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Overview of Methylotrophic Microorganisms in Agriculture

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ABSTRACT

Methylotrophs are bacteria that can utilize single carbon compounds as their sole carbon source. These methylotrophic bacteria are present in the phyllosphere as well as in the rhizosphere. These bacterial populations promote plant growth by solubilizing phosphate, nitrogen fixation, acting as biocontrol agents, biosynthesis of phytohormones, and the production of 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase and they also alleviate negative effects of salinity and are also involved in phytostimulation.

KEYWORDS: *Methylotrophs,1-aminocyclopropane-1-carboxylate, Nitrogen Fixation, Phytostimulation, Biocontrol*

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1 INTRODUCTION

India is an agricultural country. Agriculture directly and indirectly is the largest livelihood provider in India. Agriculture contributes a significant figure to the GDP. Agriculture provides food security and rural employment. But the earth's population is rapidly increasing which is putting a lot of pressure on natural resources. The excessive use of chemicals like pesticides, insecticides, and fertilizers to enhance yield has led to the progressive decline in the soil's fertility & cause water pollution, chemical burn to crops, increased air pollution, acidification of the soil, mineral depletion of the soil, introduction of heavy metals such as cadmium, zinc, lead, chromium, arsenic, etc. To limit the use of these harmful chemicals and progress toward an organic future, emphasis is to be given to sustainable agricultural practices. Sustainable agriculture is the promotion of farming practices in a manner that protects and improves the natural environment, and the social and financial situations of the farmers and

safeguards the fitness and welfare of all farmed species. Biological nitrogen fixation (BNF) is critical for farming sustainably. BNF allows retaining or enhancing the soil fertility via way of means of the usage of nitrogen that's plentiful with inside the atmosphere. Microorganisms in the soil perform the work of nitrogen fixation and phosphate solubilization. It has been demonstrated that the symbiotic association of bacteria with roots of legumes increases the root weight, length, and width, and also these bacteria act as biocontrol agents and limit the growth of pathogens. Bioinoculants are used as an alternative to chemical fertilizers to reduce environmental effects and diseases and promote plant growth. Bioinoculants that contain a consortium of microorganisms have been demonstrated to be more effective in bringing about overall plant growth. Methylotrophs are a major part of this consortium.

Methylotrophs are an important group of bacteria that can grow by consuming reduced carbon compounds containing one or more carbon atoms but do not contain carbon-carbon bonds. Methylotrophs are ubiquitous and found in air, water, soil, and plant. Methylotrophs are a major contributing member of the carbon cycle. Plants provide methylotrophs with habitat as well as compounds including growth substrates. Plant and methylotrophic bacterial interactions improve plant growth and plant health.

2. HISTORY

Methylotrophy as a phenomenon has been recognized since the late 19th century and for the duration of the 20th century, methylotrophy-directed research activities formed a small discipline inside the discipline of microbiology, formalized by a dedicated conference (C1 Conference held in 1978 and it morphed into the Gordon Research Conference format in 1998). (Ludmila Christoserdova et al., 2009)

Earlier there was only a single methylotroph called *Bacillus methanicus,* the only microorganism regarded to be able to survive on methanol or methane as a sole supply of carbon and energy. In the subsequent half-century, a few additions have been made to the listing of regarded microorganisms with methylotrophy. In the subsequent twenty years, diverse new microorganisms have been stated and their widespread distribution, taxonomic range, and physiological range have been described.

3. TYPES

Mixed Substrate	Physiological Function	Type of Organism
Glucose + Methanol	Mixed carbon/energy sources	Facultative methylotroph
Glucose + methane	Mixed carbon/energy sources	Facultative methylotroph
Glucose + methanol	Mixed energy sources	C_1-dissimating non-methylotroph
Methanol + formaldehyde	Mixed carbon/energy sources	Obligate or facultative methylotroph
Methanol + formate	Mixed energy sources	Obligate methylotroph or C_1-dissimating non-methylotroph
Methylamine + ammonia	Mixed nitrogen sources	Methazotroph

Table 1: Types of substrates for methylotrophs (Ryu, Jeong-Hyun, et al. 2006)

Based on nutrition:
Obligate Methylotrophs: Microorganisms that can grow only on single carbon substrates.

