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ORIGINAL PAPER

Characterization of 1-aminocyclopropane-1-carboxylate deaminase producing methylobacteria from phyllosphere of rice and their role in ethylene regulation

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Abstract The presence of 1-aminocyclopropane-1-carboxylate deaminase (ACCD) activity among the phyllosphere methylobacteria of rice was detected and its role in regulating plant ethylene level was assessed. Eighteen methylobacterial isolates from four different cultivars of rice were isolated and screened for ACCD. The 16S rRNA homology of ACCD positive methylobacterial isolate closely related to the species *Methylobacterium radiotolerans*. The *accD* gene sequence homology of the isolate was 98% similar to *Rhizobium leguminosarum*. Foliar spray of ACCD positive methylobacterial isolates enhanced the root and shoot length of rice and tomato seedlings under gnotobiotic condition and lower the ethylene level (60–80%) in the plant species.

Keywords ACC deaminase · Ethylene · *Methylobacterium* · Phyllosphere · PPFM · Rice

Introduction

The phytoharmone ethylene plays an important role in plant growth development including germination, root-hair initiation, leaf and flower senescence and abscission, fruit ripening, nodulation and response to wide variety of stresses (Abeles et al. 1992). The cyclopropanoid α-aminoacid, 1-aminocyclopropane-1-carboxylate (ACC) is the precursor of ethylene accumulated in the cells by conversion of S-adenosyl-met by ACC synthase (Adams and Yang 1979) which is further converted to ethylene by ACC oxidase in an oxygen-dependent process. Ethylene can promote and inhibit the plant growth depending on the cell type, plant species and stress conditions (Lehman et al. 1996; Vriezen et al. 1999; Madhaiyan et al. 2007a). Lowering the ethylene level in the plant root will elongate the root (Glick et al. 1998). To regulate the ethylene level in the root region, several plant growth promoting rhizobacteria produce ACC deaminase, a pyridoxal 5-phosphate-dependent enzyme that converts ACC to α- ketobutyrate and ammonium (Honma and Shimomura 1978; Sheehy et al. 1991; Glick et al. 1995). ACC deaminase producing soil bacterial species so far reported were *Pseudomonas* spp. (Sheehy et al. 1991; Klee et al. 1991; Campbell and Thomson 1996), *Enterobacter cloacae* (Shah et al. 1998), *Kluyvera ascorbata* (Burd et al. 1998), *Pyrococcus horikoshii* (Fujino et al. 2004), *Rhizobium leguminosoraum* bv *viciae*, *Rhizobium hedysari* (Ma et al. 2003a, b), *Mesorhizobium loti* (Sullivan et al. 2002) and *Methylobacterium oryzae* (Madhaiyan et al. 2007b). The ACC deaminase containing bacteria may be found on leaves, flowers, seeds and roots and the model based on the interaction of bacteria with seeds and roots is likely applicable to entire plant (Glick 2004).

The genus *Methylobacterium* is a group of strictly aerobic, facultative methylotrophic, gram-negative and rod-shaped bacteria able to grow on one-carbon compounds such as formate, formaldehyde, methanol, methylamine and a wide-range of multi-carbon growth substrates as sole source of carbon and energy (Green 1992; Patt et al. 1976). The genus *Methylobacterium* belongs to α2 subclass of *Proteobacteria* with validly published 26 species (Raja et al. 2008). These species are distributed in a variety of natural and man-made environments, including soil, air, dust, fresh water, marine water, water supplies, polluted soil, bathrooms, air conditioning systems and masonry (Trotsenko et al. 2001). The association of *Methylobacterium* species with plants

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seems to rely on a symbiotic relationship between the bacterium and host plants. Most common niche for synergism between *Methylobacterium* and plant is the phyllosphere, where they utilize methanol evolved from leaves as the sole source of carbon and energy (Trotsenko et al. 2001) and in response, methylobacteria may produce phytoharmones like cytokinin and auxins (Ivanova et al. 2001; Madhaiyan et al. 2005). In addition, they can fix atmospheric nitrogen (Jourand et al. 2004; Raja et al. 2006; Sy et al. 2001), regulate the ethylene level in rhizosphere by ACC deaminase (Madhaiyan et al. 2006a) and stimulate resistance against pathogens (Holland and Polacco 1994; Madhaiyan et al. 2006b). In recent years, due to demonstrated capabilities in different applications in the fields of industrial, agriculture and bioremediation, *Methylobacterium* attracted much attention (Abanda-Nkpwatt et al. 2006; Anesti et al. 2004; Fournier et al. 2005; Lee et al. 2006). Recently new ACC deaminase producing *M. oryzae* was isolated from stem tissues of rice (Madhaiyan et al. 2007b) and their inoculation to canola plant regulated the ACC and thereby the ethylene level (Madhaiyan et al. 2007a). Therefore, present study was focused to investigate the rice phyllosphere Pink-Pigmented Facultative Methylotrophs (PPFMs) for ACC deaminase and their effect on rice and tomato seedlings for ethylene regulation and growth promotion.

