J. Microbiol. Biotechnol. (2002), **12**(5), 819–825

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

Isolation and Molecular Analysis of Methanol Oxidation Genes in an Obligate Methylotrophic Bacterium, *Methylobacillus* sp. Strain SK-5

CHOI, HACK-SUN[1], JIN-KWON KIM[1], YEONGHEE AHN[2], MOONJOO KOH[3], AND SI WOUK KIM[1,4]*

[1]*Research Center for Proteineous Materials, Chosun University, Gwangju 501-759, Korea*
[2]*Department of Environmental Science and Engineering, Kwangju Institute of Science and Technology, Gwangju 500-712, Korea*
[3]*Department of Chemistry, Chosun University, Gwangju 501-759, Korea*
[4]*Department of Environmental Engineering, Chosun University, Gwangju 501-759, Korea*

Received: July 25, 2002
Accepted: September 10, 2002

Abstract Methanol dehydrogenase (MDH) is a key enzyme in the process of methanol oxidation in methylotrophic bacteria. However, information on MDH genes from genus *Methylobacillus* is limited. In this study, a 6.5-kb *Hin*dIII DNA fragment of *Methylobacillus* sp. SK-5 chromosomal DNA was isolated from the genomic library of the strain by using a degenerate oligonucleotide probe that was designed based on *N*-terminal amino acid sequence of the MDH α subunit purified from the strain. Molecular analysis of the fragment revealed four tightly clustered genes (*mxaFJGI*) involved in the methanol oxidation. The first and fourth genes were very similar to *mxaF* (77% identity for nucleotides and 78% identity for amino acids) and *mxaI* (67% identity for nucleotides and 68% identity for amino acids) genes, respectively, from *Methylovorus* sp. SS1. Genes *mxaF* and *mxaI* encode α and β subunits of MDH, respectively. The two subunits were identified from purified MDH from *Methylobacillus* sp. SK-5. A dendrogram constructed by comparison of amino acid sequences of MDH α subunits suggests that MxaF from *Methylobacillus* sp. SK-5 belongs to a subfamily cluster of MDH α subunits from β-subgroup *Proteobacteria.* The subfamily cluster is separated from the other subfamily that consists of α- and γ-subgroup *Proteobacteria*. This study provided information on MDH genes from a methylotrophic bacterium in β-subgroup *Proteobacteria*, which would aid to better develop a gene probe to detect one-carbon metabolizing bacteria.

Key words: Methanol dehydrogenase (MDH), *mxaF*, PQQ, *Methylobacillus*

Methylotrophic bacteria are capable of growth on reduced one-carbon (C1) compounds (e.g., methanol, methane, methylamine, etc.) as the sole carbon and energy source. In Gram-negative methylotrophic bacteria, methanol is oxidized to formaldehyde by a periplasmic methanol dehydrogenase (MDH, EC 1.1.99.8), which has pyrroloquinoline quinone (PQQ) as a prosthetic group and cytochrome c_L as its primary electron acceptor. MDH consists of two identical dimers of large and small subunits in an $\alpha_2\beta_2$ conformation [4, 7, 9, 14, 17].

Methanol oxidation genes have so far been analyzed mainly in α-subgroup *Proteobacteria* such as *Methylobacterium extorquens* AM1 and *Paracoccus denitrificans.* Among 32 genes known to be required for methanol oxidation [15], two chromosomal genes encode MDH; *mxaF* and *mxaI* encode large (α) and small (β) subunits of MDH, respectively.

Use of *mxaF* as a potential gene probe to detect methylotrophs and methanotrophs has been studied, based on conserved amino acid sequence identified by comparative analysis of known sequences of the MDH α subunit [18]. So far, complete *mxaF* genes have been isolated from only five methylotrophic bacteria, and their nucleotides were sequenced; *M. extorquens* AM1 [2, 3], *M. organophilum* XX [16], *P. denitrificans* PD1207 [12], *Hyphomicrobium methylovorum* GM2 [23], and *Methylophilus methylotrophus* W3A1 [27]. The former four strains belong to the α-subgroup of *Proteobacteria* while the latter belong to the β-subgroup. Despite the ecological significance of genus *Methylobacillus* as a dominant population in an aerated lagoon used for wastewater treatment [28], information on the MDH gene in the genus is limited. A complete nucleotide sequence of *mxaF* from a methylotrophic strain belonging to the genus has not been reported. Therefore, it is necessary to accumulate more information on methanol oxidation genes from phylogenetically diverse bacteria to better develop gene probes to detect methylotrophs and methanotrophs in the environment.

*Corresponding author
Phone: 82-62-230-6649; Fax: 82-62-225-6040;
E-mail: swkim@mail.chosun.ac.kr

In this study, we isolated methanol oxidation genes (corresponding to *mxaFJGI*) from a β-subgroup methylotrophic bacterium, *Methylobacillus* sp. SK-5, and analyzed them at the molecular level, particularly focusing on *mxaF* [18]. The information obtained in this study would aid to develop a functional gene probe to detect methylotrophs and methanotrophs.

