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Soluble Methane Monooxygenase Component B Gene Probe for Identification of Methanotrophs That Rapidly Degrade Trichloroethylene

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Restriction fragment length polymorphisms, Western blot (immunoblot) analysis, and fluorescence-labelled signature probes were used for the characterization of methanotrophic bacteria as well as for the identification of methanotrophs which contained the soluble methane monooxygenase (MMO) gene and were able to degrade trichloroethylene (TCE). The gene encoding a soluble MMO component B protein from *Methylosinus trichosporium* OB3b was cloned. It contained a 2.2-kb *Eco*RI fragment. With this cloned component B gene as probe, methanotroph types I, II, and X and environmental and bioreactor samples were screened for the presence of the gene encoding soluble MMO. Fragments produced by digestion of DNA with rare cutting restriction endonucleases were separated by pulsed-field gel electrophoresis and transferred to Zeta-Probe membrane (Bio-Rad) for Southern blot analysis. Samples were also analyzed for the presence of soluble MMO by Western blot analysis and the ability to degrade TCE. The physiological groups of methanotrophs in each sample were determined by hybridizing cells with fluorescence-labelled signature probes. Among twelve pure or mixed cultures, DNA fragments of seven methanotrophs hybridized with the soluble MMO B gene probe. When grown in media with limited copper, all of these bacteria degraded TCE. All of them are type II methanotrophs. The soluble MMO component B gene of the type X methanotroph, *Methylococcus capsulatus* Bath, did not hybridize to the *M. trichosporium* OB3b soluble MMO component B gene probe, although *M. capsulatus* Bath also produces a soluble MMO.

Trichloroethylene (TCE) is a solvent widely used in industries such as metal finishing and dry cleaning. Because of improper handling, inadequate disposal techniques, or accidental spillage, it is commonly found in soil and groundwater near industrial sites (5, 47). It is a significant environmental pollutant. The presence of TCE and other low-molecular-weight chlorinated aliphatic hydrocarbons in groundwater threatens drinking water supplies (30) and endangers human health because of the toxicity and suspected or demonstrated carcinogenicity of these chemicals (24). TCE is partially degraded anaerobically to vinyl chloride, which is more toxic than TCE and is a known carcinogen (27, 48). TCE is, therefore, on the Environmental Protection Agency's priority list of environmental pollutants.

Methanotrophs are bacteria which grow on methane, utilize it as a sole energy source, and assimilate formaldehyde as a major carbon source (3). They are classified as types I, II, and X methanotrophs according to their carbon assimilatory pathways, their intracytoplasmic membrane arrangements, the chain lengths of membrane phospholipid fatty acids, and the presence or absence of a complete tricarboxylic acid cycle (3, 17, 49). Methanotrophs contain methane monooxygenases (MMOs), which exist in two forms: particulate or membrane bound MMO and soluble MMO. The particulate MMO is present in all methanotrophs, whereas soluble MMO is not synthesized by all species (8, 41, 42, 45). The expression of soluble MMO is regulated by the ratio of copper ions to cell mass in culture (42). At low concentrations of copper per cell mass, soluble MMO is synthesized. MMO converts methane to methanol by introducing one oxygen atom into the methane molecule. It catalyzes the oxidation of a wide variety of aliphatic, alicyclic, and aromatic hydrocarbons (20). Soluble MMO has a broader substrate range then particulate MMO (9).

Only the type II methanotrophs which produce soluble MMO are known to degrade TCE at high rates (18, 26, 45, 46). They degrade TCE much more rapidly than bacteria that contain other nonspecific oxygenases (8). Therefore, it is desirable to have techniques that will detect bacteria that produce soluble MMO in bioreactors and environmental samples available. Soluble MMO from *Methylosinus trichosporium* OB3b has been purified and characterized previously (15, 16, 43). It is composed of three components: hydroxylase, component B, and reductase. The hydroxylase is composed of three polypeptides with molecular masses of 54 kDa (alpha subunit), 43 kDa (beta subunit), and 23 kDa (gamma subunit) (15). Component B and reductase have molecular masses of 15.8 and 39 kDa, respectively. Current knowledge indicates that all soluble MMO-producing methanotrophs are either type II or type X (8, 41). The physiological types of methanotrophs can be determined by hybridization with labelled oligodeoxynucleotide signature probes (44).