Facultative Methylotrophs: Microorganisms that can grow on substrates containing single carbon substrates as well as multicarbon

chemical compounds. Facultative methylotrophs are further divided into several types:

1. Pink Pigmented Facultative Methylotrophs (PPFMs) e.g., *Methylobacteriumpopuli sp.*
2. Non-pigmented "pseudomonads" e.g., *Pseudomonas aminovorans.*
3. Gram-positive facultative methylotrophs e.g., *Bacillus cereus.*
4. Gram-negative, non-motile rods and coccal rods e.g., *Arthrobacter rufescens.*
5. Facultative autotrophs or phototrophs thriving on methanol or formic acid e.g., *Rhodopseudomonas palustris.*
6. Hyphomicrobia e.g., *Hyphomicrobium nitrativorans.*
7. Marine bacteria thatcansurviveon methanol and methylated amines by utilizing enzyme, methanol dehydrogenase, and a few other atypical enzymes e.g., *Methylomonas thalassic.*

4. ISOLATION

Methylotrophs are cosmopolitan and can be isolated from the rhizosphere as well as from the phyllosphere of any plant species. The sample may be collected from soils from the rhizosphere region or leaves can be collected. Hyphomicrobium medium and Minimal Salts agar supplemented with filter-sterilized methanol to act as a carbon source. To prevent contamination from fungus, a fungicide (cycloheximide 20g/ml) must be added to the medium. All isolates can be identified by standard techniques described by Cowan and Steel in the manual of identification of medical bacteria.

5. DISTRIBUTION

Several methylotrophs which include genera *Methylobacterium, Methylophilus, Methylibium, and Hyphomicrobium. Methylobacterium* species represent the main genus among the leaf microbial community. These methylotrophs inhabit the phyllosphere because plants release C1 compounds including methanol and methane. Among phyllospheric microorganisms, methanol-utilizing bacteria, known as pink-pigmented facultative methylotrophs (PPFMs) are the dominant colonizers. The site of the plant greatly affects the microbial community present in the phyllosphere.

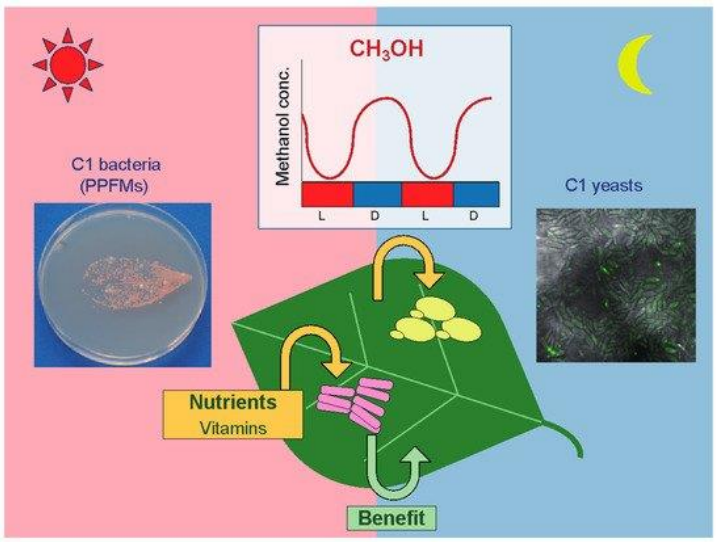

Figure 1: Colonization of methylotrophs which make use of methanol within the phyllosphere wherein methanol concentrations rise and fall diurnally. Bacteria and yeasts that make use of methanol colonize the exterior of plant leaves and gather vitamins produced via photosynthesis. Concentrations of methanol within the phyllosphere rise and fall diurnally, with decreased concentrations within the light period (L) and better concentrations within the darkish period (D). After the leaf printing at the agar medium containing methanol as the only carbon source, pink-pigmented colonies had been observed. *Candida boidinii* cells expressing the fluorescent protein Venus proliferate on *Arabidopsis thaliana* leaves. (Hiroya *et al.*)

6. PLANTGROWTH PROMOTION

Some PPFMs can promote plant growth by producing auxins, cytokinins, and gibberellins, and induce resistance against pathogens and diseases. Methylotrophic bacteria improve plant growth directly by either facilitating nutrient uptake (nitrogen fixation and phosphate solubilization), or phytostimulation and also act as biological control agents.

