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PRESENCE OF NITROGEN-FIXING METHANOTROPHIC BACTERIA ASSOCIATED WITH RICE ROOTS

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ABSTRACT: *Sequencing analysis of dinitrogenase reductase gene (nifH) amplified from mRNA of rice roots suggested presence of nitrogen-fixing methanotrophic bacteria associated with the roots. Subsequently, a traditional culture-based approach was used to isolate and confirm their presence. Three bacterial strains; CH4-K, CH4-A and CH4-C were isolated from roots of rice (Nerica type) using ammonium mineral salt medium (AMS) in presence of methane gas as sole carbon source. Gas chromatography showed depletion of methane gas within one week as a result of growth of these isolates on used medium. On the basis of 16S rDNA and nifH genes sequencing, the isolates were identified as Methylocystis parvus, methylosinus sp LW3 and Methylosinu sporium, respectively. Phylogenetic trees constructed based on both genes showed that these bacteria cluster within subdivision alpha-proteobacteria and therefore belongs to type II methanotroph that have nitrogen fixation abilities. Methane was necessary for the growth of the isolates as they were unable to grow on AMS without methane or AMS with glucose.*

Key wards: *Methane-oxidizing bacteria, nitrogen-fixing bacteria, rice, nifH, 16Sr RNA.*

INTRODUCTION

Methane-oxidizing bacteria (MOB) are a physiologically unique group of microorganisms inhabiting diverse environment and distinguished by their ability to oxidize methane and use it as sole source of carbon and energy (Hanson, 1998). Based on phylogenetic, physiological and biochemical characteristics, methanotrophs are divided into two major groups. The gamma-proteobacterial type I group that comprise members of the family *Methylococcaceae* and alpha- proteobacterial type II methanotroph group that comprise members of the family *Methylocystaceae* (Hanson and Hanson, 1996). These groups differ in their abilities to produce methane monooxygenase enzyme (MMO) and to fix nitrogen. While most type I produce only membrane bound or particular MMO (pMMO) and unable to fix nitrogen, members of type II group produce cytoplasmic enzyme or soluble MMO (sMMO) and able to fix nitrogen (Auman *et al.* 2001).

Methane is one of the significant greenhouse gases related to global warming. Wetland rice fields appear to be one of the major sources of methane emissions to the atmosphere, it accounts annually for approximately 25% of the global emissions (Minamisawa and Neue, 1994). This is because of decomposition of organic matter (root residues) to methane under O_2-limited conditions of flooded rice. On the other hand, microbial oxidation of atmospheric methane in terrestrial environments consume around 1-10% of the total emission (Dianou and Adachi, 1999). Moreover, up to 30% of methane produced in rice paddy fields is oxidized by root associated methanotrophs (Bosse and Frenzel, 1997). In this regard, the type II methane-oxidizing bacteria that include nitrogen fixer were found largely in the paddy fields as examined by molecular technique (Heyer *et al.* 2002).

Trials for direct isolation and enumeration of methanotrophs from rice field soils on artificial solidified medium was not successful (Escoffier, *et al.* 1997). Initial enrichment cultivation was prerequisite for isolation of such bacteria (Whittenbury *et al.* 1970). For enumeration purposes, most probable number (MPN) method was recommended (Escoffier *et al.* 1997). However for enrichment and isolation of these bacteria, incubation conditions similar to the natural environment should be considered (Dedysh *et al.* 1998).

As approximately 90% of the methane that is emitted from rice paddies escapes through the aerenchyma of the rice plant (Frenzel, 2000). It is expected that some of endophytic bacteria associated with rice plant tissues are benefiting from methane emitted through plant aerenchyma.

In this regard little attention has been paid to methane-oxidizing bacteria (MOB) associated with rice roots and has a nitrogen fixing abilities.

Therefore, this study aimed to examine rice roots for presence of nitrogen-fixing methanotrophic bacteria. A primer targeting the nitrogenase reductase gene was used to give an approximate picture on dominance of nitrogen-fixing methanotrophs. Subsequent isolation and characterization were done to confirm presence of these bacteria.

MATERIALS AND METHODS

Sampling

Rice (*Oryza sativa*, var. Nerica, Tetep and Sprice) was grown in flooded field conditions at the experimental farm of Japan International Research Center for Agricultural Sciences (JIRCAS) for 70 days. Rice roots were dug out from the field, washed with tap water, dried with a towel and cut into small parts. Root segments were surface sterilized using 70% ethanol for one minute then washed carefully with sterilized distilled water and saline solutions, respectively. Samples were ground to a powder in presence of liquid nitrogen then used for RNA extraction.

