Pink Pigmented Facultative Methylotrophs from Cotton Phyllosphere on Paddy Fields

**Lazarus Vijune Lawrence**

Department of Chemistry and Biosciences

SASTRA Deemed University, Srinivasa Ramanujan Centre, Kumbakonam-612 001, Tamil Nadu, India.

**Bhuvaneswari Manivel\***

Department of Chemistry and Biosciences

SASTRA Deemed University, Srinivasa Ramanujan Centre, Kumbakonam-612 001, Tamil Nadu, India.

\*Corresponding author e-mail address: [bhunz2010@gmail.com](mailto:bhunz2010@gmail.com)

ABSTRACT

Pink Pigmented Facultative Methylotrophs (PPFMs) are a unique group of bacteria recognized for their distinctive pink to reddish pigmentation, resulting from the production of carotenoid pigments like astaxanthin and canthaxanthin. Displaying a facultative lifestyle, PPFMs possess the capacity to switch between utilizing methylated compounds, such as methanol, and conventional carbon sources, demonstrating metabolic versatility in diverse environments like soil, water, and plant surfaces. PPFMs are plant-associated bacteria, frequently colonizing the rhizosphere. They engage in beneficial interactions with plants, promoting growth through the production of phytohormones like auxins, cytokinins, and gibberellins. Moreover, PPFMs enhance nutrient availability, induce systemic resistance against pathogens, and improve plant stress tolerance, making them valuable contributors to sustainable agriculture. Their bioremediation potential, ability to synthesize valuable compounds, and participation in nutrient cycling further underscore their ecological significance. PPFMs' diverse attributes highlight their role in enhancing plant health, nutrient utilization, and overall ecosystem functioning, fostering interest in their biotechnological applications and ecological implications. The cotton phyllosphere offers a unique habitat for microbial colonization and interactions, potentially influencing plant health and ecosystem functioning. This chapter highlights the presence and diversity of PPFM in the cotton phyllosphere within paddy field ecosystems. The potential implications of the study in terms of plant-microbe interactions and ecosystem sustainability are covered.

Key words: Pink Pigmented Facultative Methylotrophs; cotton phyllosphere; microbial colonization; biotechnological applications.

# INTRODUCTION

Methylotrophic bacteria are a diverse group of microorganisms that have the remarkable ability to utilize one-carbon (C1) compounds as their sole source of carbon and energy for growth. These compounds include simple molecules such as methane (CH4), methanol (CH3OH), methylamine (CH3NH2), and formaldehyde (CH2O). Methylotrophs play a significant role in the global carbon cycle, as they are involved in the degradation and recycling of various C1 compounds [1]. There are two main types of methylotrophic bacteria: (1) Obligate Methylotrophs: These bacteria are entirely dependent on C1 compounds for their carbon and energy needs and cannot utilize conventional carbon sources like sugars. Examples of obligate methylotrophs include Methylobacterium species and Methylococcus species. They are commonly found in environments where methylated compounds are abundant, such as plant surfaces and soil. (2) Facultative Methylotrophs: Unlike obligate methylotrophs, facultative methylotrophs can use both C1 compounds and conventional carbon sources like sugars. They have the flexibility to switch between these carbon sources based on environmental conditions. Pink pigmented facultative methylotrophs (PPFMs), as mentioned earlier, are an example of facultative methylotrophs [1]. Methylotrophic bacteria possess specialized enzymes called methylotrophic enzymes that enable them to assimilate C1 compounds into cellular biomass. The key enzymes involved in methylotrophic metabolism include methanol dehydrogenase (Converts methanol to formaldehyde), formaldehyde dehydrogenase (Converts formaldehyde to formate), formate dehydrogenase (Converts formate to carbon dioxide and reduces NAD+ to NADH), and Serine cycle enzymes. In biotechnology, methylotrophic bacteria are used for the production of various valuable compounds, including enzymes, vitamins, and biofuels. Moreover, they are employed in wastewater treatment and bioremediation to remove toxic substances from contaminated environments [2].

