Biocatalysis and Agricultural Biotechnology 37 (2021) 102161

Contents lists available at ScienceDirect

Biocatalysis and Agricultural Biotechnology

journal homepage: www.elsevier.com/locate/bab

Potential of pink pigmented methylotrophic bacteria on growth and physiology of cluster bean and soil microbial community

Narayanan Vijayanand [a], Sivasangari Ramya Subramanian [b], Boomibalagan Ponnerulan [c], Eswaran Saminathan [d], Uthirapandi Veerenan [e], Durairaj Kathiresan [f, *], Nagaraj Subramani [g]

[a] Department of Botany, Arumugam Pillai Seethai Ammal College, Thiruppathur, Affiliated to Alagappa University, Karaikudi, India
[b] Department of Biochemistry, Sri Sarada Niketen College for Women, Amaravathipudur, Affiliated to Alagappa University, Karaikudi, India
[c] PG and Research Department of Botany, Raja Dorasingam Government Arts College, Sivagangai, Affiliated to Alagappa University, Karaikudi, India
[d] Research Department of Botany, Pasumpon Thiru Muthuramalinga Thevar Memorial College, Kamuthi, Affiliated to Alagappa University, Karaikudi, India
[e] Department of Botany, Theni College of Arts and Science, Veerapandi, Tamil Nadu, Affiliated to Madurai Kamaraj University, Madurai, India
[f] PG Department and Research Centre in Botany, Saraswathi Narayanan College, Affiliated to Madurai Kamaraj University, Madurai, Tamilnadu, India
[g] Center for Research in Botany, Guindy Campus, Madras University, Chennai, India

ARTICLE INFO

Keywords:
Pink pigmented facultative methylobacterium
Methylobacterium tardum
16s rRNA sequence
Phylogenetic tree
Growth and yield parameters
Soil microbial community

ABSTRACT

Methylotrophs are one of the prominent microbial community which are known for utilizing methanol as well as carbon sources from various plant metabolic activities and in turn facilitates the host plant growth by synthesizing phytohormones. A pot study was conducted to examine the bio-fertilizing potential of pink pigmented methylotrophic bacteria (PPFM) on cluster bean (*Cyamopsis tetragonolaba* (L.) Taub and its impact on soil microbial communities. Foliar and soil application modes of treatment was employed for the study. Our results showed that isolated strain from *Quisqualis indica* leaf was identified as *Methylobacterium tardum* (strain IHBB) by 16s rRNA gene sequence and phylogenetic analysis confirmed its relationship with other *Methylobacterium* spp. Soil microbes such as bacterial population (9.25 CFU x 10-^{6}/g), fungal population (70.05 CFU x 10^{-3}/g) and microalgae (9.65 CFU x 10^{-4}/g) were found to be enhanced in 3% of *M. tardum* (T2) when compared to T1, T3 and control. Soil application of *M. tardum* exhibited better response on growth, biochemical and yield parameters of cluster bean than foliar application. This study confirms that *M. tardum* could be a promising candidate as microbial inoculant for plant growth and development as well as a positive driver for enriching the soil microbial community.

1. Introduction

Currently, microbial inoculants are gaining momentum due to multiple advantages such as preserving and enhancing soil ecosystem, reducing inorganic inputs, nutrient supplementation, secretion of plant growth hormone, resistance against plant pathogens etc. Natural biostimulants maintain a healthy soil atmosphere by inducing the growth of soil microbial communities prevalent in the soil such as microalgae, fungi, and bacteria. The plant-microbe interaction is greatly beneficial and climate resilient through which plant growth is not affected under biotic and abiotic stress conditions (Manish et al., 2019). Plant–microbe interaction in the soil ecosystem

* Corresponding author.
E-mail address: kathiresan.snc@gmail.com (D. Kathiresan).

https://doi.org/10.1016/j.bcab.2021.102161
Received 16 June 2021; Received in revised form 13 September 2021; Accepted 14 September 2021
Available online 17 September 2021
1878-8181/© 2021 Elsevier Ltd. All rights reserved.

makes the agriculture sector more sustainable (Rana et al., 2018) by synthesizing and exporting phytohormones through microbial community (Alirreza et al., 2014).

Plant phyllosphere harbors the growth of microbial communities in which bacterial species dominate the microbial inhabitants in the phyllosphere (Nysanth et al., 2019). Among these diverse microbial populations, methylotrophs are one among the large bacterial populations. PPFM are distinguished based on their formation of pink to red colonies on selective isolation media using both single and multicarbon compounds. PPFM utilize methanol which is produced through the hydrolysis of pectin methyl esters from the host plant, in turn synthesize and secrete phytohormones (Green et al., 2018). PPFMs are associated in rhizosphere, phyllosphere or endophytes and are thought to be phytosymbionts (Irvine et al., 2012; Lidstrom, 2006). Interactions between *Methylobacterium* spp. and host plants may be represented as symbiotic (Jourand et al., 2004), epiphytic (Omer et al., 2004) or endophytic (Lacava et al., 2004). In addition, *Methylobacterium* is also known for the production of phytohormones, atmospheric nitrogen fixation (Chanratana et al., 2017), phosphate solubilization (Verma et al., 2016), systemic resistance against plant pathogens, regulation of ethylene by ACC deaminase enzyme (Chinnadurai et al., 2009), production of Fe-chelating compounds (Verma et al., 2015) etc., Certain species of *Methylobacterium* such as *M. nodulans*, *M. radiotolerans* are directly involved in nitrogen fixation and nodule formation in the roots of leguminous plants (Sy et al., 2001; Menna et al., 2006), hormone synthesis (Meena et al., 2012) and elevating the photosynthetic activity (Cervantes-Martinez et al., 2004).

Further, growth promoting activity of PPFM was observed in wheat (Meena et al., 2012), peanut (Ghazti et al., 2014), tomato (Subhaswaraj et al., 2017), rice (Raghavendra et al., 2019; Ajaykumar and Murali, 2018; Aswathy et al., 2020), fenugreek (Anandhi et al., 2019), millet (Arun Balaji et al., 2019) and ginger (Vadivukkarasi and Suseela, 2019). The present study was aimed to evaluate the growth promoting efficiency of pink pigmented facultative methylotrophs (PPFM) on cluster bean plant and its role in soil microbial community.

2. Materials and methods

2.1. Isolation of PPFM bacteria

Pink pigmented methylotrophic bacteria were isolated from *Quisqualis indica* leaves by leaf imprint technique (Holland and Polacco, 1994). Ammonium Mineral Salt medium (AMS), a selective medium for the isolation of methylotrophs was used (Whittenbury et al., 1970). In addition, to the composition of AMS medium, 5 ml of sterile methanol (0.5%) and 0.05% cyclohexamide was added aseptically and mixed thoroughly. After incubation, pink colonies were observed, and the colonies were re-streaked for 3 times on fresh AMS medium to obtain pure culture (Fig. 1). Pure culture was once again sub-cultured in liquid medium and was stored at −20°C for further use. From the broth solution, appropriate concentrations of PPFM bacteria were prepared and used for the study.

2.2. Identification and molecular characterization of isolates

Genomic DNA was prepared from the isolated bacterial strain as described by Pospiech and Neumann 1995; Jayashree et al., 2011). The 16S rRNA genes were amplified by PCR using reaction mixtures of 2.5 U *Taq polymerase*, 1X buffer, 1 μl DNA template and 25 pmol forward primer (8F 5'AGAGTTTGATCCTGGCTCAG 3') and (1541 R 5'AGGAGGTGATCCAGCCGCA 3') with 30 cycles of 92 °C for 1 min, 55 °C for 1min and 72 °C for 1 min; followed by a final extension at 72 °C for 5 min. The PCR products run through 0.8% agarose gel electrophoresis were further sequenced at Eurofins Scientific Services, Bengaluru, Karnataka. India. Sequenced

Fig. 1. *Methylobacterium tardum* grown on AMS medium.

strain was compared with those available in the NCBI database. Phylogenetic tree was constructed by UPGMA method using MEGA 5.0 software (Tamura et al., 2011).

2.3. Quantification of auxin

Isolated strain was subjected to screening for the presence of auxin (Indole Acetic Acid) as per the method of Gordon and Weber (1951). Bacterial isolate was cultured in nutrient broth containing 100 μg/mL tryptophan for 4 days and 2 ml of Salkowsky's reagent was mixed with 1 mL of the culture supernatant. Appearance of pink colonies & colour density in the culture supernatant confirms the production of IAA.

2.4. Preparation of pot

Experiment was carried out under natural greenhouse conditions in the experimental garden. The earthenware pots (30 cm diameter and 45 cm height) containing standard soil mix were prepared. Cluster bean (*Cyamopsis tetragonoloba*) seeds were procured from Tamil Nadu Agricultural College and Research Institute, Madurai, and authenticated by a Botanist. Seeds were surface sterilized with 0.l % mercuric chloride and 10 seeds sown in each pot with seed-to-seed distance of 4.0 cm. The pots were arranged in a completely randomized block design with 5 replicates for each treatment.