RNA extraction, PCR amplification and sequencing of *nifH* gene.

The ground powder was subjected to RNA extraction using RNeasy Plant Mini Kit (QIAGEN) according the manufacture's instruction. Primers used for

PCR amplification of *nifH* gene and protocol for gene sequencing was carried out as previously described (Elbeltagy and Ando, 2005). Pure amplified *nifH* gene was ligated and cloned into the competent *Escherichia coli* JM109 to construct *nifH* library according to the technical manual (Promega company, USA). The clones were then sequenced using CEQ 8000 Genetic analysis system (Beckman Coulter Inc). The sequences of the used primers were; nH17K-F" (TAYGGNAASGGCGGTATCGGYAA) and nH139P-R" (TGGCATSGCRAARCCRCCGCAMACMACGTC), where Y represents C or T, R; A or G, S; C or G, N; A or C or G or T, M; A or C.

Culture conditions for isolation of nitrogen-fixing methanotrophic bacteria.

The ground root was serial diluted up to 10^3 and plated on a 75 mm polycarbonate microporous cell culture membrane having a 0.4 □m pour size placed on an insert (Transwell, Corning, Cambridge, MA, USA). Root suspensions were spread on the membranes while placed on a Petri dishes containing ammonium mineral salt (AMS) medium solidified with 1.2 % (w/v) agar (Difco) (Patt *et al.*, 1974). After spreading, the membrane containing insert was transferred to a corresponding Petri dishes having about 10 g agricultural soil suspended in 10 ml sterile distilled water so that the polycarbonate membrane was in contact with soil slurry (Svenning *et al.* 2003). The dishes were then placed in a gas tight jar and methane-air atmosphere was established by flushing with methane for 15 second before closing the jar, which results in 50-70 % methane in the jar (Svenning *et al.* 2003). The jar was aerated and re-flushed every 3-5 days and kept at 25°C for 40 days. Colonies (appeared on the membrane) were picked up by a capillary tube, serially diluted and streaked on AMS agar medium for further purification, then incubated in presence of methane at 25°C. Pure colonies were selected by microscope and incubated under methane-air 1:4 mixture at 25°C on AMS medium for further characterization.

Methane uptake and growth of the isolates.

Tubes containing 5 ml AMS broth medium were inoculated with equivalent cell suspensions ($O.D_{600}$ = 0.02) of the pure isolated strains. The tubes were closed with W-shaped butyl rubber stoppers. Methane gas was passed through 0.2 □m pore filter syringe and injected into each tube giving about 25 % methane in a head-gas phase, then incubated at 25°C under shaking (135 rpm/h). For comparison, two un-inoculated AMS medium was also injected with 25% methane. Rate of methane consumption and growth of the isolates were estimated simultaneously at short intervals using gas chromatograph and spectrophotometer, respectively. Samples of methane gas (0.1 ml) were taken from head space gas of each tube and injected into gas chromatograph (GC-14B Shimatdzu) equipped with Porapak-Q 80/100 column and with flame ionization detector. The peak area of methane taken from inoculated tubes and un-inoculated control were compared and calculated.

Growth of the isolates was also monitored at short intervals by measuring the optical density (O.D) at 600 nm with SmatSpec Plus Spectrophotometer (BIO-RAD). Isolates inoculated in AMS medium without addition of methane and the un-inoculated AMS medium injected with 25% methane in head space were used as controls. For comparison, growth was also checked in AMS medium separately supplemented with different carbon source; glucose and methanol (0.1 %) (as one carbon compound) in absence of methane gas.

Molecular characterization of nitrogen fixing methanotrophs using 16S rRNA and nifH genes sequencing.

Cell lysate (containing DNA) of each isolate for the PCR template was prepared as the method of Hiraishi (1992).

PCR amplification of 16S rRNA gene was conducted using universal primers that amplify the regions of bacterial DNA corresponding to positions 27 to 518 (*E. coli* positions). The primers were; 27fR (AGAGTTTGATCCTGGCTCAG) and 519rU (G(AT)ATTACC-GCGGC(GT)GCTG) (Lane 1991).

On the other hand, a part of *nifH* gene of about 390 base pair was amplified by PCR techniques using the "nH17K-F" as forward and "nH139P-R" as reverse primers as mentioned above.

