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Methane Oxidation Potentials of Rice-associated Plant Growth Promoting *Methylobacterium* Species

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Abstract

BACKGROUND: Methane is a major greenhouse gas attributed to global warming partly contributed by agricultural activities from ruminant fermentation and rice paddy fields. Methanotrophs are microorganisms that utilize methane. Their unique metabolic lifestyle is enabled by enzymes known as methane monooxygenases (MMOs) catalyzing the oxidation of methane to methanol. Rice absorbs, transports, and releases methane directly from soil water to its stems and the micropores and stomata of the plant epidermis. *Methylobacterium* species associated with rice are dependent on their host for metabolic substrates including methane.

METHODS AND RESULTS: *Methylobacterium* spp. isolated from rice were evaluated for methane oxidation activities and screened for the presence of sMMO *mmoC* genes. Qualitatively, the soluble methane monooxygenase (sMMO) activities of the selected strains of *Methylobacterium* spp. were confirmed by the naphthalene oxidation assay. Quantitatively, the sMMO activity ranged from 41.3 to 159.4 nmol min^{-1} mg of protein^{-1}. PCR-based amplification and sequencing confirmed the presence and identity of 314 bp size fragment of the *mmoC* gene showing over 97% similarity to the CBMB27 *mmoC* gene indicating that *Methylobacterium* strains belong to a similar group.

CONCLUSION(S): Selected *Methylobacterium* spp. contained the sMMO *mmoC* gene and possessed methane oxidation activity. As the putative methane oxidizing strains were isolated from rice and have PGP properties, they could be used to simultaneously reduce paddy field methane emission and promote rice growth.

Key words: Methane, Methane oxidation, Methanotrophs, *Methylobacterium* spp., Soluble methane monooxygenase

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Introduction

The increased concentration of greenhouse gases (GHGs) such as carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O) alter the energy balance of the atmosphere resulting in climate change and global warming [1]. Methane is the second most important gas contributing to global warming with 21 times more heat-trapping potency than CO_2 [2]. Globally, agriculture accounts for about 56% of the anthropogenic methane emissions due to enteric fermentation and rice paddy production [3]. Rice paddy fields are one of the largest sources in the global budget of atmospheric CH_4 [4]. More than 90% of the global emission from rice fields are from Asian countries [5].

The difference between CH_4 production and emission probably reflects CH_4 oxidation in the oxic zones around the rice roots and at the soil surface, since they contain increased numbers of methane oxidizing bacteria (MOB, methanotrophic bacteria) [6]. The enhanced methane oxidation in these areas by MOB decreases the overall methane emission in rice paddy fields [6, 7]. MOB are characterized by the capability to use CH_4 as a sole carbon and energy source. The initial oxidation of methane to methanol is catalyzed by the enzyme methane monooxygenase (MMO) classified as soluble (sMMO) and particulate methane monooxygenase (pMMO) [8].

Methylobacterium, "pink pigmented facultative methylotrophic bacteria" (PPFMs), are ubiquitous in nature, aerobic, members of the gram-negative class *Alphaproteobacteria* [9]. Although they can grow on a wide range of multi-carbon substrates, they are known to utilize single carbon compounds such as formate, formaldehyde or methanol, and methylamine [10]. Several Methylobacterial species are plant growth promoting bacteria (PGP) through the production of ACC (1-aminocyclopropane 1-carboxylate) deaminase, indole acetic acid, cytokinin, acids to release insoluble phosphate, siderophore, nitrogen fixation and pathogen antagonism [11-16]. Some novel species of *Methylobacterium* can survive using CH_4 as the sole source of carbon and energy source [17]. Considering the abundance and PGP effects of Methylobacterial strains, studying their methane utilization adds value to their application as methane oxidizing PGP for sustainable agricultural purposes.

The objective of this study is to evaluate the presence and activity of the sMMO in *Methylobacterium* spp. Nineteen *Methylobacterium* spp. strains previously isolated from rice grown in different regions of South Korea were selected for the experiment. To confirm the presence of MMO in selected strains, qualitative studies were carried out using colorimetric plate assay and a quantitative evaluation was carried out for sMMO activity. The strains were subjected to PCR assay using sMMO *mmoC* specific primers to confirm the presence of the gene. Then the PCR products were gel-eluted, reamplified, sequenced, and compared with the identified *mmoC* gene sequence. This study will pave the way for utilizing promising isolates to reduce the amount of methane gas generated in paddy fields while promoting the growth of rice and other plants.

