# Bioprospects of pink pigmented facultative methylotrophs (PPFMs)

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## Abstract

**Purpose** – The purpose of this article is to provide information about interactions between pink-pigmented facultative methylotroph (PPFM) organisms and plants, their molecular mechanisms of methylotrophic metabolism, application of PPFMs in agriculture, biotechnology and bioremediation and also to explore lacuna in PPFMs research and direction for future research.

**Design/methodology/approach** – Research findings on PPFM organisms as potent plant growth promoting organisms are discussed in the light of reports published by various workers. Unexplored field of PPFM research are detected and their application as a new group of biofertilizer that also help host plants to overcome draught stress in poorly irrigated crop field is suggested.

**Findings** – PPFMs are used as plant growth promoters for improved crop yield, seed germination capacity, resistance against pathogens and tolerance against drought stress. Anti-oxidant and UV resistant properties of PPFM pigments protect the host plants from strong sunshine. PPFMs have excellent draught ameliorating capacity.

**Originality/value** – To meet the ever increasing world population, more and more barren, less irrigated land has to be utilized for agriculture and horticulture purpose and use of PPFM group of organisms due to their draught ameliorating properties in addition to their plant growth promoting characters will be extremely useful. PPFMs are also promising candidates for the production of various industrially and medicinally important enzymes and other value-added products. Wider application of this ecofriendly group of bacteria will reduce crop production cost thus improving economy of the farmers and will be a greener alternative of hazardous chemical fertilizers and fungicides.



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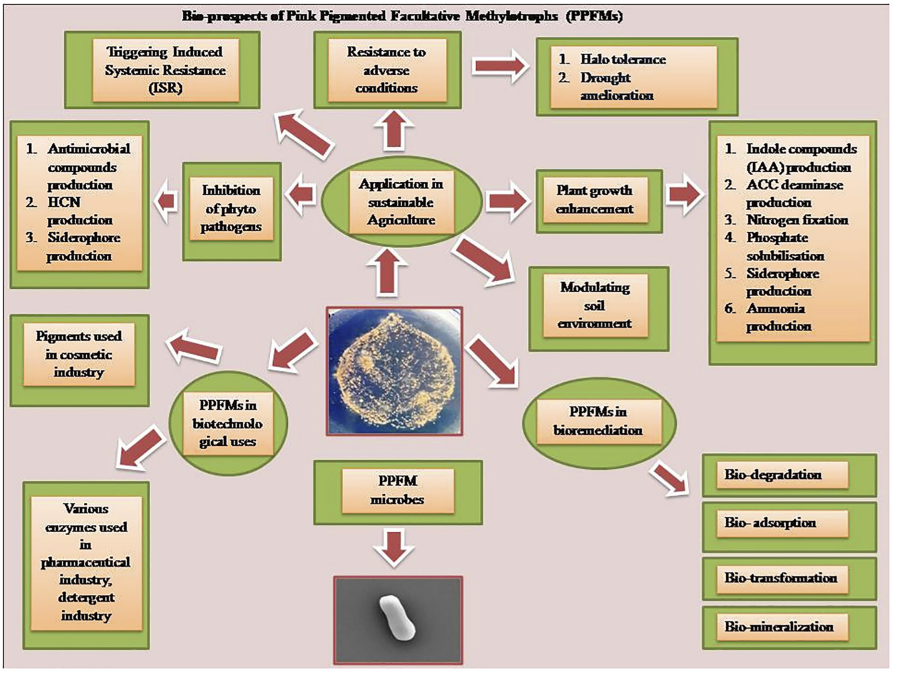


**Keywords** Draught amelioration, Methanol dehydrogenase, *mxa*F, Plant growth promotion, UV protectant
**Paper type** Literature review

## Introduction

A wide range of organisms inhabits the phyllosphere. These organisms have beneficial, harmful or neutral effects on the plant. Various physiological activities of plants are related to the interaction between such microorganisms and plants. It is hypothesized that pink pigmented facultative methylotrophs (PPFMs) of the genus *Methylobacterium* potentially dominate the phyllosphere bacterial population.

PPFMs are gram-negative, rod-shaped, aerobic, bacteria, can utilize single carbon compounds such as formate, formaldehyde and methanol as sole carbon source (Gamit, Naik, Chandarana, Chandwani, & Amaresan, 2023). They are mostly found in the phyllosphere and rhizosphere and are associated with the leaves, roots and seeds of most terrestrial plants and utilize volatile C1 compounds like methanol produced by growing plants during cell multiplication and early stages of leaf expansion (Irvine, Brigham, Suding, & Martiny, 2012). PPFMs have importance in agriculture as they promote yield and seed germination capacity, resist crops from pathogenic attack and also provide crop tolerance to manage drought stress (Nysanth, Anu Rajan, Sivapriya, & Anith, 2023). PPFMs also help in reducing global warming by taking greenhouse gases such as $CO_2$ and methane and metabolizing methanol generated by growing plant leaves (Iguchi, Yurimoto, & Sakai, 2015). They are also involved in carbon cycling (Iguchi *et al.*, 2015), phosphate acquisition (Agafonova, Kaparullina, Doronina, & Trotsenko, 2013; Jayashree *et al.*, 2011a, b), phytohormones (indole acetic acid (IAA) and cytokinins) production (Lee *et al.*, 2004), nitrogen fixation in phyllospheric and rhizospheric

regions of plants (Lee *et al.*, 2006; Sy *et al.*, 2001) and due to these reasons they are used as bioinoculants in agriculture (Kumar, Tomar, Lade, & Paul, 2016). Pigments of PPFMs have high antioxidant and UV resistant properties (Abd El-Gawad, Ibrahim, Abd El-Hafez, & Abou El-Yazied, 2015). PPFMs are potent protease (Jayashree, Annapurna, Jayakumar, Sa, & Seshadri, 2014) and cellulase producers (Jayashree *et al.*, 2011a, b). PPFMs also show effects on disease suppression by the induction of pathogenesis-related proteins (Pathogenesis related (PR)-proteins) in plants (Madhaiyan *et al.*, 2004). Ammonium mineral salt (AMS) agar media supplemented with 0.5% methanol is used to isolate PPFMs from different plant parts (Holland *et al.*, 2000). The presence of various types of pigments imparts a prominent pink color to PPFMs (Mitra, 2012). Various *Methylobacterium* spp. are isolated and reported by researchers as one of the most common PPFMs. The most interesting characteristic of PPFM organisms is their ability to oxidize methanol by using the methanol dehydrogenase enzyme (MDH), whose large subunit is encoded by *mxa*F gene which is considered as a marker gene to identify this group of bacteria (Valdivia-Anistro *et al.*, 2022). During plant host colonization, their methylotrophic metabolism is very useful as an adaptive advantage (Sy, Timmers, Knief, & Vorholt, 2005).Though many reported research work established the fact that PPFM organisms have plant growth promoting (PGP) properties and *in vitro* condition they considerably enhance crop yield; mass production of PPFMs for large scale field use is not yet done for any major corps.

