Review Article

Methylotrophic Bacteria and Their Role in Sustainable Agriculture System

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Abstract

Sustainable agriculture relies greatly on renewable resources like biologically fixed nitrogen (N). Biological nitrogen fixation (BNF) plays an important role in maintaining soil fertility. Yet beneficial legume effects in terms of increased yields in succeeding cereal crops have been reported. Such benefits are partly due to N contribution from legumes through BNF and soil N saving effect. In this paper we have reviewed the research on the contribution of methylotrophic bacteria in plant growth promotion of grain legumes. Plant growth-promoting methylotrophic bacteria are the rhizosphereic and phyllospheric bacteria that can enhance plant growth by a wide variety of mechanisms like phosphate solubilization, siderophore production, biological nitrogen fixation, production of 1-Aminocyclopropane-1-carboxylate deaminase (ACC), promoting beneficial plant-microbe symbioses, interference with pathogen toxin production etc. The potentiality of plant growth promoting methylobacteria in agriculture is steadily increased as it offers an attractive way to replace the use of chemical fertilizers, pesticides and other supplements. Growth promoting substances are likely to be produced in large quantities by these microorganisms that influence indirectly on the overall morphology of the plants. Recent progress in our understanding on the diversity of plant growth promoting methylotrophic bacteria in the rhizosphere and phyllosphere along with their colonization ability and mechanism of action should facilitate their application as a reliable component in the management of sustainable agricultural system.

Keywords: 1-Aminocyclopropane-1-carboxylate deaminase (ACC), Biological nitrogen fixation Methylotrophic bacteria, Phosphate solubilization, Siderophore production, Sustainable agricultural

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Introduction

In India, agriculture is facing various challenges which together disclose into sustainability issues. Sustainable agriculture is the dynamic production of safe, high-quality agricultural products in a way that protects and improves the natural environment, the social and economic conditions of the farmers and safeguards the health and welfare of all farmed species. It relies greatly on renewable resources and on-farm nitrogen contributions are largely achieved through biological nitrogen fixation (BNF). Biological nitrogen fixation helps in maintaining or improving soil fertility by using N_2 which is in abundance in the atmosphere. In this regards legumes have been an important component of agriculture since ancient times. They perform a crucial role in agriculture, where their ability to fix N in symbiosis makes them excellent colonizers of low N environments. They are also known as a green crop, or cover crop, provide many benefits to the soil. They improve soil quality by increasing soil organic matter, enhancing soil porosity and structure, recycling nutrients, lowering soil pH, increasing microorganisms and mitigating disease problems. Planting legumes as a cover crop also help to reduce soil erosion. Though the legume plants have been studied as single organisms but in nature, they associate with various microorganisms. The interactions between different symbiotic organisms and the host plant likely to have a great impact on the plant growth and development. Plant growth requires the synergistic activity of different life forms in a highly heterogeneous complex environment. Plant-associated microorganisms fulfill important functions for plant growth and health. Direct plant growth promotion by microbes is based on improved nutrient acquisition and hormonal stimulation. Synergistic activities of many different life forms in a heterogeneous environment affect the plant growth. Thus a field of growing plants is a group of microbial activities, containing soil microbes, atmospheric microbes, plant surface microbes, and internal plant-colonizing microbes or methylotrophs.

Methylotrophs are ubiquitous in nature and found in a variety of environments including air, water, soil, plant [1]. They are an important group of bacteria that take up greenhouse gases and therefore minimize global warming, in addition to actively participating in carbon cycleing in soil. The many functional attributes of methylotrophs in sustainable agriculture are shown in **Figure 1**. Plants provide methylotrophs with habitats as well as compounds including growth substrates. Plant and methylotrophic bacterial interactions that improve plant growth and plant fitness are becoming a topic of very important considerable interest. Among methylotrophic microorganisms, methylotrophic bacteria occupy the surface of leaves and stems as well as internal tissues such as root nodules of plants without causing the disease to their hosts. The exact population and the composition of the methylotrophic bacteria depend upon biotic and abiotic factors.