Materials and Methods

Bacterial Strains and Culture Conditions

Methylobacterium spp. from rice were selected for the study. The PGP activities of these nineteen *Methylobacterium* isolates have been previously characterized [11, 12]. These *Methylobacterium* strains are capable of nitrogen fixation, IAA and cytokinin production, and improvement of germination and plant growth. For inoculum preparation, a loop of *Methylobacterium* strain from the stock was drawn and grown on ammonium mineral salts (AMS) or nitrate mineral salts (NMS) agar medium (pH 6.8) and incubated at 28°C for 72 h. AMS and NMS are two basal media routinely used for enrichment and isolation of methane-utilizing bacteria [18]. The AMS media was prepared according to Whittenbury et al. [18] and was supplemented with 0.5% sodium succinate. The nitrate mineral salt (NMS; ATCC medium 1306) was also supplemented with 0.5% sodium succinate. Additionally, to check the growth of *Methylobacterium* strains using methane as a carbon source, 50 ml of AMS or NMS broth with 0.75g agar and without sodium succinate were prepared in 125 ml serum bottles. *Methylobacterium* sp. was streaked carefully on the slants and sealed with sterilized rubber caps. Methane at 20% v/v of headspace was injected into each bottle. The bottles were then incubated at 28°C for 4 to 7 days.

Qualitative and Quantitative Detection of Soluble Methane Monooxygenase

For qualitative detection of sMMO enzyme,

Methylotrobacterium spp. were grown on AMS and NMS medium for 72 h. A few naphthalene crystals were sprinkled on the lid of the plate and the plates were stored in an inverted position at 30°C for 1 h in air. The plates were opened followed by a spray of ortho-dianisidine dye (Fast blue B salt) (5 mg ml^{-1}) for 2-3 s. The lids were replaced and the plates were incubated for 15 min. The appearance of purple-red color upon contact with the dye is due to the production of naphthol by the colonies [19].

The naphthalene oxidation assay was followed for the quantitative determination of sMMO [20]. Each *Methylobacterium* culture was transferred in 1 ml aliquots into 20 ml test tubes and 1 ml of pre-filtered saturated naphthalene solution was added to each tube. Reaction mixtures were incubated at 200 rpm on an incubator shaker at 25°C for 1 h. After incubation, 100 μl of 4.21 mM tetrazotized-o-dianisidine solution (Fast blue B salt) was added to each tube and the intensity of the colored diazo-dye complex was monitored after 15 min by recording the A_{525} by spectrophotometry (UV-1601, Shimadzu, Japan). The intensity of diazo-dye formation is proportional to the naphthol concentration (1-naphthol and 2-naphthol). The specific activity of sMMO was expressed as nanomoles of naphthol formed per milligram of cell protein per minute. Protein estimation was carried out according to Lowry [21] using bovine serum albumin as standard and Methylobacteria grown broth used for sMMO activity was used for the enumeration of colony forming units.

Primer Design Using CBMB27 Genome

For polymerase chain reaction (PCR) amplification of sMMO genes, primers were designed using the sequences of the sMMO gene clusters of *Methylobacterium phyllosphaerae* (NCBI:locus_tag=CBMB27_028888) using Primer3 version 2.2.3 with the following parameters: primer length = 18-22 bp (Opt. 20 bp); GC% = 40-60% (Opt. 50%); temperature = 57-63°C (Opt. 60°C); and product size range = 300–550 bp. The most suitable primer was selected after running BLASTn for off-target using CLC Main Workbench. Approximately, 314 bp fragments of *mmoC* were amplified between nucleotides *mmoC*ykF and *mmoC*ykR. The primer pairs were submitted to NCBI Probe (https://www. ncbi.nlm.nih.gov/probe).

