PINK PIGMENTED FACULTATIVE METHYLOTROPHS FROM COTTON PHYLLOSPHERE ON PADDY FIELDS

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.

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I. INTRODUCTION

Methylotrophic bacteria are a diverse group of microorganisms that have the remarkable ability to utilize one-carbon (C_1) compounds as their sole source of carbon and energy for growth. These compounds include simple molecules such as methane (CH_4), methylamine (CH_3NH_2), methanol (CH_3OH), and formaldehyde (CH_2O). Methylotrophs play a significant role in the global carbon cycle, as they are involved in the degradation and recycling of various C_1 compounds [1]. There are two main types of methylotrophic bacteria: (1) Obligate Methylotrophs: These bacteria are entirely dependent on C_1 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 C_1 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), are an example of facultative methylotrophs [1].Methylotrophic bacteria possess specialized enzymes called methylotrophic enzymes that enable them to assimilate C_1 compounds into cellular biomass. The key enzymes involved in methylotrophic metabolism include methanol dehydrogenase (Converts methanol to formaldehyde), formate dehydrogenase (Converts formate to carbon dioxide and reduces NAD^+ to NADH), formaldehyde dehydrogenase (Converts formaldehyde to formate), 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 [2,3].

PPFMs are of particular interest in various applications due to their unique metabolic capabilities.PPFM can be used for the bioremediation of environments contaminated with organic pollutants such as petroleum hydrocarbons and chlorinated solvents. They can metabolize these pollutants, breaking them down into less harmful compounds. These organisms can employed in wastewater treatment processes to remove organic contaminants. They can efficiently degrade methylated pollutants present in industrial effluents. In anaerobic digesters used for biogas production, PPFM can enhance the degradation of methylated compounds like methylamines andmethanol, leading to increased biogas yield. PPFM can be used in the production of biodegradable plastics. They can convert methanol into valuable intermediates like polyhydroxyalkanoates (PHAs), which are biodegradable polymers used in bioplastics [1].PPFM can be used in the production of biofuels, particularly bioethanol. They can ferment methanol or other methylated compounds into ethanol, which can serve as an alternative to fossil fuels. In addition, PPFM can be used in microbial fuel cells (MFCs) to generate electricity from organic substrates, including methylated compounds. This has potential applications in wastewater treatment and renewable energy production. PPFM can be employed in biotechnological processes to produce enzymes or bioactive compounds. Their unique metabolic pathways make them useful in the synthesis of specific products [3].By using PPFM, it's possible to reduce the reliance on harsh chemicals and synthetic treatments in various processes. This can lead to cost savings and reduced chemical pollution. PPFM can potentially be harnessed for biological control of harmful microbes. Their ability to outcompete other microorganisms for methylated compounds can help suppress the growth of pathogenic or undesirable bacteria in certain environments. Some

PPFM strains are known to survive in extreme environments, such as acidic or saline soils. This resilience can be harnessed in applications like soil remediation in challenging conditions [2].These additional applications and advantages highlight the diverse and valuable roles that PPFM can play in environmental and industrial applications, as well as in advancing our understanding of microbial ecology and biotechnology.

II. 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 [4]. 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 [4,7].

III. PPFMs AS PLANT ASSOCIATED BACTERIA

Numerous plant species have identified to be associated with PPFMs, and these organisms are capable of developing mutualistic interactions with plants [8]. They may frequently found in the rhizosphere, which is a region of soil closest to plant roots. In the plant environment, PPFMs perform significant functions, and their interactions with plants have a number of positive effects [8].

1. **Plant Growth Promotion:** By producing plant growth-promoting (PGP) compounds including indole-3-acetic acid (IAA), gibberellins, and cytokinins, PPFMs might promote the development of plants. These substances may promote the growth of roots and shoots, increasing plant biomass [9]. PPFMs are believed to promote plant growth through a number of processes that help plants and improve their growth and development. The rhizosphere, is where these bacteria have found to form symbiotic relationships with the plants. A source of new bioactives for plant protection, polyketide synthases are found in PPFMs, which can also efficiently withstand damaging UV radiation. According to

studies, the inoculation of PPFMs can improve the development and productivity of crops including as rice and soybean. Plant growth-promoting chemicals, atmospheric nitrogen fixes, mineral phosphate solubilization, and systemic resistance against plant diseases are all produced by PPFMs. It has been discovered that PPFMs work better when used in conjunction with other helpful microorganisms than when administered alone [8,9].