The purified PCR product from both genes (*16S rRNA* and *nifH* genes) was separately ligated into pGEM-T easy Vectors. The ligation products were cloned into the competent *Escherichia coli* JM109 to construct *nifH* library according to the technical manual (Promega company, USA). The plasmids bearing *nifH* gene were extracted from grown *E. coli* according to Sambrook and Russel, (2000), then purified and sequenced using quick start kit and CEQ 8000 Genetic analysis system (Beckman Coulter Inc). Sequencing reaction mixture was prepared according the manufacture's protocol using T7 primer.

The resultant *nifH* and *16S rRNA* sequences from isolated strains were checked for their similarities to nitrogen-fixing methanotrophes by aligning them with some identified strains from DNA data bank of Japan (DDBJ). The phylogenetic trees were constructed based on FASTA program and neighbor-joining method of Saito and Nei, (1987).

RESULTS AND DISCUSSION

Molecular detection of nitrogen-fixing methanotrophic bacteria

Although the uncultured molecular detection of nitrogen-fixing methanotrophic bacteria using *nifH* gene analysis is not specific for methanotrophes. It shows whether methanotophic bacterial group is exciting among the predominant nitrogen-fixing bacteria associated with rice. PCR amplification and sequencing of *nifH* genes from rice roots and stems showed detection of 6 clones similar to methane oxidizing bacteria that has nitrogen fixing abilities (Fig 1 and Table 1). Among them, 3 clones Tet-STR28, 25 and 24 were very similar to *Methylocystis parvus* (AF484662) as the similarity reached 97.7%. The other 3 clones; Spr-ROR24, Tet-ROR24 and NE5U-ROR28 showed less similarity to *Methylocystis parvus* (AF484662) as reached 88.5%. The results also showed that the remaining two clones; NE5L-ROR28 and Tet-ROR01 were closely related to *Bradyrhizobium* sp. with similarity of 96.9 and 96.2% respectively, although it also had a considerable similarity to *Methylococcus* sp. (92.3 and 91.5 %, respectively) (Fig.1 and Table 1). Alignment of the sequenced clone with type strains, *Bradyrhizobium*, *Methylosinus* and *methylocystis* spp. showed many identical sequences in all aligned strains and little differences (bold letters in Fig. 1), which reveals the extent of relatedness between them.

In this regard, using *nifH* and *nifD* sequencing and phylogenetic analysis, Dedysh *et al.*, (2004) found that methane-oxidizing bacteria were most closely related to heterotrophic nitrogen fixing bacteria (such as *Bradyrhizobium* and *Beijerinckia* spp.), although they are metabolically different. They speculated that the two genera may be originated from a common ancestor and subsequently experienced similar evolutionary selection pressures with regard to nitrogen acquisition.

Isolation and characterization of diaztrophic methane-oxidizing bacteria.

As aforementioned un-cultural molecular results proposed presence of diazotrophic methane-oxidizing bacteria associated with rice. Mineral salt medium (AMS) along with soil substrate membrane system (Svenning *et al.* 2003) were directly used (without enrichment) to isolate such bacteria and to overcome the problems arisen from use of enrichment culture technique. In the traditional enrichment culture, the intermediate metabolites from the active cells are distributed in the liquid to support growth of other bacteria than methanotrophs (Whittenbury *et. al.*, 1970). However, when the AMS enriched membrane was placed on non-sterile soil slurries during incubation, the microorganisms present in the soil act as a buffer consuming the metabolites produced by methanotrophs and thus, reducing or preventing other bacteria from forming visible colonies (Svenning *et al.* 2003). In view of this, three bacterial strains namely; CH4-K, CH4-A and CH4-C were isolated and identified on the basis of *16S rRNA* and *nifH* genes sequencing analysis. The *16S rRNA* and *nifH* genes sequences of isolate CH4-K showed around 99.24 % and 98.5 % identity to those of *Methylocystis parvus*, respectively. For isolate CH4-A, it reached 99.2 % and 97 % to those of *methylosinus* sp. LW3, respectively. While both gene sequences from isolate CH4-C revealed 100 % identity to those of type strain *Methylosinu sporium* (Table 2).