2.5. Preparation of PPFM concentration and mode of application

Ten-day-old culture (10^{-6} CFU/mL AMS broth was taken as a stock solution. From the stock solution, different concentrations viz. 1% (T1), 3% (T2), and 5% (T3) were prepared by mixing appropriate proportions of sterilized distilled water. The same concentrations were considered for both foliar and soil applications. Foliar application was given using a hand sprayer (30 ml/Pot). During soil application, 25 ml/pot concentrations were poured near the root zone of the plant saplings. Both applications were given at an interval of two days for 15 days. Growth parameters such as total plant height, fresh and dry weight, leaf area and moisture content were observed in 30 days seedlings. Biochemical parameters such as chlorophyll content (Arnon, 1949), starch, reducing sugar (Nelson, 1944), ascorbic acid (Roe, 1954), protein (Lowry et al., 1951), nitrate reductase activity (Jaworski, 1971) amino acid (Misra et al. (1975) were also assessed on 30 days old seedlings. In case of yield parameters, number of clusters/plant, number of flowers/clusters, number of pods/plants, number of seeds/pods, pod length, and pod weight were analyzed on 45 days old seedlings.

2.6. Soil microflora analysis

For this part of the study, in addition to the treatments (T1, T2 and T3), *Methylobacterium extorquens* AM1 which was obtained from Microbial Type Culture Collection (MTCC) Chandigarh was used as reference strain (T4). As mentioned in 2.1, *M. extorquens* (T4) was cultured in AMS medium. After the appearance of pink colonies, it was re streaked and pure culture was maintained in broth medium and stored at −20 °C. From the broth culture, 3% of *M. extorquens* AM1 was prepared. Earthenware pots containing standard soil mix were prepared and for each treatment, five replicates were maintained. For control, sterilized distilled water was given. The isolate (30 mL) was poured in the marked area in each pot for 30 days. Soil samples taken at 10 cm depth were homogenized and sieved prior to serial dilution. One gram soil was dispersed in 10 mL of sterile double distilled water and thoroughly shaken. One mL of dilution was again transferred to 9 mL of sterile distilled water to form 10^{-2} dilution. Similarly, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} dilutions were made for each soil sample. An aliquot of 0.1 mL from each dilution was taken with a micropipette (0.01–0.1 mL range) and spread on selective agar medium. For bacterial culture, 10^{-6} aliquot was spread on nutrient agar medium. In case of fungal and microalgae colonies, 10^{-3} and 10^{-4} aliquot was spread on Rose Bengal Medium and BG11 medium, respectively.

2.7. Statistical analysis

Data were subjected to one-way ANOVA and the means were separated by Duncan's test ($P < 0.05$, $n = 5$). Statistical analysis was carried out using IRRISTAT ver. 4.0 (IRRI, Manila, Phillipines) (Duncan, 1965).

3. Results

3.1. Identification and molecular characterization of isolate

The isolated strain was identified as *Methylobacterium tardum* strain IHBB by 16s rRNA gene sequence analysis. The sequence was deposited in GenBank, NCBI, USA, with accession number KU749292. For identification of phylogenetic relationship, gene sequence of KU749292 and other representative sequences retrieved from BLAST analysis were taken and aligned using MUSCLE in MEGA 5.0 Software. The results revealed the presence of 99% similarity with other *Methylobacterium tardum* strains. Phylogenetic analysis of KU749292 on 16s rRNA gene sequence confirmed its relationship with other *Methylobacterium* species also (Fig. 2).

3.2. Quantification of auxin

Isolated bacteria (*M. tardum*) and reference strain (*M. extroquens,* positive control) when subjected to phytohormone screening exhibited the presence of Indole acetic acid (IAA) (Table 1).

3.3. Soil microflora analysis

The microbial population in the soils was found to be significantly influenced by the application of different concentrations (1%, 3% and 5%) of *M. tardum* and reference strain *Methylobacterium extorquens*. Among the treatments, maximum microbial population was noticed in T4 (Reference strain *M. extroquens*) followed by T2, T1, T3 and control. Reference strain (T4) significantly influenced

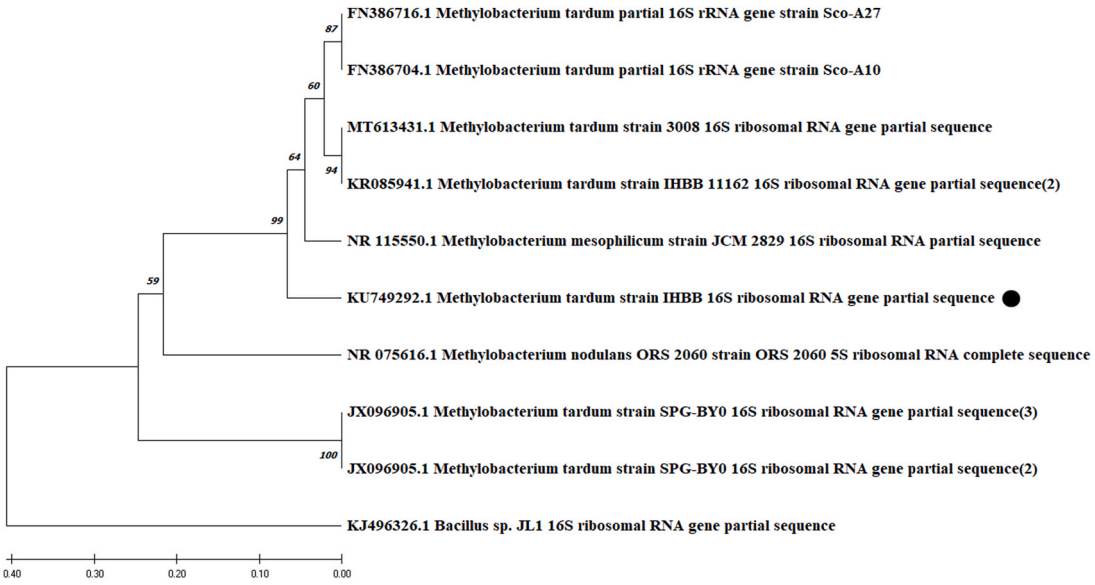

Fig. 2. Phylogenetic tree constructed based upon *16S rRNA* sequences showing the relationship of *Methylobacterium tardum* strain IHBB using UPGMA method (MEGA 5.0). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates).

Table 1
Screening of pink pigmented methylotrophic bacteria isolates for Indole Acetic Acid.

Bacterial isolate	Indole acetic acid
M. tardum (Leaf isolate)	+ + +
M. extorquens (Positive Control)	+ + + +

+ minimum + + Moderate + + + Maximum + + + + High amount.

the bacterial (12.25 × 10^6 C CFU/g), fungal (85.05 × 10^3C CFU/g), and microalgae (10.65 CFU × 10^4 C CFU/g) population when compared to control and other treatments. Moreover, T2 also influenced maximum bacterial population (9.25 CFU × 10^6 C CFU/g), fungal population (70.05 CFU × 10^3 C CFU/g) and microalgae (9.65 CFU × 10^4 C CFU/g) when compared to T1, T3 and control. Lowest microbial count of bacterial (5.33 CFU x 10^{-6} C CFU/g), fungal (29.05 CFU × 10^3 C CFU/g), and microalgae (3.33 CFU × 10^4 C CFU/g colonies were recorded in T3 soil (Table 2) (Fig. 3).

3.4. Influence of foliar application of M. tardum on growth, biochemical and yield of cluster bean

Differential responses were noticed on the growth, biochemical and yield parameters of cluster bean plants when *M. tardum* at different concentrations were administered as foliar application. In case of growth parameters, T1 (1% *M. tardum*) increased the shoot length by 20%, root length by 14% and total plant height by 16% when compared to control and other treatments (T2 & T3), however, the increase was lesser than that of T1. There was only a minimum level of increase of fresh weight by 5% in T1, 1% in T2, and 6% in T3 when compared to control. At the same time, there was an increase of 33% and 16% in dry weight in T1 and T2, respectively. Cluster bean plants which received 5% concentration of *M. tardum* (T3) did not increase the dry weight. Leaf area was found to be enhanced in T1 (10%) followed by T2 (7%) and T3 (9%). Increase in moisture content by 34% was noticed in T1 followed by T2 (31%) and T3(33%) when compared to control (Fig. 4A–C). In case of biochemical parameters, the total chlorophyll content was enhanced by 49% in T1. However, there was a 17% and 30% decrease of total chlorophyll content in T2 and T3, respectively. A similar trend was also observed in other parameters in which starch content was increased by 7% and ascorbic acid by 12% in T1 but in other treatments (T2 and T3) a decrease in level of starch and amino acid was noticed. In case of protein content, a 15% increase was noticed in T1 followed by T2 (10%) and T3 (5%). Further, reducing sugar content in leaves of cluster bean plants was enhanced by 8%

Table 2
Influence of soil application of *M. tardum* isolate on soil microbial communities.