2. **Phosphate Solubilization:** PPFMs have the ability to solubilize inorganic phosphates in the soil, increasing their availability to plants. Phosphorus is a crucial nutrient for plant growth, and more readily available phosphate ions support better root growth and basic plant health [10]. Phosphate solubilization by PPFMs is the process by which they transform insoluble forms of inorganic phosphates found in the soil into soluble ones, making them more readily absorbable by plants. For the growth and development of plants, phosphorus is an important nutrient since it is necessary for many cellular functions, such as energy transmission, nucleic acid synthesis, and enzyme activation. But phosphorus is frequently found in soil as sparingly soluble substances that are unavailable to plants, including calcium phosphate and iron phosphate [11]. The enzyme phosphatase, which is essential for the solubilization of phosphate, is present in PPFMs. By hydrolyzing both the organic and inorganic forms of phosphate, the phosphatase enzymes released by these bacteria can release soluble orthophosphate ions ($H_2PO_4^-$ and HPO_4^{2-}) into the soil solution. This method aids in releasing phosphorus from its insoluble forms so that plant roots may absorb it [12].

The capacity of PPFMs to solubilize phosphate has a number of implications for promoting plant development. Phosphates are made more available to plants by PPFMs, which solubilize them. Better root growth, cell division, and general plant growth are all aided by this. To increase soil fertility, PPFMs with phosphate solubilization properties can be utilised as biofertilizers. By decreasing the need for synthetic phosphate fertilisers, which when used excessively may be damaging to the environment, they help promote sustainable agriculture [11]. A study reported that when 13 PPFM strains were tested for phosphate solubilization from soil samples taken from rivers and forests in India, their P-solubilization indices varied from 1.1 to 2.7. After 7 days of incubation, four strains—MSF 32, MDW 80, *M. komagatae*, and MSF 34—exhibited greater phosphate solubilization. These cultures' culture filtrate showed acid phosphatase activity, demonstrating their capacity to solubilize phosphate. Phosphate also showed adhesion to the bacterial surface in the PPFMs. This concluded that the first in-depth analysis of phosphate solubilization by PPFMs, emphasising the potential of these molecules to improve plant nutrient availability. For the advantage of farmers and food production, increased phosphorus availability can result in higher crop yields and better-quality foodstuffs. The PPFMs' release of organic acids into the rhizosphere during the solubilization process is possible. By reducing the pH of the rhizosphere while stimulating the dissolution of phosphate minerals, certain organic acids, such as gluconic acid and citric acid, may also aid in phosphate solubilization [13]. Lack of phosphorus affects many agricultural soils often. This shortage can be reduced and plant productivity and health can be enhanced by the presence of PPFMs with phosphate-solubilizing properties.

3. **Production of PGP Substances:** PPFMs are known to produce various PGP 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 PGPhormones. Cytokinins influence cell division and differentiation, delay senescence 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. 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 PGP 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.

4. **Nitrogen Fixation:** A process known as nitrogen fixation is frequently correlated with a group of bacteria called diazotrophs, which possess the enzyme nitrogenase that catalyses the conversion of N_2 into ammonia. Because they can fix nitrogen as well as utilise one-carbon molecules like methanol as a source of carbon and energy, PPFMs are unique [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 source for applications in sustainable agriculture. They can contribute to improving soil fertility, enhancing plant growth, and reducing the environmental impact of nitrogen fertilizers.

5. **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 shown to enhance plant tolerance to various abiotic stresses, such as salinity, heavy metal toxicity, anddrought. 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].

6. **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, 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].

7. **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. 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 bioactive compounds (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].

8. **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 [33]. 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 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.