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1-                                                               65
YGKGGIGKSTTSQNTLAALAEMGQKILIVGCDPKADSTRLILHAKAQDTILSLAADAGSVEDLEL   Tet-STR24
YGNGGIGKSTTSQNTLAALAEMGQKILIVGCDPKADSTRLILHAKAQDTILSLAAEAGSVEDLEL   Tet-STR25
YGKGGIGKSTTSQNTLAALAEMGQKILIVGCDPKADSTRLILHAKAQDTILSLAAEAGSVEDLEL   Tet-STR28
YGKGGIGKSTTSQNTLAALAEMGQKILIVGCDPKADSTRLILHAKAQDTILSLAAEAGSVEDLEL   M. parvus
YGKGGIGKSTTSQNTLAALAQTGKKILIVGCDPKADSTRLILHAKAQDTILSLAAEAGSVEDLEI   M. sporium
YGKGGIGKSTTSQNTLAALAEMGHRILIVGCDPKADSTRLILHAKAQDTILSLAANAGSVEDLEI   Tet-ROR01
YGKGGIGKSTTSQNTLAALAEMGHRILIVGCDPKADSTRLILHAKAQDTILSLAANAGSVEDLEI   NE5L-ROR28
YGKGGIGKSTTSQNTLAALAEMGHKILIVGCDPKADSTRLILHAKAQDTILSLAANAGSVEDLEI   Bradyrhiz.
YGKGGIGKSTTSQNTLAALSELGHKILIVGCDPKADSTRLILHSKAQDTVLGLAAAKGTVEDLEL   Tet-ROR24
YGKGGIGKSTTSQNTLAALSELGHKILIVGCDPKADSTRLILHSKAQDTVLGLAAAKGTVEDLEL   Spr-ROR24
YGKGGIGKSTTSQNTLAALSELGHKILIVGCDPKADSTRLILHSKAQDTVLGLAAAKGTVEDLEL   NE5U-ROR28

66                                                               130
EDVMKIGYRDIRCVESGGPEPGVGCAGRGVITSINFLEENGAYDGVDYVSYDVLGDVVCGGFAMP   Tet-STR24
EDVMKVGYRDIRCVESGGPEPGVGCAGRGVITSINFLEENGAYDGVDYVSYDVLGDVVCGGFAMP   Tet-STR25
DDVMKVGYRDIRCVESGGPEPGVGCAGRGVITSINFLEENGAYEGVDYVSYDVLGDVVCGGFAMP   Tet-STR28
EDVMKVGFQDIRCVESGGPEPGVGCAGRGVITSINFLEENGAYEGVDYVSYDVLGDVVCGGFAMP   M. parvus
EDVMKIGFEDIRCVESGGPEPGVGCAGRGVITSINFLEEQGAYDGVDYVSYDVLGDVVCGGFAMP   M. sporium
EEVMKIGYRDIRCVESGGPEPGVGCAGRGVITSINFLEENGAYEDIDYVSYGVLGDVVCGGFAMP   Tet-ROR01
EEVMKIGYRDIRCVESGGPEPGVGCAGRGVITSINFLEENGAYEDIDYVSYDVLGDVVCGGFAMP   NE5L-ROR28
EDVMKVGYKDIRCVESGGPEPGVGCAGRGVITSINFLEENGAYEDIDYVSYDVLGDVVCGGFAMP   Bradyrhiz.
ADVVKVGFHDIKCVESGGPEPGVGCAGRGVITSINFLEENGAYDDVDYVSYDVLGDVVCGGFAMP   Tet-ROR24
ADVVKVGFHDIKCVESGGPEPGVGCAGRGVITSINFLEENGAYDDVDYVSYDVLGDVVCGGFAMP   Spr-ROR24
ADVVKVGFHDIKCVESGGPEPGVGCAGRGVITSINFLEENGAYDDVDYVSYDVLGDVVCGGFAMP   NE5U-ROR28
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Fig. (1) Alignment of 130 amino acid sequences of *nifH* clones with related strains. Bold and underlined letters indicate identical sequences in all strains.

Table (1): Homology % between selected clone sequences and related type strains

Strains	1	2	3	4	5	6	7	8
1 Tet-STR28(3)		95.4%	97.7%	94.6%	92.3%	93.1%	92.3%	87.7%
2 *Methylococcus capsulatus* (AF484671)			96.2%	93.8%	93.1%	92.3%	91.5%	87.7%
3 *Methylocystis parvus* (AF484662)				94.6%	93.8%	92.3%	91.5%	88.5%
4 *Bradyrhizobium* sp. MAFF 210318 (AB079620)					91.5%	96.9%	96.2%	87.7%
5 *Methylosinus sporium* (AF484668)						90.8%	90.0%	86.2%
6 NE5L-ROR28							99.2%	85.4%
7 Tet-ROR01								84.6%
8 Spr-ROR24 Tet-ROR24 NE5U-ROR28								

Table (2): Similarity percentages of *16S rRNA* and *nifH* gene sequences of the isolated strains to related type strains.