Organisms	Control	T1	T2	T3	T4
Bacteria	9 × 10^{-6} ab	6.35 × 10^{-6} b	9.45 × 10^{-6} d	5.33 × 10^{-6} c	12.25 × 10^{-6} de
Fungi	4 × 10^{-3} a	27.55 × 10^{-3} b	70.15 × 10^{-3} e	17.05 × 10^{-3} b	85.05 × 10^{-3} ef
Microalgae	1 × 10^{-4} a	2.05 × 10^{-4} b	9.45 × 10^{-4} d	3.33 × 10^{-4} b	10.65 × 10^{-4} d

T1- (1% of *M. tardum*), T2- (3% of *M. tardum*), T3-(5% of *M. tardum*), T4-Reference strain (M. extroquens, MTCC – 7279, Control: Distilled water. Different letters (a-d) in a data set are significantly different (P ≤ 0.05 level). Different letters followed in each row are statistically significant based on DMRT.

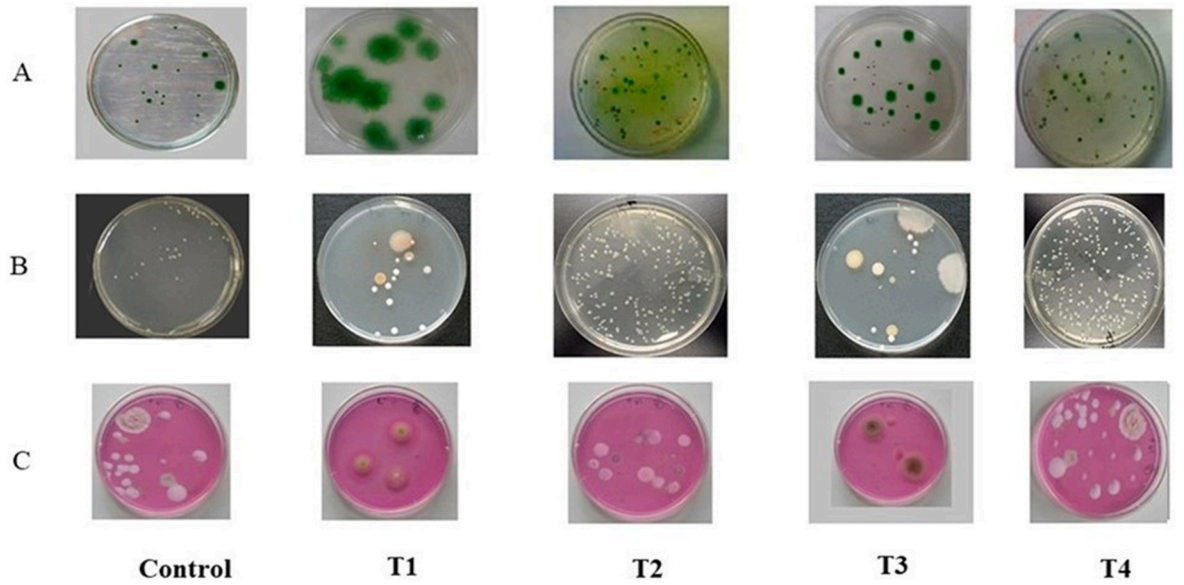

Fig. 3. Picture depicts the growth of microbial colonies on soil application of *M. tardum* isolate.
A: Microalgae B: Bacterial Colonies C: Fungal Colony
T1: (1% of *M. tardum*), T2: (3% of *M. tardum*), (5% of *M. tardum*); Control: Distilled water. Different letters (a–d) in a data set are significantly different ($P \leq 0.05$ level). Values above each bar are percent over control. Different letters present above each bar are statistically significant based on DMRT.

and 1% in T1 and T2, respectively. An increase of 22% in amino acid content was seen in followed by T2 (19%) and T3 (3%). Nitrate reductase activity was found to be maximum in the leaves of cluster bean by 24%, 20% & 18% in T1, T2 & T3, respectively (Table 3). In case of yield parameters, plants governed under T1 enhanced the number of clusters/plant (32%), number of flowers/plant (25%) & number of pods/plant (57%), whereas, though T2 & T3 plants also enhanced the yield parameters, comparatively lesser than the control. Pod length was found to be elevated in T1 plants by 22% followed by T2 (8%) & T3 (3%). Pod weight was increased by 54% in T1, whereas in T2 and T3 the increase in weight of the pod was 25% & 5% respectively. The increase in number of seeds/pods were found to be maximum by 24% in T1 followed by 10% in T2 and 1% in T3 (Fig 5 A and B). In general, T1 (1% of *M. tardum*) enhanced the growth, biochemical and yield parameters of cluster bean when compared to other treatments (T2 & T3) and control. However, as the concentration increased, there was a decline noticed on T2 (3% of *M. tardum*) and T3 (5% of *M. tardum*) when compared to T1 plants; that assumed only a minimum statistical difference between treated and control sets.

3.5. Influence of soil application of M. tardum on growth, biochemical and yield of cluster bean

Soil application of different concentrations of *M. tardum* exhibited positive response in growth, biochemical and yield parameters. Unlike foliar application (Refer 3.4), 3% concentration of *M. tardum* (T2) exhibited better response in terms of growth, biochemical and yield parameters when compared to 1% concentration (T1), and 5% concentration (T3) and control. Plant height was found to be increased by 37% in T2, whereas other treatments T1 and T3 also elevated the plant height by 11% and 5 % respectively, however lesser than T2. Similarly, fresh weight (32%) and dry weight (49%) was found to increase in T2 than T1, T3, and control. Leaf area was found to be enhanced by 12% in all treatments. Further, T2 plants accounted for an increase in moisture content by 32% followed by T3 (30%) and T1 (29%) (Fig. 6A–C). A positive response on biochemical parameters was noticed in all the treatments, while T2 plants recorded maximum increase when compared to other treatments (T1, T3 and control) The total chlorophyll content was found to be elevated by 137% in T2 plants followed by 32% in T1 and 22% in T3. Protein content was also found to be enhanced in T2 plants (18%), whereas only 9% and 4% increase was noticed in T1 and T3. As for reducing sugar, T2 plants recorded an increase of 6% whereas T1 and T3 plants recorded an increase of 5% and 3% respectively. There was an increase in starch and amino acid content in T2 plants by 33% and 50% respectively. In T2 plants, a 32% increase of ascorbic acid content in the leaves was noticed, while in other treatments (T1 & T3) only 7% increase was noticed. There was 35% increase in nitrate reductase activity in T2 plants whereas only 17% increase was observed in T1 & T3 (Table 4). On yield parameters, T2 plants enhanced the formation of number of clusters/plant by 21% and number of flowers/plant by 80%. There was 119% increase in number of pods/plant in T2 whereas T1 and T3 increased the number of pods by 31% and 19%. Maximum increase in pod length (58%) and pod weight (35%) was observed in T2 than other treatments. Increase in the number of seeds/plant (19%) was observed in T2 plants followed by T1 (3%) and T3(1%) (Fig 7 A and B). In general, T2 (3% of *M. tardum*) promoted the growth, biochemical and yield parameters of cluster bean plants when compared to other treatments T1 (1% of *M. tardum*) & T3 (5% of *M. tardum*). There was a significant statistical difference among the treatments and control.