MATERIALS AND METHODS

Strains and Plasmid

Methylobacillus sp. SK-5 was previously isolated from soil by virtue of its ability to utilize methanol as a carbon and energy source [13]. The strain was grown in NMS mineral salt solution [26] with 1% (v/v) methanol at 30°C. *E. coli* DH5α cells were cultured at 37°C on LB medium and used as a host strain for the gene cloning experiment [20, 21, 22]. Vector plasmid pUC19 was used for the cloning experiment and recombinant DNA was introduced into *E. coli* DH5α by electroporation [8]. Transformed *E. coli* DH5α cells were grown on LB medium supplemented with ampicillin (50 μg/ml).

Chemicals and Enzymes

All chemicals were purchased from Sigma Chemical Company (St. Louis, U.S.A.) unless otherwise specified. DEAE-Toyopearl was obtained from Supelco (Bellefonte, U.S.A.) while Mono-S and Superose 12 were from Amersham Biosciences (Uppsala, Sweden). Standard proteins for SDS-PAGE were purchased from Sigma.

[α-^{32}P]dCTP purchased from Amersham Biosciences was used for radiolabeled gene probe preparations. Restriction endonucleases, T_4 DNA ligase, and bacterial alkaline phosphatase were obtained from Takara Shuzo Co., Ltd. (Shiga, Japan).

Enzyme Purification

Methylobacillus sp. SK-5 cells were harvested when the optical density of the culture reached 1.0 at 600 nm. Harvested cells were washed with 20 mM Tris-HCl buffer (pH 8.0) and disrupted by two passages through French Pressure Cell (SLM Instruments, Urbana, U.S.A.). Cell debris was removed by centrifugation at 15,000 ×*g* for 15 min, while the membrane fraction was removed by further centrifugation at 100,000 ×*g* for 60 min at 4°C. The supernatant was used for enzyme (MDH) purification at 4°C. Ammonium sulfate was slowly added to the supernatant to give 60% saturation. After removal of the precipitate by centrifugation (15,000 ×*g*, 60 min), $(NH_4)_2SO_4$ was further added to give 85% saturation. The precipitated proteins after contrifugation were dissolved in 20 mM Tris-HCl (pH 8.0) buffer and subjected to dialysis. The dialysate was concentrated with Centricon (Millipore, Bedford, MA, U.S.A.) and applied to a DEAE-Toyopearl column equilibrated with 20 mM Tris-HCl buffer. Pre-eluted MDH with 20 mM Tris-HCl buffer was applied to a Mono-S column that was equilibrated with phosphate buffer (pH 6.0) and eluted with a 0–100 mM linear gradient of NaCl in phosphate buffer. Fractions containing the enzymatic activities were pooled, concentrated, and applied to a FPLC Superose 12 HR 10/30 column which was equilibrated with 20 mM Tris-HCl buffer containing 0.15 M NaCl. The purified MDH was determined by 15% SDS-PAGE.

Enzyme Assay

MDH activity was determined by a modified spectrophotometric method [4] by measuring decrease of absorbance of the substance, 2,6-dichlorophenol indophenol (DCPIP), at 600 nm. Protein concentration was determined by the Bradford method [6].

Determination of *N*-Terminal Amino Acid Sequence

Purified MDH was separated into its subunits by 15% SDS gel electrophoresis and blotted to a polyvinylidene fluoride membrane. *N*-Terminal amino acid sequence was determined by a sequencer (Model 473A, Applied Biosystems) method of Edman and Begg [9].

DNA Extraction and Cloning

Genomic DNA of *Methylobacillus* sp. SK-5 was extracted by the method of Goldberg and Ohman [10] and plasmid DNA was isolated by the alkaline lysis method [5]. Extracted genomic DNA was partially digested with *Hin*dIII and subjected to agarose gel electrophoresis. DNA fragments in the desired size range (approximately 5–10 kb) were cut out from the agarose gel and extracted using a QIAGEN Gel Extract Kit (QIAGEN, Valencia, CA, U.S.A.). *Hin*dIII-digested plasmid pUC19 was treated with bacterial alkaline phosphatase and ligated to fractionated genomic DNA with T_4 ligase. Ligated DNA was transformed into competent *E. coli* DH5α cells.

Transformants (ampicillin-resistant white colonies) containing gene *mxaF* were screened using a degenerate oligonucleotide probe, 1F [AACCA(A/G)GA(C/T)CTGCA (A/G)AACCTG]. The probe was designed based on *N*-terminus amino acid sequence of the MDH α subunit purified from *Methylobacillus* sp. SK-5. Probe F1 (100 pmol) was 5'-end labeled with [α-^{32}P] ATP and T_4 kinase according to the manufacturer's instruction. Colony hybridization was performed using the labeled F1 probe. Hybridization was performed at 42°C for 16 h with hybridization buffer containing 6× SSC (0.15 M sodium chloride and 0.015 M sodium citrate), 1× Denhardt solution, 100 μg/ml yeast tRNA, and 0.05% sodium pyrophosphate. Final washes were performed at increasing temperatures (55 to 65°C) in a 1× SSC. Autoradiographs were obtained by exposing hybridized filters to Fuji RX films for 16 to 24 h.