Materials and methods

Bacterial strains and culture condition

The PPFMs were isolated using Ammonium Mineral Salt (AMS) medium as described earlier (Holland and Polacco 1994) from the phyllosphere of rice grown at Tamil Nadu Agricultural University, Coimbatore, India. The 30 days old rice plants were selected randomly, 4th and 5th leaves of the main tiller were collected in sterile polybags and PPFM bacteria were isolated by leaf imprinting technique on the same day (Holland and Polacco 1994). Standard culture of ACC deaminase producing *M. oryzae* CBMB20 (kindly provided by T. Sa, Korea), The ACC deaminase positive *Pseudomonas fluorescens* Pf1 obtained from Department of Plant Pathology, Tamil Nadu Agricultural University and for regular experiments, the standard strains *Methylobacterium fujisawaense* TNAU14, *M. aminovorans* TNAU9 and *M. thiocyanatum* TNAU7 of this department were used. The *Methylobacterium* isolates were grown in 0.1% (v/v) methanol-supplemented AMS medium (MMS) at 28°C.

Authentication of PPFMs

The presence of *mxaF* gene in the isolates, encoding methanol dehydrogenase, a key enzyme for PPFM was used for authentication. The presence of *mxaF* gene in the isolates was detected by partial amplification of the gene using specific primers (McDonald and Murrell 1997). The forward primer mxaf1003 (5′GCG GCA CCA ACT GGG GCT GGT3′) and reverse primer mxar1561 (5′GGG CAG CAT GAA GGG CTC CC3′) were used for amplification of *mxaF* gene (550 bp). The 40 µl PCR reaction mixture contain 50 ng DNA template, 1×Taq buffer, 0.2 mM of each of dNTP mixture, 1 µM of each primers, 2.5 mM $MgCl_2$ and 2U of *Taq* DNA polymerase (Bangalore Genei, India). PCR amplification was performed in a thermocycler (Eppendorf Master cycler, Germany) using the following conditions: initial denaturation at 95°C for 5 min, 35 cycles consisting of 95°C for 1 min (denaturation), 55°C for 1 min (annealing), 72°C for 1 min (primer extension) and final extension at 72°C for 10 min. PCR products were separated by 1.5% agarose gels stained with ethidium bromide and documented in Alpha imager TM1200 documentation and analysis system.

Carbon utilization test

All the PPFM isolates were analyzed for growth in different carbon substrate *viz*., acetate, citrate, fructose, D-glucose, L-glutamate, methylamine, D-xylose, thiocyanate, tartarate and methanol. These carbon compounds were substituted for methanol in AMS liquid medium at 1.0% (w/v) level. Presence of growth was observed after 15 days of incubation at 30°C in an orbital shaking incubator and growth was compared to a negative control containing no added carbon source. Carbon utilization profiles of the isolates were compared with that of standard type cultures (Jourand et al. 2004; Van-Aken et al. 2004; Wood et al. 1998) and grouped based on the similarity.

Screening of PPFM isolates for ACC deaminase gene by PCR

The screening of ACC deaminase containing PPFM isolates from rice phyllosphere was done based on the amplification of ACC deaminase gene. Presence of *accD* gene in the isolates was detected by partial amplification of the gene using specific primers. The degenerated primers designed by Hontzeas et al. (2005) (DegACCF 5′-GGB GGV AAY AAR MYV MGS AAG CTY GA-3′ and DegACCR5′-TTD CCH KYR TAN ACB GGR TC-3′) were used for amplification of *accD* gene (750 bp). The 30 µl PCR reaction mixture containing DNA template 50 ng, 1×Taq buffer, 0.2 mM of each of dNTP mixture, 1 µM of each primers, 2.5 mM $MgCl_2$ and 2U of *Taq* DNA polymerase (Bangalore Genei, India). PCR amplification was performed in a thermocycler (Eppendorf Master cycler, Germany) using conditions: initial denaturation at 95°C for 5 min, 35 cycles consisting of 94°C for 1 min

(denaturation), 60°C for 1 min (annealing), 72°C for 1 min (primer extension) and final extension at 72°C for 5 min. PCR products were separated by 1.5% agarose gels stained with ethidium bromide and documented in Alpha imager TM1200 documentation and analysis system.