Genes encoding the soluble MMO of *Methylococcus capsulatus* Bath have been cloned, mapped, and sequenced (25, 28, 39, 40). The MMOs of *M. capsulatus* Bath and *M. trichosporium* OB3b are closely related (12, 15, 50). Using the genes encoding soluble MMO of *M. capsulatus* Bath as probes, Cardy et al. (10) isolated and sequenced the soluble MMO gene cluster of *M. trichosporium* OB3b.

Pulsed-field gel electrophoresis (PFGE) was designed and used for the fractionation of DNA fragments larger than 50 kb (11, 34, 35). In combination with the use of rare cutting restriction enzymes, PFGE produces DNA restriction fragment patterns which have been used to differentiate closely

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TABLE 1. Methanotrophs and environmental samples used in this study

Methanotroph or sample	Source or description[a]	Reference or source
Methanotrophs		
Methylococcus capsulatus Bath	R. Whittenbury	49
Methylocystis parvus OBBP	R. Whittenbury	49
Methylocystis pyriformis 14	Y. Trotsenko	17
Methylomonas albus BG8	R. Whittenbury	49
Methylomonas methanica	R. Whittenbury	49
Methylomonas rubra	P. Green	Personal communication
Methylosinus methanica 81Z	S. J. Zhao	17
Methylosinus sporium 27	Y. Trotsenko	Personal communication
Methylosinus sp. strain B	This laboratory	
Methylosinus trichosporium OB3b	R. Whittenbury	49
Sample source		
Lake Mendota[b]	Bacteria isolated from the metalimnion of Lake Mendota, Madison, Wis.; sampled in 1990 by B. J. Bratina	
Bioreactor	Mixed methanotrophic culture initially seeded with effluent from a laboratory column of aquifer material from Moffett Field Naval Air Station, Mountain View, Calif. (2)	2
Plant isolate[b]	Pure culture isolated by Greg Brusseau from *L. minor*	
Garden soil[b]	Bacteria isolated from garden soil	
Peat[b]	Bacteria isolated from a peat bog sample from northern Minnesota	

[a] For the growth conditions for pure cultures, see Tsien et al. (44).
[b] Enriched with 25% methane and air mixture at 30°C in a mineral salt medium.

related bacterial species, such as *Agrobacterium*, *Rhizobium*, and *Bradyrhizobium* spp. (7, 23, 33, 37).

When a specific gene probe was used in Southern hybridization, the lengths of DNA fragments that hybridized to the probe were characteristic of a species, strain, or isolate. In this report, we describe the use of PFGE-restriction fragment length polymorphisms and the use of a cloned DNA fragment carrying the component B gene to detect soluble MMO genes from methanotrophs on Southern blots prepared from gels on which large DNA restriction fragments were separated by PFGE. This technique, when combined with fluorescence-labelled oligodeoxynucleotide signature probes and Western blot (immunoblot) analysis, becomes a very useful tool in the characterization of methanotrophs and in the detection of methanotrophs which can synthesize soluble MMO and can therefore degrade TCE.

MATERIALS AND METHODS

Microorganisms and culture conditions. The methanotrophs and their sources are listed in Table 1. Cells were harvested during the mid- or late-exponential growth phase. The growth media used and the conditions for growth of pure cultures have been described previously (45). For enrichment cultures, a mineral salt medium described by Cornish et al. (13) was used. The cultures were grown at 30°C under a 25% methane–75% air mixture on a rotary shaker. The conditions that allow expression of the soluble MMO (copper limitation) have been described previously (45). Methane-utilizing bacteria were isolated from the surfaces of *Lemna minor* plants (little duckweed) obtained from Lake Minnetonka, Minn., and the metalimnion of Lake Mendota, Madison, Wis., with the mineral salts medium described by Cornish et al. (13) and an atmosphere of 25% methane–75% air (vol/vol).

Isolation of bacteria from environmental samples. The sources of environmental samples are listed in Table 1. The method described by Holben et al. (21) was employed to separate bacteria from soil particles. The bacterial cell suspensions were used to prepare samples for PFGE.

Preparation of bacterial and plasmid DNAs. Methods for the preparation of bacterial and plasmid DNAs described by Allen and Hanson (1) and Machlin et al. (22) were used.

Cloning the gene encoding soluble MMO component B. Polyclonal antibodies prepared against the purified component B of soluble MMO were prepared as described previously (45) and were used for the screening of a genomic library of *M. trichosporium* OB3b. An oligodeoxynucleotide probe of 24 bases was designed on the basis of the N-terminal amino acid sequence of component B (14). The sequence of the oligodeoxynucleotide probe is:

5′-AAGGCITTCGCIGAIGAITTCTTC-3′ (I = inosine)

The gene encoding soluble MMO component B was cloned into the *Eco*RI site of pBluescript II and transformed into XL-1 Blue cells (Stratagene, La Jolla, Calif.). Procedures for cloning and preparation of genomic libraries described by Sambrook et al. (31) and Ausubel et al. (4) were followed.