PPFMs are a group of microorganisms that possess the unique ability to utilize methylated compounds as a carbon and energy source. Methylotrophs are a diverse group of bacteria that can metabolize one-carbon compounds such as methanol, methane, and methylated amines [3]. PPFMs are characterized by their pink to reddish pigmentation, which is due to the production of carotenoid pigments, such as astaxanthin and canthaxanthin. These pigments serve various functions in the microorganisms, including protection against oxidative stress and protection from harmful ultraviolet (UV) radiation [4]. PPFMs have been found in various environments, including soil, water, and plant surfaces. Their ability to metabolize methylated compounds makes them important players in the global carbon cycle, as they contribute to the breakdown of these compounds and their subsequent incorporation into biomass. These microorganisms have also attracted attention in biotechnology and environmental applications due to their unique metabolic capabilities. For instance, they can be employed in bioremediation processes to remove organic pollutants and toxins from contaminated environments. Additionally, PPFMs have potential applications in the production of carotenoids and other valuable metabolites. As with any microorganism, understanding the physiology, ecology, and genetics of pink pigmented facultative methylotrophs is essential to harness their potential for various biotechnological applications and gain insights into their ecological roles in different ecosystems [3].

# CAROTENOID PIGMENT PRODUCTION BY PPFMs

PPFMs are known for their distinctive pink coloration, which is attributed to the production of carotenoid pigments. Carotenoids are a class of pigments found in various organisms, including bacteria, plants, and algae. These pigments play important roles in light harvesting, photoprotection, and antioxidant activities [4]. The primary carotenoid pigment responsible for the pink color in PPFMs is astaxanthin. Astaxanthin is a red carotenoid that belongs to the xanthophyll group of carotenoids. It is synthesized by PPFMs as a part of their response to environmental stressors, such as high light intensity and oxidative stress. Astaxanthin serves as a potent antioxidant, helping to neutralize harmful reactive oxygen species (ROS) generated during stressful conditions. This property makes astaxanthin an essential protective pigment for PPFMs and other organisms that produce it [5]. The production of astaxanthin in PPFMs is regulated by various factors, including light, nutrient availability, and oxidative stress [6]. When exposed to stress, PPFMs induce the synthesis of astaxanthin to protect their cells from damage caused by ROS. The accumulation of astaxanthin gives these bacteria their characteristic pink color, which is particularly noticeable in cultures and colonies [6]. The pink pigmentation of PPFMs not only serves as a marker for their identification but also highlights their ability to respond to environmental challenges and protect themselves from oxidative stress. Additionally, the production of astaxanthin by PPFMs may have broader applications, as astaxanthin is a valuable compound with various potential health benefits for humans and animals. It is a powerful antioxidant with anti-inflammatory properties and is used as a dietary supplement and food colorant [7].

# PPFMS AS PLANT ASSOCIATED BACTERIA

PPFMs are a group of bacteria that have the ability to utilize one-carbon compounds, such as methanol, as a sole carbon and energy source. PFMs have been found to be associated with various plant species and can establish mutualistic relationships with plants [8]. They are often found in the rhizosphere, which is the region of soil immediately surrounding plant roots. PPFMs play important roles in the plant ecosystem, and their interactions with plants have several beneficial effects [8]

**A. Plant Growth Promotion**

PPFMs can promote plant growth by producing plant growth-promoting substances like indole-3-acetic acid (IAA), cytokinins, and gibberellins. These compounds can stimulate root and shoot development, leading to increased plant biomass. Plant growth promotion by PPFMs are attributed to several mechanisms that benefit plants and enhance their growth and development. These bacteria have found to establish symbiotic relationships with plants, particularly in the rhizosphere, which is the region of soil surrounding plant roots [9].

**a. Phosphate Solubilization**

PPFMs can solubilize inorganic phosphates present in the soil, making them more available to plants. Phosphorus is an essential nutrient for plant growth, and increased availability of phosphate ions helps improve root development and overall plant health [10]. Phosphate solubilization by PPFMs refers to their ability to convert insoluble forms of inorganic phosphates present in the soil into soluble forms, making them more accessible to plants for uptake. Phosphorus is an essential nutrient required for various cellular processes, including energy transfer, nucleic acid synthesis, and enzyme activation, making it crucial for plant growth and development. However, phosphorus often exists in soil as sparingly soluble compounds, such as calcium phosphate and iron phosphate, which are not readily available to plants [11]. PPFMs possess the enzyme phosphatase, which plays a key role in phosphate solubilization. The phosphatase enzymes secreted by these bacteria can hydrolyze the organic and inorganic forms of phosphate, releasing soluble orthophosphate ions (H2PO4- and HPO42-) into the soil solution. This process helps in liberating phosphorus from its insoluble forms, making it available for absorption by plant roots [12].