Sequencing analysis

The ACC deaminase gene was amplified as described earlier and the band of the expected size was gel-purified using spin columns (Bangalore genei, India) according to the manufacturer's instructions and cloned using pTZ57R/T vector supplied with T/A cloning kit (Fermentas, USA) prior to sequencing. Sequencing reactions were performed using ABI prism terminator cycle sequencing ready reaction kit and electrophoresis of the products were carried out on an Applied Biosystems (Model 3100) automated sequencer. The 16S rRNA gene of ACC deaminase producing PPFM was also sequenced using universal bacterial primers as described earlier by Sy et al. (2001) and Raja et al. (2008). The 16S rRNA and ACC deaminase (*accD*) nucleotide sequences determined in this study have been deposited in the NCBI database under the accession numbers FJ624148 and FJ624149 respectively.

Phylogenetic analysis

The identity of ACC deaminase gene sequence was established by performing a similarity search against the GenBank database (website: http.www.ncbi.nih.gov/BLAST). The phylogenetic tree was constructed with existing *accD* gene sequences from different eubacteria. The phylogenetic tree was constructed by neighbor-joining method of Saitou and Nei (1987) using MEGA 4.0 and the tree file was analyzed using treeview (Tamura et al. 2007).

ACC deaminase activity

All the PPFM isolates and standard cultures were grown in DF mineral salt medium (Dworkin and Foster 1958) supplemented with 10 μM of ACC (Sigma–Aldrich, USA). ACC deaminase activity was quantified spectrophotometrically by following procedure discussed by Honma and Shimomura (1978) and expressed as α-ketobutyrate produced per mg of protein per h.

Inoculation studies

To assess the impact of ACC deaminase producing bacterial inoculants on germination and seedling growth, gnotobiotic studies using paper-towel and pro-tray culturing techniques were used for rice and tomato respectively. The whole experimental set up was provided with light and dark at 12 h intervals. Watering was done using sterile distilled water at 4 days interval. ACC deaminase producing bacterial inoculants as cell suspensions in distilled water (10^9 cfu per ml) was sprayed at 7, 14 and 21 days. After 25 days of inoculation, the root and shoot length of the seedlings were recorded. To assess the impact of ACC deaminase producing bacterial inoculants on growth and ethylene regulation in rice and tomato, an experiment was carried out under pot culture under green house conditions. Fertilizers were applied at 75% of the recommended dose, for rice 150:50:50 (N: P: K kg per ha) and for tomato 100:60:60 (N: P: K kg per ha). Overnight pre-soaked paddy seeds and 12 days old tomato seedlings were used for this study. The ACC deaminase producing *Methylobacterium* strains and *Psedomonas fluorescens* were grown in AMS and LB broth respectively till the population reached to 10^{10} cells per ml. The cell pellets washed with sterile water, resuspended in sterile water to get a final concentration of 10^8 cells per ml was used as liquid inoculant for this experiment. Pre-soaked paddy seeds and roots of tomato seedlings were imbibed with above said bacterial inoculants for 1 h and planted in the pots. All the bacterial inoculants were also sprayed on the leaves at early morning to have uniform wetting at weekly interval. The ethylene level of the plants (2 months old) was measured by following standard procedure using a gas chromatograph. In brief, a known weight of seedlings was placed in 250 ml vials capped with a rubber septum and following 4 h incubation, 1 ml of head space was sampled from each vial and the ethylene content was measured in the gas chromatograph (Shimadzu GC14B) packed with Poropak-Q column at 70°C equipped with flame ionization detector. The carrier gas was N_2 at a flow rate of 50 ml per min and air containing 100 μM ethylene was used as standard. The amount of ethylene emitted from the plant was expressed as μM of ethylene per g fresh weight of the plant per h.