## Interaction between PPFMs and plants

Interaction between PPFMs and host plants varies widely, right from rhizospheric symbiotic (Jourand *et al.*, 2004) to epiphytic (Omer, Tombolini, & Gerhardson, 2004) and endophytic (Lacava, Araújo, Marcon, Maccheroni, & Azevedo, 2004). PPFMs are isolated by using leaf impinting method in AMS agar media (Corpe, 1985) from various plants like mustard (Subhaswaraj, Jobina, Parasuraman, & Siddhardha, 2017) neem (Kumar & Lee, 2009), rice (Joel, Latha, Gopal, & Sreedevi, 2023), cotton (Ismail & Mohammed, 2023), capsicum (Santosh, Santosh, & Sreenivasa, 2019), bamboo (Madhaiyan and Poonguzhali, 2014) and also from human nasal cavity and hair scalp (Uy *et al.*, 2013).Various studies have reported that PPFMs colonize plant surfaces in a mucilaginous layer (Rossetto *et al.*, 2011). PPFMs have the ability to form biofilms (Rossetto *et al.*, 2011; Chowdhury, Basak, & Islam, 2023). *Methylobacterium* strains are capable of producing quorum sensing inducers like N-acyl homoserine lactones (AHLs) (Pomini, Cruz, Gai, Araújo, & Marsaioli, 2009). It is reported that PPFMs may also interact with other microorganisms inside the host including phytopathogens (Lacava, Li, Araújo, Azevedo, & Hartung, 2006). During plant colonization, PPFMs may regulate gene coordination by quorum sensing system to ensure efficient plant colonization. Proteomic study of *Methylobacterium extorquens* reveals that in the phyllosphere region of *Arabidopsis thaliana*, they help to over express the proteins related to the antioxidant system and decrease the over expression of Phy R regulator that helps in colonizing in the phyllospheric region.

## Methylotrophic metabolism of PPFMs

PPFMs can utilize C1 compounds like methanol, formate and formaldehyde (Santosh *et al.*, 2019) as a sole carbon source. They are also able to utilize multicarbon compounds which are with or without carbon-carbon bonds. This ability of PPFMs to utilize several carbon sources permits them to inhabit different environments, including the phyllosphere of plants (Abanda-Nkpwatt *et al.*, 2006). Plant releases methanol by stromata during plant growth, cell expansion due to pectin breakdown by pectin methylesterase (Rossetto *et al.*, 2011). Methanol concentration in the phylloplane of plants may also change according to various environmental conditions, plant age (Šmejkalová, Erb, & Fuchs, 2010) and physiological state because in mature (yellow) leaves and during abscission, methanol release increases

significantly (Sun, Copolovici, & Niinemets, 2012). Thus, PPFMs take advantages during plant colonization in presence of methanol released by plants than other plant-associated bacteria because genes related to methylotrophy (Gamit *et al.*, 2023), such as *mxa*F are expressed in presence of methanol.

Methylotrophic metabolism of PPFMs occurs in the periplasm, where the key MDH is present and it oxidizes methanol into formaldehyde. MDH is a well-studied enzyme. It has four subunits ($\alpha 2\beta 2$), two substrate-binding sites, calcium atom as a cofactor and pyrroloquinoline quinone (PQQ) as a prosthetic group (Zhang *et al.*, 2008). Formaldehyde (the main intermediate of methylotrophic metabolism) is generated during methanol oxidation. Formaldehyde is utilized in the serine cycle for cellular utilization or for the generation of energy they finally oxidize to $CO_2$. One molecule of adenoisine triphosphate (ATP) is generated by one molecule of methanol oxidation.

It was reported that *M. extorquens* AM1 contains 70 methylotrophic metabolism-related genes and these genes are located in eight regions of the bacterial chromosome (Chistoserdova, Chen, Lapidus, & Lidstrom, 2003). The first of these loci contains a cluster of 12 known genes: *mxa*FJGIRSACKLDB (Morris, Kim, Perkins, & Lidstrom, 1995), there is another gene viz., *mxa*W adjacent to *mxa*F which undergoes through divergent transcription (Xu, Viebahn, & Hanson, 1993). To sense the presence of methanol in phyllosphere five genes are thought to be required for transcription initiation of other genes related to methanol oxidation in PPFM strains and these putative regulatory genes include *mxc*QE, *mxb*DM both of which encode a putative sensor-regulator pair and *mxa*B (Springer, Auman, & Lidstrom, 1998). sensor-regulator pair MxcQE control expression of the sensor-regulator pair MxbDM and MxbDM in turn control expression of a number of genes involved in methanol oxidation (Springer, Morris, & Lidstrom, 1997). MDH has two large (66 kDa) and two small (8.5 kDa) subunits. *mxa*F and *mxa*I genes encode large and small subunits, respectively. *mxa*G gene encodes cytochrome C which is the primary electron acceptor for MDH (McDonald and Murrell, 1997). Four of the *mxa* genes, *mxa*ACKL are required for insertion of calcium into MDH (Anthony, Ghosh, & Blake, 1994; Morris *et al.*, 1995). The functions of the remaining *mxa* genes, including *mxa*W, are unknown. Periplasmic alcohol dehydrogenase has 50% sequence similarity with *mxa*F gene and is encoded by *xox*F gene. It is also reported that two copies of *xox*F gene are present in the genome of *M. extorquens*. The most interesting observation related to *xox*F gene is that when both *xox*F genes are absent, the strain loses methanol dehydrogenase activity and is unable to grow in methanol as the sole carbon source instead of the presence of *mxa*F gene in the genome. This observation strongly supports that *xox*F acts as a regulatory complex (Skovran, Palmer, Rountree, Good, & Lidstrom, 2011) and plays an important role in methanol metabolism.

## PPFMs in agriculture

In agriculture, an increasing demand and decreasing supply of irrigation water is observed in recent years due to insufficient rainfall and indiscriminate use of groundwater which results in lowering of water tables. The scarcity of irrigation water will be more severe in the upcoming days. So to fight that inevitable drought condition is a big challenge to scientists. Apart from this, chemical fertilizers which are now-a-days used on a large scale for increasing crop yield are highly detrimental to the environment. They increase production cost, decrease soil fertility, jeopardize plant-microbe interaction and make plants more prone to pathogenic attack. The performance of biofertilizers, available in the market, can't solve all these problems independently in most instances. So, scientists are desperately searching for more potent biofertilizers that are eco-friendly and have nitrogen fixation, phosphorus acquisition, phytohormones (IAA, cytokinins etc.) production and iron-chelating properties. PPFMs may be handy in solving all those problems as from the reported research work it is an established

fact that they are eco-friendly and not only help plants to ameliorate drought stress effects, promote seed germination, phosphate solubilization, iron chelation, nitrogen fixation, 1-aminocyclopropane-1-carboxylate (ACC) deaminase production and increase phytohormones production, but also protect them against various potent plant pathogenic microorganisms.

*Phytohormones production*

PPFM organisms produce phytohormones like IAA, cytokinins which help in plant growth stimulation. Cytokinin and auxin produced by *Methylobacterium* strains help plants to promote cell division and elongation, respectively (Gamit *et al.*, 2023). It is reported that *Methylobacterium extorquens* produces adenine derivatives that may act as precursors in the cytokinin biosynthesis pathway (Pirttilä, Joensuu, Pospiech, Jalonen, & Hohtola, 2004). Another study also reported that *M. oryzae* CBMB20 have two *mia*A genes (Kwak *et al.*, 2014). These genes are essential for the production of cytokinin (mainly zeatin). The IAA hormone promotes the root development of plants (Aloni, Aloni, Langhans, & Ullrich, 2006). As PPFMs are able to produce IAA (Ivanova, Doronina, & Trotsenko, 2001), their inoculation to plants induces plant growth by increasing plant IAA concentration. It is reported that auxin biosynthesis-related genes of various essential enzymes like aldehyde dehydrogenase, cyanide hydratase, amine oxidase, nitrile hydratase, N-acyltransferase, amidase is present in *Methylobacterium* genus (Kwak *et al.*, 2014; Tani *et al.*, 2012).