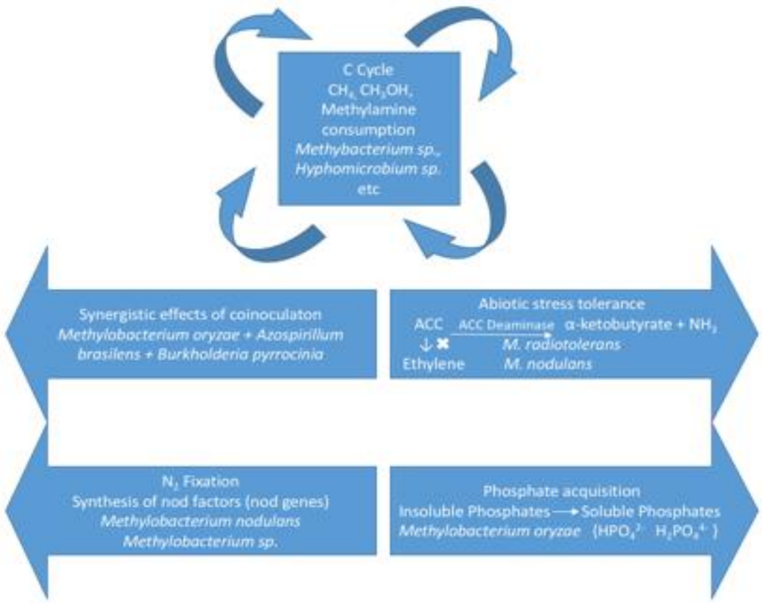

Figure 1 Various functions of methylotrophs in sustainable agriculture

History of Methylotrophs

Earlier there was a single methylotroph called *Bacillus methanicus*, sole representative in the history of the microorganisms capable of growth on methanol or methane as a sole source of carbon and energy [2]. In the next half century, some additions were made to the list of known bacteria with these growth characteristics. [3] were able to quote only seven references to the bacteria growing on methanol in their first paper on *Pseudomonas*. In the next twenty years, various new bacteria were reported and their widespread distribution, taxonomic diversity and physiological diversity was described [4], [5]. A measure of the growth of interest in this type of microorganisms and their metabolism is shown by the holding of International Symposia on Microbial Growth on C1 compounds; the first informal symposium was in Edinburgh in 1973 and since then more formal symposia have been held in Tokyo, Putschino on Oka and in San Diego, U.S.A.

Definition and types of methylotrophs

Colby, J. and Zatman, L.J. (1972) [6] provided a generally accepted definition of methylotrophs *i.e.* those microorganisms which are able to grow at the expense of reduced carbon compounds containing one or more carbon atoms but do not contain carbon-carbon bonds. Methylotrophs are mainly of 2 types: (1) Obligate methylotrophs, include microorganisms able to grow only on C1 substrates and (2) Facultative methylotrophs, include microorganisms able to grow on C1 substrates as well as a variety of multicarbon compounds like, dimethylamine, dimethyl ether, tetramethylammonium, trimethylamine, trimethyl sulphonium, trimethylamine N-oxide and other organic carbon compounds like acetate, lactate, hydroxybutyrate, pyruvate and carboxylic acids [7]. The facultative methylotrophs are further divided into many types: (1) The pink facultative methylotrophs (*e.g. Methylobacterium extorquens*), (2) The non-pigmented "pseudomonads" (*e.g. Pseudomonas aminovorans*), (3) Gram-positive facultative methylotrophs (*e.g. Bacillus cereus*), (4) Gram-negative (or variable), non-motile rods and coccal rods (*e.g. Arthrobacter rufescens*), (5) Facultative autotrophs or phototrophs growing on methanol or formic acid (*e.g*; *Rhodopseudomonas palustris*), (6) The Hyphomicrobia (*e.g. Hyphomicrobium sp*.) and (7) Marine bacteria able to grow on methanol and methylated amines with the help of enzyme, methanol dehydrogenase and few more unique enzymes (*e.g. Methylomonas thalassics*) [8].