Results and discussion

The bacterium produce the enzyme known as ACC deaminase which cleaves the plant ethylene precursor ACC and thereby lower the ethylene level in developing plants (Liu et al. 1984; Jacobson et al. 1994; Li et al. 1996; Zhao et al. 2003; Hontzeas et al. 2004). Unfortunately this enzyme could not be produced by the plants and hence it is essential that presence of ACC deaminase producing bacteria to regulate the ethylene level and most of the plants harbour the ACC deaminase containing bacteria in seed, root and leaves (Glick 2005). Present study was focused on the ACC deaminase property of the common plant growth promoting phyllosphere bacteria, PPFMs. PPFMs were isolated from phyllosphere of different rice cultivars and

Table 1 Occurrence of PPFMs in phyllosphere of different rice cultivars

Rice cultivars	Population (cfu/sq cm)
White ponni	6.00 ± 1.53
Coimbatore local	10.00 ± 2.08
ADT 39	5.67 ± 1.86
ADT 39[a]	11.22 ± 1.82
ADT 43	12.33 ± 2.03

Values are mean ± SE of 3 replicates

[a] Same cultivar from different location

screened for ACC deaminase by PCR as well as enzyme assay. Five different cultivars of rice *viz*., White ponni, Coimbatore local, ADT 39 and ADT43 from wetland and ADT 39 from Plant Breeding Station of Tamil Nadu Agricultural University, were used for isolation of PPFMs. All the samplings were done at active tillering stage of the crop and isolation of PPFM using leaf impression technique was done on the same day. The result revealed that all the rice cultivars harbor PPFMs in their phyllosphere (Table 1). The PPFM colonies appeared as dark pink-colored, raised with spherical in shape in AMS medium after 5 days of incubation. However, the population of PPFM is very low in general and among the five different cultivars, ADT43 and ADT39 collected from wetland recorded the maximum PPFM population of 12.33 ± 2.03 and 11.22 ± 1.82 cfu per cm^2 respectively. The result indicated that there is no correlation between cultivars and PPFM population. The well-characterized pigmented PPFM colonies isolated from above experiment were further purified and used throughout the experiment. The genus *Methylobacterium* is one of the phyllosphere bacteria isolated from several plant species (Corpe and Rheem 1989) and about ten different species of *Methylobacterium* are so far reported as plant-associated PPFMs (Sy et al. 2001; Van-Aken et al. 2004; Idris et al. 2006; Kang et al. 2007; Madhaiyan et al. 2007b). Similarly, the diversity of PPFMs in phyllosphere of cotton, maize, sunflower, soybean and mentha were well-documented (Raja et al. 2008; Balachandar et al. 2008).

The number of colonies of methylobacteria per sq cm of leaf surface was relatively low in this study, which may be due to several agronomic and environmental factors. However all methylobacterial colonies were purified and authenticated by colony morphology and PCR studies. Most of the phyllosphere methylobacteria produce pigments while using methanol as carbon source. All the isolates of this study were able to produce pink pigmentation in AMS medium. Hence, all the well-characterised colonies were subjected to authentication by detection of *mxaF* gene. The presence of *mxaF* in the isolates was detected by partial amplification of gene using specific primers as described by McDonald et al. (1995) and could amplify about 550 bp portion of *mxaF*. In the present study, all the 18 PPFM isolates harbor the gene and thereby methylotrophy. The authenticated methylobacterial isolates were further subjected for differential carbon-substrate utilization profiling in order to grouping the isolates from different cultivars of rice (Table 2). The carbon-substrate utilization profiling of all the species belonging to *Methylobacterium* are well-documented as this genus contains a limited number of species with difference in substrate utilization pattern. Such biochemical studies are of immense help even for the tentative species level identification (Jourand et al. 2004; Van-aken et al. 2004).

In the present, carbon substrate utilization profile of majority of the isolates coincided with already reported profiles of methylobacterial type cultures. Further, the data obtained from this profiling were subjected for cluster analysis of PPFM isolates. The result revealed that all the 18 isolates were clustered into 5 different groups and different groups of PPFMs are evenly distributed among the rice cultivars. The diversity and species richness of *Methylobacterium* in phyllosphere of rice were not influenced by their difference in cultivars (Yang et al. 2001; Lambais et al. 2006; Opeft et al. 2007).