*Atmospheric nitrogen fixation*

One of the limiting nutrients for plant growth is nitrogen. But atmospheric nitrogen is unavailable to plant metabolism. The conversion of unavailable nitrogen to ammonia by the process of nitrogen fixation helps plants to use nitrogen. The biological nitrogen fixation is performed by some microorganisms with the presence of the nitrogenase enzyme (Menna *et al.*, 2006). Few PPFMs like *Methylobacterium nodulans* isolated from *Crotalaria podocarpa* (Sy *et al.*, 2001), *Methylobacterium* sp. MV10 (Raja, Uma, & Sundaram, 2006), *Methylobacterium* sp. CBMB 20 (Madhaiyan *et al.*, 2004) is so far reported to fix atmospheric nitrogen to ammonia. *Methylobacterium* sp. MV10 (Raja *et al.*, 2006), *Methylobacterium nodulans* ORS 2060 (Jourand *et al.*, 2004) are reported to contain the *nif* H gene (involved in nitrogen fixation). As *Methylobacterium nodulans* can utilize methanol generated through catabolic activities of the host plant in addition to host photosynthetic products, it has a competitive advantage during plant colonization and nodule formation over their counterparts (Renier *et al.*, 2011). It is also reported that loss of methylotrophic function of the *Methylobacterium nodulans* affects plant development because nonmethylotrophic mutants of the bacteria decrease the total root nodule number per plant and nitrogen fixation of *C podocarpa*. It is also observed that total dry plant biomass is reduced compared with the wild-type strain (Jourand *et al.*, 2005).

*Phosphate solubilization*

Phosphorus, an essential nutrient, is present in the soil. Though total phosphorus present in the soil is in high concentrations, it binds to iron, calcium or aluminum or immobilizes in organic matter such as myo-inositol hexakisphosphate, phytic acid, etc.(collectively called phytate). All these bound phosphorus are not readily available to plants. Soluble ionic phosphate forms that are $HPO_4^{2-}$, $H_2PO_4^{-}$, are mainly assimilated by bacteria but in soil, the soluble ionic phosphate concentration is very low. In phosphate metabolism, many PPFMs (*Methylobacterium* sp.) have the ability to dissolve inorganic phosphates which are utilized by both microorganisms and plants (Agafonova *et al.*, 2013). Three different types of microbial enzymes are involved in phosphate solubilization that is a non-specific acid phosphatase, phytase, and C-P lyase (or phosphatase). All these enzymes finally release phosphate.

Phosphate is released from phosphoric ester or phosphoric anhydride by nonspecific acid phosphatases. Phosphate is released by phytase and C-P lyase from phytic acid and organophosphates, respectively. It is reported that *M oryzae* have all three types of phosphate-releasing enzyme-producing genes (Kwak *et al.*, 2014).

*ACC deaminase production*

Ethylene is an important compound that regulates root growth and development (Madhaiyan *et al.*, 2006a, b, c). The concentration of ethylene production is related to the biosynthesis pathway of auxin (HarDOIm, van Overbeek, & van Elsas, 2008). A high concentration of ethylene has a negative effect on plant growth and root elongation as it imparts stress conditions in plants which accelerate abscission, aging and senescence (Glick, 1995). The precursor of ethylene hormone in the ethylene biosynthesis pathway is ACC (aminocyclopropane-1-carboxylic acid). The precursor of ACC is S-adenosylmethionine (SAM). SAM is converted to ACC by the enzyme ACC synthase and ACC is converted to ethylene by the enzyme ACC oxidase. Various biotic and abiotic factors regulate transcriptionally both of these enzymes (Madhaiyan *et al.*, 2006a, b, c; HarDOIm *et al.*, 2008). The interesting finding is that the ACC activity of plants is increased when bacterial IAA production is also increased. This phenomenon indicates the similarity between these two pathways. According to researcher (HarDOIm *et al.*, 2008), for the maintenance of endophytic bacterial plant colonization, the fundamental process is the balance between IAA and ethylene. The *acd*S gene, which encodes an ACC deaminase enzyme, is present in various PPFM organisms. This enzyme converts ACC into ammonia ($NH_3$) and α-ketobutyrate. The whole-genome analysis of various *Methylobacterium* sp. reveals that in *Methylobacterium oryzae*, *Methylobacterium nodulans*, *Methylobacterium radiotolerans* ACC deaminase gene is present (Kwak *et al.*, 2014). *Methylobacterium nodulans* and *Methylobacterium radiotolerans* have the ability to utilize ACC as a sole nitrogen source (Kwak *et al.*, 2014). They break ACC and reduce the ethylene levels (Fedorov, Ekimova, Doronina, & Trotsenko, 2013). As a result, the stress ethylene response is also decreased in the host plant.

*Siderophores production*

Siderophores are low molecular weight compounds that have a high affinity for iron. It helps bacteria to solubilize iron to promote its efficient uptake. As iron is necessary for various biological processes, iron is required in almost all forms of life. Iron presents mainly as insoluble $Fe^{+3}$ in the environment (Rajkumar, Ae, Prasad, & Freitas, 2010). So, bacteria acquire iron by releasing siderophores. In this way, they make iron available for plant uptake, contributing to plant growth (Bar-Ness, Hadar, Chen, Shanzer, & Libman, 1992). Iron uptake genes *iuc* A and *iuc* C have been found in 35 strains of PPFMs including *Methylobacterium extorquens* strains AM1,PA1,DM4 and CM4, and *Methylobacterium populi*. (Tani *et al.*, 2012).

*PPFMs as a bio control agents*

The presence of PPFM organisms ensures plant protection against pathogenic attack (Benhamou, Gagné, Le Quéré, & Dehbi, 2000) and improves plant health. Wide varieties of the large spectrum of antimicrobial compounds are synthesized by various PPFMs (Ryan, Germaine, Franks, Ryan, & Dowling, 2008), that reduce competition for nutrients with pathogens (Berg, 2009) or up-regulate systemic resistance (induced systemic resistance (ISR)) (Nigris, Baldan, Zottini, Squartini, & Baldan, 2013), and by this way, they protect host plants. Volatile organic compounds released from some PPFMs (Naznin, Kimura, Miyazawa, & Hyakumachi, 2013) induce ISR and some cell wall degrading enzymes such as pectinase, cellulases and hemicellulases, and glycosidases (Lee *et al.*, 2006; Madhaiyan *et al.*, 2006a, b, c).

It is also reported that at low-density *Methylobacterium* sp. IMBG290 inoculum activates the plant antioxidant system and induces resistance of potato against *Pectobacterium atrosepticum* but at high density, it results in susceptibility to the pathogen (Pavlo, Leonid, Iryna, Natalia, & Maria, 2011).

It is reported that PPFMs with their various biocontrol efficiencies inhibit various plant pathogens like *Xanthomonas campestris*, *Selerotium rolfsii*, *Fusarium oxysporum*, *Colletotrichum capsici* and *Cercospora capsici*, measured by the zone of inhibition (Savitha, Sreenivasa, & Nirmalnath, 2015; Gamit *et al.*, 2023). It also reported that PPFM isolates also inhibit the growth of wilt causing plant pathogen *Fusarium oxysporum* (Savitha *et al.*, 2015). Another study also reported that treatment of four different *Methylobacterium* strains combinations inhibits phytopathogen *Ralstonia solanacearum* by inducing plant defense responses against this pathogen (Yim, Seshadri, Kim, Lee, & Sa, 2013). Low ethylene levels due to reduction of ACC accumulation were responsible for reduced disease symptoms. *Methylobacterium* sp showed significant biocontrol activity against *Aspergillus niger* and *Scterotium rolfsii* in groundnuts in a pot culture experiment (Madhaiyan *et al.*, 2006a, b, c).