Nucleotide Sequence Determination and Analysis

The isolated 6.5-kb *Hin*dIII DNA fragment was sequenced by a DNA sequencer (Applied Biosystems Model 373A, Foster City, U.S.A.) using a *Taq* DyeDeoxy Terminater Cycle sequencing kit. Custom-made oligonucleotides were used for primer walking. The resulting DNA sequence was screened against GenBank database. BLAST (version 2.0) [1] was used to search the most similar sequences in the database. DNA sequences were aligned and analyzed by DNASIS program (Hitachi, Tokyo, Japan). Evolutionary distances were calculated using the program CLUSTAL X and a dendrogram was constructed by using the program CLUSTAL X [24].

RESULTS AND DISCUSSION

Properties of MDH

The native MDH had a molecular mass of 130 kDa and consisted of two identical subunits in an $\alpha_2\beta_2$ conformation. Each of the α and β subunits had molecular masses of 63 and 8 kDa, respectively. Purified MDH from *Methylobacillus* sp. SK-5 showed broad substrate specificity with alcohols. Compared to its activity with methanol, its activity on alcohols containing more than three carbons was relatively high, while its activity on formaldehyde was relatively low (only 7% of MDH activity with methanol) (Table 1). MDH from other strains showed 78% or more activity with formaldehyde, compared to its activity with methanol [11]. Activity of MDH from *Methylobacillus* sp. SK-5 increased with carbon number in the alcohols tested in this study, showing the highest activity with 1-octanol. MDH from *Methylocystis* sp. GB25 showed decreasing activity with higher carbon number in the alcohols [11].

Isolation of Methanol Oxidation Genes of *Methylobacillus* sp. SK-5

The genomic library of *Methylobacillus* sp. SK-5 was constructed. A total of 1,200 recombinant clones containing

Table 1. Substrate specificity of methanol dehydrogenase from *Methylobacillus* sp. SK-5.

Substrate[a]	Relative activity (%)[b]	Substrate[a]	Relative activity (%)[b]
Methanol	100	1-Hexanol	144
Ethanol	100	1-Heptanol	152
1-Propanol	97	1-Octanol	159
1-Butanol	118	Formaldehyde	7
1-Pentanol	119		

[a]Final concentration of each substrate was 6.7 mM.
[b]100% corresponds to 1.5 unit/mg of protein. One unit is defined as the amount of enzyme that catalyzes the reduction of 1 μmol DCPIP per minute. Enzyme activities were obtained as average values from three independent experiments.

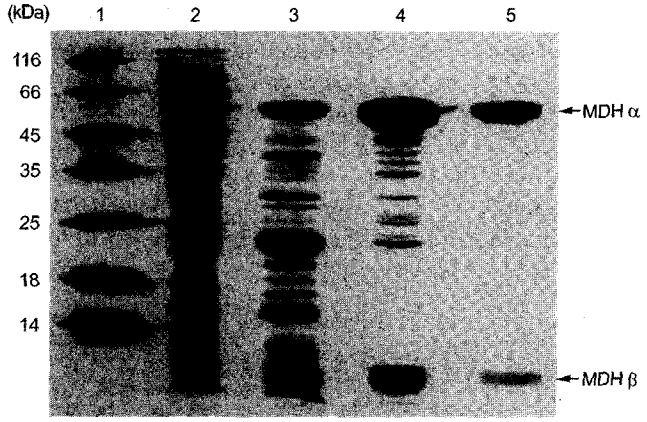

Fig. 1. SDS-polyacrylamide gel electrophoresis of MDH from *Methylobacillus* sp. SK-5 during purification steps.
Lane 1, size markers; lane 2, cell free extracts obtained from ultracentrifugation of *Methylobacillus* sp. SK-5 lysate; lane 3, DEAE-Toyperal pool; lane 4, Mono-S pool; lane 5, purified MDH after gel filtration.

*Hin*dIII DNA fragments from the strain were screened by colony hybridization using the probe 1F, a degenerate oligonucleotide probe designed based on the *N*-terminal amino acid sequence of MDH α subunit purified from *Methylobacillus* sp. SK-5 (Fig. 1). One clone showing a positive signal from the hybridization was obtained as a result of the screening. The clone was designated pMDH and used for further study.

Clone pMDH contained a *Hin*dIII DNA insert in the size of approximately 6.5 kb (Fig. 2). A restriction map of pMDH was obtained. Nucleotide sequence of the entire 6.5-kb *Hin*dIII DNA fragment was determined as described in Materials and Methods.