PCR Amplification and Sequencing of sMMO *mmoC* Genes

The PCR amplification of sMMO *mmoC* genes was performed using primer sets *mmoC*ykF (5'-CACACT GGAGCTGATGATCGA-3') and *mmoC*ykR (5'-AGAA GTTGGGATCGGCTTCC-3'); NCBI, Probe: (Pr032826540) selected from the five primers designed earlier (Table 1). Colony PCR was performed using *Methylobacterium* sp. A fresh bacterial colony was transferred to a microcentrifuge tube containing 20 μL sterile water. The PCR reaction mixture consisted of a 2 μL template colony mixture, 2 μL of F and R primers each, 10 μL Taq Mix (Dong Sheng Biotech, South Korea) with 2X PCR buffer, 0.4 mM dNTPs, 0.25 U μ L^{-1} Taq DNA polymerase, and made up to 20 μL using sterile water. The PCR reactions were carried out in a Thermocycler (ThermalCycler, BIO-RAD, USA) with conditions: 95°C for 5 min, 35 cycles consisting of 95°C for 30 sec denaturation, 59°C for 30 sec annealing, and 72°C for 1 min extension with a final extension of 72°C for 15 min.

Gel eluted sMMO *mmoC* gene products of *Methylobacterium* spp. were sequenced at Solgent,

Table 1. List of primer sets designed in this study

	Candidate		Primer sequence	Product size (bp)
MCBMB27_ 02888 (sMMO target gene)	1	F	GTGATTGAGTCGGCGATCGA	547
		R	TCGCAAGATGCAGGGAGAAG	
	2	F	GGCACACTGGAGCTGATGAT	431
		R	TTGGAGCCGCAGAGGAAGA	
	3	F	CTGGAGCTGATGATCGAGGC	315
		R	CAGGGAGAAGTTGGGATCGG	
	4	F	CACACTGGAGCTGATGATCGA	314
		R	AGAAGTTGGGATCGGCTTCC	
	5	F	AGCTGATGATCGAGGCCCT	315
		R	GATGCAGGGAGAAGTTGGGA	

Daejeon, Korea. The sequence outputs were aligned using CLC Main Workbench 6.8.4 (http://clcbio.com). Similarity searches were carried out at the Nucleotide BLAST, NCBI (https://www.ncbi.nlm.nih.gov). A phylogeny tree was constructed using MEGA 7.0.26. Sequences of the *mmoC* gene were deposited in GenBank and the accession numbers of the entries were OM396913 – OM396931.

Statistical Analysis

Significant differences of the sMMO activity of *Methylobacterium* spp. strains were checked by conducting analysis of variance and Tukey's test using SAS Version 9.4.

Results

Growth of *Methylobacterium* spp. and Detection of sMMO Activity

Methylobacterium spp. strains were evaluated for their growth with methane as a sole carbon source and their qualitative and quantitative sMMO activity (Table 2). All of the strains grew well in AMS medium and some strains grew slowly in NMS medium. In the plate assay, the oxidation of naphthalene to 1-

and 2-naphthol by the sMMO gene turned the media to deep purple or brownish indicating the production of naphthol by the bacteria (Fig. 1; Fig. 2). All strains showed positive activity in the plate assay. In the quantitative determination, sMMO activity ranged from 41.3 to 159.4 nmol min^{-1} mg of protein^{-1} observed in *Methylobacterium* sp. CBMB48 and *Methylobacterium* sp. CBMB17, respectively. The population ranged from 0.38×10^8 - 3.36×10^{11} cfu mL^{-1}.

Detection of sMMO *mmoC* Gene in *Methylobacterium* spp.

For the detection of sMMO *mmoC* genes, primers were designed using the sequences of the sMMO gene clusters of *Methylobacterium phyllosphaerae*. In the primer designing process, the repetitive DNA, as well as duplicate sequences were excluded and primers were selected from the unique genomic regions. After the off-target of compared primer sequence and *mmoC* gene sequence is checked, the most suitable primer was selected. As a result, five different primer sets were designed (Table 1). After comparison, the most suitable primer set was selected *mmoCykF* (5'-CACACTGGAGCTGATGATCGA-3') and *mmoCykR*

Table 2. Growth in AMS and NMS media with methane as a sole carbon source and determination of sMMO activities of selected *Methylobacterium* spp. strains