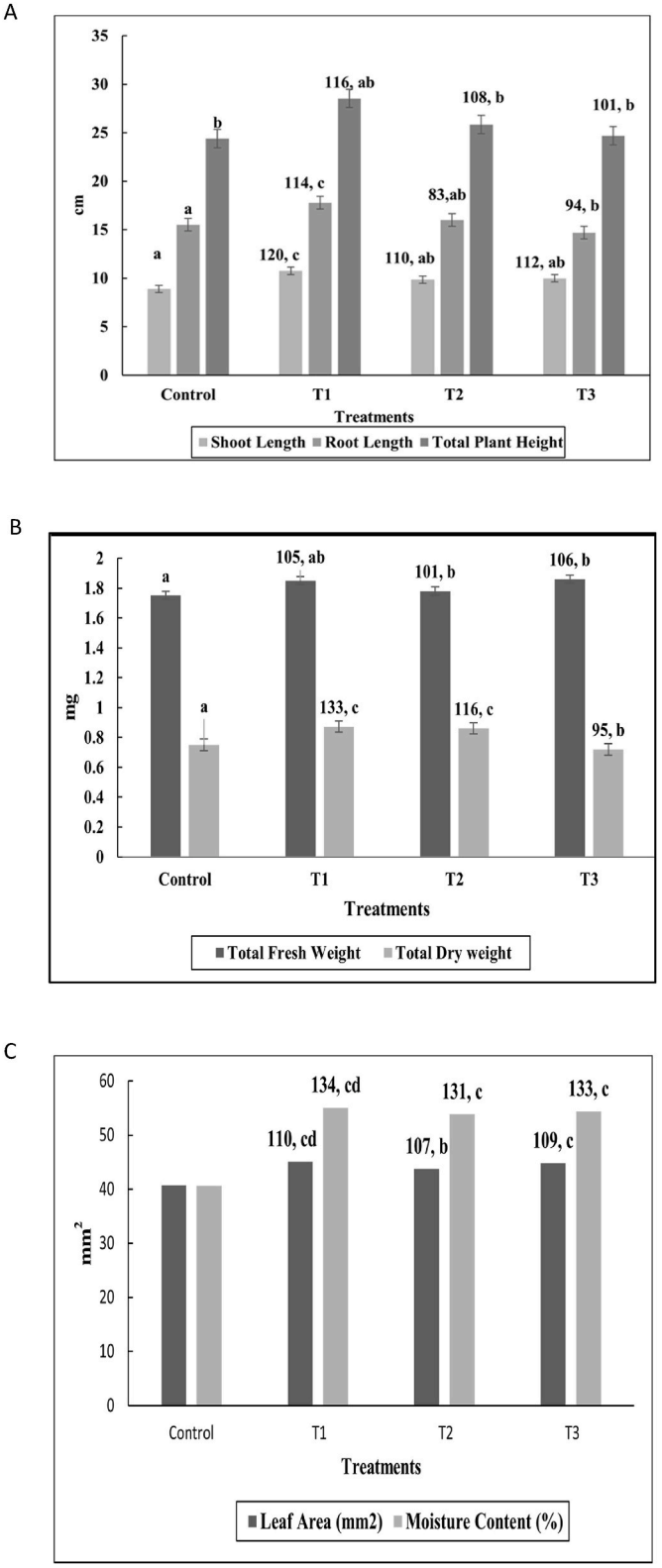

Fig. 4. Foliar application of *M. tardum* isolate on growth characteristics of cluster bean.

A- Total Plant Height, B- Total fresh and Dry weight, C- Leaf area and Moisture Content. T1: (1% of *M. tardum*), T2: (3% of *M. tardum*), (5% of *M. tardum*); Control: Distilled water. Different letters (a–d) in a data set are significantly different (P ≤ 0.05 level). Values above each bar are percent over control. Different letters present above each bar are statistically significant based on DMRT.

Table 3
Foliar application of *M. tardum* isolate on biochemical characteristics of cluster bean.

Treatments	Chlorophyll-a (mg.g-1fr.wt)	Chlorophyll-b (mg.g-1fr.wt)	Total chlorophyll (mg.g-1fr.wt)	Protein (mg.g-1fr.wt)	Reducing sugar (mg.g-1fr.wt)	Starch (mg.g-1fr.wt)	Amino acid (mg.g-1fr.wt)	Ascorbic acid (mg.g-1fr.wt)	NRA (μ moles No-2/gm.fr.wt/hr)
Con	0.329 a	0.350 a	0.679 b	17.50 b	40.32 a	25.09 a	12.32 a	0.779 a	1.07 a
T1	0.547 c (**166**)	0.470 c (**134**)	1.017 cd (**149**)	20.33 cd (**115**)	43.75 b (**108**)	26.96 b (**107**)	15.07 c (**122**)	0.873 a (**112**)	1.33 b (**124**)
T2	0.223 b (**67**)	0.345 bc (**98**)	0.568 bc (**83**)	19.47 cd (**110**)	41.02 ab (**101**)	22.70 bc (**90**)	14.77 b (**119**)	0.654 ab (**83**)	1.29 ab (**120**)
T3	0.205 b(**62**)	0.272 ab (**77**)	0.477 bc (**70**)	18.50 d (**105**)	40.21 ab (**99**)	20.30 a (**80**)	12.78 a (**103**)	0.509 ab (**65**)	1.27 ab (**118**)

T1: (1% of *M. tardum*), T2: (3% of *M. tardum*), (5% of *M. tardum*); Control: Distilled water. Different letters (a-d) in a data set are significantly different (P ≤ 0.05 level). Values in bracket are percent over control. Different letters followed in each column are statistically significant based on DMRT.

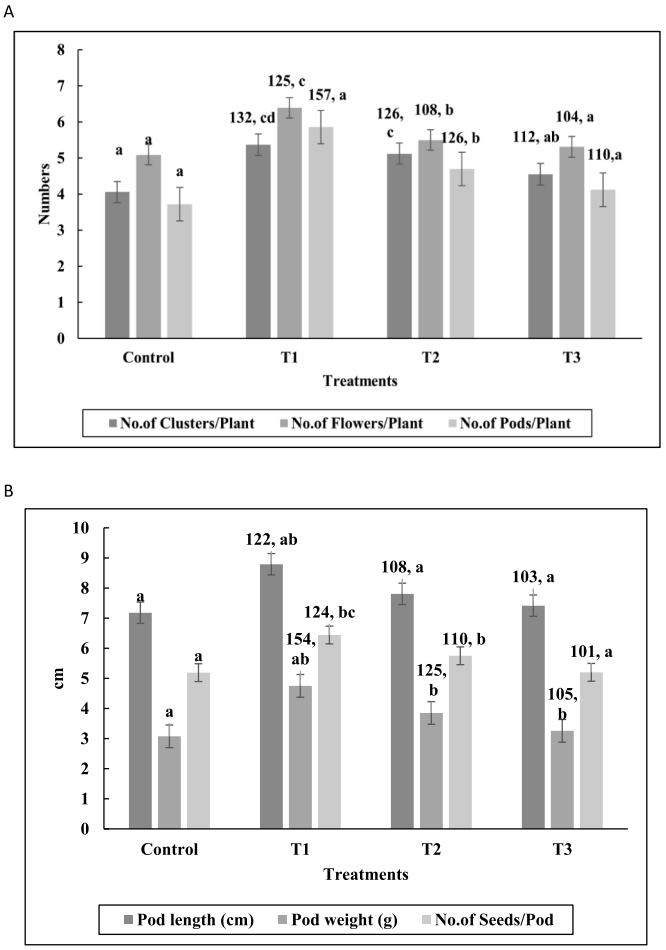

Fig. 5. Foliar application of *M. tardum* isolate on yield characteristics of cluster bean.
A- Number of clusters/plant, Number of flowers/plant, Number of pods/plant, B- Pod length, Pod weight, Number of seeds/pod.
T1: (1% of *M. tardum*), T2: (3% of *M. tardum*), (5% of *M. tardum*); Control: Distilled water. Different letters (a–d) in a data set are significantly different (P ≤ 0.05 level). Values above each bar are percent over control. Different letters present above each bar are statistically significant based on DMRT.