Slot blotting of plasmid DNA and bacterial DNA. One microgram of plasmid DNA containing the soluble MMO component B gene insert or 2 μg of *M. trichosporium* OB3b DNA and twofold diluted samples of each were applied to successive wells of a slot blotter (Bio-Rad Laboratories, Richmond, Calif.). Membranes, as recommended by the manufacturer (Zeta-Probe membrane; Bio-Rad), were fixed by baking 30 to 60 min at 80°C in a vacuum oven, hybridized at 65°C overnight with ^{32}P-labelled B gene probe, washed three times at 65°C, and autoradiographed (22, 44). The hybridization and washing conditions were identical to those used for Southern hybridization (see below).

Preparation of DNA samples for PFGE. The procedures for the preparation of DNA agarose inserts for prokaryotes described by Smith et al. (36) were followed. To make 10 inserts containing approximately 8 μg of DNA per insert, exponential-growth-phase cultures (50 to 100 ml) containing approximately 10^{10} cells were harvested by centrifugation at

6,000 × *g* for 20 min at 4°C. Inserts to isolate DNA from cells were prepared and treated as described by Smith et al. (36).

For restriction enzyme digestion, protocols provided by enzyme manufacturers were followed. Before digestion, agarose gel inserts containing DNA samples were washed twice with 1 mM phenylmethylsulfonyl fluoride in TE (10 mM Tris-HCl [pH 7.4], 0.1 M EDTA) and three times with TE. For each washing, 1 ml of the solution was added to agarose inserts and incubated for 2 h to overnight at room temperature with gentle shaking. A total of 5 U of restriction enzyme per μg of DNA was added to the buffer and inserts in a volume of 400 μl, which was incubated at 37°C for 16 h. The inserts were washed once with ES (EDTA-Sarcosine; 36) and once with ESP (ES and proteinase K) for 2 h to overnight at 50°C. The compositions of ES and ESP buffers have been described by Smith et al. (36).

PFGE. A contour-clamped homogeneous electric field electrophoresis system (CHEF-DRII; Bio-Rad) was used to separate DNA fragments by PFGE. A total of 1 g of SeaKem LE agarose (FMC BioProducts), in 100 ml of 0.5× TBE buffer (0.1 M Tris base, 0.1 M boric acid, 0.2 mM disodium EDTA), was used to form a gel with dimensions of 12.5 by 14 cm. One-fourth or one-eighth of a DNA insert was electrophoresed at 12 to 14°C for 30 to 40 h with pulse time varying continuously from 0.1 to 20 s. A constant voltage of 200 V was used. Lambda concatemer (Bio-Rad), yeast chromosome (Bio-Rad), and *Hin*dIII-digested lambda DNA were used as size markers. After electrophoresis, the gel was stained with 0.5 μg of ethidium bromide per ml for 1 h to overnight and photographed.

Southern hybridization. The technique of Southern transfer (38), as described in the instruction manual of either Schleicher & Schuell, Inc. (32), or Bio-Rad Laboratories (6), was used with the following modifications. DNA was depurinated by soaking the gel in 200 ml of 0.25 M HCl for 20 to 30 min. DNA was denatured twice in 200 ml of 0.5 M NaOH–1.5 M NaCl for 20 min. Southern capillary transfer was accomplished according to the instruction manual of Schleicher & Schuell, Inc. (32). An alkaline transfer medium containing 0.4 M NaOH and 0.6 M NaCl was used. Either a Nytran nylon membrane (Schleicher & Schuell, Inc.) or a Zeta-Probe blotting membrane (Bio-Rad Laboratories) was used as solid support. After blotting overnight to 48 h, the membrane was neutralized in 200 ml of 5× SSC (1× SSC is 0.15 M NaCl plus 15 mM sodium citrate [pH 7.0]) for 20 min and then rinsed in 200 ml of 2× SSC for 10 min. The blot was air dried and was ready for hybridization after baking at 80°C in a vacuum oven for 60 min. The blot was prehybridized with 0.07 ml of hybridization buffer per cm^2 of membrane in a Seal-a-Meal bag for 2 to 4 h. ^{32}P-labelled soluble MMO component B gene from *M. trichosporium* OB3b (1×10^6 to 3×10^6 cpm/ml of hybridization buffer) was added to the bag, and the bag was resealed. Hybridization and washing of Southern blots were in a shaking water bath as described previously (22). The hybridization and washing temperature for the MMO component B gene probe was 65°C.