The PCR based detection of ACC deaminase was used to screen the methylobacterial isolates. For this, the degenerated primers and the protocol for PCR amplification as described by Hontzeas et al. (2005) were used. The result revealed that the *accD* gene positive isolates could produce a partial amplification (750 bp) of *accD*. This was further confirmed by positive control of *P. fluorescens* strain Pf1. This PCR based detection is relatively easy to screen the isolates than the enzyme assay. Among 18 isolates, only two could show presence of *accD* gene by PCR amplification, indicating low percentage of ACC deaminase producing methylobacteria among the isolates. Further the partial *accD* gene was characterized by DNA sequencing and compared with existing *accD* genes of eubacteria. The results indicated that the *accD* of *Methylobacterium* isolated in this study was very close to *Rhizobium leguminosarum accD* (Fig. 1). Using 16S rRNA gene sequence homology, the strain COLR1 was identified as *M. radiotolerans* (100% homology with strain DSM1819^T). This result further indicated the possibility of horizontal gene transfer among the *Proteobacteria*. Both *Methylobacterium* and *Rhizobium* are soil inhabitants and belong to same phylogenetic group, alpha proteobacteria, hence the possibility for horizontal gene transfer of *accD* gene between these two genera cannot be ruled out (Hontzeas et al. 2005). In earlier, the rhizobacteria are the predominant group of ACC deaminase producers including *E. cloacae* (Penrose and Glick 2001), *Rhizobium* (Ma et al.

Table 2 Differential carbon-substrate utilization profiling of methylobacterial isolates from rice cultivars

Isolates	Carbon sources[a,b]									
	Glutamate	Glucose	Acetate	Citrate	Fructose	Thiocynate	Xylose	Methylamine	Tartarate	Methanol
WP 1	+	−	−	+	+	−	+	−	−	+
WP 2	−	−	+	−	−	−	+	+	−	+
WP 3	+	+	+	+	−	−	+	+	+	+
CO L–1	+	+	+	+	−	+	+	−	−	+
CO L–2	−	−	+	−	−	+	+	+	+	+
CO L–3	−	−	+	+	+	−	−	+	+	+
CO L–4	+	+	+	−	+	−	−	+	+	+
CO L–5	−	−	−	+	+	−	−	+	+	+
ADT 39–1	+	−	+	−	+	−	−	−	−	+
ADT 39–2	+	+	+	−	+	−	−	+	−	+
ADT 39–3	−	−	−	−	+	+	−	+	+	+
ADT 39[c]–1	−	+	+	−	−	−	+	+	+	+
ADT 39[c]–2	+	−	+	−	+	+	+	−	+	+
ADT 39[c]–3	−	+	+	+	+	−	−	−	−	+
ADT 43–4	+	−	−	+	+	−	−	+	+	+
ADT 43–5	+	+	+	−	−	+	+	+	+	+
ADT 43–6	−	+	−	+	+	+	+	+	−	+
ADT 43–7	+	−	−	+	+	+	+	−	+	+

[a] The carbon substrates were supplemented in AMS medium at 1% (w/v). + Presence of growth; −Absence of growth

[b] Mean of three replicates

[c] Same cultivar from different location

2003b), *Pseudomonas*, *Variovorax*, *Alcaligenes*, *Bacillus* etc. (Preisfeld et al. 2001) in roots of higher plants. *Methylobacterium oryzae* was reported as endophyte of rice (Madhaiyan et al. 2007b).

The *accD* positive methylobacterial isolates, *Methylobacterium radiotolerans* COLR1 and *Methylobacterium* sp. WP1 were assayed for ACC deaminse activity with standard strains *viz*., *M. oryzae* CBMB20 and *P. fluorescens* Pf1 and the spectrophotometry values are presented in Table 3. The two isolates (COLR1 and WP1) from this study recorded higher ACC deaminase activity than the standard methylobacterial and rhizobacterial strains. This result also indicated that there is a potential source of ACC deaminase in phyllosphere of rice by *Methylobacterium*. It is suggested that enhancing their activity in phyllosphere may also be helpful for ethylene regulation. The presence of ACC deaminase activity among the phyllosphere-PPFMs of rice reported in the present study is the first of its kind. The present study is in accordance with the earlier studies reported in various plant-associated rhizobacterial species (Madhaiyan et al. 2007b; Ma et al. 2003a; Sullivan et al. 2002).