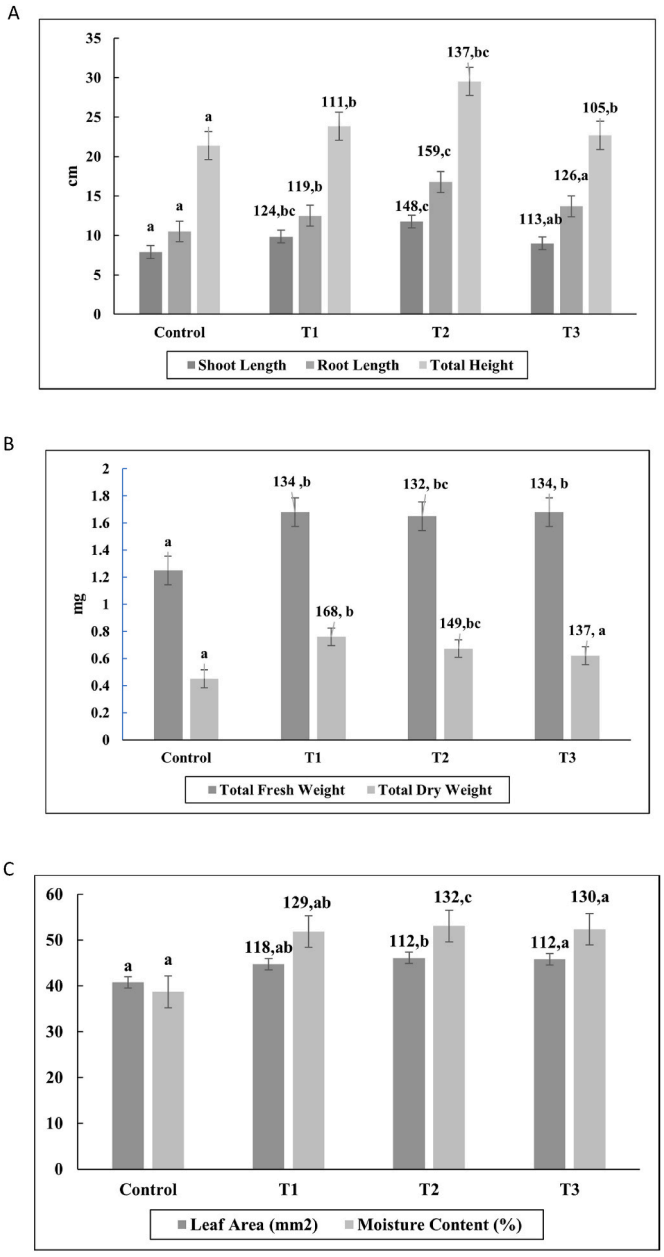

Fig. 6. Soil application of *M. tardum* isolate on growth characteristics of cluster bean
A- Total Plant Height, B- Total fresh and Dry weight, C- Leaf area and Moisture content.
T1: (1% of *M. tardum*), T2: (3% of *M. tardum*), (5% of *M. tardum*); Control: Distilled water.
Different letters (a–
d) in a data set are significantly different ($P \leq 0.05$ level). Values above each bar are percent over control.
Different letters present above each bar are statistically significant based on DMRT.

4. Discussion

Phyllospheres serve as one of the main hubs for the microbial community. Bacterial species continue to dominate the microbial community when compared to other microbes. Many researchers have paid attention towards biodiversity of the genus, colonization, and pathogenicity of bacteria on the host plant than plant growth promoting factors (Hubbard et al., 2015; Meena et al., 2017). Methylotrophic bacteria are phytosymbionts that consume waste products such as methanol produced by the plants (Sy et al., 2005) and have the potential to synthesize growth hormones (Jayashree et al., 2011; Mizuno et al., 2013; Chanratana et al., 2017; Kaparullina et al., 2017; Rangel et al., 2017). *Methylobacterium* spp also play a vital role in carbon cycling, nitrogen fixation, inducing stress tolerance and synergistic behavior with other microbiotas for plant growth and development (Kumar et al., 2016).

Table 4
Soil application of *M. tardum* isolate on biochemical characteristics of cluster bean.

Treatments	Chlorophyll-a (mg.g-1fr.wt)	Chlorophyll-b (mg.g-1fr.wt)	Total chlorophyll (mg.g-1fr.wt)	Protein (mg.g-1fr.wt)	Reducing sugar (mg.g-1fr.wt)	Starch (mg.g-1fr.wt)	Amino acid (mg.g-1fr.wt)	Ascorbic acid (mg.g-1fr.wt)	NRA (μ moles No-2/gm.fr.wt/hr)
Con	0.319 a	0.420 b	0.428 b	19.50 a	39.30 a	20.48 a	11.32 a	0.700 a	1.11 a
T1	0.413 a **(129)**	0.600 c **(142)**	0.568 b **(132)**	21.47 b **(109)**	42.02 b **(105)**	23.70 b **(116)**	17.77 c **(156)**	0.754 ab **(107)**	1.30 ab **(117)**
T2	0.747 b **(233)**	0.770 cd **(183)**	1.017 cd **(237)**	23.33 bc **(118)**	42.75 c **(106)**	27.96 bc **(133)**	17.07 d **(150)**	0.930 b **(132)**	1.50 b **(135)**
T3	0.405 a **(126)**	0.425 b **(101)**	0.522 c **(122)**	20.50 a **(104)**	41.21 a **(103)**	20.30 b **(99)**	14.78 bc **(130)**	0.754 a **(107)**	1.30 a **(117)**

T1: (1% of *M. tardum*), T2: (3% of *M. tardum*), (5% of *M. tardum*); Control: Distilled water. Different letters (a-d) in a data set are significantly different ($P \leq 0.05$ level). Values in bracket are percent over control. Different letters followed in each column are statistically significant based on DMRT.

In our study, foliar and soil application of *M. tardum* increased the growth and physiology of cluster bean plants. Even though 1% foliar application (T1) was found to be effective in enhancing plant growth, the statistical difference between the treatments (T1, T2, T3) and control was found to be minimum. This may be due to ineffective colonization with the host plant, inability to penetrate protective layers of the leaves such as the cuticle, and other layers present in the epidermal cells. Positive response to plant growth upon foliar administration of PPFM depends on the thickness of cuticle layer, surface roughness, and leaf hairs to reach the internal cells of the plant (Fernández and Brown, 2013). Wettability of plant leaves as well as the ability of leaves to absorb and translocation of nutrients also play a crucial role in foliar fertilization (Puente and Baur, 2011). However, successful foliar efficacy depends upon the physiological status of the plant, morphological features of leaf shape, leaf chemistry, cuticle composition, surface wax architecture and mobility of the nutrients or phytohormones within the plant (Fernández et al., 2014).

Several authors have observed that *Methylobacterium* spp showed appreciable increase in plant growth attributes in soyabean (Radha et al., 2009), coleus (Pattanashetti et al., 2012), snapbean (Abd-El- Gawad et al., 2015), tomato (Subhaswaraj et al., 2017), barnyard millet (Arun Balaji et al., 2019); fenugreek (Anandhi et al., 2019); rapeseeds (Roodi et al., 2020), rice (Aswathy et al., 2020). In ginger, *Methylobacterium* spp. (IISRGPPFM13) increased plant growth parameters, such as germination, number of pseudostems, length of pseudostems, number of leaves, length of leaves, width of leaves, length of roots, vigour when administered as soil drenching cum foliar spraying (Vadivukarasi et al., 2018). IAA producing phyllosphere methylotrophic bacteria (*Methylobacterium extorquens* MM2) isolated from mustard leaves improved the shoot and root length in tomato when compared to control (Subhaswaraj et al., 2017). In another study, methylotrophic strains isolated from the phylloshere of groundnut promoted IAA and siderophore production, ACC deamination, nitrogen fixation, sulphur oxidation and solubilization of insoluble minerals and concluded that bacterial habitat on the phyllosphere directly influences the growth and yield of the groundnut plants (Krishnamoorthy et al., 2018). Similarly, chlorophyll content in soyabean (Meenakshi and Salvage, 2009), coleus (Pattanashetti et al., 2012), snapbean (Abd-El-Gawad et al., 2015), paddy (Nysanth et al., 2019), rice (Anagha et al., 2020) was found to be elevated when *Methylobacterium* species were administered as bioinoculant. Additionally, Ivanova et al. (2001) reported that enhanced leaf chlorophyll, carotenoids, and fruit sugars were observed when methylotrophic bacteria were induced in leaf phyllosphere. Madhaiyan et al. (2015) observed that foliar spray of L2-4 *Methylobacterium* sp. led to successful colonization on both leaf surface subsequently increased the chlorophyll content in leaves of *Jatropha* sp. In pot culture experiment, foliar application of *Methylobacterium* spp. (1% v/v concentration) exhibited a significant increase in total chlorophyll, total sugar, total reducing sugar and total phenolic content in tomato plant (Senthilkumar and Krishnamoorthy, 2017).

In a pot study, Pattanashetti et al. (2012) observed that *Coleus forskohlii* tuber yield was found to be maximized when PPFM50 pink pigmented facultative methylotrophic bacteria was given. Yield components viz., number of pods per plant, grain yield was found to be significantly higher in soya bean plants which received Methylotrophic spp. + *Bradyrhizobium* when compared to reference strain of methylotrophic spp. + *Bradyrhizobium* (Radha et al., 2009). In another study, grain yield of soybean was found to be enhanced by combined inoculation of rhizobium + PPFMs + foliar spray consortium of PPFMs +100 % N treatment when compared to other treatments (Govekar and Navale, 2020). Along with other bioinoculants, *Methylobacterium* sp. enhanced the number of tillers/plants, number of grains/plants, grain size and grain weight in rice crop (Anagha et al., 2020).