^{32}P labelling of the soluble MMO component B gene probe. Either a nick translation system or a random-primer DNA labelling system was used to label the purified MMO component B gene probe. Labelling kits, purchased from Bethesda Research Laboratories Life Technologies, Inc., Gaithersburg, Md., were used. [α-^{32}P]dCTP was used as the source of $^{32}PO_4$. The labelling protocol recommended by Bethesda Research Laboratories was also followed. Specific activities of 2×10^7 to 4×10^7 and 1×10^9 to 2×10^9 cpm/μg for nick translation and random priming, respectively, were routinely obtained.

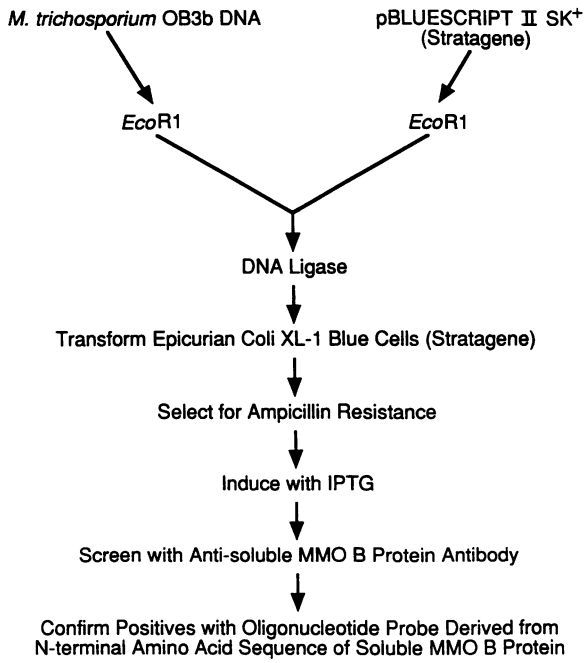

FIG. 1. Cloning strategy for soluble MMO component B gene of *M. trichosporium* OB3b. Epicurian Coli competent cells are *E. coli* UM109 cells that transform at high frequencies.

Synthesis, labelling, and in situ cell hybridization of oligodeoxynucleotide probe. The design, synthesis, and fluorescent-dye labelling of oligodeoxynucleotide signature probes 9-alpha and 10-gamma for the identification of type I and type II methylotrophs have been described previously (44). The sequences of oligodeoxynucleotide signature probes and in situ hybridization with cells on microscope slides were also described previously (44).

Preparation of antibodies against soluble MMO components, SDS-PAGE, and Western blot analysis. The production of antibodies and purification of immunoglobulin G fractions have been described previously (45). A discontinuous sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gel (45) was used for the fractionation of cell proteins. A 10% vertical slab gel was used. Fractionated proteins were transferred to a nitrocellulose membrane by electroblotting according to the protocol supplied by the manufacturer (Bio-Rad). Western blotting and subsequent immunoreactions were also done as described before (45).

RESULTS

Cloning the gene encoding soluble MMO component B protein from *M. trichosporium* OB3b. The strategy used for cloning component B gene from *M. trichosporium* OB3b DNA is summarized in Fig. 1. *M. trichosporium* OB3b DNA was digested with restriction enzyme *Eco*RI, cloned into the *Eco*RI site of pBluescript SK II (Stratagene), and transformed into XL-1 Blue cells (Stratagene). Antibodies against soluble MMO component B protein were used for screening the genomic library. A positive clone was identified. SDS-PAGE and Western blot analysis showed that, upon induction with isopropyl-β-D-thiogalactopyranoside (IPTG), this clone produced a protein that specifically cross-reacted with