IV. PPFMs FROM COTTON PHYLLOSPHERE ON PADDY FIELDS

Bacteria found in the cotton phyllosphere can be used to increase cotton crop productivity. These microorganisms, including *Acinetobacter* sp., *Pseudomonas chlororaphis*, *Pseudomonas stutzeri*, *Bacillus mojavensis*, and *Enterobacterasburiae*, have been reported to produce ACC deaminase and to be drought tolerant. It has been demonstrated that exposing cotton seeds to these bacteria improves the plants’ nutrition, seed vigour, and germination. Additionally, with the inoculation of phyllosphere bacteria, it has been discovered that the root length, fresh weight, proline content, and number of bolls in cotton plants all increase. In cotton plants, PPFMs are the main phyllospherecolonisers. Red clover and winter wheat have both been discovered to be colonised by Methylobacterium, a genus of PPFMs, in agricultural fields. When combined with other bioinoculants, these PPFMs have been demonstrated to stimulate plant growth and boost agricultural output. The phyllosphere is a challenging environment, but methylotrophic bacteria and yeasts that use methanol have special molecular and cellular mechanisms to adapt to it. In general, it has been demonstrated that PPFMs from the cotton phyllosphere have positive impacts on plant development and may be employed as bio-inoculants in field crops [22,33]. The role of phyllosphere bacteria in improving cotton growth and yield under drought conditions is a topic of growing importance in agricultural research. Cotton, a major global crop, is particularly susceptible to drought stress, which can significantly reduce yield. Phyllosphere bacteria, which inhabit the above-ground parts of plants, have the potential to impact cotton cultivation during periods of water scarcity positively. Phyllosphere bacteria can influence a plant's water use efficiency. They can promote the closure of stomata, the tiny openings on leaves through which plants lose water via transpiration. By regulating stomatal conductance, these bacteria help the cotton plant retain more water during drought [22]. Some phyllosphere bacteria can solubilize nutrients in the leaf surface, making them more accessible to the plant. This nutrient mobilization can maintain plant vigour and growth even when water is limited. Phyllosphere bacteria can produce PGP hormones, such as auxins and cytokinins [14]. These hormones stimulate root development and plant growth, helping cotton plants cope with drought stress. Certain beneficial phyllosphere bacteria can protect cotton plants from foliar diseases that may become more prevalent under drought conditions [33]. This disease resistance can help maintain the health and productivity of cotton crops. Drought stress often leads to the production of harmful ROS in plant tissues. Phyllosphere bacteria can help scavenge ROS and mitigate oxidative damage, thereby preserving the plant's physiological functions. Some phyllosphere bacteria can form biofilms on plant surfaces, acting as a protective barrier against environmental stresses, including drought. These biofilms can help maintain adequate hydration of plant tissues [36].

In nature, PPFMs may found in a wide range of environments, including soil, dust, fresh water lake sediments, leaf surface, and nodules. These organisms survive on substances with one carbon atom [36]. By generating plant growth regulators like Zeatin and related cytokinins, these bacteria impact seed germination and seedling development. Therefore, co-inoculating with PPFMs can improve the efficacy of the traditional bio inoculants. Because of this, an effort has made to extract PPFM from the cotton phyllosphere (CV.LRA 5166). Fresh leaf was gathered, impregnated, and added to a medium of ammonium mineral salts with 0.5% cyclohexamide. Here, the carbon source is methanol, 0.5%. Pink colonies of the genus Methyl bacteria, known as PPFM, emerged after seven days of incubation at room temperature. The vigour index of cotton CV.LRA 5166 was studied using these PPFMs isolated from LRA 5166 and other growth-promoting rhizobacteria. The findings showed that cotton seeds soaked in *Azospirillumlipoferum* and PPFMs isolated from the phyllosphere of LRA 5166 produced significantly higher vigour index than other rhizobacteria. The effect of plant growth regulators and PPFM in reducing the effects of drought stress on tomato. The study found that the PPFM and plant growth regulators might significantly increase the capacity of tomato crops to withstand drought. PPFM (2%) is superior to other plant growth regulators and the various concentrations of PPFM utilised in enhancing RWC, photosynthetic rate, SPAD value, and proline content. The antioxidant enzyme catalase, whose activity increased by treatments with salicylic acid (100 ppm) and PPFM (2%) can shield plants from abiotic stress by preventing oxidative damage.Under drought, PPFM (2%) followed by brassinolide (2%) maintained the soluble protein content [36].