Further, soil application of *M. tardum* was found to be effective in growth and physiology of cluster bean when compared to foliar application. Growth promoting efficiency of *M. tardum* may be due to the ability to produce phytohormones such as IAA, cytokinin etc., which could have stimulated cell division or plant elongation. Several studies suggested that PPFM mainly improves plant growth and productivity through enhanced nutrient accumulation (Madhaiyan et al., 2010) and increased plant indole acetic acid, and cytokinin production (Lee et al., 2006; Madhaiyan et al., 2007; Chanratana et al., 2017; Pattnaik et al., 2017). In a study, methylotrophic bacteria isolated from different species of herbs, shrubs, and trees were found to have the ability to synthesize auxins (Kaparullina et al., 2017). Methylotrophs have the potential of playing multiple roles in plant growth development such as nitrogen fixation (Menna et al., 2006; Sy et al., 2001; Raja and Sundaram, 2006), inhibit plant pathogens (Lacava et al., 2004) induce higher photosynthetic activity (Cervantes Martinez and Rodriguez garay, 2004). In a study, Grossi et al. (2020) reported that *Methylobacterium* sp. 2A increased the plant growth by decreasing the plant ethylene production during stress conditions. Based on our results, we can confer that soil application of *M. tardum* could have influenced the biofilm formation in the roots, enabling root colonization,

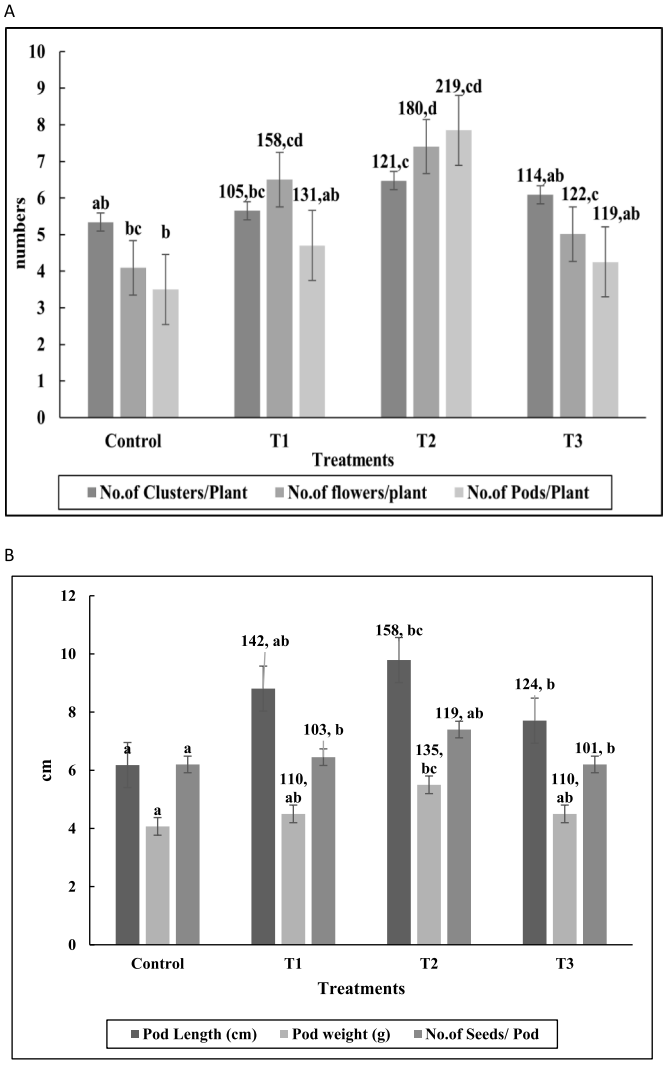

Fig. 7. Soil application of *M. tardum* isolate on yield characteristics of cluster bean.
A- Number of clusters/plant, Number of Flowers/plant, Number of pods/plant.
B- Pod length, Pod weight, Number of seeds/pod
T1: (1% of *M. tardum*), T2: (3% of *M. tardum*), (5% of *M. tardum*); Control: Distilled water.
Different letters (a–
d) in a data set are significantly different ($P \leq 0.05$ level).
Values above each bar are percent over controlDifferent
letters present above each bar are statistically significant based on DMRT.

formation of root nodules, phytohormone secretion etc. nutrient mineralization and breakdown the soluble and insoluble organic matter into useable forms for the plants.

Rhizosphere environment hosts a complex microorganism network which interacts very closely with plant roots by establishing an intimate symbiotic association and capable of stimulating the plant growth and soil ecosystem. Most of the microbial inoculants play a key role in maintaining soil fertility by solubilizing inorganic minerals into useable form, nitrogen fixation, phytohormone production, ACC deaminase activity, siderophore production. (Ahmed et al., 2014; Abeer et al., 2016). Methylotrophs are a crucial group of bacteria that are known to play a vital role in soil fertility. Several studies have reported that pigmented facultative methylotrophic bacteria such as *M. nodulans* can increase the number of nodules through nitrogen fixation due to NifH genes (Madhaiyan et al., 2006; Chanaratna et al., 2017. Moreover, *Methylobacterium* genus including *M. extorquens* and *M. mesophilicum*, form biofilms on the root surface which favours the growth of the bacteria (Rossetto et al., 2011). Rodriquez et al., 2006 reported that *Methylobacterium* species can dissolve inorganic phosphates, which in turn promotes phosphate metabolism in both microorganisms and plants. Jeyajothi et al. (2014) reported that foliar application of PPFM along with a biofertilizer enhanced the microbial population in soil, making nutrients more available to the plants. It can be concluded that *M. tardum* could have involved in the conversion of soil nitrate and inorganic phosphates to useable forms for both plants and to resident microbes.

In our study, reference strain (3% of *M. extorquens*) recorded the highest number of colonies of soil microbial community (bacterial, fungal and microalgal colonies) followed by T2 (3% of *M. tardum*), T3 (5% of *M. tardum*) and T1 (1% of *M. tardum*) when compared to control (supplemented only with water). This enhancement may be attributed to the fact that the bioinoculants could have released or involved in the biosynthesis of essential secondary metabolites which might have nurtured the resident microbiota populations (Richardson et al., 2009) or prevented the growth of pathogenic microbes affecting the resident microbes (Saharan and Nehra, 2011). Moreover, PPFMs synthesize and secrete vitamin B12, for which many algal species are auxotrophic which may be the basis for symbiosis (Grossman, 2017). Kelly et al. (2015) reported that PPFMs produce auxin (Doroninqa et al., 2002), cytokinin (Holland et al., 2002), and gibberellin (Siddikee et al., 2010) and inhibit the production of ethylene by producing an ACC deaminase (Madhaiyan et al., 2006), all of which influenced faster algal growth.

5. Conclusion

From our study, we can confer that soil application of *M. tardum* exhibited a positive response in terms of growth, biochemical and yield of cluster bean when compared to foliar application. It may be due to efficient root colonization, biofilm formation, nitrogen fixing ability, production of phytohormones, decreased ethylene production and phosphate solubilization, etc. However, the addition of adjuvants or wetting agents or humectants along with foliar application may also a vital role for plant growth development by effective colonization, reducing pathogenicity and mobility of nutrients. But concentration of the PPFM must be optimized while using for commercial purposes. In future, designing a microbial consortium using PPFM with suitable formulations and delivery approaches is essential. Soil microbial community was also found to be increased when soil application of PPFM was given. But a thorough understanding of the interaction between resident soil microbial community and bioinoculant must be explored which will foster the development of eco-friendly microbial agricultural techniques for sustainable agriculture.

Funding source

This project was supported by University Grants Commission, UGC, New Delhi under Minor Research Project (MRP- 5267/14 SERO/UGC).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

Authors express sincere to University Grants Commission, New Delhi for providing financial support for this research project.

References

Abd, E.l., Gawad, A., El-Hafez, Abd, Abou El-Yazie, D.A., 2015. Contribution of pink pigmented facultative methylotrophic bacteria in promoting antioxidant enzymes, growth and yield of snap bean. Am.-Eurasian J. Agric. Environ. Sci. 15 (7), 1331–1345.

Abeer, H., Salwa, A.A., Alqarawi, A.A., Allah, E.E., Egamberdieva, D., 2016. Arbuscular mycorrhizal fungi enhance basil tolerance to salt stress through improved physiological and nutritional status. Pakistan J. Bot. 48, 37–45.

Ahmed, E.A., Hassan, E.A., El Tobgy, K.M., Ramadan, E.M., 2014. Evaluation of rhizobacteria of some medicinal plants for plant growth promotion and biological control. Ann. Agric. Sci. 59, 273–280.

Ajaykumar, R., Murali Krishnasamy, S., 2018. Effect of PPFM and PGRs on crop growth rate in transplanted rice under moisture stress condition. J. Pharmacogn. Phytochem. 7 (4), 2366–2368.

Alirreza, H.G., Varahram, R., Mohammed, N.S.V., Masoud, E., Farhad, 2014. Effects of foliar application of methylobacterium and methanol on growth and yield of peanut (Arachis hypogea L. cv. NC2). Adv. Environ. Biol. 8 (21), 1256–1262.

Anagha, K., Tarun Agarwal, A., Jhansi Narmada Reddy Kotamreddy, B., Raktim Bhattacharya, B., Adinpunya Mitra, B., Tapas, K., Maiti, A., Mrinal, K., 2020. Impact of seed-transmitted endophytic bacteria on intra- and inter-cultivar plant growth promotion modulated by certain sets of metabolites in rice crop. Microbiol. Res. 241, 126582.