*Heavy metal tolerance*

Methylotrophic bacteria have the ability to tolerate the high amount of several heavy metals, such as cadmium (Cd), cobalt (Co), chrome (Cr), nickel (Ni), zinc (Zn), (Idris *et al.*, 2006), arsenic (As), lead (Pb) (Dourado, Ferreira, Araújo, Azevedo, & Lacava, 2012) and mercury (Hg) (Fernandes, Albergaria, Oliva-Teles, Delerue-Matos, & De Marco, 2009). In tomato plants, *Methylobacterium oryzae*, it is reported that genes related to heavy metal tolerance (uptake and efflux), copper translocating P-type ATPase which is responsible for copper resistance, genes related to the cation efflux system protein Czc A which are responsible for zinc-cobalt-cadmium resistance, several ABC transporters involved in zinc and nickel uptake, and chromate transport protein are present (Kwak *et al.*, 2014). Therefore, this genus has increased plant tolerance to heavy metals and decreased plant stresses and thus imparts a role in plant growth promotion and inhibition of plant pathogens.

## PPFMs in bioremediation

According to various reported studies, PPFMs have the ability to degrade a variety of organic toxic compounds. It is reported that within 10 to 55 days, *Methylobacterium* sp. strain BJ001 degrades several toxic explosives such as hexahydro-1,3,5-trinitro-1,3,5 triazine (RDX), 2,4,6 trinitrotoluene (TNT) and octahydro-1,3,5,7-tetranitro-1,3,5 tetrazine (HMX) in vitro (Van Aken, Yoon, & Schnoor, 2004). Industrially used and produced volatile, toxic, halogenated solvent, dichloromethane ($CH_2Cl_2$) is also degraded by *Methylobacterium extorquens* DM4 (Muller *et al.*, 2011) by converting dichloromethane into methylotrophic metabolic growth intermediate formaldehyde (Kayser, Ucurum, & Vuilleumier, 2002). *Methylobacterium populi* VP2 was able to degrade industrially treated toxic compounds, polycyclic aromatic hydrocarbons(PAHs) (Ventorino *et al.*, 2014). It is reported that *Methylobacterium* sp. showed a high (99.9%) efficiency of methyl-tert-butyl-ether (MTBE) degradation (Zhang, Chen, & Fang, 2008). *Methylobacterium* spp. a combination of a few bacteria had the ability to degrade MTBE and trichloroethylene (TCE), soil pollutants in presence of heavy metals at high efficiency (Fernandes *et al.*, 2009). These reports suggest that PPFMs can be effectively used to bioremediate the contaminated environments.

## PPFMs in biotechnological uses

PPFMs have the ability to produce several industrial products and biodegradable compounds. Methylobacterium spp. is able to produce biodegradable plastics such as

biodegradable polyesters polyhydroxy butyric acid (PHB) and polyhydroxyalkanoate (PHA). To increase the production of PHB and PHA using methanol as a substrate *Methylobacterium extorquens* was genetically modified (Höfer, Vermette, & Groleau, 2011). Under nitrogen limitation, *Methylobacterium organophilum* was also produced in PHB and PHA using methane which is a greenhouse gas (Yezza, Fournier, Halasz, & Hawari, 2006).

Glyoxylate is an important compound in perfume manufacture and it is also produced as an intermediate in drug and pesticide production. It is reported that a genetically modified *Methylobacterium* sp. strain that is able to over express a key enzyme component in the serine cycle that is hydroxy pyruvate reductase enzyme, leading to glyoxylate accumulation (Shen and Wu, 2007).

In vitro production of two furanoid compounds such as 2,5 dimethyl-4 methoxy-2H-furanone and 2,5 dimethyl-4 hydroxy-2H-furanone (DMHF) are promoted by *Methylobacterium extorquens* DSM 21961 (Verginer *et al.*, 2010). These two furanoid compounds are responsible for strawberry flavor and thus the bacteria influence the fruit quality. This report was also reinforced by another study and showed that the expression of the alcohol dehydrogenase enzyme by the bacteria and the flavor components (DMHF) in the same tissues (Nasopoulou, Pohjanen, Koskimäki, Zabetakis, & Pirttilä, 2014). PPFMs are potent protease (Jayashree *et al.*, 2014) and cellulase producers (Jayashree *et al.*, 2011a, b) and their pigments have high antioxidant properties (Gamit *et al.*, 2023).

## Omics studies of the PPFM organisms

Modern techniques of molecular biology and the advancement of next-generation sequencing make it possible to sequence many bacterial genomes. Many PPFM organisms such as *Methylobacterium extorquens* (AM1, DM4, PA1, DSM13060, CM4), *Methylobacterium nodulans* ORS 2060, *Methylobacterium populi* BJ001, *Methylobacterium radiotolerans* JCM2831, *Methylobacterium mesophilicum* SR1.6/6 sequenced genomes are available in National Center for Biotechnology Information (NCBI) database.

A recent study showed that *Methylobacterium extorquens* strain PA1 isolated from Arabidopsis showed similarity in GC contents with *Methylobacterium extorquens* AM1 strain. GC content of PA1 and AM1 is 68.2% and 68.5% respectively and most of the genes (>90%) of these two strains, involved with methylotrophy, showed 95% of sequence identity at the amino acid level. According to the author, these two strains have similar modules during C1 growth but a difference in growth rate was observed when they used different substrates (Nayak and Marx, 2014).

Genomes of nine PPFM organisms analyzed and compared and divided the strains into three groups with significant features (Kwak *et al.*, 2014). *Methylobacterium nodulans* and *Methylobacterium* sp. 4–46 which contained genes for nitrogen fixation were included in the first group. *Methylobacterium oryzae* and *Methylobacterium radiotolerans* which had genes related to ACC deaminase and phytase included in the second group. *Methylobacterium extorquens* which lacked these previous genes were included in the third group.

Another studies reported that proteome of *Methylobacterium extorquens* AM1 differs largely under methylotrophic growth conditions than when grown on succinate (Bosch *et al.*, 2008).

Thus, these approaches give us essential clues to understand the interaction processes at the biochemical and molecular levels and help to explore the biotechnological potential in different areas of interest.

## Lacuna in PPFMs research

The biochemical and molecular basis of interaction of this group of organisms with their host plants to impart improved seed germination, protection of host plant from pathogenic attack is not well understood yet.

Phylloplane microbiome is highly dynamic. Physical parameters like temperature, sunlight, air current, humidity and concentration of various metabolites produced by both the host and residing microbes on phylloplane region change with time in different growth phase of the host plant which affect the diversity of the residing microbial population, interaction between the phylloplane microbes within themselves and with the host plant. So, for a clearer understanding of the interaction between phylloplane environment and PPFMs more in-depth research is necessary. Genomics study of PPFM organisms reveals that almost 70 methylotrophy related genes are available in their genome (Chistoserdova *et al.*, 2003). The actual roles of many of those genes are still unknown. So, further research may reveal the presence of many industrially important proteins in future.

On the way to utilize methanol through serine cycle pathway to generate energy, PPFM organisms generate many value added byproducts which may be further explored for their commercial use. For this purpose PPFM organisms can be genetically modified to utilize those useful by products through bioprocess technology. More research is required to develop methanol-based bioprocess technology.

## Direction for future research

PPFM organisms possess all the PGP features. It is known from the reported research work that they are eco-friendly and not only help plants to ameliorate drought stress effects but also promote seed germination, enhance yield, make host plants resistant to plant pathogens. Study of phylloplane metagenome of host plant before and after PPFM treatment will give an idea about the interaction of PPFM organisms and non PPFM organisms on phylloplane. Study of phylloplane metagenome and proteome will throw new lights on phylloplane community dynamics study and will also help in detecting the groups of bacteria get that are selected after PPFM treatment, proteins that are responsible for their selection and also their role in the phylloplane. Comparative genomics study of PPFM bacteria will help to identify the marker characters of this group of bacteria. Whole genome analysis of the PPFM isolates will give insight into its important genes and genetic regulatory systems which will be helpful in exploitation of the isolated strains more efficiently.