Nucleotide Sequence Analysis of MDH Gene and Intervening Genes

Nucleotide sequence analysis revealed that the *Hin*dIII DNA fragment of pMDH contained four complete open reading frames (ORF) involved in methanol oxidation (Figs. 2, 3). The four ORF were designated genes *mxaF*, *mxaJ*, *mxaG*, and *mxaI*, since they showed high similarity to genes *mxaFJGI* from *Methylovorus* sp. SS1 (unpublished, GenBank accession No. AAD56237) in the GenBank

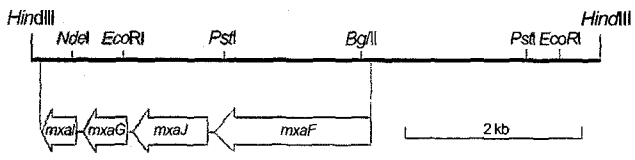

Fig. 2. Location of genes *mxaFJGI* in 6.5-kb *Hin*dIII DNA fragment isolated from *Methylobacillus* sp. SK-5 chromosome.
Arrows indicate transcriptional directions of the genes. Restriction sites in the fragment are indicated. The *lac* promoter on the cloning vector pUC19 is located on the left of the map.

database. The putative *mxaF* (encoding MDH α-subunit) and *mxaI* (encoding MDH β-subunit) showed the highest similarities with *mxaF* and *mxaI* from the strain SS1: *mxaF*, 77% identity for nucleotides and 78% identity for amino acids; *mxaI*, 67% identity for nucleotides and 68% identity for amino acids. Genes *mxaF* and *mxaI* from *Methylobacillus* sp. SK-5 were separated by two intervening genes, *mxaJ* (encoding a protein with unknown function) and *mxaG* (encoding cytochrome c_L). Their identity with the strain SS1 was as follows: *mxaJ*, 55% for nucleotides and 47% for amino acids; *mxaG*, 67% for nucleotides and 68% for amino acids. Cytochrome c_L is the electron acceptor for MDH [7].

The newly identified *mxaF*, *mxaJ*, *mxaG*, and *mxaI* gene ORF were 1.8, 0.8, 0.5, and 0.3-kb long, respectively (Fig. 3). They were transcribed in the same direction (Fig. 2).