Anandhi, M., Ramar, A., Jegadeeswari, V., Srinivasan, S., 2019. Effect of bio-stimulants and growth regulators on plant growth and herbage yield of fenugreek (*Trigonella foenum- graecum* L). J. Pharmacogn. Phytochem. 8 (3), 3364–3367.

Arun Balaji, G., Sakthivel, S., Chinnusamy, C., Muthuramu, S., 2019. Effect of nutrient management and PPFM application on growth and productivity of barnyard millet under rain-fed ecosystem. J. Pharmacogn. Phytochem. 8 (3), 3031–3033.

Aswathy, J.C., Shalini Pillai, P., Jacob, John, Meenakumari, K.S., 2020. Effect of pink pigmented facultative methylotrophs (PPFM) on growth and growth attributes of rice (*Oryza sativa* L.). Int. J. Chem. Stud. 8 (4), 3209–3213.

Cervantes-Martinez, J., Lopez-Diaz, S., Rodriguez-Garay, B., 2004. Detection of the effects of Methylobacterium in *Agave tequilana* Weber var. Azul by laser-induced fluorescence. Sci. 166, 889–892.

Chanratana, M., Han, G.H., Roy Choudhury, A., Sundaram, S., Halim, M.A., Krishnamoorthy, R., Kang, Y., Sa, T., 2017. Assessment of *Methylobacterium oryzae* CBMB20 aggregates for salt tolerance and plant growth promoting characteristics for bio-inoculant development. Amb. Express 2017 (1), 208.

Chinnadurai, C., Balachandar, D., Sundaram, S., 2009. Characterization of 1-aminocyclopropane-1-carboxylate deaminase producing methylobacteria from phyllosphere of rice and their role in ethylene regulation. World J. Microbiol. Biotechnol. 25, 1403–1411. https://doi.org/10.1007/s11274-009-0027-1.

Doroninqa, N.V., Ivanova, E.G., Trotsenko, I., 2002. New evidence for the ability of methylobacteria and methanotrophs to synthesize auxins. Microbiology 71, 116–118.

Duncan, B.D., 1965. Multiple range and multiple test. Biometrics 11, 1–42.

Fernández, V., Brown, P.H., 2013. From plant surface to plant metabolism: the uncertain fate of foliar applied nutrients. Front. Plant Sci. 4, 289. https://doi.org/10.3389/fpls.2013.00289.

Fernández, V., Guzmán, P., Peirce, C.A.E., McBeath, T.M., Khayet, M., McLaughlin, M.J., 2014. Effect of wheat phosphorus status on leaf surface properties and permeability to foliar-applied phosphorus. Plant Soil 384, 7–20. https://doi.org/10.1007/s11104-014-2052-6.

Gordon, S.A., Weber, R.P., 1951. Colorimetric estimation of indoleacetic acid. Plant Physiol. 26 (1), 192.