Isolation of methylotrophs

Holland, M.A. (1997) [9] isolated methylotrophic bacteria by leaf print method in which fully expanded leaves were collected and samples were handled aseptically then firmly pressed on to the surface of ammonium mineral salt (AMS) medium [10]. [11] isolated the methylotrophic bacteria from root nodules of *Crotalaria juncea* and *Sesbania aculeate* by surface sterilization with 0.1% mercuric chloride and 70% alcohol followed by serial washing in sterilized distilled water and finally by plating the suspension of crushed nodules on AMS medium. [12] isolated *Bacillus methylotrophicus* strain from rhizoplane of rice (*Oryza sativa* L. cv O-dae) from samples collected by the Chungbuk Provincial Agricultural Research and Extension Services (Cheongwon, Republic of Korea). The strain was isolated on selective ammonium mineral salts (AMS) medium supplemented with filter-sterilized cycloheximide (10 μg/ml) and methanol 0.5 % (v/v) at 28 °C for 5 days.

Distribution of methylotrophs

Methanol oxidizers derived from soil are primarily facultative methylotrophs. About 31% of the soil species are restricted to methanol and or other C1 compounds. Bacteria that come under the genus *Methylobacterium* are facultative methylotrophs which are able to grow on methanol and methylamine as well as C2, C3, and C4 compounds. They belong to the alpha-*Proteobacteria* and sometimes they are referred to as pink-pigmented facultative methylotrophs (PPMF). *Methylobacterium* strains mostly found in association with plants, specifically with leaf surfaces, have been hypothesized to potentially dominate the phyllosphere bacterial community [13]. PPFMs are not only restricted to the phylloplane but also associated with other parts of the plant, particularly at the actively growing portions. [14] harvested *Methylobacterium* from the roots and the aerial portions of the plants. [15] reported a decrease in the population of *Methylobacterium* on red clover in the field from the spring towards summer but then increase again towards the end of the cropping season. [16] described that in the case of soybean up to 42.8% of the microbial community in phyllosphere are alpha-proteobacteria, including *Methylobacterium* species as one of the main components. [17] reported that plant species and, more specifically, location influence the *Methylobacterium* community composition in the phyllosphere. [18] suggested that phyllosphere-colonizing methylobacteria, which have undergone a coevolution together with plants have evolved into generalists rather than specialists in their ability to colonize plants.

Biodiversity of methylotrophs

Methylotrophic bacteria in a plant host are not restricted to a single species but they comprise of several genera and species. Methylotrophic bacteria have been isolated from a large diversity of plants. It has been shown that leaf surfaces of most of the temperate plant species are occupied by active methylotrophs, which constitute about 14–20% of the total microbial community of the phyllosphere [16, 19-22]. However, the upper soil layers of grasslands and forests are also well supplied with oxygen, and belowground parts of plants are possible sources of methanol. Utilization of methanol needs different metabolic pathways that differentiate methylotrophs from other aerobic heterotrophs. Methylotrophs oxidize methanol sequentially to carbon dioxide.

Strains of *Methylobacterium* are ubiquitous and have been reported from various plants. They are able to influence plant growth promotion with the help of plant growth regulators [15, 23, 24] and induced systemic resistance against diseases [25]. [26] isolated *Methylobacterium* from various tissues of different crops and first time reported the symbiotic association of *Methylobacterium nodulans* with plants. [27] isolated pink-pigmented facultatively methylotrophic bacterium BJ001T from internal poplar tissues (*Populus deltoidesnigra* DN34) and they found that the strain BJ001T was related to *M. thiocyanatum*, *M. extorquens*, *M. zatmanii* and *M. rhodesianum*.

Schauer, S. and Kutschera, U. (2008) [28] isolated methylobacteria from three different habitats: yellow ligulate florets, phytosynthetically active green leaves and side-roots and identified *as M. mesophilicum*, *M. extorquens* and *M. radiotolerans,* respectively. In all the three habitats the species *M. mesophilicum* was found in abundance of 18-32%. Similarly, *M. radiotolerans* was also present in all three habitats but at much lower percentages (3-10%). On the other hand the species *M. extroquens* dominated the bacterial microflora of rhizoplane (36%) and 15% of the ligulate florets microbial population. [7] isolated pink-pigmented facultative methylotrophs from the phyllosphere of cotton, maize and sunflower plants raised in the experimental plots of Tamil Nadu Agricultural University, Coimbatore, India.