PPFMs have the capacity to utilize methanol as a carbon source. They produce pink color in AMS media in presence of 0.01% methanol. Pigment synthesis is also induced by 0.01% methanol in presence of low concentration of ethanol but when ethanol is present as carbon source in the media without methanol supplementation, they cannot produce the pigment (unpublished data). So, potent PPFMs isolate can be used as a methanol biosensor to detect the presence of methanol in various types of country liquors which will help in reducing death toll in underdeveloped and developing countries like India.

Moreover, PPFMs are reported to be useful source of antimicrobial agents and source of various hydrolytic enzymes like protease, cellulase etc which in turn, can be used in pharmaceutical and biotechnology industries. So, further research with PPFMs in these regards is required to get more potent isolates. Carotenoids, isolated from PPFMs show high UV absorbing and antioxidant properties that protect the host plants from strong sunshine. Such pigments of PPFMs may be purified, characterized and patented for their use in cosmetic industries as UV protectant and antioxidant to reduce reactive oxygen species in human.

Instead of using single PPFM strain, use of a cocktail of different PPFM strains isolated from the same host but from different places may be more effective in improving their PGP traits.

## Conclusion

PPFM organisms are drawing increasing attention of researchers in recent times due to their strong PGP activities and their crucial role in alleviating the adverse impacts of climate stress

on plants. Molecular studies on PPFM organisms reveals the presence of beneficial genes and their regulatory systems. Further proteomics and metabolomics studies will help us to understand molecular mechanisms related to plant interaction, explaining how PPFM organisms induce plant growth, protect plants from various plant pathogens and how various secondary metabolites produced by them manage draught, salinity, heavy metals stress, nutrient deficiency and help in the bioremediation of contaminated soils. Those findings will also help us in using this group of organisms as plant growth promoter and modulator in a more effective way as bioactive compounds and secondary metabolites are crucial in modern agriculture. Treatment of seeds during germination, seedling root before transplantation and foliar spray with isolated PPFM organisms may be helpful in amelioration of draught stress and in increasing yield and vigor of major crops of draught infested areas including the coastal region where the crop plants get insufficient water through their root system due to physiologically dry soil. So, further research on PPFM organisms is needed to use these organisms more effectively as plant growth promoter for improved productivity of various crops, spices and vegetables.

## References


Abanda-Nkpwatt, D., Müsch, M., Tschiersch, J., Boettner, M., & Schwab, W. (2006) Molecular interaction between Methylobacterium extorquens and seedlings: Growth promotion, methanol consumption, & localization of the methanol emission site. *Journal of Experimental Botany*, *57*(15), 4025–4032. doi:10.1093/jxb/erl173.

Abd El-Gawad, H. G., Ibrahim, M. F., Abd El-Hafez, A. A., & Abou El-Yazied, A. (2015). Contribution of pink pigmented facultative methylotrophic bacteria in promoting antioxidant enzymes, growth and yield of Snap Bean. *American–Eurasian Journal of Agricultural and Environment Science*, *15*, 1331–1345. doi: 10.5829/idosi.aejaes.2015.15.7.12709.

Agafonova, N. V., Kaparullina, E. N., Doronina, N. V., & Trotsenko, Y. A. (2013). Phosphate-solubilizing activity of aerobic methylobacteria. *Microbiology*, *82*(6), 864–867. doi: 10.1134/S0026261714010020.

Aloni, R., Aloni, E., Langhans, M., & Ullrich, C. I. (2006). Role of cytokinin and auxin in shaping root architecture: Regulating vascular differentiation, lateral root initiation, root apical dominance and root gravitropism. *Annals of Botany*, *97*(5), 883–893. doi: 10.1093/aob/mcl027.

Anthony, C., Ghosh, M., & Blake, C. C. (1994). The structure and function of methanol dehydrogenase and related quinoproteins containing pyrrolo-quinoline quinine. *Biochemical Journal*, *304*(3), 665–674. doi: 10.1042/bj3040665.

Bar-Ness, E., Hadar, Y., Chen, Y., Shanzer, A., & Libman, J. (1992). Iron uptake by plants from microbial siderophores: A study with 7-nitrobenz-2 oxa-1, 3-diazole-desferrioxamine as fluorescent ferrioxamine B analog. *Plant Physiology*, *99*(4), 1329–1335. doi: 10.1104/pp.99.4.1329.

Benhamou, N., Gagné, S., Le Quéré, D., & Dehbi, L. (2000). Bacterial-mediated induced resistance in cucumber: Beneficial effect of the endophytic bacterium *Serratia plymuthica* on the protection against infection by *Pythium ultimum*. *Phytopathology*, *90*(1), 45–56. doi: 10.1094/PHYTO.2000.90.1.45.

Berg, G. (2009). Plant–microbe interactions promoting plant growth and health: Perspectives for controlled use of microorganisms in agriculture. *Applied Microbiology and Biotechnology*, *84*(1), 11–18. doi: 10.1007/s00253-009-2092-7.

Bosch, G., Skovran, E., Xia, Q., Wang, T., Taub, F., Miller, J. A., . . . Hackett, M. (2008). Comprehensive proteomics of *Methylobacterium extorquens* AM1 metabolism under single carbon and nonmethylotrophic conditions. *Proteomics*, *8*(17), 3494–3505. doi: 10.1002/pmic.200800152.

Chistoserdova, L., Chen, S. W., Lapidus, A., & Lidstrom, M. E. (2003). Methylotrophy in Methylobacterium extorquens AM1 from a genomic point of view. *Journal of Bacteriology*, *185*(10), 2980–2987. doi: 10.1128/JB.185.10.2980-2987.2003.

Chowdhury, A. A., Basak, N., & Islam, E. (2023). Removal of uranium from water using biofilm of uranium sensitive Methylobacterium sp. *Journal of Hazardous Materials Advances*, *10*, 100296. doi: 10.1016/j.hazadv.2023.100296.

Corpe, W. A. (1985). A method for detecting methylotrophic bacteria on solid surfaces. *Journal of Microbiological Methods*, *3*(3-4), 215–221. doi: 10.1016/0167-7012(85)90049-1.

Dourado, M. N., Ferreira, A., Araújo, W. L., Azevedo, J. L., & Lacava, P. T. (2012). The diversity of endophytic methylotrophic bacteria in an oil-contaminated and an oil-free mangrove ecosystem and their tolerance to heavy metals. *Biotechnology Research International*, *2012*, 759865. 8. doi: 10.1155/2012/759865.

Fedorov, D. N., Ekimova, G. A., Doronina, N. V., & Trotsenko, Y. A. (2013). 1-aminocyclopropane-1-carboxylate (ACC) deaminases from *Methylobacterium radiotolerans* and *Methylobacterium nodulans* with higher specificity for ACC. *FEMS Microbiology Letters*, *343*(1), 70–76. doi: 10.1111/1574-6968.12133.

Fernandes, V. C., Albergaria, J. T., Oliva-Teles, T., Delerue-Matos, C., & De Marco, P. (2009). Dual augmentation for aerobic bioremediation of MTBE and TCE pollution in heavy metal-contaminated soil. *Biodegradation*, *20*(3), 375–382. doi: 10.1007/s10532-008-9228-9.

Gamit, H. A., Naik, H., Chandarana, K. A., Chandwani, S., & Amaresan, N. (2023). Secondary metabolites from methylotrophic bacteria: Their role in improving plant growth under a stressed environment. *Environmental Science and Pollution Research*, *30*(11), 28563-28574. doi: 10.1007/s11356-023-25505-8.

Glick, B. R. (1995). The enhancement of plant growth by free-living bacteria. *Canadian Journal of Microbiology*, *41*(2), 109–117. doi: 10.1139/m95-015.