Most related strains based on used *16S rRNA* and *nifH* genes	Similarity (%)		
	CH4-K	CH4-C	CH4-A
Methylosinus sporium (*16S rRNA* gene)	99.2	100	98.3
Methylosinus sporium (*nifH* gene)	93.8	100	96.1
Methylocystis parvus (*16S rRNA* gene)	99.24	98.8	98.3
Methylocystis parvus (*nifH* gene)	98.5	93.8	92.3
Methylosinus sp. Lw3 (*16S rRNA* gene)	98.5	99.0	99.2
Methylosinus sp. Lw3 (*nifH* gene)	nd	nd	97.0

nd; not detected

The difficulty in obtaining methanotrophic pure cultures and the lack of discriminating phenotypic characteristics have resulted in classification

problems in taxonomy of methanotrophic bacteria (Dianou and Adachi, 1999). Therefore, in order to reveal the taxonomic position of the isolated strains, the phylogenetic trees of both gene (*nifH* and *16s rRNA*) sequences were constructed using different treeing method and compared. The results showed that the clusters formed by the *nifH* sequences were corresponded well with those obtained by 16s rRNA. Moreover, the three isolates were gathering with *methylosinus* and *methylocystis* spp cluster by both gene sequences, regardless of the treeing method as shown in Fig 2 (A and B), and Fig 3 (A and B). These bacteria belong to the group of alpha proteobacteria, type II methanotroph, that has nitrogen-fixation abilities and include *Rhizobium* and *Bradyrhizobium* spp. (Hanson and Hanson, 1996).
The study based on genomic characteristics and 5S rRNA and 16S rRNA of Bowman *et al.* (1993) have supported our results. They found that the group II methanotroph genera *methylosinus* and *methylocystis* are closely related but distinctly different groups within type II methanotroph.
However, this group was phylogenetically far from type I methanotrophs that include *methylomonas* and *methylococcus* spp. and belongs to group of gamma proteobacteria Fig 2 (A and B) and 3 (A and B).

Type II methanotroph are generally present in rice ecosystem, landfill, freshwater lakes and soil (Hanson and Hanson 1996, and Svenning et al. 2003). The strains, *Methylosinus sporium* and *methylocystis* sp., has been isolated from rice field rhizosphere and from soil, respectively, and showed nitrogen-fixing capabilities (Dianou and Adachi, 1999 and Takeda, 1988).

Growth and methane oxidizing activity

To confirm methane-oxidizing activity of the isolated strains, the growth was monitored over time in presence and absence of methane gas as sole carbon source for one week. The isolate CH4-C identified as, *methylosinus sporium*, showed fastest growth and methane consumption rates. The growth of this isolate reached log phase within 16 h and reached stationary phase after almost 96 h, which was also the time observed for methane depletion (Fig 4 A and B). The isolate CH4-K identified as, *Methylocystis parvus*, showed similar trend with relatively slower rate of growth (as reached log phase after 22 h) and methane depletion. On the other hand, the isolate CH4-A identified as, *methylosinus* sp. LW3, was the slowest one in the growth and methane consumption rates. Up to 140 h, the isolate was slowly and steady growing and did not reach the stationary phase (Fig 3A and B), while still around 11% of methane gas was remaining in the headspace of the tube at that time.

On the other hand, the isolates did not show any growth in AMS without methane gas or in AMS medium with glucose instead (Table 3). However, the growth was observed in AMS medium supplemented with methanol (instead of methane) revealing necessities of using one carbon compound such as methane or methanol as sole carbon source for growth of such bacteria (Table 3).