Podolich, O., et al (2009) [29] isolated methylotrophic bacteria from potato tissue and classified as *Methylobacterium sp.* by a phylogenetic analysis having the closest homology with the type strain of *M. radiotolerans.* [30] isolated four methylotrophic strains from the sediments of Lonar Lake using minimal salt medium containing all required salts and trace elements with 2% methanol as the single source of carbon and energy. Four methylotrophic strains were identified as *Acinetobacter baumani*, *Achromobactrum xylosoxidans*, *Ochromobactrum tritici* and *Pseudomonas aeruginosa*. [31] isolated a facultative methylotrophic bacterium JT1T from thallus of the liverwort *Marchantia polymorpha* L and comparative 16S rRNA gene sequence analysis placed the strain in a clade with *M. adhaesivum* AR27T, *M. fujisawaense* DSM 5686T, *M. radiotolerans* JCM 2831T and *M. jeotgali* S2R03-9T.

A Pink Pigmented Facultative Methylotrophic (PPFM) bacterium was isolated from the rhizosphere region of Western Ghats by serial dilution method on minimal salts agar medium supplemented with 2% methanol. The isolate was morphologically and biochemically characterized and identified as a member of the genus *Methylobacterium* [32]. [33] collected a total of twelve samples comprising of sediment, matt and water samples from Lonar lake and isolated methylotrophic bacteria from the collected samples. The isolated bacterium was identified by 16S rRNA sequencing from NCCS, Pune and identified as *Ochrobactrum oryzae*. [34] isolated thirty PPFMs from cotton, datura, snap bean, castor oil plant and peanut plants from three different locations of Egypt. Identification results showed that the selected PPFM isolates were mostly related to the genus *Methylobacterium.*

Plant growth promotion by methylotrophs

Methylotrophs are able to help in plant growth and development using a wide range of mechanisms, including synthesizing compounds to promote plant growth or increasing the uptake of nutrients and working as biocontrol agents by overpowering plant pathogens. In addition, plant growth-promoting methylotrophic bacteria could help in the remediation process since these microorganisms can both degrade and mineralise organic xenobiotic compounds, enabling them to work directly as contaminant degraders or in combination with plants. They enhance seed germination and root biomass accumulation through the biosynthesis of phytohormones. Usually, the plant growth promoting methylotrophic bacteria improve plant growth directly by either facilitating nutrient acquisition (nitrogen and phosphorus), phytostimulation, or indirectly in the forms of biocontrol agents.

Nutrient acquisition for plant growth and promotion

Methylotrophic bacteria with plant growth promoting characteristics could improve the availability of certain nutrients for plant uptake. Nitrogen and phosphorus are the two most important nutrients which cannot be utilized by plants directly in an adequate amount. The methylotrophic bacteria have the capability to fix the atmospheric nitrogen and make it available for the plant. On the other hand, they can solubilize and convert the insoluble phosphorus present in the soil into the soluble plant utilizable form.

Nitrogen Fixation: Nitrogen (N) is the most important nutrient for plant growth and productivity. Although there is about 78% N_2 in the atmosphere, it is unavailable to the growing plants. In the case of soil, nitrogen exists in three general forms: organic nitrogen compounds, ammonium (NH_4^+) ions, and nitrate (NO_3^-) ions. This nitrogen is not directly available to plants, but some can be converted into plant-utilizable forms by biological N_2 fixation (BNF) performed by nitrogen-fixing microorganisms using a complex enzyme system known as nitrogenase [35].

Sy, A., ei al (2001) [36] isolated *Methylobacterium nodulans* showing nitrogenase activity and nodulation ability from *Crotalaria* species and described it under the fourth rhizobial branch in α-Proteobacteria. The nodA gene, encoding a key enzyme in Nod factor biosynthesis present in all legume-nodule-forming bacteria, induces legume nodulation [37], [38]. [26] reported that *M. nodulans* ORS2060 isolated from *Crotalaria sp.* contained the nif H gene (involved in nitrogen fixation) to induce nitrogen-fixing nodules on the roots of legumes by the nodA gene. Subsequently, [39] characterized pigmented methylotrophic nitrogen fixing isolates from the root nodules of a legume *Lotononsis bainesii* (Papilionoideae, Crotalarieae) with its closest phylogenetic neighbor as *M. nodulans* showing a sequence homology to 98%. [11] isolated and revealed that two out of twenty-three root nodule isolates from tropical legumes represented two distinct strains of *Methylobacterium*, with *M. nodulans* as their closest neighbor. This investigation proved and extended the capability of *Methylobacterium* to effectively nodulate leguminous plants thus proving the polyphyletic origin of rhizobia within α-Proteobacteria.