Strains	A	B	C	D	sMMO activity (E)	Population (F)
Methylobacterium fujisawaense CBMB12	+++	+++	+	+	106.5 ± 8.3 bc	1.07×10^9
Methylobacterium fujisawaense CBMB13	+++	+++	+	+	62.3 ± 1.0 de	0.77×10^9
Methylobacterium sp. CBMB15	+++	+++	+	+	85.7 ± 8.9 cd	0.73×10^{10}
Methylobacterium sp. CBMB17	+++	+++	+	+	159.4 ± 17.5 a	0.61×10^9
Methylobacterium sp. CBMB19	++	++	+	+	60.8 ± 2.5 de	2.89×10^{11}
Methylobacterium oryzae CBMB20	+++	+	+	+	82.8 ± 9.4 cd	1.20×10^9
Methylobacterium phyllosphaerae CBMB27	++	++	+	+	107.1 ± 6.0 bc	0.98×10^9
Methylobacterium fujisawaense CBMB31	+++	+	+	+	60.6 ± 5.3 de	1.15×10^8
Methylobacterium sp. CBMB35	+++	++	+	+	128.4 ± 11.9 ab	0.53×10^{11}
Methylobacterium fujisawaense CBMB37	+++	+++	+	+	82.3 ± 9.9 cd	0.62×10^9
Methylobacterium sp. CBMB38	++	+	+	+	86.9 ± 7.7 cd	0.74×10^{11}
Methylobacterium fujisawaense CBMB45	+++	+++	+	+	65.1 ± 3.4 de	1.19×10^9
Methylobacterium fujisawaense CBMB46	++	+++	+	+	57.8 ± 3.5 de	1.02×10^8
Methylobacterium sp. CBMB48	+++	+++	+	+	41.3 ± 2.1 e	0.38×10^8
Methylobacterium fujisawaense CBMB50	+++	++	+	+	55.2 ± 0.7 de	0.44×10^8
Methylobacterium fujisawaense CBMB53	+++	+	+	+	56.1 ± 5.5 de	0.58×10^8
Methylobacterium oryzae CBMB110	+++	++	+	+	79.8 ± 2.0 cde	0.33×10^{10}
Methylobacterium suomiense CBMB120	+++	+++	+	+	78.3 ± 5.9 cde	3.04×10^{11}
Methylobacterium rhodinum CBMB130	+++	+++	+	+	78.1 ± 5.7 cde	3.36×10^{11}

A - Growth of *Methylobacterium* spp. in AMS with methane as a sole carbon source; B - Growth of *Methylobacterium* spp. in NMS with methane as a sole carbon source; C - Qualitative detection of sMMO activity using plate assay on AMS medium; D - Qualitative detection of sMMO activity using plate assay on NMS medium; E - Naphthalene oxidation nmol min^{-1} mg of protein^{-1}; F - cfu mL^{-1}; For A and B: + - low, ++ - moderate, +++ - high growth; For C and D: + positive, - not detected. Values in each column are the means of three replications ± S.E. Values in each column followed by the same letter are not significantly different at 0.05% (Tukey's).

(5'-AGAAGTTGGGATCGGCTTCC-3'); NCBI, Probe: Pr032826540.