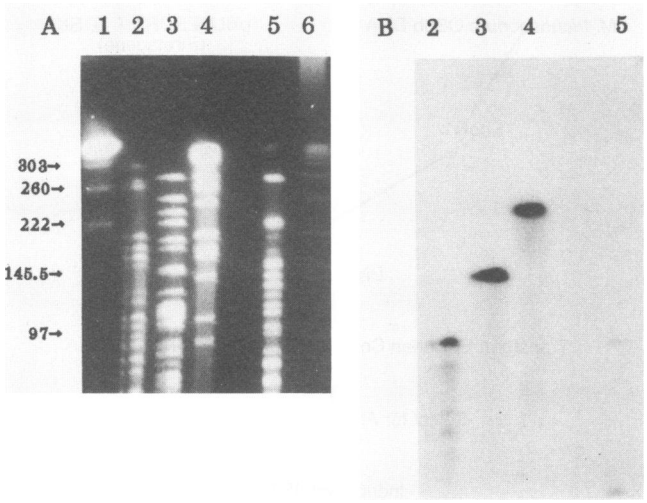

FIG. 2. PFGE separation of restriction enzyme digests of *M. trichosporium* OB3b chromosomal DNA and Southern hybridization with the soluble MMO component B gene probe. (A) Restriction fragmentation patterns of chromosomal DNA digested with *Ase*I (lane 2), *Dra*I (lane 3), *Spe*I (lane 4), and *Xba*I (lane 5). Lanes 1 and 6 are DNA size standards, yeast chromosomes, and lambda concatemer. Numbers with arrows at left indicate sizes of DNA fragments in kilobases. (B) Soluble MMO component B gene fragments identified positively by hybridization with component B gene probe after Southern blotting.

the antibody against component B and comigrated with the purified component B protein. The identity of the DNA insert in the pBluescript clone was subsequently confirmed by hybridization with the oligodeoxynucleotide probe derived from the N-terminal amino acid sequence of the component B protein. The plasmid carrying the component soluble MMO component B gene was digested by restriction enzyme *Eco*RI, analyzed by agarose gel electrophoresis, Southern blotted, and hybridized with the oligodeoxynucleotide probe. The cloned component B gene was contained on a 2.2-kb DNA fragment which hybridized positively with the oligodeoxynucleotide probe for the component B gene on Southern blots.

Sensitivity of detection with the B gene probe. Samples (50 μl), each with 45 ng of cloned plasmid DNA containing the soluble MMO component B gene or 2 μg of *M. trichosporium* OB3b total DNA, were applied to the first well of a slot blotter. Twofold dilutions were applied to subsequent wells until a 250-fold dilution of each sample was applied to the final wells in a series. With the oligonucleotide probe or the 2.2-kb soluble MMO component B gene probe labelled by nick translation, the MMO B gene in the plasmid containing the 2.2-kb fragment can be detected with 0.5 to 1 ng of plasmid DNA, whereas the detection limit for the gene in total *M. trichosporium* OB3b DNA is 15 to 30 ng of total DNA.

PFGE and Southern blot analysis of chromosomal DNA of *M. trichosporium* OB3b after digestion with rare cutting restriction endonuclease. The chromosomal DNA of *M. trichosporium* OB3b was digested with rare cutting restriction endonuclease. Five restriction enzymes, *Ase*I, *Dra*I, *Spe*I, *Ssp*I, and *Xba*I, all of which have recognition sequences rich in A and T, were selected. All restriction enzymes except *Ssp*I cut *M. trichosporium* OB3b DNA, producing between 20 and 30 fragments (Fig. 2A). *Ssp*I cut OB3b DNA to a large number of small fragments which moved faster in the gel under the conditions used. Southern hybridization revealed that the lengths of B gene-carrying fragments vary, depending on the restriction enzyme used (Fig. 2B). The restriction enzyme *Xba*I produced two fragments (180 kb and 50 kb) which hybridized with the B gene probe.

Restriction fragment length polymorphism of methanotroph DNAs analyzed by PFGE after restriction digestion with *Ase*I and Southern hybridization with component B gene probe. A collection of methanotrophs, including pure cultures, enriched cultures, and samples from environments, were prepared for PFGE and treated with restriction enzyme *Ase*I. DNA fragments of methanotrophic bacteria were well separated by PFGE after digestion with enzyme *Ase*I. The restriction fragment patterns of these DNAs are shown in Fig. 3A. Different patterns for each DNA sample were observed. Restriction fragments produced from DNAs isolated from *M. trichosporium* OB3b and *Methylosinus* sp. strain B were very similar in size, indicating that they are closely related isolates. All environmental samples produced a smeared DNA strip representing the presence of DNA fragments of all sizes. It is believed that the DNA molecules in the environmental sample are from a mixed population of diverse organisms.

Hybridization with the B gene probe after Southern transfer revealed that four methanotrophs, *M. trichosporium* OB3b, *Methylosinus methanica* 81Z, *Methylosinus sporium*, and a bioreactor sample, BioR, hybridized positively with the soluble MMO component B gene probe (Fig. 3B). It indicated that these methanotrophs contain a gene encoding soluble MMO component B protein. The sizes of DNA fragments which harbor the soluble MMO B gene are listed in Table 2.