The phosphate solubilization ability of PPFMs has several implications for plant growth promotion. By solubilizing phosphates, PPFMs enhance the availability of this essential nutrient to plants. This, in turn, supports better root development, cell division, and overall plant growth. PPFMs with phosphate solubilization capabilities can be used as biofertilizers to improve soil fertility. They contribute to sustainable agriculture by reducing the need for chemical phosphate fertilizers, which can be environmentally harmful when overused [11]. Increased phosphorus availability can lead to higher crop yields and better quality produce, benefiting farmers and food production. The solubilization process may lead to the release of organic acids by PPFMs into the rhizosphere. These organic acids, such as gluconic acid and citric acid, can further aid in phosphate solubilization by lowering the pH of the rhizosphere and promoting the dissolution of phosphate minerals [13]. Phosphorus deficiency is a common problem in many agricultural soils. The presence of PPFMs with phosphate-solubilizing abilities can help alleviate this deficiency and improve plant health and productivity.

**b. Production of Plant Growth-Promoting Substances**

PPFMs are known to produce various plant growth-promoting substances that positively influence the growth and development of plants. PPFMs can synthesize and secrete the phytohormone IAA, which is a type of auxin. IAA plays a critical role in stimulating cell elongation, division, and differentiation, leading to increased root and shoot growth. It also promotes the formation of lateral roots, which helps plants access nutrients and water more efficiently [14]. PPFMs can produce cytokinins, which are another class of plant growth-promoting hormones. Cytokinins influence cell division and differentiation, delay senescence (aging) in plant tissues, and promote lateral bud development. This results in increased branching, improved plant architecture, and overall enhanced growth [15]. PPFMs may also produce gibberellins, which are plant hormones involved in regulating stem elongation, seed germination, and flowering. Gibberellins promote elongation of internodes and stems, leading to taller and more vigorous plants [16]. Some PPFMs can modulate ethylene levels in plants. Ethylene is a plant hormone that can either promote or inhibit growth, depending on the concentration. By regulating ethylene levels, PPFMs can help maintain a balanced growth response in plants [17].

Certain PPFMs possess the enzyme 1-Aminocyclopropane-1-Carboxylate (ACC) deaminase, which reduces the levels of ethylene precursor (ACC) in plants. By doing so, these bacteria can mitigate the negative effects of ethylene-induced stress, such as root inhibition and premature senescence [18]. PPFMs may produce and release vitamins and nutrients, such as B vitamins, iron, and zinc, that can enhance the nutritional status of plants and support their growth. PPFMs can secrete enzymes and metabolites that promote nutrient uptake, such as phosphate solubilizing enzymes, siderophores for iron uptake, and nitrogenase for nitrogen fixation [19]. These plant growth-promoting substances and mechanisms enable PPFMs to establish mutualistic relationships with plants, especially in the rhizosphere. By stimulating growth, improving nutrient uptake, and providing protection against stresses, PPFMs contribute to the overall health and productivity of plants. Harnessing the potential of these bacteria as biofertilizers or bioenhancers can be a promising approach to sustainable agriculture and environmental management.

**c. Nitrogen Fixation**

Nitrogen fixation is a process that is typically associated with certain bacteria known as diazotrophs, which have the enzyme nitrogenase to catalyze the conversion of N2 into ammonia. PPFMs are unique because they possess both the ability to fix nitrogen and to utilize one-carbon compounds, such as methanol, as a carbon and energy source [20]. By fixing nitrogen and making it available to plants, PPFMs play an essential role in nitrogen cycling and nutrient availability in ecosystems. This process is particularly beneficial for plants growing in nitrogen-poor soils since it provides them with a renewable and sustainable nitrogen source, reducing their dependency on external nitrogen fertilizers [21]. The ability of PPFMs to both fix nitrogen and utilize one-carbon compounds makes them promising candidates for applications in sustainable agriculture. They can contribute to improving soil fertility, enhancing plant growth, and reducing the environmental impact of nitrogen fertilizers.

**d. Enhanced Nutrient Uptake**

PPFMs can protect plants from pathogenic microorganisms through competition for resources and the production of antimicrobial compounds. By suppressing harmful pathogens, they help reduce disease incidence and improve plant health [22]. PPFMs have been shown to enhance plant tolerance to various abiotic stresses, such as drought, salinity, and heavy metal toxicity. They can produce stress-related enzymes and metabolites, which help plants cope with adverse environmental conditions [23]. Some PPFMs have been reported to enhance photosynthetic efficiency in plants, leading to increased carbon assimilation and improved plant growth [23].

