* RB1 and 2-aminomuconic semialdehyde dehydrogenase from *P. pseudoalcaligenes* JS45 fall into a subfamily in the dendrogram. 2-HMS dehydrogenase from *B. cepacia* G4 showed identities ranging from 29-41% in the amino acid sequence with other NAD(P)$^+$-dependent dehydrogenases.

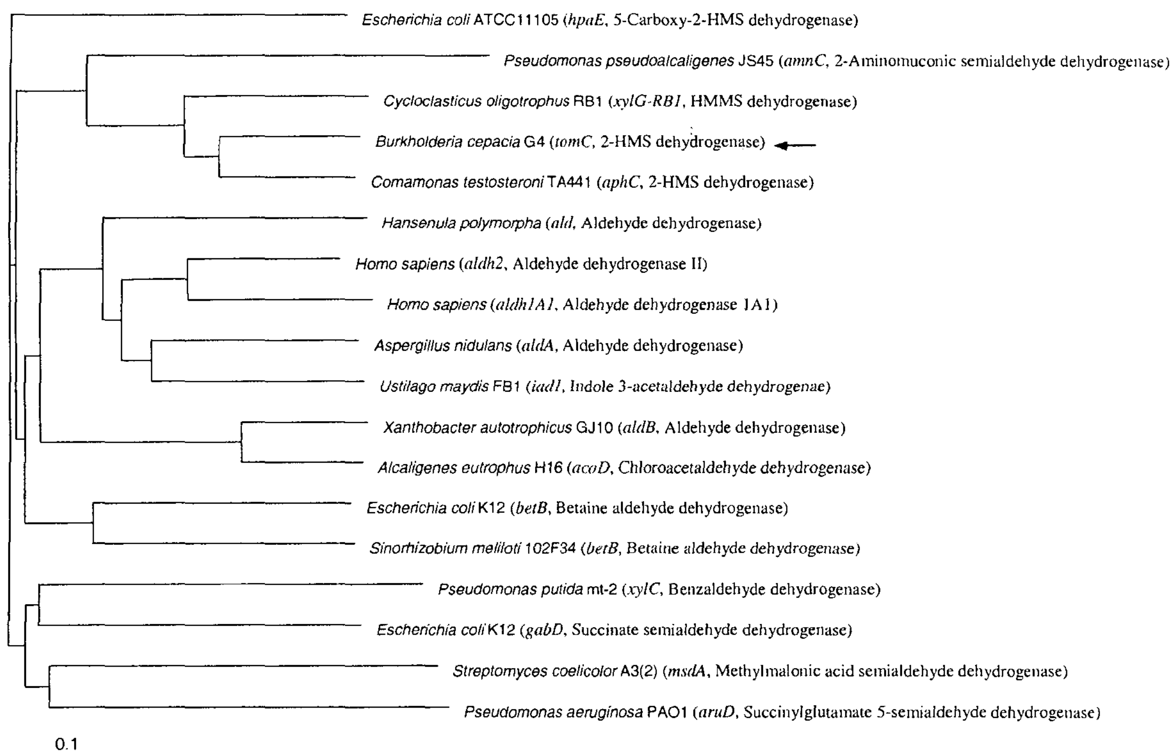


Fig. 3. Phylogenetic relationship of 2-HMS dehydrogenase with 17 homologues. The bar indicates a 10% difference. GenBank accession numbers of the dehydrogenase genes are as follows: *hpaE* (Z37980), *amnC* (U93363), *xylG-RB1* (U51165), *tomC* (AY278205), *aphC* (AB029044), *ald* (U40996), *aldh2* (M26760), *aldh1A1* (NM000689), *aldA* (M16197), *iad1* (U74468), *aldB* (AF029734), *acoD* (M74003), *betB* from *E. coli* K12 (M77739), *betB* from *S. meliloti* 102F34 (U39940), *xylC* (U15151), *gabD* (NC000913), *msdA* (L48550) and *aruD* (AF011922).

However, 2-HMS dehydrogenases from *B. cepacia* G4 exhibited significant phylogenetic relationship, as judged by the amino acid sequence similarities, with aldehyde dehydrogenase, indole 3-acetaldehyde dehydrogenase, betaine aldehyde dehydrogenase, benzaldehyde dehydrogenase, succinate semialdehyde dehydrogenase, methylmalonic acid semialdehyde dehydrogenase, and succinylglutamate 5-semialdehyde dehydrogenase from bacteria, fungi, and human beings, even though some organisms are very distant in the phylogenetic tree (Fig. 3).