By PFGE and Southern blot analysis, the soluble MMO component B gene was found in a methanotroph isolated from an aquatic plant, *L. minor* (electrophoresis data not shown) (Table 3). The restriction fragment length for the *L. minor* isolate is 50 kb (Table 2).

Synthesis of soluble MMO and degradation of TCE by methanotrophs. Cultures of bacteria were grown under conditions to promote the expression of soluble MMO (45). Samples from these cultures and from a bioreactor sample were tested for the presence of soluble MMO, hydroxylase, and component B by SDS-PAGE and Western blot analysis and for the ability to degrade TCE. Some environmental samples were also tested. For environmental samples, suspensions of bacteria isolated without enrichment were lysed and used directly for PFGE. As shown in Table 3, only methanotrophs possessing soluble MMO are able to degrade TCE at reasonably high rates. Among pure cultures, five methanotrophs, *M. trichosporium* OB3b, *M. methanica* 81Z, *M. sporium*, *Methylosinus* sp. strain B, and *Methylococcus capsulatus* Bath, are capable of producing soluble MMO and of degrading TCE. All of them synthesized soluble MMO which cross-reacts with antibodies prepared against the hydroxylase of *M. trichosporium* OB3b. *M. capsulatus* Bath, however, did not produce a protein which was cross-reactive to antibody against component B protein (Fig. 4). Other than methanotrophic pure cultures, the bioreactor sample and the isolate from the aquatic plant *L. minor* synthesized soluble MMO hydroxylase and component B (Table 3).

Determination of physiological group of soluble MMO-producing methanotrophs by oligodeoxynucleotide signature probes targeting 16S ribosomal RNA. Cells of methano-

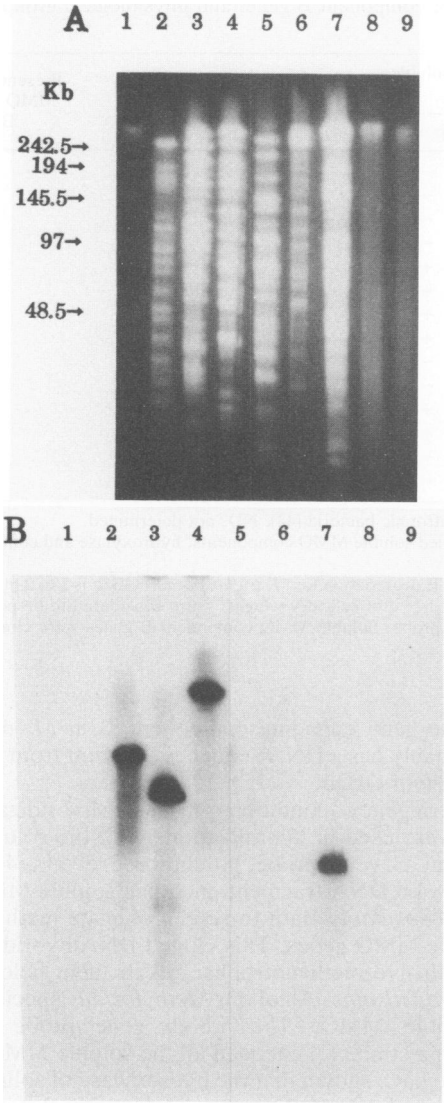

FIG. 3. Detection of bacteria from pure cultures, a bioreactor, and environmental samples containing sequences homologous to that of the soluble MMO B gene with the 2.2-kb soluble MMO component B gene probe. PFGE separation (A) and Southern blot hybridization (B) of DNA preparations digested by restriction enzyme *Ase*I. Lanes: 1, DNA samples of lambda concatemer; 2, *M. trichosporium* OB3b; 3, *M. methanica* 81Z; 4, *M. sporium*; 5, *Methylocystis parvus* OBBP; 6, *M. capsulatus* Bath; 7, bioreactor sample; 8, bacteria isolated from the surface of *L. minor*; 9, environmental sample from Lake Mendota. Numbers to the left indicate DNA fragment sizes. The Southern blot hybridization shows that *M. trichosporium* OB3b, *M. methanica* 81Z, *M. sporium*, and the bioreactor sample hybridized positively with the component B gene probe.

trophs, pure and enrichment cultures, environmental isolates, and a bioreactor sample were examined for their ability to bind with fluorescence-labelled signature probes as determined by fluorescence microscopy (44). *M. capsulatus* Bath, a type X methanotroph similar to type I methanotrophs, employs the ribulose monophosphate (RuMP) pathway as the major route of formaldehyde assimilation. This bacterium hybridized positively with signature probe 10-gamma, a type I signature probe. With this exception, all other soluble MMO-producing methanotrophs bound probe 9-alpha, a type II signature probe (Table 3).