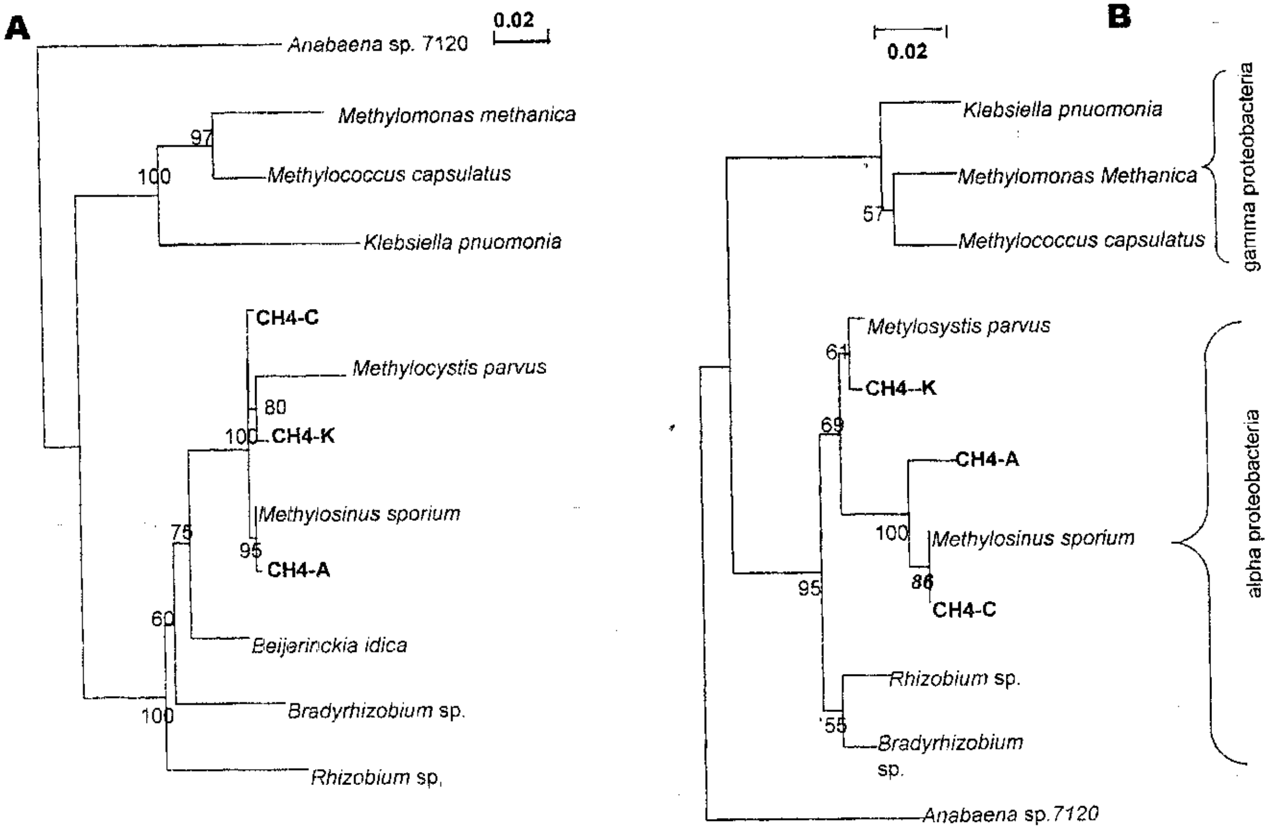

Fig (2). Phylogenetic rooted trees showing relatedness of isolated strains to their relatives of type strains based on aligned sequences of 16S rRNA (A) and nifH (B) genes. Bootstrap vlaues > 50% are indicated on the tree nodes. The strain *Anabaena* sp. 7120 was used as outgroup.