Experiments were conducted to study the effect of ACC deaminase producing bacterial inoculants on the growth of rice and tomato seedlings by roll-towel and pro-tray culturing methods respectively. All the ACC deaminase producing bacterial inoculants enhanced shoot and root length of rice than uninoculated control (Table 4). Inoculation of strains COLR1 and WP1 recorded the maximum shoot and root length than the other two standard inoculants. When same bacterial inoculants enforced to tomato seedlings under pro-tray culturing condition also reported the same trend of root and shoot length enhancement. The pot culture experiment was carried out in rice and tomato with the ACC deaminase producing bacteria in the presence of 75% of fertilizer source of nutrients. The result revealed that the introduced bacterial inoculants reduced the ethylene level to a tune of 60–80% than control plants (Table 5) and enhanced the shoot and root length and plant biomass (data not shown) after 60 days. Introducing ACC deaminase containing bacterium through seed or root reduced the plant ACC level and thereby the ethylene level (Penrose et al. 2001; Grichko and Glick 2001; Mayak et al. 2004). A study in *Arabidopsis thaliana* with PGPR, *Paenibacillus polymyxa* conducted by Timmusk and Wagner (1999) revealed that ACC deaminase bacterium down-regulated the gene involved in ethylene induced stress response and up-regulated the gene involved in plant growth. The microarray data also suggested that lower the plant ACC level resulted in an increase in the transcription

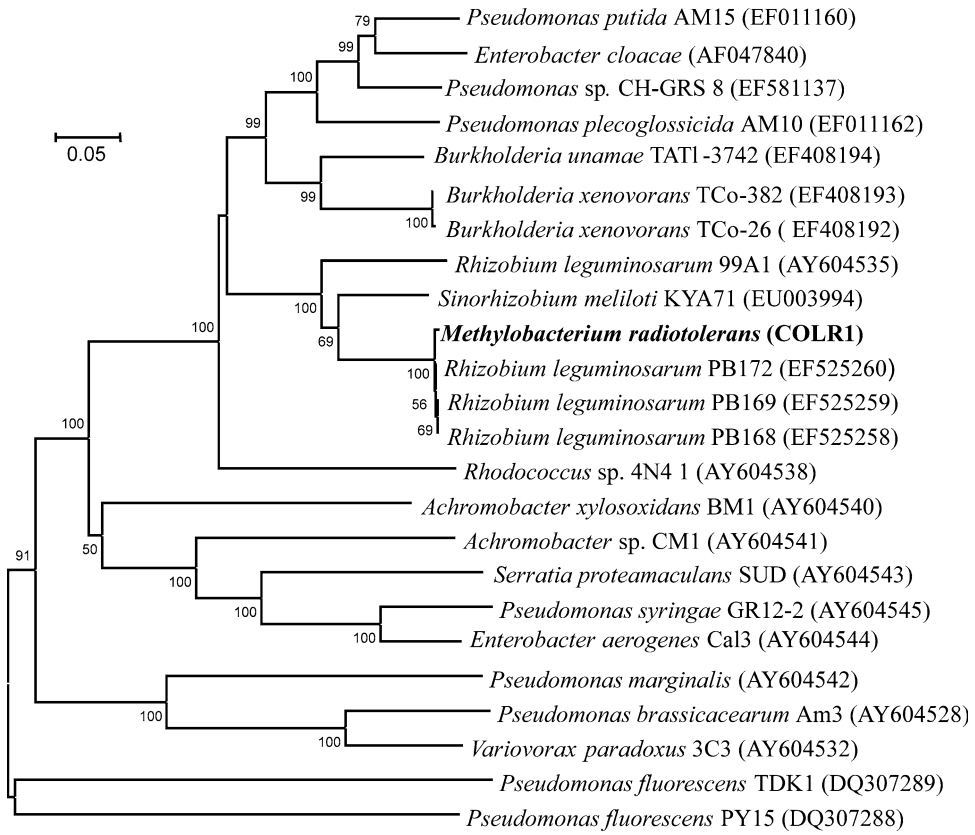

Fig. 1 Phylogenetic tree based the *accD* partial sequence (450 bp) from methylobacterial isolate of phyllosphere of rice using neibhour-joining method. The data of other *accD* sequences of soil bacteria were from GenBank. The bacterial species, strain and GenBank accession number used in this analysis are given in the figure. The boot-strap values of 500 and above are shown as per cent at the nodes