Phosphate Solubilization: Phosphorus (P), the another major plant growth-limiting nutrient after nitrogen, is sufficiently present in soils in both organic and inorganic forms [40]. The amount of available forms of P to plants is generally lower. Because the majority of soil P is found in insoluble forms, while the plants absorb it only in soluble forms [41]. Microorganisms are an essential part of the soil phosphorus (P) cycle and are necessary for the transfer of P among different pools of soil P [42].

There is a considerable population of phosphate solubilizing bacteria in soil and in plant rhizospheres which have the ability to bring sparingly insoluble organic or inorganic phosphate into soluble forms by secreting organic acids

[43]. Among those phosphate solubilizing bacteria, *Methylobacterium spp*. Have the capability to dissolve inorganic phosphates, which may be further connected to phosphate metabolism in both microorganisms and plants [44]. Nonspecific acid phosphatase, phytase and C-P lyase (or phosphonatase) are three different type of microbial enzymes that solubilize organic phosphate. All three of these release phosphate, the first from phosphoric ester or phosphoric anhydride, the second from phytic acid, and the third from organophosphates. [45] reported that *M. oryzae* have genes encoding all three phosphatase enzymes.

Jayashree, S., et al (2011) [46] isolated thirteen PPMFs and reported P-solubilization index of the PPFMs grown for 7 days ranging from 1.1 to 2.7. [47] reported four different strains of facultative methylotrophs that solubilised tricalcium phosphate in pikovskaya's agar medium and solubilization index ranged from 1.28 to 1.85.

Phytostimulation

The phenomenon of phytostimulation profits especially from manipulating the complex and a balanced network of plant hormones or hormone-like compounds that directly regulate growth or stimulate root formation. Methylotrophic bacteria able to alter plant physiology directly often synthesize and exudate plant hormones. The most profoundly studied examples of phytostimulants involve ACC deaminase (1-aminocyclopropane-1-carboxylate) which helps in lowering plant hormone ethylene levels and indole acetic acid (IAA) production.

ACC utilization by methylotrophic bacteria: Ethylene is a vital metabolite for the normal growth of plants [48]. This plant growth hormone is important in inducing various physiological changes in plants and produced endogenously by almost all plants. Apart from being a plant growth regulator, ethylene has also been discovered as a stress hormone [49]. Under stress conditions, the endogenous level of ethylene is significantly increased which negatively alters the overall plant growth. The high concentration of ethylene may lead to reduced performance of the crop [49], [41]. Plant growth promoting methylotrophic bacteria which possess the enzyme, 1-aminocyclopropane-1- carboxylate (ACC) deaminase, promote plant growth and development by decreasing the levels of ethylene in plants (Glick *et al*., 1998). Such methylotrophic bacteria take up the ethylene precursor ACC and convert it into α-ketobutyrate and ammonia [50].

Glick, B.R. (2004) [51] reported that the ACC deaminase containing bacteria may be found on roots, leaves, flowers and seeds. [52] isolated *M. oryzae* from stem tissues of rice producing ACC deaminase. Their inoculation to canola plant regulated the ACC and thereby the ethylene level [53]. [54] revealed that methylobacteria present in the phyllosphere of rice produce the enzyme ACC deaminase and thereby control the ethylene level in the rice plant. In earlier investigations, *Methylobacterium* strains were found to have ACC deaminase activity and tested for their potential in plant growth promoting traits in various crops [55], [56], [57] and [58].

Production of indole acetic acid: It is strongly proved that many of the soil and plant associated bacterial groups are able to synthesize the phytohormone indole-3-acetic acid (IAA) [59]. The methylotrophic bacteria are soil and plant-associated bacteria that are distributed ubiquitously on plant surfaces [60]. [23] showed that many aerobic methylotrophs synthesize IAA in the range of 5 to 120 μg ml^{-1} into the medium. The increase in the plant growth, biomass, and yield of groundnut were observed by [61] due to the production of both auxins and cytokinins. Further, he also mentioned that balanced growth between root and shoot of groundnut was due to the action of both auxin and cytokinin respectively.