To determine the specificity of the PCR primers designed to amplify regions of the *mmoC* gene of the sMMO gene cluster in all the *Methylobacterium* spp., strains were subjected to PCR amplification (Fig. 3). The same amplicon size of 314 bp was observed in all the strains and thus the presence of *mmoC* gene was confirmed in the *Methylobacterium* spp. The gel eluted PCR re-amplification again confirmed the presence of *mmoC* gene in *Methylobacterium* spp. (Fig. 4). The obtained sequences were compared for similarity with the *Methylobacterium phyllosphaerae* CBMB27 methane monooxygenase component C (APT32179.1) using BLAST in GenBank at NCBI. To identify the similarity a phylogenetic tree was constructed using the sequences of all the strains (Fig. 5). The sequences had over 97% similarity indicating that they belong to a similar group of *Methylobacterium* sp. (Table 3).
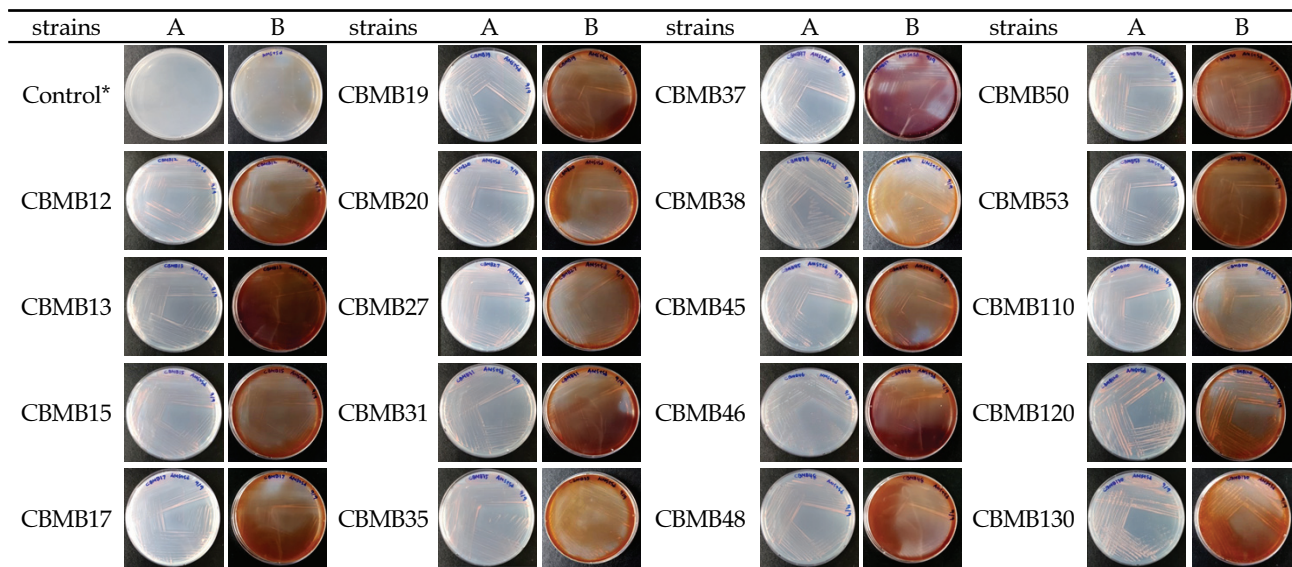
Growth of *Methylobacterium* spp. in (A): AMS medium, (B): *Methylobacterium* spp. in AMS medium showing sMMO activity, (*) : Only AMS medium.

Fig. 1. Qualitative detection of sMMO activity using plate assay on AMS medium.
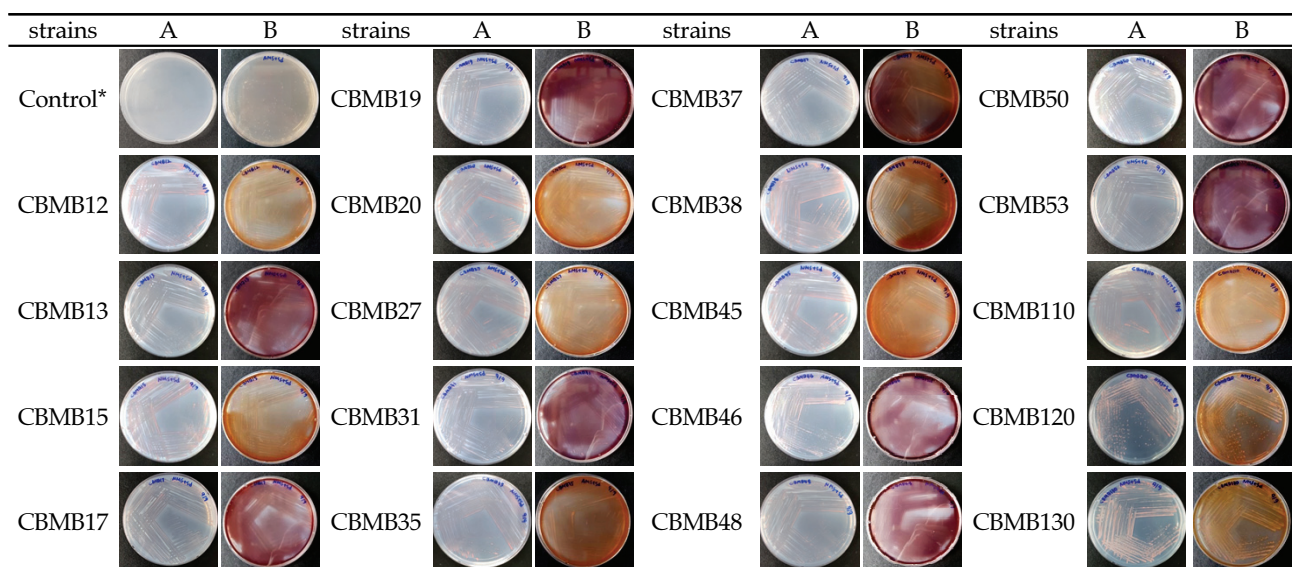
Growth of *Methylobacterium* spp. in (A) NMS medium, (B) *Methylobacterium* spp. in NMS medium showing sMMO activity, (*) : Only NMS medium.