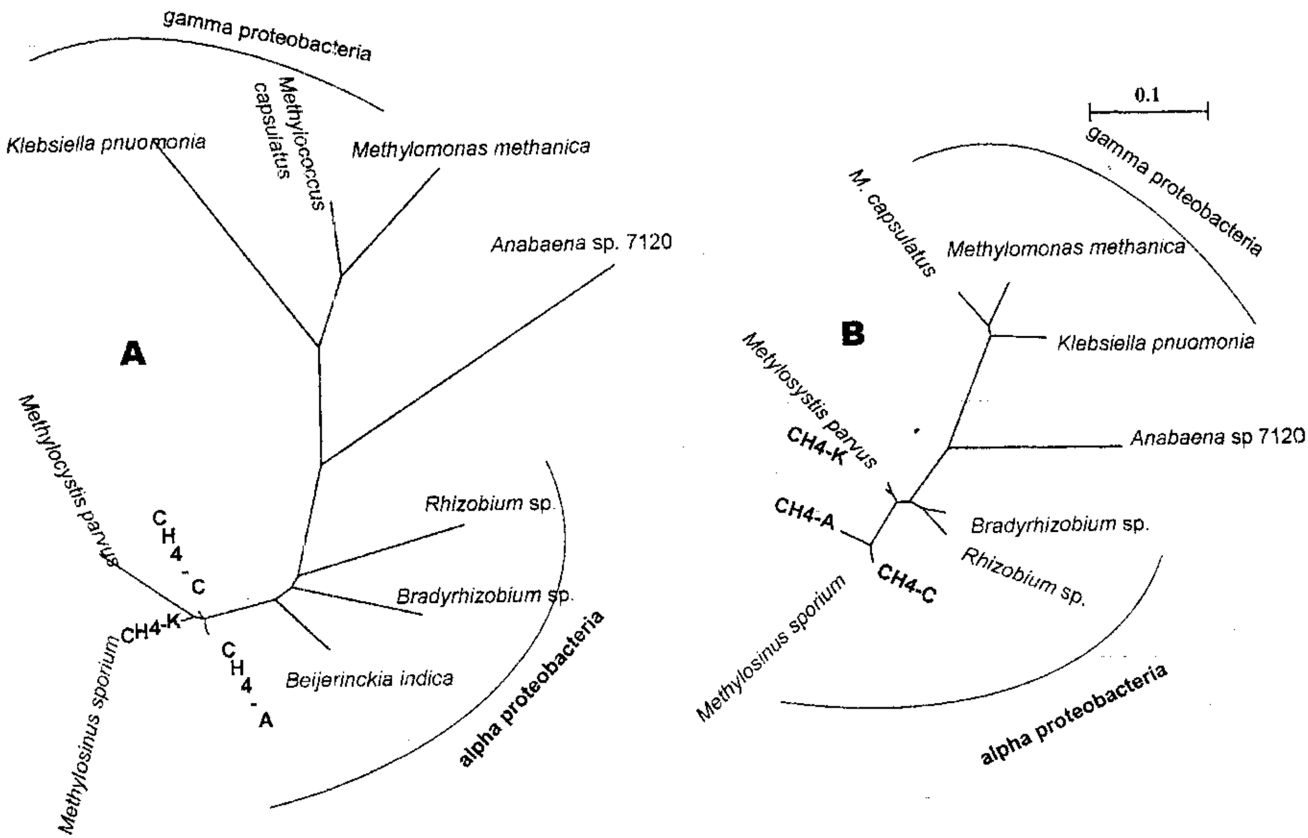

Fig (3). Phylogenetic un-rooted trees showing relatedness of isolated strains to their relatives of type strains based on aligned sequences of 16S rRNA (A) and nifH (B) genes. The strain *Anabaena* sp. 7120 was used as outgroup.