TABLE 2. Sizes of *Ase*I restriction fragments which hybridize with the *M. trichosporium* OB3b MMO component B probe

Methanotroph sp. or source	Approx size of component B gene fragment (kb)
M. trichosporium OB3b	160–165
M. methanica 81Z	120–125
M. sporium	285–290
Bioreactor sample	65–70
Aquatic plant isolate[a]	55–60

[a] Methanotrophic pure culture isolated from *L. minor*.

DISCUSSION

Antibodies prepared against the soluble MMO component B were used to screen the *M. trichosporium* OB3b gene bank. We successfully cloned a 2.2-kb DNA fragment harboring the gene encoding soluble MMO component B protein. Upon IPTG induction, the cloned gene was expressed in the transformation *Escherichia coli* host. The expressed protein cross-reacted with the antibody prepared against soluble MMO component B protein and comigrated with the purified component B protein. The 2.2-kb DNA fragment hybridized positively with the oligodeoxynucleotide probe which was synthesized by using the N-terminal amino acid sequence of soluble MMO component B protein.

The DNAs of type II methanotrophs have G+C contents of 62.5 to 64% (17). We therefore selected restriction enzymes with recognition sequences rich in A and T residues, expecting that these enzymes would cut DNA from type II methanotrophs infrequently. Restriction endonuclease *Ase*I cuts DNA from type II methanotrophs, producing a limited number of fragments ranging in size from 10 to several hundred kb, whereas it cuts DNA from type I methanotrophs to many small fragments in the range of 5 to 50 kb. This phenomenon can be used in preliminary differentiation of type I and type II methanotrophs. The G+C contents of type I methanotrophs are 50 to 54% (17, 49).

Under appropriate conditions, all methanotrophs containing the soluble MMO component B gene can synthesize soluble MMO and degrade TCE under the appropriate growth conditions. All of them are type II methanotrophs, as determined by fluorescence microscopy after hybridizing with fluorescence-labelled signature probes (Table 3).

M. capsulatus Bath cells are capable of synthesizing soluble MMO and degrading TCE. The component B gene of this bacterium is located within the soluble MMO gene cluster between genes encoding the beta and gamma subunits of hydroxylase. However, the chromosomal DNA from *M. capsulatus* Bath does not hybridize with the cloned component B gene probe from *M. trichosporium* OB3b. This can be explained by the results of SDS-PAGE and Western blot analysis. We demonstrated that *M. capsulatus* Bath does not produce a protein which cross-reacts with the antibody against the soluble MMO component B of *M. trichosporium* OB3b. This is an indication that the soluble MMO component B protein of *M. capsulatus* Bath is different from that of *M. trichosporium* OB3b. The difference is probably not limited to the antigenic determinant of the protein; it may extend to the amino acid sequence. As a

TABLE 3. Correlation of soluble MMO, TCE degradation, the presence of component B gene, and physiological group for methanotrophic bacteria

Methanotroph sp. or source	Physiological group[a]	Presence of the following soluble MMO components[b]:		TCE degradation[c]	Presence of soluble MMO component B gene[d]
		Hydroxylase	Component B		
M. albus BG8	I	−	−	−	−
M. methanica	I	−	−	−	−
M. rubra	I	−	−	−	−
M. capsulatus Bath	X	+	−	+	−
M. methanica 81Z	II	+	+	+	+
M. sporium	II	+	+	+	+
Methylosinus sp. B	II	+	+	+	+
M. trichosporium OB3b	II	+	+	+	+
M. parvus OBBP	II	−	−	−	−
M. pyriformis 14	II	−	−	−	ND
Bioreactor sample	II	+	+	+	+
Isolate from *L. minor*	ND	+	+	+	+
Garden soil	ND	−	−	−	−
Peat sample	ND	−	−	−	−