Fig. 2. Qualitative detection of sMMO activity using plate assay on NMS medium.

Table 3. Identities of *mmoC* gene sequence of *Methylobacterium* spp

| Strains	Identities
Methylobacterium fujisawaense CBMB12	99%
Methylobacterium fujisawaense CBMB13	98%
Methylobacterium sp. CBMB15	98%
Methylobacterium sp. CBMB17	99%
Methylobacterium sp. CBMB19	98%
Methylobacterium oryzae CBMB20	99%
Methylobacterium phyllosphaerae CBMB27	100%
Methylobacterium fujisawaense CBMB31	97%
Methylobacterium sp. CBMB35	99%
Methylobacterium fujisawaense CBMB37	99%
Methylobacterium sp. CBMB38	99%
Methylobacterium fujisawaense CBMB45	98%
Methylobacterium fujisawaense CBMB46	99%
Methylobacterium sp. CBMB48	99%
Methylobacterium fujisawaense CBMB50	99%
Methylobacterium fujisawaense CBMB53	99%
Methylobacterium oryzae CBMB110	99%
Methylobacterium suomiense CBMB120	99%
Methylobacterium rhodinum CBMB130	99%
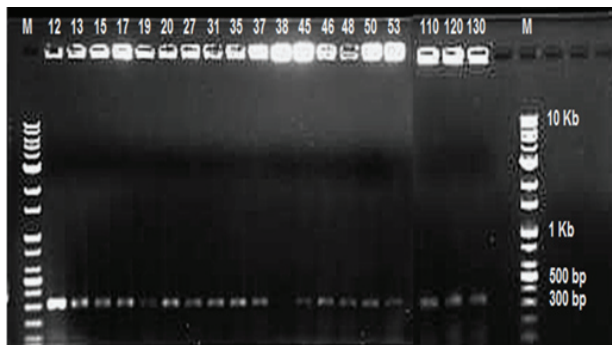
Fig. 3. PCR amplification and detection of soluble methane monooxygenase (sMMO) in *Methylobacterium* spp. using the *mmoC* specific primers (*mmoCykF* and *mmoCykR*) designed in this study. Lane M - 1 kb Plus DNA Ladder (SolGent). The other lanes contained the PCR products obtained after amplification with *mmoC* specific primers from different *Methylobacterium* sp. as follows - CBMB12, CBMB13, CBMB15, CBMB17, CBMB19, CBMB20, CBMB27, CBMB31, CBMB35, CBMB37, CBMB38, CBMB45, CBMB46, CBMB48, CBMB50, CBMB53, CBMB110, CBMB120 and CBMB130.