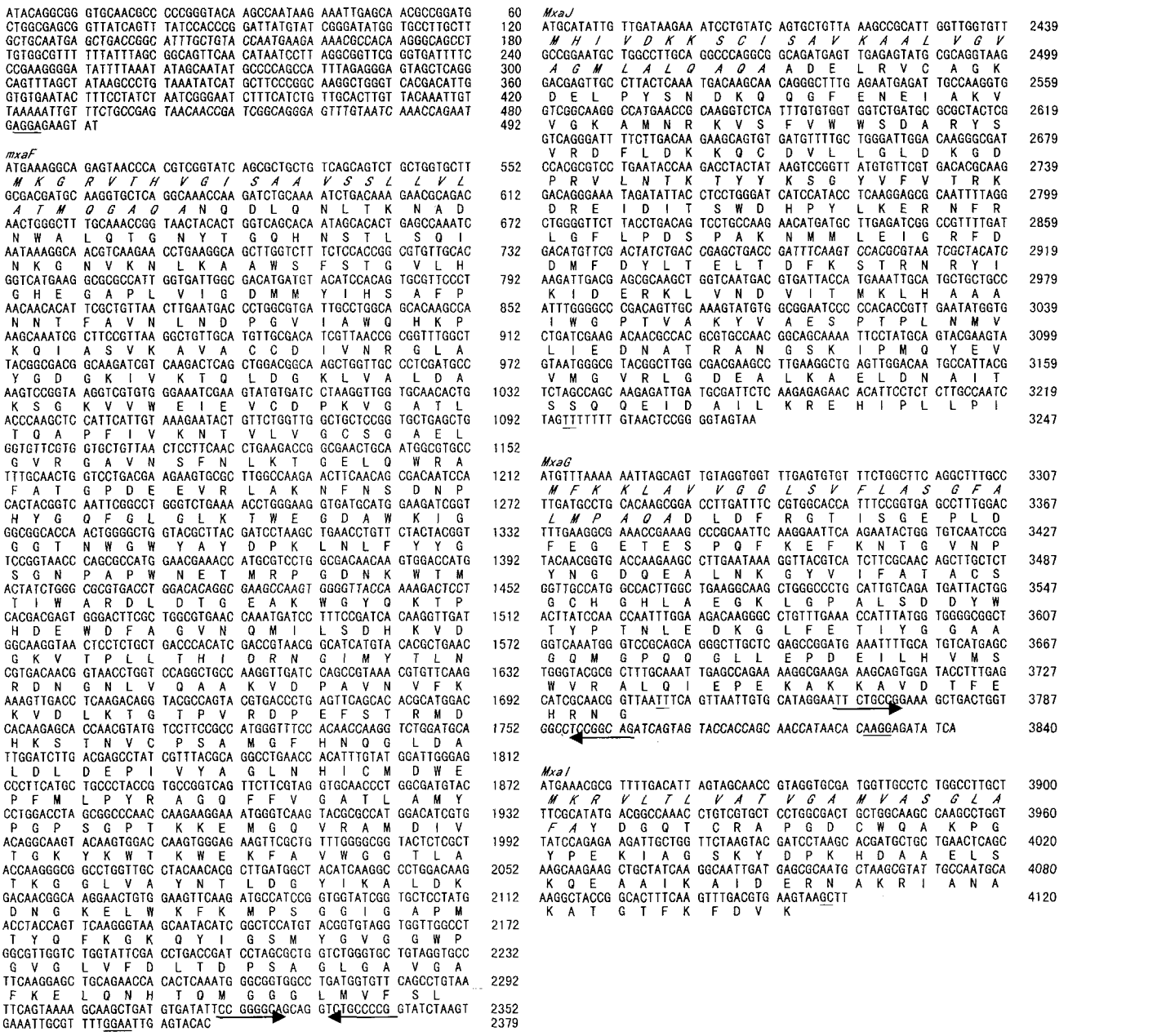

Fig. 3. The complete nucleotide sequence of genes *mxaFJGI*.

The genes *mxaF*, *mxaJ*, *mxaG*, and *mxaI* encode methanol dehydrogenase (MDH) α subunit, a protein with unknown function, cytochrome c_L, and MDH β subunit, respectively. The nucleotide sequences of the genes were deposited in GenBank under the accession no. AF494424. Underli ned nucleotide sequences are putative ribosomal binding sites. Putative signal sequence of each gene is italicized and arrows indicate inverted repeats. The asterisks indicate stop codons.

The intergenic regions separating *mxaFJGI* were 87-, 25-, and 98-bp long, respectively. An inverted repeat was found in each of the intervening regions between *mxaF* and *mxaJ* and between *mxaG* and *mxaI* (Fig. 3). They might form a step-loop or hairpin structure in the mRNA.

The gene order, *mxaFJGI* found in *Methylobacillus* sp. SK-5, were identical with those in the other four strains belonging to α-subgroup of *Proteobacteria* (*M. extorquens* AM1 [2, 3], *M. organophilum* XX [16], *P. denitrificans* PD1207 [12], *H. methylovorum* GM2 [23]). Gene *mxaF* in *P. denitrificans* forms a gene cluster with the *mxaJ*, *mxaG*, *mxaI*, *mxaR*, and *mxaS* genes in that order [25]. Genes *mxaFJGIRS* in *M. extorquens* AM1 were found upstream of gene cluster *mxaACKLD* [19]. Gene *mxaF* forms a gene cluster with the *mxaJGIRSA* in *H. methylovorum* GM2 [23].

Deduced amino acids of gene *mxaF* from *Methylobacillus* sp. SK-5 were 599 amino acids (Fig. 3). The *N*-terminal amino acid sequence (NQDLQNLTKNADNWALQTG) of purified MDH α subunit was identified in the region of N29-G47 of the gene *mxaF*. The *N*-terminal region, M1-A28, had a feature of the signal sequence previously found in *mxaF* from *H. methylovorum* GM2 [23], which serves to translocate the enzyme into the periplasm during biosynthesis.