B. **Methylotrophy and Methanol Metabolism**

PPFMs are a group of bacteria that possess the unique ability to utilize one-carbon compounds, such as methanol, as their sole carbon and energy sources. Methylotrophy is the process by which these bacteria metabolize and utilize one-carbon compounds for their growth and energy needs [24]. PPFMs have specialized transport systems that allow them to efficiently uptake methanol from their environment. Once inside the cell, methanol is oxidized to formaldehyde by the enzyme methanol dehydrogenase (MDH). This is the first step in the breakdown of methanol, and formaldehyde is an essential intermediate in methylotrophic metabolism [25]. Formaldehyde is a toxic compound, but PPFMs possess enzymes, such as formaldehyde-activating enzymes, that facilitate the assimilation and incorporation of formaldehyde into various metabolic pathways [26]. PPFMs can use different metabolic pathways to convert formaldehyde into biomass. One of the common pathways is the serine cycle, which involves a series of enzymatic reactions to produce serine, an amino acid used for cell growth and biosynthesis. Another pathway used by some PPFMs is the ribulose monophosphate (RuMP) pathway [27]. Throughout these metabolic processes, the breakdown of methanol and assimilation of formaldehyde yield energy that PPFMs use to sustain their growth and cellular activities. Methylotrophy provides a unique ecological niche for PPFMs, allowing them to thrive in environments rich in one-carbon compounds, such as the phyllosphere (leaf surfaces), soil, and marine environments. It is essential to note that the pink pigmentation observed in these bacteria is due to the synthesis of the red carotenoid pigment astaxanthin, which serves various functions, including protection against oxidative stress [24].

**C. Biocontrol**

PPFMs have recognized for their potential as biocontrol agents in agriculture. Biocontrol refers to the use of beneficial microorganisms to suppress plant diseases caused by pathogenic microorganisms. PPFMs exhibit biocontrol properties through various mechanisms. PPFMs can outcompete pathogenic microorganisms for nutrients and space in the rhizosphere, the region of soil surrounding plant roots. By utilizing available resources efficiently, they limit the growth and establishment of harmful pathogens [28]. PPFMs can synthesize and release antimicrobial compounds, such as antibiotics and secondary metabolites that inhibit the growth and development of pathogenic microorganisms. These compounds act as natural defense mechanisms against pathogens [29]. Some PPFMs can induce systemic resistance in plants. When exposed to these bacteria, plants activate their defense mechanisms, leading to enhanced resistance against a broad range of pathogens [30]. PPFMs can influence the levels of plant hormones, such as jasmonic acid and salicylic acid, which play important roles in the plant defense response. By modulating these hormones, PPFMs can enhance plant immunity against pathogens. PPFMs possess enzymes that can detoxify harmful compounds produced by pathogens, protecting the plant from their toxic effects [31]. By promoting plant growth and health, PPFMs indirectly improve the plant's ability to defend itself against pathogens. Healthy and vigorously growing plants are generally more resistant to diseases. Some PPFMs produce surface-active compounds that prevent pathogen attachment and colonization on plant surfaces, reducing the chances of infection [32].

**D. Stress Tolerance**

PPFMs can enhance plant stress tolerance against various environmental stresses, such as drought, salinity, and heavy metal toxicity. This often achieved by the production of stress-resistance-inducing metabolites. PPFMs have found to exhibit stress tolerance, which is the ability to withstand and survive various adverse environmental conditions. These bacteria possess several mechanisms that enable them to cope with stresses in their habitats, including the rhizosphere and other soil environments [33]. PPFMs have shown to enhance drought tolerance in plants by producing osmoprotectants and antioxidants. Osmoprotectants help maintain cellular water balance, while antioxidants help neutralize reactive oxygen species (ROS) that are produced under drought stress. PPFMs can improve plant tolerance to salinity stress by producing osmoprotectants like proline and glycine betaine. These compounds help to regulate cellular water balance and prevent ion imbalances caused by high salt levels [34]. Some PPFMs are capable of tolerating heavy metal toxicity in the soil. They can accumulate and sequester heavy metals within their cells, reducing their toxic effects on the plant and soil environment [35]. PPFMs have the ability to grow and survive in a wide range of temperatures, allowing them to thrive in diverse environments. PPFMs can tolerate a broad pH range, which is beneficial in fluctuating soil conditions.