Discussion

Methylobacterium spp. occupy different habitats due to their great phenotypic plasticity. In meristematic tissues, they can reach populations of 10^4 to 10^6
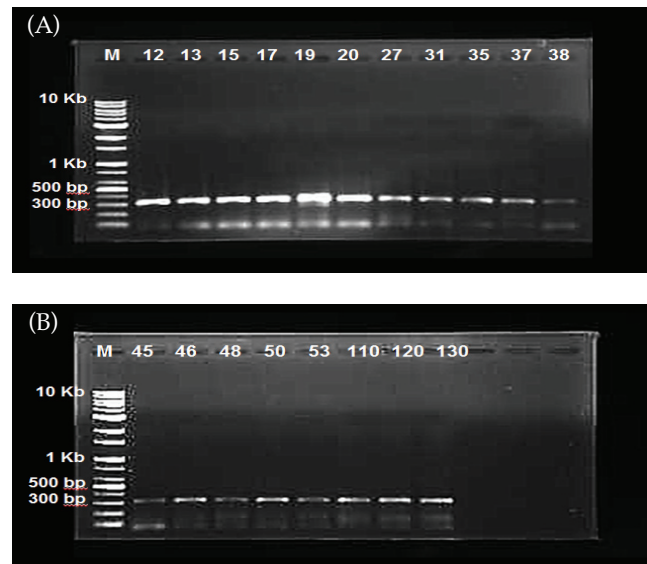

Fig. 4. Reamplification of the gel eluted products for confirmation. 1% agarose gel electrophoresis confirmed the presence of sMMO *mmoC* gene in all the *Methylobacterium* spp. Lane M - 1 kb Plus DNA Ladder (SolGent). The other lanes contained the PCR products obtained after amplification with *mmoC*-specific primers from different *Methylobacterium* sp. as follows − A: CBMB12, CBMB13, CBMB15, CBMB17, CBMB19, CBMB20, CBMB27, CBMB31, CBMB35, CBMB37, CBMB38, B: CBMB45, CBMB46, CBMB48, CBMB50, CBMB53, CBMB110, CBMB120 and CBMB130.
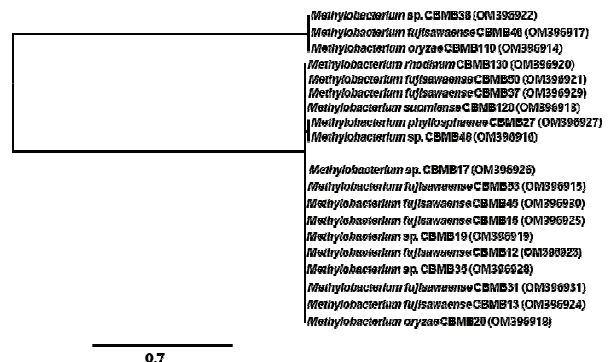

Fig. 5. Phylogenetic tree based on sMMO *mmoC* gene sequences from nineteen *Methylobacterium* spp. strains constructed using the neighbor-joining method.

colony-forming units (CFU) per gram of plant tissue. They also form biofilms and use methylotrophic metabolism as an adaptive advantage during plant host colonization [22]. *Methylobacterium* spp. from various habitats are also proven plant growth promoters [11, 12]. However, there was limited screening for methane utilization of the isolated strains.

Methylobacterium sp. can grow on one-carbon

compounds such as formate, formaldehyde, and methanol as the sole source of carbon and energy, as well as on a wide range of multi-carbon growth substrates [10]. The type species of this genus, *Methylobacterium organophilum*, was originally described as a facultative methane-utilizing bacterium [23]. The *Methylobacterium* sp. strain CRL-26 grown in a fermenter was reported to contain methane monooxygenase activity in soluble fractions [24]. Another type strain, *Methylobacterium populi*, utilizes methane as the sole source of carbon and energy [17]. To study methane oxidation, Dedysh et al. [25] recommended checking purity, growth on methane, identification of formaldehyde assimilation pathway, observation of intracytoplasmic membrane, MMO activity measurement, and detection of gene encoding MMO. Dedysh et al. [26] also demonstrated that members of *Methylobacterium* could grow on substrates containing one carbon or multi carbon compounds. They reported that the growth rate and carbon conversion efficiency were higher on acetate than on methane and that acetate was preferred by the bacteria when both substrates were provided in excess and methane oxidation was shut down. With this, they concluded that not all methanotrophic bacteria are limited to growing on one-carbon compounds and that this could have major implications for understanding the factors controlling methane fluxes in the environment.