[a] Determined by oligodeoxynucleotide signature probes for physiological groups of methylotrophic bacteria (44). ND, not determined.
[b] Determined by SDS-PAGE and Western blot probed with antibodies prepared against purified soluble MMO components, hydroxylase and component B, of *M. trichosporium* OB3b (45). +, presence; −, absence.
[c] Determined by colorimetric assay and gas chromatographic assay (8). The typical rate of TCE degradation for *M. trichosporium* OB3b is 1 to 2 mmol · h^{-1} · g of cell (dry weight)$^{-1}$ (8). Only methanotrophs with TCE degradation rates exceeding 0.1 mM h^{-1} g of cell (dry weight)$^{-1}$ are considered to be positive.
[d] Determined by PFGE and Southern hybridization with cloned 2.2-kb component B gene probe. Soluble MMO component B genes were cloned from *M. trichosporium* OB3b. +, presence; −, absence; ND, not determined.

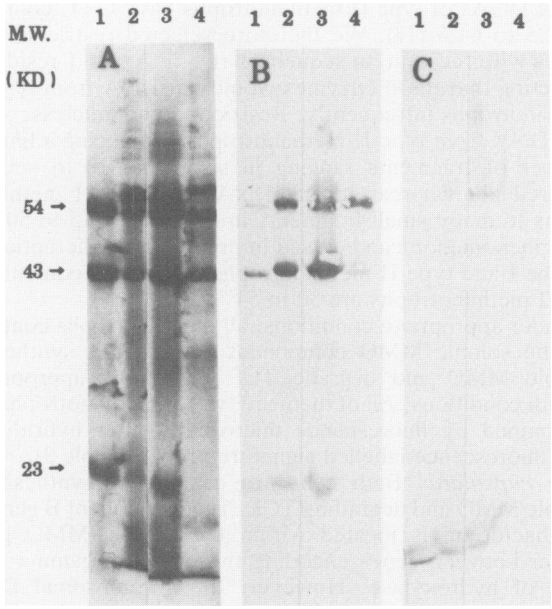

FIG. 4. SDS-PAGE and Western blot analyses of soluble MMO hydroxylase and component B of bioreactor samples and plant isolate. Shown are SDS-PAGE of solubilized proteins stained with Coomassie blue (A) and Western blot analyses of the same samples probed with antibodies prepared against purified hydroxylase (B) and component B (C). The samples from lanes 2 to 4 are *M. trichosporium* OB3b, the plant isolate, and *M. capsulatus* Bath, respectively. The sample from lane 1 in panels A and B is purified hydroxylase and that from lane 1 in panel C is purified component B protein. Component B is absent from *M. capsulatus* Bath. MW, molecular weight.

result, the gene encoding component B in *M. capsulatus* Bath probably has a DNA sequence different from that of *M. trichosporium* OB3b.

High-stringency conditions in both hybridization and washes were used in hybridizations with the soluble MMO component B gene probe. Stainthorpe et al. (41) used a cloned 5.8-kb DNA fragment encoding soluble MMO genes from *M. capsulatus* Bath to screen obligate methanotrophs for soluble MMO genes. This cloned DNA hybridized positively with five methanotrophs. All of them belong to the genus *Methylosinus*. All of our *Methylosinus* species synthesize soluble MMO. The 5.8-kb gene probe used by Stainthorpe et al. (41) encoded all the soluble MMO components. We have shown that the hydroxylase of soluble MMO of *M. capsulatus* Bath cross-reacts with the antibodies against the hydroxylase of *M. trichosporium* OB3b and that it comigrates with the purified hydroxylase of *M. trichosporium* OB3b (Fig. 4). For this reason, the high degree of homology between two sets of genes which encode soluble MMO in *M. capsulatus* Bath and *M. trichosporium* OB3b is possibly in a region other than the region encoding the component B protein.

A DNA probe for the detection of specific microorganisms in the soil bacterial communities has been used successfully by Holben et al. (21). They reported that *Bradyrhizobium japonicum* at the density of 4.3×10^4 cells per g (dry weight) of soil is sufficient for a positive identification. There are very few ecological studies concerning the populations of methanotrophic bacteria in environments (19). The results of these studies were complicated by the methodological problem that the recovery of control cells added to the environment was not quantitative (29). Therefore, the number of methanotrophs in bacterial communities is virtually unknown.

We concluded from this study that, when used together with phylogenetic staining and SDS-PAGE Western blot analysis, cloned soluble MMO component B gene has been proven to be effective as a gene probe for the detection and

classification of methanotrophs that contain soluble MMO genes and that have the potential to degrade TCE. *M. capsulatus* Bath was the only methanotroph capable of degrading TCE that was not detected by this probe.

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