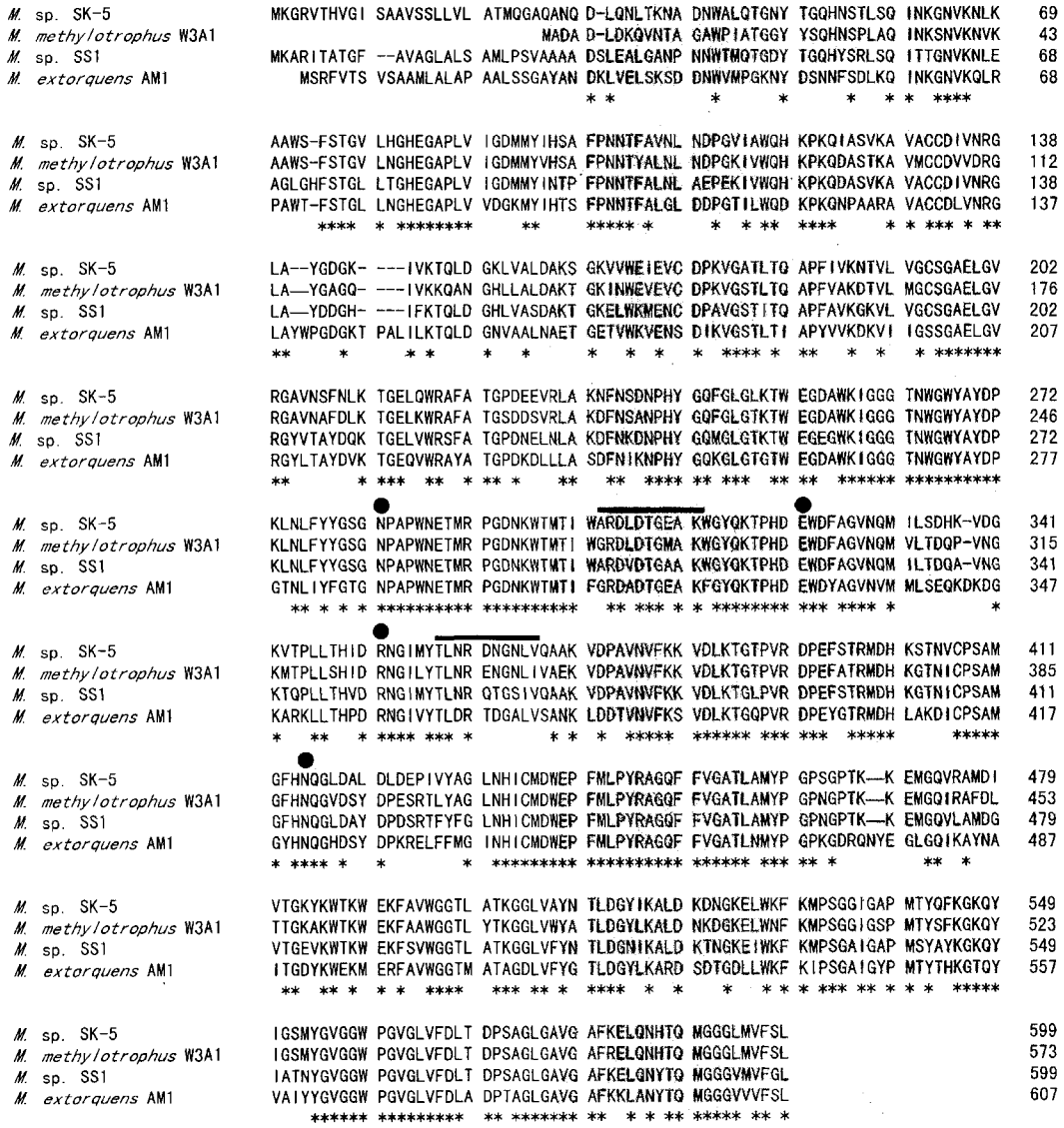

Fig. 4. Amino acid sequence alignment of four methanol dehydrogenase α subunits.
Source strain of each sequence is as follows: *M.* sp. SK-5, *Methylobacillus* sp. SK-5; *M. methylotrophus* W3A1, *Methylophilus methylotrophus* W3A1; *M.* sp. SS1, *Methylovorus* sp. SS1; *M. extorquens* AM1, *Methylobacterium extorquens* AM1. Symbol • indicates conserved amino acids in the active sites of all methanol dehydrogenases. Overlined residues indicate potential amino acids involved in tryptophan-docking motifs. The asterisks indicate conserved amino acid residues in the alignment.

The cleavage site occurred after the sequence Ala-Xxx-Ala, a feature conserved in all of the signal peptides for periplasmic Mxa polypeptides [3]. The putative *mxaF* promoter sequence (-35 -AAAGAGC-, -10 -TAGAA-) observed in *M. extorquens* AM1, *M. organophilum*, and *P. denitrificans* was not found in the 5' upstream region of *mxaF* isolated from *Methylobacillus* sp. SK-5 [23].

The deduced amino acid sequence of gene *mxaG* encoding cytochrome c_L showed the typical heme-binding site (-CSGCH-) at position 79 through 83 (Fig. 3), which was formed by two closely linked cysteine residues and a consecutive histidine residue [20].

Comparative Analysis of Deduced Amino Acid Sequences of *mxaF* Genes

A methylotrophic bacterium *Methylobacillus* sp. SK-5 is an obligate methanol-oxidizer [13]. MDH is involved in a key step in methanol oxidation, catalyzing conversion of methanol to formaldehyde that serves as an intermediate of both assimilative and dissimilative metabolism in methylotrophs. Considering that MDH is a tetramer composed of $\alpha_2\beta_2$ and its active site is in α subunit, the gene encoding the α subunit is a potential source to develop a functional gene probe that can be used to detect C1-utilizing bacteria.

The deduced amino acid sequences of genes corresponding to *mxaF* were aligned for comparison (Fig. 4). The amino acid sequences showed key amino acids (Asn 283, Glu 323, Arg 352, and Asn 415) at the active site of the MDH α subunit. The key amino acids in the active site are completely conserved among all MxaF sequences reported. Potential amino acids involved in the tryptophan docking motifs were also found. They are suggested to form a planar stabilizing girdle of interactions around the periphery of the subunit [18].