# PPFMs FROM COTTON PHYLLOSPHERE ON PADDY FIELDS

PPFMs are ubiquitous in nature found in variety of habitats including soil, dust, fresh water lake sediments, leaf surface and nodules. These organisms are capable of growing on compounds containing one carbon [36]. These bacteria influence the seed germination and seedling growth by producing plant growth regulators like Zeatin and related cytokinins. Hence, there is a possibility of increasing the effectiveness of the conventional bio inoculants by co-inoculating with PPFMs. For this attempt has made to isolate PPFM from the phyllosphere of cotton (CV.LRA 5166). Fresh leaf collected and impregnated on Ammonium mineral salt medium supplemented with 0.5 % cyclohexamide. Here 0.5 % methanol used as carbon source. After seven days of incubation at room temperature, pink colonies of PPFM appeared which belongs to the genus Methyl bacterium. These PPFM s isolated from LRA 5166 ad other growth promoting rhizobacteria were used to study the vigour index of cotton CV.LRA 5166 and the results revealed that cotton seeds soaked in Azospirillum lipoferum and PPFMs isolated from the phyllosphere of LRA 5166 produced significantly higher vigor index over other rhizobacteria. The impact of PPFM (Pink Pigmented Facultative Methylotroph) and PGRs on alleviating the drought stress effects in tomato. The study indicated that the PPFM and PGRs could effectively improving drought tolerance capacity of tomato crop under drought. Among the PGRs and different concentrations of PPFM used, PPFM (2%) found to superior in improving RWC, photosynthetic rate, SPAD value and proline content. The antioxidant enzyme, catalase activity enhanced by PPFM (2%) and salicylic acid (100 ppm) treatments, which has the ability to protect the plant under abiotic stress by nullifying oxidative damage.The soluble protein content, maintained by brassinolide followed by PPFM (2%) under drought [36].

**A. Microorganisms**

The genus, Methyl bacterium, 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 the sole source of carbon and energy [37]. The genus Methyl bacterium is composed of a variety of pink pigmented facultative methylotrophs i.e. (PPFM) and non pigmented facultative methylotrophs i.e. (NPFM) which are capable of growing on C1 compounds such as formate, formaldehyde, methanol and methylamine as well as on a wide range of multi carbon growth substrates such as C2, C3 and C4 compound. Isolation, purification and characterization of Pink Pigmented Facultative Methylotrophs (PPFM) from phyllosphere of *Coleus forskohlii* plants

1. To study the functional diversity of these PPFM isolates.
2. To study the effect of the efficient PPFM isolates on growth and tuber yield of *Coleus forskohlii* plants. (Pot culture studies).

**B. PPFMs**

The genus Methyl bacterium belongs to alpha-2 subclass of *Proteobacteria* with validly published 26 species [38], namely as few follows.

1. *M. aquaticum* [39]
2. *M. chloromethanicum*
3. *M. dichloromethanicum*
4. *M. extorquens* [40]
5. *M. fujisawaense* [41]

The species of Methylotrophs 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 [42]. Several species of methylotrophic bacteria are found in association with terrestrial and aquatic plants, colonizing roots, leaf surfaces, and growing buds [43].

**1. Morphological characteristics**

All *Methylobacterium* strains are rods (0.8 - 1.0 × 1.0 - 8.0 µm), which occur singly or occasionally in rosettes [44]. They are often branched or pleomorphic, especially in older stationary phase cultures. They exhibit polar growth or budding morphology. All strains are motile by a single polar, subpolar or lateral flagellum, although some strains are not vigorously motile [45]. Cells often contain large sudanophilic inclusions (Poly hydroxybutyrate) and sometimes volutin granules. They are gram negative, although many strains stain as gram variable [46]. Colonies on glycerol peptone agar are < 1 to 3 mm in diameter and pale pink to bright orangered in colour while, colonies on methanol salts agar are uniformly pale pink. The pigment is insoluble and probably carotenoid. In the static liquid media strains grow as a pink surface ring or pellicle [47].

**2. Biochemical characteristics**

A study demonstrated that all isolates were aerobes producing catalase and oxidase. As per biochemical study of Green (1992) [48], all *Methylobacterium* strains were catalase and oxidase positive. They are chemoorganotrophs and facultative methylotrophs capable of growing on a variety of C1 compounds. Most of the strains do not degrade or hydrolyse starch, gelatin, cellulose, lecithin or DNA. All these strains have weak lipolytic activity. The enzymes ı-galactosidase, Lornithine decarboxylase, L-lysine decarboxylase and L-arginine dihydrolase are not produced. The methyl red and Voges-Proskauer tests are negative, although some strains reduce nitrate to nitrite. Thangamani (2005) [49] reported that methylotrophs are positive for urease test and indole production.