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REFERENCES

Arai, H., Ohishi, T., Chang, M. Y., and Kudo, T., Arrangement and regulation of the genes for *meta*-pathway enzymes required for degradation of phenol in *Comamonas testosteroni* TA441. *Microbiology,* 146, 1707-1715 (2000).

Folson, B. R. and Chapman, P. J., Performance characterization of a model bioreactor for the biodegradation of trichloroethylene by *Pseudomonas cepacia* G4. *Appl. Environ. Microbiol.,* 57, 1602-1608 (1991).

Gibson, D. T.. Hensely, M., Yoshioka, H., and Mabry, T. J., Formation of (+)-*cis*-dihydroxy-1-methylcyclohexa-4,6-diene form toluene by *Pseudomonas putida. Biochemistry,* 9, 1626-1630 (1970).

Harayama, S., Lehrbach, P. R., and Timmis, K. N., Transposon mutagenesis analysis of *meta*-cleavage pathway operon genes of the TOL plasmid of *Pseudomonas putida* mt-2. *J. Bacteriol.,* 160, 251-255 (1984).

Hayase, N., Taira, K., and Furukawa, K., *Pseudomonas putida* KF715 *bphABCD* operon encoding biphenyl and polychlorinated biphenyl degradation: cloning, analysis and expression in soil bacteria. *J. Bacteriol.,* 172, 1160-1164 (1990).

He, Z., Davis, J. K., and Spain, J. C., Purification, characterization, and sequence analysis of 2-aminomuconic 6-semialdehyde dehydrogenase from *Pseudomonas pseudoalcaligenes* JS45. *J. Bacteriol..* 180, 4591-4595 (1998).

Inoue, J., Shaw, J. P., Rekik, M., and Harayama, S., Overlapping substrate specificities of benzaldehyde dehydrogenase (the *xylC* gene product) and 2-hydroxymuconic semialdehyde dehydrogenase (the *xylG* gene product) encloded by TOL plasmid pWWO of *Pseudomonas putida. J. Bacteriol.,* 177, 1196-1201 (1995).

Kang, E., Oh, J. M., Lee, J., Kim, Y. C., Min, K. H., Min, K. R., and Kim, Y., Genetic structure of the *bph*G gene encoding 2-hydroxymuconic semialdehyde dehydrogenase of *Achromobacter xylosoxidans* KF701. *Biochem. Biophys. Res. Commun.,* 246, 20-25 (1998).

Kim, S., Shin, H. J., Kim, Y., Kim, S. J., and Kim, Y. C., Nucleotide sequence of the *Pseudomonas* sp. DJ77 *phn*G gene encoding 2-hydroxymuconic semialdehyde dehydrogenase. *Biochem. Biophys. Res. Commun.,* 240, 41-45 (1997).

Kukor, J. J. and Olsen, R. H., Genetic organization and regulation of a *meta* cleavage pathway for catechols produced from catabolism of toluene, benzene, phenol, and cresols by *Pseudomonas pikettii* PKO1. *J. Bacteriol.,* 173, 4587-4594 (1991).

Nelson, M. J. K., Montgomery, S. O., ONeil, E. J., and Pritchard, P. H., Aerobic metabolism of trichloroethylene by a bacterial isolate. *Appl. Environ. Microbiol.,* 52, 383-384 (1986).

Oh, J. M., Kang, E., Min, K. R., Kim, C. K., Kim, Y. C., Lim, J. Y., Lee, K. S., Min, K. H., and Kim, Y., Structure of catechol 2,3-dioxygenase gene encoded in TOM plasmid of *Pseudomonas cepacia* G4. *Biochem. Biophys. Res. Commun.,* 234, 578-581 (1997).

Shields, M. S., Reagin, M. J., Gerger, R. R., Campbell, R., and Somerville, C., TOM, a new aromatic degradative plasmid from *Burkholderia (Pseudomonas) cepacia* G4. *Appl. Environ. Microbiol.,* 61, 1352-1356 (1995).

Shingler, V., Powlowski, J., and Marklund, U., Nucleotide sequence and functional analysis of the complete phenol/3,4-dimethylphenol catabolic pathway of *Pseudomonas* sp. strain CF600. *J. Bacteriol.,* 174, 711-724 (1992).

Whited, G. M. and Gibson, D. T., Toluene-4-monoxygenase, a three component enzyme system that catalyzes the oxidation of toluene to *p*-cresol in *Pseudomonas mendocina* KR1. *J. Bacteriol.,* 173, 3010-3016 (1991).

Worsey, M. J. and Williams, P. A., Metabolism of toluene and xylenes by *Pseudomonas putida* (arvilla) mt-2: evidence for a new function of the TOL plasmid. *J. Bacteriol.,* 124, 7-13 (1975).