For the first time, *Methylobacterium organophilum* (CZ-2) was reported to have the ability to grow on methane and accumulate polyhydroxybutyrate (PHB) under nitrogen limitation [27]. In the present study, some of the *Methylobacterium* sp. grew well and some could grow slowly when methane was used as a sole carbon source in AMS and NMS media. The growth of selected strains was better in both AMS and NMS media with few strains showing lesser growth in the NMS medium. Though *Methylobacterium* strains can be enriched in NMS medium [28], the lower growth of some strains observed in the present study could be due to nitrate as a nitrogen source. Conversely, there are more reports on the use of AMS medium than NMS in enriching methanotrophs [18].

The naphthalene oxidation assay has been used to evaluate the activity of sMMO. The cytoplasm bound sMMO is capable of oxidizing naphthalene to 1- and 2-naphthol [29] and the color intensity stabilizes to a brown color [30]. In this study, some isolates visually

changed the media to brown and purple color. Koh et al. [31] quantitatively reported the sMMO activity of *Methlyomonas methanica* 68-1 and *Methylosinus trichosporium* OB3b as 551 ± 27 and 321 ± 16 nmol h^{-1} mg of protein^{-1}, respectively. However, there was little genetic homology between OB3b and 68-1 indicating an evolutionary diversification of the sMMOs. In the present study, the sMMO activity of the *Methylobacterium* spp. strains were confirmed qualitatively and quantitatively and varied from 41.3 to 159.4 nmol min^{-1} mg of protein^{-1}. The presence of the sMMO *mmoC* gene in all the strains also supports their potential to oxidize methane quantitatively. Interestingly, Jhala et al. [20] demonstrated an sMMO activity of 22.5, 37.0, 30.0, 26.7 nmol min^{-1} mg of protein^{-1} in *Rhizobium* sp., *Pseudomonas illinoisensis*, *Bacillus megaterium*, and *Methylobacterium extorquens*, respectively. These support our study on the sMMO activity in both NMS medium and AMS medium, and also indicate that diverse groups of bacteria possess sMMO activity.

Shigematsu et al. [32] determined the DNA sequence of the gene cluster that codes for the sMMO proteins for three methanotrophs by detecting six genes viz. *mmoX, mmoY, mmoB, mmoZ, orfy,* and *mmoC.* This enzyme complex consists of three components: a hydroxylase component (MMOH), a reductase component (MMOR), and a regulatory protein B (MMOB). While MMOH is encoded by *mmoX, mmoY,* and *mmoZ* genes, MMOR and MMOB are encoded by *mmoC* and *mmoB*, respectively [32, 33]. While designing PCR primers by using the DNA sequences of the soluble methane monooxygenase gene clusters of *Methylosinus trichosporium* OB3b and *Methylococcus capsulatus* (Bath), McDonald and Coworkers [8] have found that a large number of different methanotrophs are present in peat samples and also that there is a high level of variability in the *mmoC* gene which codes for the reductase component of the sMMO, while the *mmoX* gene, which codes for the alpha subunit of the hydroxylase component of this enzyme complex appears to be highly conserved in methanotrophs. They designed a primer set of *mmoC* gene and obtained a PCR product of 446 bp. In this study *mmoC* gene primers were synthesized based on the full genome sequence of *Methylobacterium phyllosphaerae* CBMB27. Interestingly all 19 *Methylobacterium* spp. strains responded positively when subjected to PCR amplification and all the

mmoC gene sequences were deduced and presented here.

This study opens new areas for investigation, as native or synthetic industrially relevant Methylotrophs could potentially host for expressing MMO and convert methane to various chemicals and fuels, and their potential use in agriculture particularly with mitigation of methane emission through enhanced methane oxidation [34, 35].

Conclusion

Methylobacterium spp. in this study could grow in the presence of methane as a sole carbon source. PCR amplification with gene sequencing molecular analysis confirms the presence and identity of the *mmoC* gene of sMMO strengthening the methane oxidation potentials observed in the studied *Methylobacterium* spp. Thus, the study supports the methane oxidation capabilities of *Methylobacterium* spp. which could be explored in line with their plant growth promotion for sustainable agriculture and mitigation of global climate change. Though the strains may not fall into the obligate methanotroph category, further intensive studies based on all MMO activity measurements, hybridization, and identification of pathways used for assimilation of methane, methanol, formaldehyde, and formate will confirm their nature.

Note

The authors declare no conflict of interest.

Acknowledgement

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