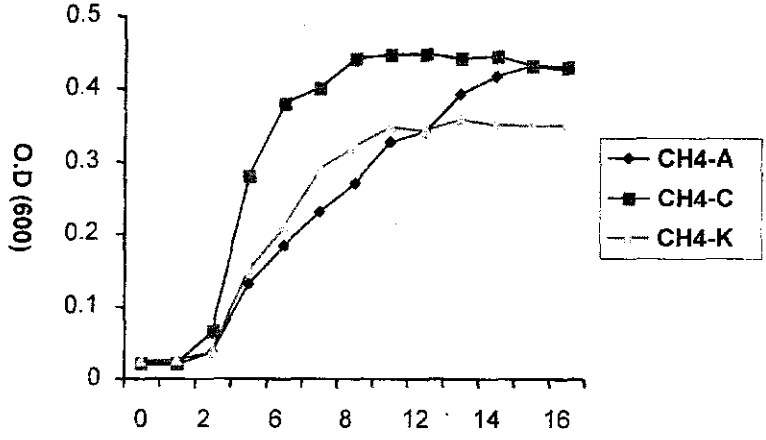

Fig. (4 A). Growth in shaking culture of isolated strains CH4-A, C and K at 25°C

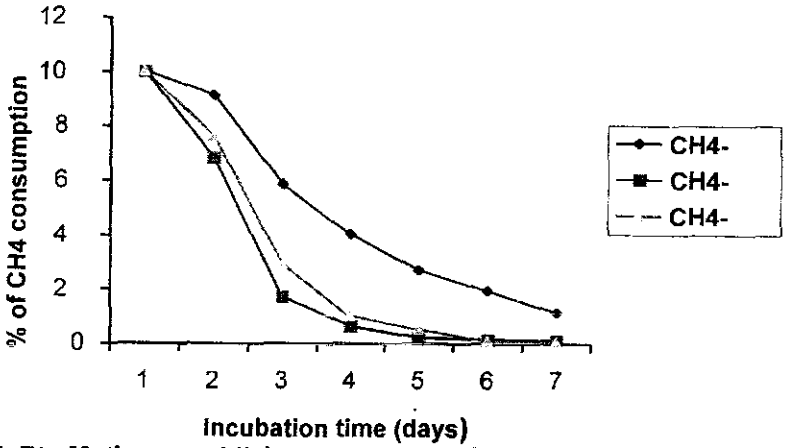

Fig. (4 B). Methane oxidizing activity in shaking culture of isolated strains CH4-A, C and K.

Table (3): Growth characteristics of isolated strains

Characteristics	Isolates CH4-K	CH4-C	CH4-A
Growth on AMS + Methane	+	+	+
Growth on AMS + methanol	+	+	+
Growth on AMS + glucose	-	-	-
Colony color on AMS medium	White or buff	Buff to brown	Buff
Presence of nitrogen fixation gene	+	+	+

In support to these results, Hanson and Hanson, (1996) indicated that the methanotrophic bacteria grew well on one carbon compound (methane or methanol but not in ethanol or glucose. Dianuo and Adachi, (1999) found the isolated strains (*Methylosinus* spp.) were able to grow in nitrate salt medium with methane or methanol and not with glucose as sole carbon source.

Regarding to the distribution of group I and II methanotrophs in nature, Amaral *et al.* (1995) reported that methanotrophs that grew in a zone of low CH_4 and high O_2 concentrations were generally from group I, and those of high CH_4 and low O_2 concentration were from group II. On the other hand, methanotrophs associated with the rhizosphere of aquatic plants were found to be largely group II (Adachi, 2001). In these habitats, CH_4 is present in large concentrations while O_2 diffusing via roots is kept low in the rhizosphere by microbial consumptions (Sebacher *et al.* 1985). These results are consistent with each other assuming that the majority of methanotrophs in waterlogged rice fields may belong to group II.

The potential for nitrogen fixation and methane oxidation by these isolates confer advantage in using such bacteria in the technology aiming to clean up the environment.

CLONE SEQUENCES ACCESSION NUMBERS.

Sequences of *nifH* clones; Tet-STR24, 25, 28, Spr-ROR24, Tet-ROR24, NE5U-ROR28, NE5L-ROR28 and Tet-ROR01 were deposited in the DNA Data Bank of Japan (DDBJ) under the accession numbers; AB208364, Ab208365, AB208367, AB208271, AB208329, AB208417, AB208383 and AB208312, respectively.

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تواجد بكتريا مثبتة للأزوت ومحبة للميثان مرتبطة بجذور الأرز

عادل البلتاجى

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الملخص العربى

أعطى تحليل تتابعات قواعد الجين المشفر لأنزيم الداى نتروجينيز ريدكتيز (نيف اتش) المعزول من الحمض النووى الريبوسومى الرسول انطباعا على وجود مجموعة من البكتريا محبة للميثان ومثبتة للأزوت فى جذور الأرز.

وبناءا عليه استخدمت طريقة مزرعية لعزل وتأكيد وجود هذه البكتريا، حيث تم عزل ٣ سلالات بكتيرية وهى CH4K, A, C من جذور الأرزعلى بيئة ملح الأمونيوم مع الأملاح المعدنية فى وجود غاز الميثان كمصدروحيد للكربون.

ولقد أظهر التحليل الكروماتوجرافى نفاذ غاز الميثان بعد أسبوع واحد نتيجة لنمو هذة العزلات.

ولقد تم تعريف هذه البكتريا عن طريق دراسة تتابعات قواعد جين نيف اتش وجين ال١٦ اس الريبوسمى، حيث عرفت الأولى بالمثيلوسيستس بارفاس والثانية بالميثيلوسينس ، والثالثة بال بالميثيلوسينس سبوريم.

وبدراسة تطور هذه السلالات وجد أنها تقع تحت قسم ألفا بروتيو بكتريا المنتمية الى المجموعة الثانية من البكتريا المحبة للميثان والتى لها القدرة على تثبيت الأزوت.

وقد أثبتت النتائج أن وجود غاز الميثان ضرورى لنمو هذه البكتريا، حيث أنها لم تسطع النمو بدونه أو فى وجود الجلكوز كمصدر وحيد للكربون بديلا عن الميثان.