Long, R.L. (2000) [62] suggested that growth of seedling was promoted by methylotrophic bacteria not only by synthesizing cytokinins but also by other phytohormones like auxins. [15] reported that three out of sixteen isolated pink-pigmented facultative methylotrophic bacteria (PPFMs) have shown the presence of indole-3-acetic acid (IAA) in the culture filtrate during a colorimetric assay. [63] have shown that *M. mesophilicum* strain, maintained in the laboratory since 1973, produced only about 1/3 the amount of auxin compared with two unidentified, plant-associated "wild-type" isolates of the genus *Methylobacterium*. [64] reported production of IAA by methylotrophs ranging from 22.47 μg/ml to 29.97 μg/ml of culture filtrate. [65] observed variability among PPFM isolates in producing IAA (0.14 to 25.12 μg/ml). [66] isolated eleven methylotrophic isolates and all the isolates produced IAA, in the range of 3.48μg ml^{-1} to 8.77μg mL^{-1}.

Biocontrol

Biological control is the suppression of phytopathogens by one or more beneficial organisms, which may lead to the plant growth promotion either in direct or indirect manner. Several mechanisms may be involved in this process, including the production of siderophores.

Siderophore production: Iron is extensively required by all living cells. In aerobic environments, it is found as highly insoluble ferric hydroxide complexes. To acquire iron, microorganisms synthesize and secrete siderophores. Siderophores are defined as relatively low molecular weight extracellular compounds with a high specific chelating affinity for ferric iron. They sequester ferric iron in a form that cannot be utilized by the pathogen [67]. Ability to produce siderophores by an organism to chelate iron under iron limiting conditions can promote plant growth. They directly provide iron for plant utilization and remove iron from the environment which is required for the growth of phytopathogens thereby reducing their competitiveness and enhance the plant growth and promotion.

Siderophores can be grouped broadly into three classes depending upon the group that donates oxygen ligands for Fe^{3+} coordination. Maximum of the bacterial siderophores are catecholates (*i.e*. enterobactin), and some are carboxylates (*i.e*. rhizobactin) and hydroxamates (*i.e*. ferrioxamine B) [68]. However, there are also several types of bacterial siderophores that contain a mix of the main functional groups (*i.e*. pyoverdine) [69]. Siderophore production was evaluated by [70] in 37 *Methylobacterium sp*. strains and observed that all the strains which were tested could produce hydroxamate-like siderophores, but not catechol-like siderophores, suggesting that these strains were able to bind specific metals. [71] described the iron uptake genes iucA and iucC in 35 strains of *Methylobacterium* genus, including *M. extorquens* strains AM1, PA1, DM4, and CM4 and *M. populi*.

Tani, A., (2012 b) [72] analyzed 190 unique strains of *Methylobacterium* species collected from leaf samples of many host plants using WC-MS (whole-cell matrix-assisted laser desorption ionization-time of flight mass spectrometry), for siderophore production and found that 35 *Methylobacterium* isolates were positive for siderophore production.

HCN production: A beneficial microbial community plays an important role in growth and development of the plant host [73] and includes plant growth promoting (PGP) methylotrophic bacteria. These bacteria regulate plant developmental processes, increase nutrient availability, inhibit the growth of phytopathogens, and fix nitrogen. Cyanide is a potential inhibitor of enzymes involved in major plant metabolic processes like respiration, carbohydrate metabolism and may also block photosynthetic electron transport [74]. Microbial cyanide biosynthesis has been demonstrated in many species of bacteria. [75] reported the production of HCN as a most common trait of *Pseudomonas.* Various investigations attribute a disease protective effect to HCN, *e.g.* in the suppression of black rot and root-knot in tobacco and tomato root caused by the nematodes *Thielaviopsis basicota* and *Meloidogyne javanica,* respectively [76]. [77] revealed that HCN can control subterranean termite *Odontotermes obesus,* which is an important pest in forestry and agricultural crops in India.