HarDOIm, P. R., van Overbeek, L. S., & van Elsas, J. D. (2008). Properties of bacterial endophytes and their proposed role in plant growth. *Trends in Microbiology*, *16*(10), 463–471. doi: 10.1016/j.tim.2008.07.008.

Höfer, P., Vermette, P., & Groleau, D. (2011). Introducing a new bioengineered bug: *Methylobacterium extorquens* tuned as a microbial bioplastic factory. *Bioengineered Bugs*, *2*(2), 71–79. doi: 10.4161/bbug.2.2.15009.

Holland, M. A., Davis, R., Moffitt, S., O'Laughlin, K., Peach, D., Sussan, S., . . . Tayman, B. (2000). Using "leaf prints" to investigate a common bacterium. *American Biology Teacher*, *62*(2), 128–131. doi: 10.1662/0002.

Idris, R., Kuffner, M., Bodrossy, L., Puschenreiter, M., Monchy, S., Wenzel, W. W., & Sessitsch, A. (2006). Characterization of Ni-tolerant methylobacteria associated with the hyperaccumulating plant *Thlaspi goesingense* and description of *Methylobacterium goesingense* sp. nov. *Systematic and Applied Microbiology*, *29*(8), 634–644. doi: 10.1016/j.syapm.2006.01.011.

Iguchi, H., Yurimoto, H., & Sakai, Y. (2015). Interactions of methylotrophs with plants and other heterotrophic bacteria. *Microorganisms*, *3*(2), 137–151. doi: 10.3390/microorganisms3020137.

Irvine, I. C., Brigham, C. A., Suding, K. N., & Martiny, J. B. (2012). The abundance of pink-pigmented facultative methylotrophs in the root zone of plant species in invaded coastal sage scrub habitat. *PLOS ONE*, *7*(2), e31026. doi: 10.1371/journal.pone.0031026.

Ismail, S., & Mohammed, F. (2023). Effect of foliar spraying with pink pigmented facultative methylotrophic bacteria on the growth and productivity of strawberry. *Arab Universities Journal of Agricultural Sciences*, *31*(1), 1-14. doi: 10.21608/AJS.2023.135514.1479.

Ivanova, E. G., Doronina, N. V., & Trotsenko, Y. A. (2001). Aerobic methylobacteria are capable of synthesizing auxins. *Microbiology*, *70*(4), 392–397. doi: 10.1023/A:1010469708107.

Jayashree, S., Lalitha, R., Vadivukkarasi, P., Kato, Y., & Seshadri, S. (2011a). Cellulase production by pink pigmented facultative methylotrophic strains (PPFMs). *Applied Biochemistry and Biotechnology*, *164*(5), 666–680. doi: 10.1007/s12010-011-9166-6.

Jayashree, S., Vadivukkarasi, P., Anand, K., Kato, Y., & Seshadri, S. (2011b). Evaluation of pink-pigmented facultative methylotrophic bacteria for phosphate solubilisation. *Archives of Microbiology*, *193*(8), 543–552. doi: 10.1007/s00203-011-0691-z.

Jayashree, S., Annapurna, B., Jayakumar, R., Sa, T., & Seshadri, S. (2014). Screening and characterization of alkaline protease produced by a pink pigmented facultative methylotrophic (PPFM) strain, MSF 46. *Journal of Genetic Engineering and Biotechnology*, *12*(2), 111–120. doi: 10.1016/j.jgeb.2014.11.002.

Joel, G. V. V., Latha, P. C., Gopal, A. V., & Sreedevi, B. (2023). Isolation and characterization of pink pigmented facultative methylotrophic bacteria: An in-vitro evaluation of the isolates for plant growth promotion on rice. *Biological Forum – An International Journal*, *15*(2), 1167-1179.

Jourand, P., Giraud, E., Béna, G., Sy, A., Willems, A., Gillis, M., … de Lajudie, P. (2004). *Methylobacterium nodulans* sp. nov., for a group of aerobic, facultatively methylotrophic, legume root-nodule-forming and nitrogen-fixing bacteria. *International Journal of Systematic and Evolutionary Microbiology*, *54*(6), 2269–2273. doi: 10.1099/ijs.0.02902-0.

Jourand, P., Renier, A., Rapior, S., Miana de Faria, S. M., Prin, Y., Galiana, A., … Dreyfus, B. (2005). Role of methylotrophy during symbiosis between *Methylobacterium nodulans* and *Crotalaria podocarpa*. *Molecular Plant–Microbe Interactions*, *18*(10), 1061–1068. doi: 10.1094/MPMI-18-1061.

Kayser, M. F., Ucurum, Z., & Vuilleumier, S. (2002). Dichloromethane metabolism and C1 utilization genes in *Methylobacterium* strainsThe GenBank accession numbers for the sequences determined in this work are AJ421476 and AJ421477. *Microbiology*, *148*(6), 1915–1922. doi: 10.1099/00221287-148-6-1915.

Kumar, R., & Lee, A. C. (2009). Isolation and characterization of pink-pigmented, facultative methylotrophic (PPFM) bacteria from leaves of neem, *Azadirachta indica* A. *Philippine Journal of Systematic Biology*, *3*(1), 8–16. doi:10.3860/pjsb.v3i1.1009.

Kumar, M., Tomar, R. S., Lade, H., & Paul, D. (2016). Methylotrophic bacteria in sustainable agriculture. *World Journal of Microbiology and Biotechnology*, *32*(7), 120. doi: 10.1007/s11274-016-2074-8.

Kwak, M. J., Jeong, H., Madhaiyan, M., Lee, Y., Sa, T. M., Oh, T. K., & Kim, J. F. (2014). Genome information of *Methylobacterium oryzae*, a plant-probiotic methylotroph in the phyllosphere. *PLOS ONE*, *9*(9), e106704. doi: 10.1371/journal.pone.0106704.

Lacava, P. T., Araújo, W. L., Marcon, J., Maccheroni, Jr., W., & Azevedo, J. L. (2004). Interaction between endophytic bacteria from citrus plants and the phytopathogenic bacteria *Xylella fastidiosa*, causal agent of citrus-variegated chlorosis. *Letters in Applied Microbiology*, *39*(1), 55–59. doi: 10.1111/j.1472-765X.2004.01543.x.

Lacava, P. T., Li, W. B., Araújo, W. L., Azevedo, J. L., & Hartung, J. S. (2006). Rapid, specific and quantitative assays for the detection of the endophytic bacterium *Methylobacterium mesophilicum* in plants. *Journal of Microbiological Methods*, *65*(3), 535–541. doi: 10.1016/j.mimet.2005.09.015.

Lee, K. H., Munusamy, M., Kim, C. W., Lee, H. S., Selvaraj, P., & Sa, T. (2004). Isolation and characterization of the IAA producing methylotrophic bacteria from phyllosphere of rice cultivars (*Oryza sativa* L.). *Korean Journal of Soil Science and Fertilizer*, *37*, 235–244.

Lee, H. S., Madhaiyan, M., Kim, C. W., Choi, S. J., Chung, K. Y., & Sa, T. M. (2006). Physiological enhancement of early growth of rice seedlings (*Oryza sativa* L.) by production of phytohormone of $N_2$-fixing methylotrophic isolates. *Biology and Fertility of Soils*, *42*(5), 402–408. doi: 10.1007/s00374-006-0083-8.

Madhaiyan, M., Poonguzhali, S., Senthilkumar, M., Seshadri, S., Chung, H., Jinchul, Y. A., … Tongmin, S. A. (2004). Growth promotion and induction of systemic resistance in rice cultivar Co-47 (*Oryza sativa* L.) by *Methylobacterium* spp. *Botanical Bulletin of Academia Sinica*, *45*, 315–324.