The deduced amino acid sequence of gene *mxaF* from *Methylobacillus* sp. SK-5 was compared with those of other MDH α subunits to construct a dendrogram to show their phylogenetic relationship (Fig. 5). The dendrogram contained two subfamilies of the proteins. One subfamily (lower part of the dendrogram) contained MDH α subunits of α- and γ-subgroups *Proteobacteria*, while the other (upper part of the dendrogram) contained those of β-subgroups *Proteobacteria*. The dendrogram clearly showed a separate cluster of MDH α subunits of β-subgroup *Proteobacteria*.

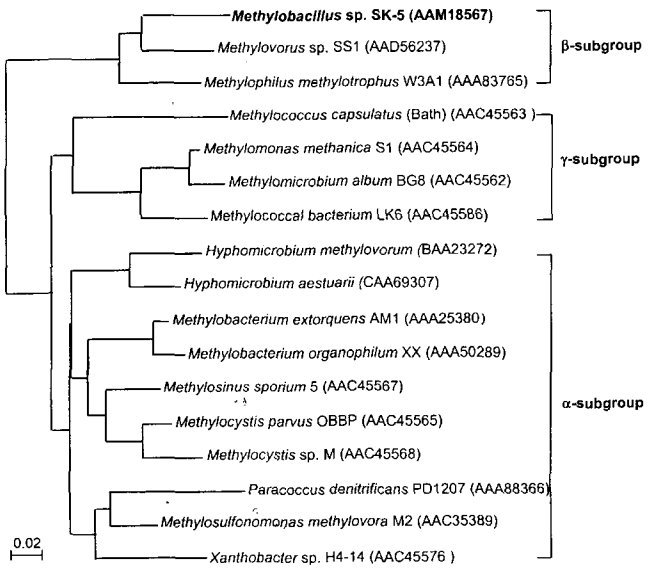

Fig. 5. Phylogenetic relationship of *mxaF* gene products (partial, 172 amino acids; accession number in brackets).
The dendrogram was generated by comparison of derived amino acid sequences of methanol dehydrogenase (MDH) α subunits, using the program CLUSTAL X. Since only 5 complete sequences of MDH α subunits have been reported, the partial sequence (172 amino acids) of each source was compared. The sequences were retrieved from the GenBank database. Scale bar represents 2% sequence divergence. Phylogenetic positions of each strain based on 16S rDNA genes are indicated by α, β, and γ for the subgroups of *Proteobacteria*.

Acknowledgments

This work was supported by research grants from the Ministry of Environment as "The Eco-technopia 21 project (No. 2001-44002-3)" and ERC grants from Ministry of Science and Technology and KOSEF in Korea in the year of 2002.

REFERENCES

1. Altschul, S. F., T. L. Madden, A. A. Schäffer, J. Zhang, Z. Zhang, W. Miller, and D. J. Lipman. 1997. Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. *Nucleic Acids Res.* **25:** 3389–3402.
2. Amaratunga, K., P. M. Goodwin, C. D. Oconnor, and C. Anthony. 1997. The methanol oxidation genes *mxaFJGIR(S)ACKLD* in *Methylobacterium extorquens* AM1. *FEMS. Microbiol. Lett.* **146:** 31–38.
3. Anderson, J. D., C. J. Morris, D. N. Nunn, C. Anthony, and M. E. Lidstrom. 1990. Nucleotide sequence of the *Methylobacterium extorquens* AM1 *moxF* and *moxJ* genes involved in methanol oxidation. *Gene* **90:** 173–176.
4. Anthony, C. and L. J. Zatman. 1964. The microbial oxidation of methanol. Purification and properties of methanol dehydrogenase of *Pseudomonas* sp. M27. *Biochem. J.* **92:** 614–621.
5. Birnboim, A. and J. Doly. 1979. A rapid alkaline extract procedure for screening recombinant plasmid DNA. *Nucleic Acid Res.* **7:** 1513–1523.
6. Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72:** 248–254.