1. **Microorganisms:** The Methyl bacterium genus consists of gram-negative, facultatively methylotrophic, strictly aerobic, rod-shaped bacteria that can grow on a variety of multi-carbon growth substrates as well as one-carbon compounds like formate, formaldehyde, methanol, and methylamine as their sole source of carbon and energy [37]. The genus Methyl bacterium consists of a variety of PPFM and non pigmented facultative methylotrophs (NPFM) that can grow on a variety of multicarbon growth substrates, including C_2, C_3, and C_4 compounds, as well as on C_1 compounds like formate, formaldehyde, methanol, and methylamine. PPFM was isolated, purified, and characterised from *Coleus forskohlii* plants' phyllospheres.
   - To research the range of functions these PPFM isolates possess.
   - To examine how the effective PPFM isolates affect the development and tuber production of *Coleus forskohlii*plants. (Studies on pot cultures).

2. **PPFMs:** The genus Methyl bacterium belongs to α-2 subclass of *Proteobacteria* with validly published 26 species [38], namely as few follows.
   - *M. aquaticum*[39]
   - *M. dichloromethanicum*
   - *M. chloromethanicum*
   - *M. extorquens*[40]
   - *M. fujisawaense*[41]

   The methylotroph species are found in both natural and man-made environment, such as air, soil,dust, fresh water,water sources, sea water, contaminated soil, washrooms, air conditioning systems, and construction [42]. There are a number of methylotrophic bacterial species that colonise the roots, leaf surfaces, developing buds of both terrestrial and aquatic plants [43].

   - **Morphological Characteristics:** The rod-shaped Methylobacterium strains all appear singly or irregularly in rosettes and range in size from 0.8 - 1.0 mm - 8.0 µm [44]. Particularly in older stationary phase cultures, they are often branching or pleomorphic. They have morphologies known as polar growth or budding. Although certain strains are not actively motile, all strains are motile via a single polar, subpolar, or lateral flagellum [45]. Large sudanophilic inclusions (Poly hydroxybutyrate) and volutin granules are frequently seen in cells. Despite the fact that many strains stain as gram variable, they are gram negative [46]. While colonies on methanol salts agar are consistently light pink, those on glycerol peptone agar range in size from <1 to 3 mm and range in colour from pale pink to bright orange-red. The insoluble pigment is probably a carotene. Strains develop as a pink surface ring or pellicle in static liquid environments [47].

- **Biochemical Characteristics:** All isolates were found to be aerobes that produced catalase and oxidase, according to a research. All Methylobacterium strains were catalase and oxidase positive, according to Green's biochemical investigation from 1992 [48]. They can survive on a range of C_1 chemicals as facultative methylotrophs and chemoorganotrophs. The majority of the strains do not hydrolyze or digest DNA, lecithin, cellulose, gelatin, or starch. These strains are all lipolytically inactive. There is no production of the enzymes -galactosidase, Lornithine decarboxylase, L-lysine decarboxylase, or L-arginine dihydrolase. Even if certain bacteria convert nitrate to nitrite, the methyl red and Voges-Proskauer tests come out negative. The urease test and indole synthesis of methylotrophs are both positive, according to Thangamani, (2005) [49].

- **Carbon Utilization Tests:** They can grow on C_1 compounds as their only source of carbon and energy. They also can also grow on a variety of multicarbon substrates, making them facultatively methylotrophic, according to a study that classified the various *Methylobacterium* species based on the patterns of compounds they used as a source of carbon and energy. Over 95% of *Methylobacterium* strains used a variety of compounds, including trimethylamine, methylamine, acetate, L-glutamate,citrate, D-glucose, D-xylose, betaine and fructose. According to the carbon source utilisation patterns of the 12 species of *Methylobacterium* that are now known, the majority of the strains primarily utilised malonate, glycerol,succinate, fumarate, and α-ketoglutarate as carbon and energy sources [50]. None of the strains progressed to use any of the investigated sugar alcohols or disaccharides.