Madhaiyan, M., Poonguzhali, S., Ryu, J., & Sa, T. (2006a). Regulation of ethylene levels in canola (*Brassica campestris*) by 1-aminocyclopropane-1-carboxylate deaminase-containing *Methylobacterium fujisawaense*. *Planta*, *224*(2), 268–278. doi: 10.1007/s00425-005-0211-y.

Madhaiyan, M., Poonguzhali, S., Sundaram, S. P., & Sa, T. (2006b). A new insight into foliar applied methanol influencing phylloplane methylotrophic dynamics and growth promotion of cotton (*Gossypium hirsutum* L.) and sugarcane (*Saccharum officinarum* L.). *Environmental and Experimental Botany*, *57*(1-2), 168–176. doi: 10.1016/j.envexpbot.2005.05.010.

Madhaiyan, M., Suresh Reddy, B. V., Anandham, R., Senthilkumar, M., Poonguzhali, S., Sundaram, S. P., & Sa, T. (2006c). Plant growth–promoting *Methylobacterium* induces defense responses in groundnut (*Arachis hypogaea* L.) compared with rot pathogens. *Current Microbiology*, *53*(4), 270–276. doi: 10.1007/s00284-005-0452-9.

Madhaiyan, M., & Poonguzhali, S. (2014). *Methylobacterium pseudosasicola* sp. nov. and *Methylobacterium phyllostachyos* sp. nov., isolated from bamboo leaf surfaces. *International Journal of Systematic and Evolutionary Microbiology*, *64*(7), 2376–2384. doi: 10.1099/ijs.0.057232-0.

McDonald, I. R., & Murrell, J. C. (1997). The methanol dehydrogenase structural gene mxaF and its use as a functional gene probe for methanotrophs and methylotrophs. *Applied and Environmental Microbiology*, *63*(8), 3218–3224. doi: 10.1128/aem.63.8.3218-3224.1997.

Menna, P., Hungria, M., Barcellos, F. G., Bangel, E. V., Hess, P. N., & Martínez-Romero, E. (2006). Molecular phylogeny based on the 16S rRNA gene of elite rhizobial strains used in Brazilian commercial inoculants. *Systematic and Applied Microbiology*, *29*(4), 315–332. doi: 10.1016/j.syapm.2005.12.002.

Mitra, B. (2012). Standardization of cultivation parameters for the extraction of carotenoid from pink pigmented facultative methylotrophic (PPFM) bacteria. *Asian Journal of Pharmaceutical and Clinical Research*, *5*, 52–57.

Morris, C. J., Kim, Y. M., Perkins, K. E., & Lidstrom, M. E. (1995). Identification and nucleotide sequences of mxaA, mxaC, mxaK, mxaL, and mxaD genes from Methylobacterium extorquens AM1. *Journal of Bacteriology*, *177*(23), 6825–6831. doi: 10.1128/jb.177.23.6825-6831.1995.

Muller, E. E., Hourcade, E., Louhichi-Jelail, Y., Hammann, P., Vuilleumier, S., & Bringel, F. (2011). Functional genomics of dichloromethane utilization in *Methylobacterium extorquens* DM4. *Environmental Microbiology*, *13*(9), 2518–2535. doi: 10.1111/j.1462-2920.2011.02524.x.

Nasopoulou, C., Pohjanen, J., Koskimäki, J. J., Zabetakis, I., & Pirttilä, A. M. (2014). Localization of strawberry (Fragaria x ananassa) and *Methylobacterium extorquens* genes of strawberry flavor biosynthesis in strawberry tissue by in situ hybridization. *Journal of Plant Physiology*, *171*(13), 1099–1105. doi: 10.1016/j.jplph.2014.03.018.

Nayak, D. D., & Marx, C. J. (2014). Genetic and phenotypic comparison of facultative methylotrophy between *Methylobacterium extorquens* strains PA1 and AM1. *PLOS ONE*, *9*(9), e107887. doi: 10.1371/journal.pone.0107887.

Naznin, H. A., Kimura, M., Miyazawa, M., & Hyakumachi, M. (2013). Analysis of volatile organic compounds emitted by plant growth-promoting fungus *Phoma* sp. GS8-3 for growth promotion effects on tobacco. *Microbes and Environments*, *28*(1), 42–49. doi: 10.1264/jsme2.ME12085.

Nigris, S., Baldan, E., Zottini, M., Squartini, A., & Baldan, B. (2013). Is the bacterial endophyte community, living in Glera (Vitis vinifera) plants, active in biocontrol? In *Endophytes for plant protection: The state of the art* (*12*). Padova: CABI Digital Library. Department of Biology, University of Padova.

Nysanth, N. S., Anu Rajan, S., Sivapriya, S. L., & Anith, K. N. (2023). Pink pigmented facultative methylotrophs (PPFMs): Potential bioinoculants for sustainable crop production. *Journal of Pure and Applied Microbiology*, *17*(2), 660-681, 10.22207/jpam.17.2.17.

Omer, Z. S., Tombolini, R., & Gerhardson, B. (2004). Plant colonization by pink-pigmented facultative methylotrophic bacteria (PPFMs). *FEMS Microbiology Ecology*, *47*(3), 319–326. doi: 10.1016/S0168-6496(04)00003-0.

Pavlo, A., Leonid, O., Iryna, Z., Natalia, K., & Maria, P. A. (2011). Endophytic bacteria enhancing growth and disease resistance of potato (*Solanum tuberosum* L.). *Biological Control*, *56*(1), 43–49. doi: 10.1016/j.biocontrol.2010.09.014.

Pirttilä, A. M., Joensuu, P., Pospiech, H., Jalonen, J., & Hohtola, A. (2004). Bud endophytes of Scots pine produce adenine derivatives and other compounds that affect morphology and mitigate browning of callus cultures. *Physiologia Plantarum*, *121*(2), 305–312. doi: 10.1111/j.0031-9317.2004.00330.x.

Pomini, A. M., Cruz, P. L., Gai, C., Araújo, W. L., & Marsaioli, A. J. (2009). Long-chain acyl-homoserine lactones from *Methylobacterium mesophilicum*: Synthesis and absolute configuration. *Journal of Natural Products*, *72*(12), 2125–2129. doi: 10.1021/np900043j.

Raja, P., Uma, S., & Sundaram, S. (2006). Non-nodulating pink-pigmented facultative *Methylobacterium* sp. with a functional nifH gene. *World Journal of Microbiology and Biotechnology*, *22*(12), 1381–1384. doi: 10.1007/s11274-006-9199-0.

Rajkumar, M., Ae, N., Prasad, M. N., & Freitas, H. (2010). Potential of siderophore-producing bacteria for improving heavy metal phytoextraction. *Trends in Biotechnology*, *28*(3), 142–149. doi: 10.1016/j.tibtech.2009.12.002.

Renier, A., De Faria, S. M., Jourand, P., Giraud, E., Dreyfus, B., Rapior, S., & Prin, Y. (2011). Nodulation of *Crotalaria podocarpa* DC. by *Methylobacterium nodulans* displays very unusual features. *Journal of Experimental Botany*, *62*(10), 3693–3697. doi: 10.1093/jxb/err083.

Rossetto, P. B., Dourado, M. N., Quecine, M. C., Andreote, F. D., Araújo, W. L., Azevedo, J. L., & Pizzirani-Kleiner, A. A. (2011). Specific plant induced biofilm formation in *Methylobacterium* species. *Brazilian Journal of Microbiology*, *42*(3), 878–883. doi: 10.1590/S1517-83822011000300006.

Ryan, R. P., Germaine, K., Franks, A., Ryan, D. J., & Dowling, D. N. (2008). Bacterial endophytes: Recent developments and applications. *FEMS Microbiology Letters*, *278*(1), 1–9. doi: 10.1111/j.1574-6968.2007.00918.x.