Methylotrophic bacteria in field application

The bioinoculants used as alternatives to fertilizer usually target only the rhizosphere, and less attention has been paid to the phyllosphere. Methylotrophic bacteria are commonly found in soils, as well as on the surfaces of leaves, seeds and in the rhizosphere of a wide variety of plants, with highest numbers, present on actively growing and meristematic tissue, sometimes averaging 10^4-10^6 colony forming units (cfu) per leaflet [78]. They have been reported to influence seed germination and seedling growth by producing plant growth regulators like zeatin and related cytokinins and auxins [9], [23], [15] and to alter agronomic traits like branching, seedling vigor, rooting and heat/cold tolerance [9], [79]. Methylotrophs are known to play an important role in increasing crop yield and land fertility. Their phosphate acquisition and nitrogen fixation abilities make them promising candidates as biofertilizers. They are very effective when applied with a small amount of chemical fertilizer to field. The application of PPFM and *Pseudomonas* foliar spray along with a biofertilizer enhanced the microbial population in soil, making nutrient more available to plants [80]. Methylotrophs indirectly reduce or prevent the deleterious effects of pathogenic microorganisms, through induced systemic resistance [25], and their inoculation was found to increase the photosynthetic activity by enhancing the number of stomata, chlorophyll concentration and malic acid content of crops [81]. Hence there is a possibility of increasing the effectiveness of the conventional bioinoculants by coinoculating with methylotrophic bacteria.

Many studies proved the positive effect of methylotrophic bacteria on the growth and yield of plants. [9] reported an increase in the productivity of a plant by spraying pink pigmented facultative methylotrophic (PPFM) bacteria on it. Also, the PPFM could promote red pepper plant growth and yield [82]. Inoculation with PPFM had significant positive effects on growth and yield of several plant species, *i.e*. soybean [83] and peanut [84]. [85] found that PPFM improved plant growth and development of mungbean. The PPFM enhanced seed germination, seedling length, and vigor of wheat [86]. [87] found that PPFM sample inoculation increased seedling vigor, dry matter production and yield of cotton. In this respect, [88] reported that the inoculation of PPFM along with chemical fertilizers resulted in significant increase in growth parameters of cotton. [89] concluded that application of PPFM increased the productivity as well as quality parameters of baby corn. [24] revealed that inoculation of PPFM resulted in significant increase in sugar cane plant growth, cane yield, and sugar quality and these effects might be mediated by the production or synthesis of plant hormones.

Madhaiyan, M., et al (2010a) [90] described the positive effect of *M. oryzae* co-inoculation with the nitrogen-fixing and phosphate solubilizing bacteria on the growth and nutrient uptake of several crops that often exceeded the effect of single treatments. [64] studied the effect of selected methylotrophs on the growth and yield of *Coleus forskohlii* under pot culture conditions and found 216.10 % increase in the tuber yield due to the inoculation with PPFM against uninoculated control and 136.07 % against reference strain. [34] performed a two-year field experiment in different seasons and elucidated the contribution of PPFM community to the enhancement of antioxidants activities, growth and yield of snap bean crops. [91] studied the effect of salt-tolerant [91] studied the effect of salt-tolerant *Methylobacterium organophium* on seed germination and plant growth of mungbean and examined its use as a bioinoculant and biofertilizer in agricultural fields. The use of *Methylobacterium* biofertilizer (PPFMs) was also advocated for the protection of crops in heat and drought stree conditions [92].

Conclusion

The application of rhizospheric and non rhizospheric methylotrophs as bioinoculants is common, and their use as alternative to chemical fertilizers is also increasing. Their association with plant growth can be exploited for ecofriendly and cost effective practices to promote sustainable agriculture. They employ multiple mechanisms to promote plant growth like phytohormone production, nodulation, nitrogen fixation and nutrient acquisition. Similarly, methylotrophic bacteria offer the alternative of biocontrol, plant growth enhancement by nitrogen fixation, phytohormone production, ACC deaminase production and phosphate solubilization. These beneficial methylotrophic bacteria either as an individual inoculant or as co-inoculant with other plant beneficial microorganisms have the potential to increase the plant growth and development in sustainable agricultural systems.

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