Govekar, Y.R., Navale, A.M., 2020. Combine inoculation effect of pink pigmented facultative methylotrophs and *B. japonicum* on nutrient status of soybean. Ind. J. Pure App. Biosci. 8 (5), 429–435. https://doi.org/10.18782/2582-2845.8368.
Green, P.N., Ardley, J.K., 2018. Review of the genus Methylobacterium and closely related organisms: a proposal that some Methylobacterium species be reclassified into a new genus. Methylorubrum gen. nov. Int. J. Syst. Evol. Microbiol. 68, 2727–2748.
Grossi, C.E.M., Fantino, E., Serral, F., Zawoznik, M.S., Fernandez Do Porto, D.A., Ulloa, R.M., 2020. Methylobacterium sp. 2A is a plant growth-promoting rhizobacteria that has the potential to improve PotatoCrop yield under adverse conditions. Front. Plant Sci. 11, 71. https://doi.org/10.3389/fpls.2020.00071.
Grossman, A., 2017. Nutrient acquisition: the generation of bioactive vitamin B12 by microalgae. Curr. Biol. 26, R319–R337. https://doi.org/10.1016/j.cub.2016.02.047.
Holland, M.A., Polacco, J.C., 1994. PPFMs and other covent contaminants: is there more to plant physiology than just plant. Annu. Rev. Plant Physiol. Plant Mol. Biol. 45, 197–208.
Holland, M.A., Long, R.L.G., Polacco, J.C., 2002. Methylobacterium spp.: phylloplane bacteria involved in cross-talk with the plant host? In: Lindow, S.E., Hecht-Poinar, E.I., Elliot, V.J. (Eds.), Phyllosphere Microbiology. APS Press, St. Paul, MN, pp. 125–135.
Hubbard, A., Lewis, C.M., Yoshida, K., Ramirez-Gonzalez, R.H., de Vallavieille-Pope, C., Thomas, J., Kamoun, S., Bayles, R., Uauy, C., Saunders, D.G., 2015. Field pathogenomics reveals the emergence of a diverse wheat yellow rust population. Genome Biol. 16, 23. https://doi.org/10.1186/s13059-015-0590-8.
Ivanova, E.G., Doronina, N.V., Trotsenko, Y.A., 2001. Aerobic methylobacteria are capable of synthesizing auxins. Microbiology 70, 392–397.
Jaworski, E.G., 1971. Nitrate reductase in intact plant tissue. Biochem. Biophys. Res. Commun. 43, 1274–1279.
Jayashree, S., Vadivukkarasi, P., Anand, K., Kato, Y., Seshadri, S., 2011. Evaluation of pink-pigmented facultative methylotrophic bacteria for phosphate solubilization. Arch. Microbiol. 193, 543–552. https://doi.org/10.1007/s00203-011-0691-z.
Jeyajothi, R., Subbalakshmi, L., Nalliah, D., 2014. Effect of PPFM application on microbial population in transplanted Tice. Trends Biosci. 7, 3573–3574.
Jourand, P., Giraud, E., Béna, G., et al., 2004. Methylobacterium nodulans sp. nov., for a group of aerobic, facultatively methylotrophic, legume root-nodule-forming and nitrogen-fixing bacteria. Int. J. Syst. Evol. Microbiol. 5 (6), 2269–2273.
Kaparullina, E.N., Doronina, N.V., Mustakhimov, I.I., Agafonova, N.V., Trotsenko, Y.A., 2017. Biodiversity of aerobic Methylobacteria associated with the phyllosphere of the southern moscow region. Microbiology 86, 113–118. https://doi.org/10.1134/S0026261717010076.
Kelly, S.M., 2015. Altering Growth Rates and Nutritional Qualities of Microalgal Feedstocks with Symbiotic Bacteria Masters Thesis. Salisbury University, Salisbury, MD.
Krishnamoorthy, R., Soon Wo, Kwon, Kumutha, K., Senthilkumar, M., Ahmed, S., Tongmin, S.A., Anandham, R., 2018. Diversity of culturable methylotrophic bacteria in different genotypes of groundnut and their potential for plant growth promotion. 3 Biotech 8, 275 2018.
Kumar, M., Tomar, R.S., Lade, H., Paul, D., 2016. Methylotrophic bacteria in sustainable agriculture. World J. Microbiol. Biotechnol. 32, 120. https://doi.org/10.1007/s11274-016-2074-8.
Lacava, P.T., Araújo, W.L., Marcon, J., Maccheroni, W., Azevedo, J.L., 2004. Interaction between endophytic bacteria from citrus plants and the phytopathogenic bacteria Xylella fastidiosa, causal agent of citrus-variegated chlorosis. Lett. Appl. Microbiol. 39 (1), 55–59.
Lidstrom, M.E., 2006. Aerobic methylotrophic prokaryotes. In: Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, K H., Stackebrandt, E. (Eds.), The Prokaryotes. Springer, New York, NY. https://doi.org/10.1007/0-387-30742-7_20.
Lowry, O.H., Rosebrough, H., Farr, A.L., Randall, R.J., 1951. Protein measurement by folin-phenol reagent. J. Biol. Chem. 193, 265–275.
Madhaiyan, M., Poonguzhali, S., Kang, B.G., Lee, Y.-J., Chung, J.-B., Sa, T.-M., 2010. Effect of co-inoculation of methylotrophic Methylobacterium oryzae with Azospirillum brasilense and Burkholderia pyrrocinia on the growth and nutrient uptake of tomato, red pepper and rice. Plant Soil 328, 71–82.
Madhaiyan, M., Poonguzhali, S., Lee, H.S., Hari, K., Sundaram, S.P., 2005. Pink-pigmented facultative methylotrophic bacteria accelerate germination, growth and yield of sugarcane clone Co86032 (*Saccharum officinarum* L.). Biol. Fertil. Soils 41, 350–358.
Madhaiyan, M., Poonguzhali, S., Sa, T., 2007. Influence of plant species and environmental conditions on epiphytic and endophytic pink-pigmented facultative methylotrophic bacterial populations associated with field-grown rice cultivars. J. Microbiol. Biotechnol. 17, 1645–1654.
Manish, Kumar, Divjot, Kour, Ajar Nath, Yadav, Raghvendra, Saxena, Pankaj Kumar, Rai, Anurag, Jyoti, Rajesh Singh, Tomar, 2019. Biodiversity of methylotrophic microbial communities and their potential role in mitigation of abiotic stresses in plants. Biologia 74, 287–308.
Meena, K.K., Kumar, M., Kalyuzhnaya, M.G., et al., 2012. Epiphytic pink-pigmented methylotrophic bacteria enhance germination and seedling growth of wheat (*Triticum aestivum*) by producing phytohormone. Antonie Leeuwenhoek 101 (4), 777–786.
Meena, P.D., Gupta, R., Meena, H.S., Sharma, P., Jambhulkar, S., 2017. Pathogenic variability within Indian *Alternaria brassicae* isolates using seed, cotyledon and leaf of Brassica species. J. Phytopath 165, 238–248. https://doi.org/10.1111/jph.12555.
Menna, P., Hungria, M., Barcellos, F.G., Bangel, E.V., Hess, P.N., Martínez-Romero, E., 2006. Molecular phylogeny based on the 16S rRNA gene of elite rhizobial strains used in Brazilian commercial inoculants. Syst. Appl. Microbiol. 29 (4), 315–332.
Misra, P.A., Mertz, E.T., Glover, D.V., 1975. Studies on corn proteins VIII. Free amino acid content of opaque-2 double mutants. Cereal Chem. 52, 844–848.
Mizuno, M., Yurimoto, H., Iguchi, H., Tani, A., Sakai, Y., 2013. Dominant colonization and inheritance of Methylobacterium species strain OR01 on Perilla plants. Biosc. Biotech. Biochem. 77, 1533–1538.
Nelson, N., 1944. A photometric adaptation of Somogyis method for the determination of reducing sugar. Anal. Chem. 31, 426–428.
Nysanth, N.S., Meenakumari, K.S., Elizabeth, K., Syriac, R., Beena, 2019. Screening of pink pigmented facultative methylotrophs for growth enhancement in paddy Biocatalysis and. Agricult. Biotechnol. 18, 101055 2019.
Omer, Z.S., Tombolini, R., Gerhardson, B., 2004. Plant colonization by pink-pigmented facultative methylotrophic bacteria (PPFMs). FEMS (Fed. Eur. Microbiol. Soc.) Microbiol. Ecol. 47 (3), 319–326 2004.
Pattanashetti, S.R., 2012. Isolation and Characterization of Pink Pigmented Facultative Methylotrophs from Coleus Forskohlii and Their Influence on Growth and Tuber Yield M.Sc. (Ag) Thesis. University of Agricultural Sciences, Dharwad, p. 73p.
Vadivukkarasi, Ponnusamy, Suseela Bhai, R., 2019. Phyllosphere-associated Methylobacterium: a potential biostimulant for ginger (Zingiber officinale Rosc.) cultivation. Arch. Microbiol. 202 (2), 365–375. https://doi.org/10.1007/s00203-019-01753-6.
Pospiech, A., Neumann, B., 1995. A versatile quick prep of genomic DNA from gram positive bacteria. Trends Genet. 11, 217–218.
Puente, D.W.M., Baur, P., 2011. Wettability of soybean (Glycine max L.) leaves by foliar sprays with respect to developmental changes. Pest Manag. Sci. 67, 798–806. https://doi.org/10.1002/ps.2116.
Radha, T.K., Savalgi, V.P., Alagawadi, A.R., 2009. Effect of methylotrophs on growth and yield of soybean (*Glycine max* (L.) Merrill)* *Karnataka*. J. Agric. Sci. 22 (1), 118–121.
Raghavendra, J., Santhosh, G.P., Mahadevaswamy, Shubha, S., Ravi, M.V., 2019. Isolation and identification of PPFM bacterial isolates isolated from direct seeded rice growing areas of hyderabad-Karnataka. India Int. J. Curr. Microbiol. App. Sci. 8 (7), 152–163.
Raja, P., Sundaram, S., 2006. Combined inoculation effect of pink pigmented facultative *Methylobacterium* (PPFM) and other bioinoculants on cotton. Asian J. Bio. Sci. 1 (2), 39–44.
Rana, K.L., Kour, D., Sheikh, I., Yadav, N., Yadav, A.N., Kumar, V., Singh, B.P., Dhaliwal, H.S., Saxena, A.K., 2018. Biodiversity of endophytic fungi from diverse niches and their biotechnological applications. In: Singh, B.P. (Ed.), Advances in Endophytic Fungal Research. Springer, Switzerland. https://doi.org/10.1007/978-3-030-03589-1.
Rangel, W.M., De Oliveira Longatt, S.M., Ferreira, P.A.A., Bonaldi, D.S., Guimarães, A.A., Thijs, S., Weyens, N., Vangronsveld, J., Moreira, F.M.S., 2017. Leguminosae native nodulating bacteria from a gold mine as-contaminated soil: multi-resistance to trace elements, and possible role in plant growth and mineral nutrition. Int. J. Phytoremediation 10, 925–936.
Richardson, A.E., Barea, J.-M., McNeill, A.M., Prigent-Combaret, C., 2009. Acquisition of phosphorus and nitrogen in the rhizosphere and plant growth promotion by microorganisms. Plant Soil 321, 305–339.
Roe, J.H., 1954. Chemical determination of ascorbic, dehydroascorbic and diketogluconic acids. Methods Biochem. Anal. 1, 115–139.
Roodi, D., Millner, J.P., McGill, C., Johnson, R.D., Jauregui, R., Card, S.D., 2020. Methylobacterium, a major component of the culturable bacterial endophyte community of wild Brassica seed. PeerJ 8, e9514. https://doi.org/10.7717/peerj.9514.

Rossetto, P.B., Dourado, M.N., Quecine, M.C., et al., 2011. Specific plant induced biofilm formation in Methylobacterium species. Braz. J. Microbiol. 42 (3), 878–883.
Saharan, B., Nehra, V., 2011. Plant growth promoting rhizobacteria: a critical review. Life Sci. Med. Res. 21, 1–30.
Senthilkumar, M., Krishnamoorthy, R., 2017. Isolation and characterization of tomato leaf phyllosphere *methylobacterium* and their effect on plant growth. Int. J. Curr. Microbiol. App. Sci. 6 (11) xx-xx (Page No).
Siddikee, A., Hamayun, M., Han, G.-H., Sa, T.-M., 2010. Optimization of gibberellic acid production by *Methylobacterium oryzae* CBMB20 Md. Korean J. Soil. Sci. Fert. 43 (4), 522–527.
Subhaswaraj, P., Jobina, R., Parasuraman, P., Siddhardha, B., 2017. Plant growth promoting activity of pink pigmented facultative methylotroph-*Methylobacterium extorquens* MM2 on *Lycopersicon esculentum* L. J. Appl. Biol. Biotechnol. 5 (01): 042-046 https://doi.org/10.7324/JABB.2017.50107.
Sy, A., Giraud, E., Jourand, P., et al., 2001. Methylotrophic Methylobacterium bacteria nodulate and fix nitrogen in symbiosis with legumes. J. Bacteriol. 183 (1), 214–220.
Sy, A., Timmers, A.C., Knief, C., Vorholt, J.A., 2005. Methylotrophic metabolism is advantageous for *Methylobacterium extorquens* during colonization of *Medicago truncatula* under competitive conditions. Appl. Environ. Microbiol. 71, 7245–7252.
Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., Kumar, S., 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol. Biol. Evol. 28 (10), 2731–2739.
Verma, P., Yadav, A.N., Khannam, K.S., Mishra, S., Kumar, S., Saxena, A.K., Suman, A., 2016. Appraisal of diversity and functional attributes of thermotolerant wheat associated bacteria from the peninsular zone of India. Saudi J. Biol. Sci. 26 (7), 1882–1895. https://doi.org/10.1016/j.sjbs.2016.01.042.
Verma, P., Yadav, A.N., Khannam, K.S., Panjiar, N., Kumar, S., Saxena, A.K., Suman, A., 2015. Assessment of genetic diversity and plant growth promoting attributes of psychrotolerant bacteria allied with wheat (*Triticum aestivum*) from the northern hills zone of India. Ann. Microbiol. 65, 1885–1899. https://doi.org/10.1007/s13213-014- 1027-4.
Whittenbury, R., Davies, S.L., Wilkinson, J.F., 1970. Enrichment, isolation and some properties of methane-utilizing bacteria. J. Gen. Microbiol. 61, 205–218.