**3. Carbon utilization tests**

Based on the pattern of compounds they utilize as carbon and energy source, a study differentiated *Methylobacterium* species, concluded that they are capable of growing on C1 compounds as sole source of carbon and energy and can also grow on wide range of multicarbon substrates making them facultatively methylotrophic. The compounds that were used by more than 95 per cent of *Methylobacterium* strains include, methylamine, trimethylamine, acetate, citrate, L-glutamate, D-glucose, D-xylose, fructose and betaine and carbon source utilization pattern by 12 known species of the genus *Methylobacterium* revealed that none of the strains appear to use any of the disaccharides or sugar alcohols examined but most of m*ethylobacterium* strains used glycerol, malonate, succinate, fumarate, ı-ketoglutarate as carbon and energy sources [50].

**Growth characteristics**

Except for some, most of the *Methylobacterium* strains grow slowly on nutrient agar. After seven days of incubation at 30℃, colonies on GP agar are 1 to 3 mm in diameter and pale pink to bright orange red, whereas colonies on methanol mineral salts (MMS) agar are more uniform pale pink. The pigment is non-diffusible, non-fluorescent and probably a carotenoid compound [47]. The optimum growth temperature for all *Methylobacterium* strains is in the range of 250 to 300℃. Some strains will grow at 510℃ or less and some will grow at or above 370℃. The growth is optimal around neutrality, although some strains can grow at pH of 4.0 to 10.0. External growth factors are not known to be required for growth and development of *Methylobacterium* strains [48].

**C. Beneficial effect on plant**

* Fasten seed germination and seedling growth
* Accelerate vegetative growth
* Increase leaf area index and chlorophyll content
* Earliness in flowering, fruit set and maturation
* Improves fruit quality, color and seed weight
* Yield increase by 10%
* Mitigate drought

**D. Method of Application**

* Seed treatment –  Imbibe seed in 1.0 % volume for 5-10min (depending on seed)
* Foliar Spray of 1% PPFM
* Spray during morning or evening
* Recommended for  all crops
* Spray at critical stage of crop growth  (or)  30days interval
* Precaution: Do not mix with pesticide / fungicide.

**Table 1: Different types of crops associated Methylotrophs with activities**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **S. No** | **Crop plants** | **Associated Methylotrophs** | **Source** | **Activity** |
| 1 | Wheat | *Methylobacterium sp* | Phyllosphere | Cytokinin Production |
| 2 | Rice | *M. extorquens, M. fujisawaense* | Phyllosphere | IAA |
| 3 | Sugarcane | *Methylobacterium sp* | Rhizosphere | IAA,PGP activities |
| 4 | Rice | *Methylobacterium sp* | Rhizosphere | N2 Fixation |
| 5 | Red Pepper | *M. suomiense* | Rhizosphere | Root colonise |
| 6 | Tomato | *M. suomiense* | Rhizosphere | Root colonise |
| 7 | Soya bean | PPFM | Phyllosphere | IAA |
| 8 | Groundnut | PPFM | Phyllosphere | IAA |
| 9 | Mung bean | *M. organophilum* | Mud | Bio fertilizer |
| 10 | Green gram | PPFM | Phyllosphere | Bio fertilizer |

# CONCLUSION

Rhizospheric and non-rhizospheric methylotrophs associated with plant growth can exploited for eco-friendly and cost-effective practices to promote sustainable agriculture. The application of methylotrophs as bioinoculants and in microbial sprays to crops is common and their use as alternatives to chemical fertilizers also increasing. Methylotrophs employ multiple mechanisms to promote plant growth, which makes them a suitable and promising candidate for use in sustainable agriculture. They regulate and govern biogeochemical cycling in soil ecosystems, making the land more amenable to crop production. Additionally, phytohormone production, plant growth promotion, nodulation, nitrogen fixation, and nutrient acquisition all make methylotrophs a promising substitute to chemical fertilizers for crops. In summary, methylotrophic bacteria offer the alternative of biological control, plant growth enhancement by nitrogen fixation, phosphate solubilization, phytohormone production, and ACC deaminase production in the rhizosphere, along with balanced carbon cycling. These beneficial methylotrophs can play a significant role in sustainable agriculture.

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