Table 3 ACC deaminase activity of methylobacterial isolates and standard strains

Cultures	ACC deaminase (nmoles of α-ketobutyrate produced/ mg protein/h)
Methylobacterium oryzae CBMB20	298.27 ± 4.82
Pseudomonas fluorescens Pf1	325.11 ± 8.23
Methylobacterium radiotolerans COLR1	339.39 ± 5.53
Methylobacterium sp. WP1	444.59 ± 26.25

Values are mean ± SE of three replicates

of gene involved in the IAA biosynthesis. Similar studies conducted with *M. oryzae* in canola plant also regulated the ethylene level. Inoculation of this bacterium as seed treatment in canola plant enhanced the IAA production, cytokine synthesis, germination percentage and root length in canola roots to several fold. About 10 fold decrease in ethylene level and above two fold decrease in ACC level were noticed. This bacterial inoculation also reduced the ACC oxidase but enhanced the ACC synthase of the plant (Madhaiyan et al. 2006a). The present result revealed that there was significant increase in shoot and root length of rice and tomato seedlings due to ACC deaminase producing bacterial inoculum. This finding is in agreement with earlier studies conducted by Ghosh et al. (2003) and Madhaiyan et al. (2006a) in different plant species. The present result also confirmed a 60–80% reduction in ethylene emission in both the plants than the respective controls. This result is in accordance with the earlier

Table 4 Effect of ACC deaminase producing bacterial inoculation on root and shoot length of rice cultivar (PMK3) and tomato cultivar (PMK1)

Treatments	Rice (cultivar PMK3)		Tomato (cultivar PMK1)	
	Shoot length (cm)	Root length (cm)	Shoot length (cm)	Root length (cm)
T1–Control	3.69 ± 1.05	4.89 ± 1.20	3.87 ± 0.44	5.60 ± 0.64
T2–*Methylobacterium oryzae* CBMB20	9.10 ± 1.06	13.99 ± 1.60	5.55 ± 0.15	7.55 ± 0.20
T3–*Pseudomonas fluorescens* Pf1	9.68 ± 0.82	13.36 ± 0.60	5.18 ± 0.13	6.94 ± 0.90
T4–*Methylobacterium radiotolerans* COLR1	9.85 ± 0.18	16.25 ± 0.44	4.82 ± 0.13	6.50 ± 0.16
T5–*Methylobacterium* sp. WP1	9.87 ± 1.79	16.71 ± 1.92	5.96 ± 0.16	7.60 ± 0.30

Values are mean ± SE of ten replicates

Table 5 Ethylene emission in rice and tomato influenced by ACC deaminase producing bacterial inoculation

Treatments	Ethylene level in whole plant (μmol/g fresh weight/h)[a]	
	Rice (cultivar PMK3)	Tomato (cultivar PKM1)
T1–Control	166.42 ± 4.17	1.99 ± 0.23
T2–75% RDF + Uninoculated control	117.59 ± 3.18	1.23 ± 0.26
T3–75% RDF + *M. oryzae* CBMB20	54.91 ± 3.02	0.30 ± 0.06
T4–75% RDF + *P. fluorescens* Pf1	57.40 ± 4.23	0.39 ± 0.03
T5–75% RDF + *Methylobacterium* COLR1	40.23 ± 2.35	0.36 ± 0.04
T6–75% RDF + *Methylobacterium* WP1	32.51 ± 1.87	0.22 ± 0.03

RDF Recommended Dose of Fertilizer for rice (150:50:50 kg NPK/ha) and tomato (100:60:60 kg NPK/ha)

[a] The ethylene level in whole plant was measured at 60 DAS, data are mean ± SE of three replicates

findings reported in *M. oryzae* (Madhaiyan et al. 2007a) and *P. putida* (Mayak et al. 1999; Hontzeas et al. 2004).

Present study revealed that methylobacteria present in the phyllosphere of rice produce the enzyme ACC deaminase and thereby regulates the ethylene level in rice plant. The present investigation also confirmed the presence of *accD* gene in *Methylobacterium*, which is phylogenetically close to *R. leguminosarum accD*. Further, experiment conducted on rice and tomato seedlings revealed that inoculation of ACC deaminase producing *Methylobacterium* enhanced the growth of seedlings and reduced the ethylene level in plants. Hence it is concluded that ACC deaminase producing phyllosphere methylobacteria are also a potential candidate for regulation of plant ethylene.

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