- **Growth Characteristics:** On nutrient agar, most *Methylobacterium* strains only grow poorly with a few exceptions. Colonies on GP agar are 1 to 3 mm in diameter and range in colour from pale pink to brilliant orange red after seven days of incubation at 30°C. In contrast, colonies on methanol mineral salts (MMS) agar are a more consistent light pink. The pigment is not luminous or diffusible and is most likely a carotenoid complex [47]. All *Methylobacterium* strains survive best at temperatures between 250 and 300 °C. Some strains can grow at 510°C or less, while others can develop at 370°C or above. Although certain strains can thrive at pH of 4.0 to 10.0, neutrality is the region where growth is most favourable. *Methylobacterium* strains are known to grow and thrive without external growth agents [48].

3. **Positive Effects on Plants:** The positive effects on plants include accelerating vegetative growth, accelerating seed germination, and hastening flowering, fruit set, and maturation. It also improves fruit quality, colour, and seed weight, increases yield by 10%, and mitigates drought.

4. Application Techniques

- Seed treatment: ingest seeds in 1.0% volume for 5–10 minutes, depending on the seed.
- Foliar spray of 1% PPFM
- Recommended for all crops
- Should applied in the morning or evening at crucial stages of crop growth or every 30 days.
- Caution: Do not combine with pesticides or fungicides.

Table 1: Different types of crops associated Methylotrophs with activities

S.No	Plants	Associated Methylotrophs	Source	Activity
1	Rice	*M.extorquens, M.fujisawaense*	Phyllosphere	IAA
2	Wheat	*Methylobacteriumsp*	Phyllosphere	Cytokinin Production
3	Rice	*Methylobacteriumsp*	Rhizosphere	N_2 Fixation
4	Red Pepper	*M.suomiense*	Rhizosphere	Root colonise
5	Tomato	*M.suomiense*	Rhizosphere	Root colonise
6	Sugarcane	*Methylobacteriumsp*	Rhizosphere	IAA,PGP activities
7	Soyabean	PPFM	Phyllosphere	IAA
8	Groundnut	PPFM	Phyllosphere	IAA
9	Mung bean	*M.organophilum*	Mud	Bio-fertilizer
10	Green gram	PPFM	Phyllosphere	Bio-fertilizer

V. SUSTAINABLE AGRICULTURE AND PPFMs

Sustainable agriculture, which aims to balance the needs of current and future generations for food security and nutrition with the sustainable management of natural resources, can benefit significantly from the presence and activities of PPFM. PPFM can enhance nutrient availability in the soil by solubilizing nutrients like phosphorus and iron. This can reduce the need for synthetic fertilizers, which are a significant expense in conventional agriculture and can lead to nutrient runoff and water pollution [3].PPFM's ability to make nutrients more accessible to plants can lead to improved nutrient uptake and use efficiency. The nutrients applied to the soil are more effectively utilized by crops, reducing waste and potential environmental harm [51].Some PPFM strains produce plant growth-promoting hormones and protect plants from diseases. This can result in healthier and more resilient crops, reducing the need for chemical pesticides and promoting long-term sustainability [52].PPFM can help plants regulate water use by influencing stomatal conductance. This can be particularly valuable in water-scarce regions where efficient water use is critical for sustainable agriculture. PPFM's ability to stimulate root development and enhance stress resistance in plants can contribute to crop resilience in the face of drought, a growing concern due to climate change [51].PPFM contribute to the microbial diversity and activity in the rhizosphere, which can improve soil structure and nutrient cycling. Healthy soils are a cornerstone of sustainable agriculture. Sustainable agriculture often seeks to maintain or enhance biodiversity. PPFM, by promoting healthier plant growth and reducing the need for chemical inputs, can contribute to the preservation of natural ecosystems and the species that inhabit them. Sustainable agriculture practices that incorporate PPFM can lead to economic benefits for farmers. Reduced input costs, improved yields, and access to eco-friendly markets can all contribute to the financial sustainability of farming operations [52].