7. Dispirito, A. A. 1990. Soluble cytochrome c from *Methylomonas* A4. *Meth. Enzymol.* **188:** 289–297.
8. Dower, W. J., J. F. Miller, and C. W. Ragsdale. 1988. High efficiency transformation of *E. coli* by high voltage electroporation. *Nucleic Acids Res.* **16:** 6127.
9. Edman, P. and G. Begg. 1967. A protein sequenator. *Eur. J. Biochem.* **1:** 80–91.
10. Goldberg, J. B. and D. E. Ohman. 1984. Cloning and expression in *Pseudomonas aeroginosa* of a gene involved in the production of alginate. *J. Bacteriol.* **158:** 1115–1121.
11. Grosse, S., K.-D. Wendlandt, and H.-P. Klebler. 1997. Purification and properties of methanol dehydrogenase from *Methylocystis* sp. GB 25. *J. Basic Microbiol.* **37:** 269–279.
12. Harms, N., G. E. De Vries, K. Maurer, J. Hoogendijk, and A. H. Stouthamer. 1987. Isolation and nucleotide sequence of methanol dehydrogenase structure gene from *Paracoccus denitrificans*. *J. Bacteriol.* **169:** 3969–3975.
13. Kim, S. W. and Y. H. Park. 1993. Characteristics of a new obligate methanol-oxidizing bacterium. *Kor. J. Microbiol.* **31:** 261–266.
14. Koh, M., C. S. Kim, Y. A. Kim, H. S. Choi, E. H. Cho, E. Kim, Y. M. Kim, and S. W. Kim. 2002. Properties of electron carriers in the process of methanol oxidation in a new restricted facultative marine methylotrophic bacterium, *Methylophaga* sp. MP. *J. Microbiol. Biotechnol.* **12:** 476–482.
15. Lidstrom, M. E., C. Anthony, F. Biville, F. Gasser, P. Goodwin, R. S. Hanson, and N. Harms. 1994. New unified nomenclature for genes involved in the oxidation of methanol in Gram-negative bacteria. *FEMS. Microbiol. Lett.* **117:** 103–106.
16. Machlin S. M. and R. S. Hanson. 1988. Nucleotide sequence and transcriptional start site of the *Methylobacterium organophilum* XX methanol dehydrogenase structural gene. *J. Bacteriol.* **170:** 4739–4747.
17. Matsushita, K., K. Takahashi, and O. Adachi. 1993. A novel quinoprotein methanol dehydrogenase containing an additional 32-Kilodalton peptide purified from *Acetobacter methanolicus*: Identification of the peptide as a MoxJ product. *Biochemistry* **32:** 5576–5582.
18. McDonald, I. R. and J. C. Murrell. 1997. The methanol dehydrogenase structural gene *mxaF* and its use as a functional gene probe for methanotroph and methylotroph. *Appl. Environ. Microbiol.* **63:** 3218–3224.
19. Morris C. J., Y. M. Kim, K. Perkins, and M. E. Lidstrom. 1995. Identification and nucleotide sequences of *mxaA*, *mxaC*, *mxaK*, *mxaL*, and *mxaD* genes from *Methylobacterium extorquens* AM1. *J. Bacteriol.* **177:** 6825–6831.
20. Park, J.-H., J.-H. Lee, Y.-S. Kim, Y.-K. Hong, and I.-S Kong. 2001. Molecular cloning and expression of a sodium-driven flagellar motor component gene (*motX*) from *Vibrio fluvialis*. *J. Microbiol. Biotechnol.* **11:** 973–978.
21. Ryou, C., T. Chung, and M. Kwon. 2001. Molecular cloning and hyperexpression of a Bt gene, *cryIAc*, in *Escherichia coli* DH5α: Production and usage of anti-cryIAc antibody. *J. Microbiol. Biotechnol.* **11:** 1093–1098.
22. Shin, J.-H., D.-H. Roh, G.-Y. Heo, G.-J. Joo, and I.-K. Rhee. 2001. Purification and characterization of a regulatory protein XylR in the D-xylose operon from *Escherichia coli*. *J. Microbiol. Biotechnol.* **11:** 1002–1010.
23. Tanaka, Y., T. Yoshida, K. Watanabe, Y. Izumi, and T. Mitsunaga. 1997. Cloning and analysis of methanol oxidation genes in the methylotroph *Hyphomicrobium methylovorum* GM2. *FEMS Microbiol. Lett.* **154:** 397–401.
24. Thompson, J. D., T. J. Gibson, F. Plewniak, F. Jeanmougin, and D. G. Higgins. 1997. The CLUSTAL X windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res.* **25:** 4876–4882.
25. Van Spanning, R. J. M., C. W. Wansell, T. De Boer, J. H. Marten, H. Anazawa, N. Harms, L. F. Oltmann, and A. H. Stouthamer. 1991. Isolation and characterization of the *moxJ*, *moxG*, *moxI*, and *moxR* genes of *Paracoccus denitrificans*: Inactivation of *moxJ*, *moxG*, and *moxR* and the resultant effect on methylotrophic growth. *J. Bacteriol.* **173:** 6948–6961.
26. Whittenbury, R. and H. Dalton. 1981. The methylotrophic bacteria, pp. 894–902. *In* Starr, M. P., H. Stolp, H. G. Truper, A. Balows, and H. G. Schlegel (eds.), *The Prokaryotes*. Springer-Verlag, Berlin.
27. Xia, Z.-X. X., W.-W. Dai, Y.-F. Zhang, S. A. White, G. D. Boyd, and F. S. Mathews. 1996. Determination of the gene sequence and the three-dimensional structure at 2.4 Å resolution of methanol dehydrogenase from *Methylophilus* W3A1. *J. Mol. Biol.* **259:** 480–501.
28. Yu, Z. and W. W. Mohn. 2001. Bacterial diversity and community structure in an aerated lagoon revealed by ribosomal intergenic spacer analyses and 16S ribosomal DNA sequencing. *Appl. Environ. Microbiol.* **67:** 1565–1574.