Santosh, S., Santosh, H. B., & Sreenivasa, M. N. (2019). Assessment of native pink pigmented facultative methylotrophs of chilli (*Capsicum annuum* L.) for their plant growth promotional abilities. *International Journal of Current Microbiology and Applied Sciences*, *8*(1), 1196–1205. doi: 10.20546/ijcmas.2019.801.126.

Savitha, P., Sreenivasa, M. N., & Nirmalnath, J. P. (2015). In vitro screening for biocontrol activity of pink pigmented facultative methylotrophs against phytopathogens. *Karnataka Journal of Agricultural Sciences*, *28*, 286–287.

Shen, P. H., & Wu, B. (2007). Over-expression of a hydroxypyruvate reductase in *Methylobacterium* sp. MB200 enhances glyoxylate accumulation. *Journal of Industrial Microbiology and Biotechnology*, *34*(10), 657–663. doi: 10.1007/s10295-007-0238-0.

Skovran, E., Palmer, A. D., Rountree, A. M., Good, N. M., & Lidstrom, M. E. (2011). XoxF is required for expression of methanol dehydrogenase in *Methylobacterium extorquens* AM1. *Journal of Bacteriology*, *193*(21), 6032–6038. doi: 10.1128/JB.05367-11.

Šmejkalová, H., Erb, T. J., & Fuchs, G. (2010). Methanol assimilation in Methylobacterium extorquens AM1: Demonstration of all enzymes and their regulation. *PLOS ONE*, *5*(10), e13001. doi: 10.1371/journal.pone.0013001.

Springer, A. L., Morris, C. J., & Lidstrom, M. E. (1997). Molecular analysis of mxbD and mxbM, a putative sensor-regulator pair required for oxidation of methanol in Methylobacterium extorquens AM1. *Microbiology*, *143*(5), 1737–1744. doi: 10.1099/00221287-143-5-1737.

Springer, A. L., Auman, A. J., & Lidstrom, M. E. (1998). Sequence and characterization of mxaB, a response regulator involved in regulation of methanol oxidation, & of mxaW, a methanol-regulated gene in Methylobacterium extorquens AM1. *FEMS Microbiology Letters*, *160*(1), 119–124. doi: 10.1111/j.1574-6968.1998.tb12900.x.

Subhaswaraj, P., Jobina, R., Parasuraman, P., & Siddhardha, B. (2017). Plant growth promoting activity of pink pigmented facultative methylotroph–*Methylobacterium extorquens* MM2 on *Lycopersicon esculentum* L. *Journal of Applied Biology and Biotechnology*, *5*, 42–46. doi: 10.7324/JABB.2017.50107.

Sun, Z., Copolovici, L., & Niinemets, Ü. (2012). Can the capacity for isoprene emission acclimate to environmental modifications during autumn senescence in temperate deciduous tree species Populus tremula?. *Journal of Plant Research*, *125*(2), 263–274. doi: 10.1007/s10265-011-0429-7.

Sy, A., Giraud, E., Jourand, P., Garcia, N., Willems, A., De Lajudie, P., ... Dreyfus, B. (2001). Methylotrophic Methylobacterium bacteria nodulate and fix nitrogen in symbiosis with legumes. *Journal of Bacteriology*, *183*(1), 214–220. doi: 10.1128/JB.183.1.214-220.2001.

Sy, A., Timmers, A. C., Knief, C., & Vorholt, J. A. (2005). Methylotrophic metabolism is advantageous for *Methylobacterium extorquens* during colonization of Medicago truncatula under competitive conditions. *Applied and Environmental Microbiology*, *71*(11), 7245–7252. doi: 10.1128/AEM.71.11.7245-7252.2005.

Tani, A., Takai, Y., Suzukawa, I., Akita, M., Murase, H., & Kimbara, K. (2012). Practical application of methanol-mediated mutualistic symbiosis between *Methylobacterium* species and a roof greening moss, *Racomitrium japonicum*. *PLOS ONE*, *7*(3), e33800. doi: 10.1371/journal.pone.0033800.

Uy, M. M., Uy, J., Carvajal, T. M., Castro, C. Z., Ho, H. T., & Lee, A. C. (2013). Pink pigmented facultative methylotrophic (PPFM) bacteria isolated from the hair scalp and nasal cavity. *Philippine Journal of Systematic Biology*, *7*, 13–21.

Valdivia-Anistro, J., Cruz-Córdova, A., Souza, V., & Rosas-Pérez, I. (2022). Diversity of cultivated methylotrophs from the extremely oligotrophic system in the Cuatro Cienegas Basin, Mexico: An unexplored ecological guild. *Journal of Microbiology and Experimentation*, *10*(6), 208-214, 10.15406/jmen.2022.10.00375.

Van Aken, B., Yoon, J. M., & Schnoor, J. L. (2004). Biodegradation of nitro-substituted explosives 2,4,6-trinitrotoluene, hexahydro-1,3,5-trinitro-1,3,5-triazine, and octahydro-1,3,5,7-tetranitro-1,3,5-tetrazocine by a phytosymbiotic Methylobacterium sp. associated with poplar tissues (Populus deltoides x nigra DN34). *Applied and Environment Microbiology* 70:508-517, *70*(1), 508–517. doi: 10.1128/AEM.70.1.508-517.2004.

Ventorino, V., Sannino, F., Piccolo, A., Cafaro, V., Carotenuto, R., & Pepe, O. (2014). *Methylobacterium populi* VP2: Plant growth-promoting bacterium isolated from a highly polluted environment for polycyclic aromatic hydrocarbon (PAH) biodegradation. *The Scientific World Journal*, *2014*, 931793–11. doi: 10.1155/2014/931793.

Verginer, M., Siegmund, B., Cardinale, M., Müller, H., Choi, Y., Míguez, C. B., … Berg, G. (2010). Monitoring the plant epiphyte *Methylobacterium extorquens* DSM 21961 by real-time PCR and its influence on the strawberry flavour. *FEMS Microbiology Ecology*, *74*(1), 136–145. doi: 10.1111/j.1574-6941.2010.00942.x.

Xu, H. H., Viebahn, M., & Hanson, R. S. (1993). Identification of methanol-regulated promoter sequences from the facultative methylotrophic bacterium Methylobacterium organophilum XX. *Journal of General Microbiology*, *139*(4), 743–752. doi: 10.1099/00221287-139-4-743.

Yezza, A., Fournier, D., Halasz, A., & Hawari, J. (2006). Production of polyhydroxyalkanoates from methanol by a new methylotrophic bacterium *Methylobacterium* sp. GW2. *Applied Microbiology and Biotechnology*, *73*(1), 211–218. doi: 10.1007/s00253-006-0458-7.

Yim, W., Seshadri, S., Kim, K., Lee, G., & Sa, T. (2013). Ethylene emission and PR protein synthesis in ACC deaminase producing *Methylobacterium* spp. inoculated tomato plants (*Lycopersicon esculentum* Mill.) challenged with *Ralstonia solanacearum* under greenhouse conditions. *Plant Physiology and Biochemistry*, *67*, 95–104. doi: 10.1016/j.plaphy.2013.03.002.

Zhang, L. L., Chen, J. M., & Fang, F. (2008). Biodegradation of methyl t-butyl ether by aerobic granules under a cosubstrate condition. *Applied Microbiology and Biotechnology*, *78*(3), 543–550. doi: 10.1007/s00253-007-1321-1.

## Further reading

Nysanth, N. S., Meenakumari, K. S., Syriac, E. K., & Subha, P. (2018). Isolation, characterization, and evaluation of pink pigmented facultative methylotrophs (PPFMs) associated with paddy. *International Journal of Current Microbiology and Applied Sciences*, *7*(7), 2187–2210. doi: 10.20546/ijcmas.2018.707.258.

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