VI. ECOLOGICAL IMPORTANCE OF PPFMs

PPFMs important ecological role in a variety of habitats due to their distinct metabolic capacities and interactions with the environment. Methane is a strong greenhouse gas, and PPFM are engaged in the cycling of this gas. By oxidising methane to carbon dioxide, which has less potency as a greenhouse gas, they play a significant part in reducing methane emissions. This procedure contributes to the Earth's carbon cycle and reduces the effect of methane on global warming by regulating the amount of methane in the atmosphere [53].Many PPFM strains have the ability to biodegrade aromatic and methylated contaminants. For bioremediation activities aiming at cleaning up polluted settings, this biodegradation capacity is crucial. Pollutants can be transformed by PPFM into less hazardous or non-toxic forms, assisting in the recovery of harmed ecosystems [54].Some PPFM strains have antagonistic action towards plant pathogens, which promotes plant health and the control of disease. As a result, less demand for chemical pesticides in agriculture may arise, and more ecologically friendly pest control techniques may be encouraged [55]. PPFMs have a variety of ecological activities, including nutrient cycling, bioremediation, and the control of greenhouse gases. They significantly influence the health, sustainability, and resilience of ecosystems by their interactions with the surrounding environment, plants, and other microbes. Understanding their ecological significance can help develop solutions for sustainable agriculture, bioremediation, and environmental preservation.

VII. FUTURE DIRECTIONS

Future research may focus on manipulating the metabolic pathways of PPFM to enhance their efficiency in utilizing methylated compounds. This could lead to the development of more efficient bioremediation and bioconversion technologies. Synthetic biology techniques could be applied to engineer PPFM strains with specific functions, such as the production of high-value chemicals or biofuels from methanol or other methylated substrates. Ongoing genomic studies of PPFM can provide insights into their metabolic capabilities and ecological roles. Comparative genomics can help identify key genes and pathways for potential biotechnological applications. Research on PPFM's role in carbon and nutrient cycling within ecosystems may expand. Understanding their contributions to these processes can have implications for ecosystem management and climate change mitigation. PPFM could be further utilized as bioindicators in environmental monitoring. Monitoring changes in their populations can provide valuable information about environmental health and pollution levels.Continued research may lead to the development of novel biodegradable materials and bioplastics using PPFM as microbial factories for the production of biopolymers. PPFM-based bioenergy technologies, such as microbial fuel cells and biogas production, could see increased research and development as sustainable energy sources gain importance. PPFM are part of various microbiomes, and future research may explore their interactions with other microorganisms in complex environments, shedding light on microbial community dynamics. PPFM may find applications in the biotechnological production of enzymes, specialty chemicals, and pharmaceuticals due to their unique metabolic capabilities. PPFM's ability to metabolize methane makes them relevant in astrobiology. Future space exploration missions might investigate the potential for these bacteria to thrive in extraterrestrial environments.

VIII. CONCLUSION

To enhance sustainable agriculture, both rhizospheric and non-rhizospheric to plant development, methylotrophs can used eco-friendly and economical. Methylotrophs are frequently used as bioinoculants and in microbial sprays on crops, and their usage as natural fertilisers is increasing. Methylotrophs exploit a variety of ways to stimulate plant growth, making them appropriate and attractive competitors for usage in sustainable agriculture. They control biogeochemical cycling in soil ecosystems and improve the land's suitability for crop production. Additionally, methylotrophs are a possible alternative to artificial fertilisers for crops due to their capacity to produce phytohormones, promote plant development, nodulate, fix nitrogen, and acquire nutrients. In conclusion, methylotrophic bacteria provide a biological control option promoting balanced carbon cycling, plant growth enhancement through nitrogen fixation, phosphate solubilization, phytohormone synthesis, and ACC deaminase production in the rhizosphere. These helpful methylotrophs have an enormous potential for contributing to sustainable agriculture.

In conclusion, phyllosphere bacteria have a multifaceted role in improving cotton growth and yield under drought conditions. Their ability to enhance water use efficiency, nutrient availability, hormonal balance, and disease resistance makes them valuable allies for cotton farmers facing water scarcity challenges. Further research into specific bacterial strains, their mechanisms of action, and their compatibility with different cotton varieties will contribute to more efficient and sustainable cotton cultivation in regions prone to drought stress.

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