(a) Nutrient acquisition for plant growth and promotion

Certain methylotrophic bacteria with plant growth-promoting properties can take atmospheric nitrogen and convert it into organic compounds containing nitrogen. Nitrogen is present in the soil in forms like nitrate and ammonium ions which are converted to organic forms.

Jourand *et al.* (2004) reported that *Methylobacterium nodulans* ORS2060 isolated from *Crotalaria* species had the nif H gene which induces nitrogen-fixing nodules on the roots of legumes by the nodA gene.

Phosphate Solubilization: The second most important nutrient whose deficiency restricts plant growth is phosphorus. Phosphorus is present in soils in both organic and inorganic forms. The majority of phosphorus present in the soil is in insoluble forms, while the plants can absorb it only in soluble forms (Bhattacharyya, P.N. & Jha, D.K. 2012). There is a big populace of bacteria that make phosphate soluble within the soil and in rhizospheres thatcanconvertless soluble phosphate to soluble phosphate by emanating organic acids. In this population, *Methylobacterium* species can dissolve inorganic phosphates, which may be further connected to phosphate metabolism in both microorganisms and plants. Nonspecific acid phosphatase, phytase, and C-P lyase (or phosphonatase) are three different types of microbial enzymes that solubilize organic phosphate. All three of these release phosphates, the first from phosphoric ester or phosphoric anhydride, the second from phytic acid, and the third from organophosphates. Kwak et al. (2014) stated that *Methylobacterium oryzae* has genes encoding all the three phosphatases.

(b) Phytostimulation

Phyto-stimulation gets increased by plant hormones or by chemicals which are like plant hormones that promote the growth of roots. Methylotrophic bacteria that can modify the physiology of plants directly generally synthesize and emanate plant hormones. ACCdeaminase is the Phyto-stimulant that has been studied in the greatest detail. It helps in reducing the levels of plant hormone ethylene and indole acetic acid (IAA) production.

(c) Biocontrol

Biological control is the use of naturally occurring substances or natural enemies to inhibit the growth of pests and pathogens. The goal is to control disease and maximize the yield. Biological control is the suppression of phytopathogens by one or more beneficial organisms, which may lead to plant growth promotion either directly or indirectly. Several mechanisms may be involved in this process, including the production of siderophores.

Siderophore production: All cells require iron for various metabolic processes. In environments where oxygen is present, iron is present in the ferric hydroxide complex which is not soluble. In order to intake iron microorganisms use siderophores. Siderophores are low molecular weight extracellular chemicals which have a unique binding capacity for ferric iron. Siderophores make iron unavailable to pathogens (Neilands, J.B. & Leong, S.A., 1986). Since iron becomes unavailable to the pathogens, their growth is inhibited and more iron is available for plants.

HCN production: Cyanide has been verified to inhibit enzymes that participate in primary metabolic processes and cyanide may block electron transport during photosynthesis (Grossman, K. 1996). Several species of bacteria produce cyanide. HCN protects the plant from diseases e.g., control of black rot in tobacco caused by *Thielaviopsis basicola* and root-knot in tomato root caused by *Meloidogyne javanica.* (Siddiqui, Z.A. 2006)

7. FIELD APPLICATION OF METHYLOTROPHIC BACTERIA

Methylotrophs as biofertilizers

Methylotrophs can be used as foliar spray or seed inoculants present alone or in a consortium of microorganisms like *Burkholderia pyrrocinia* CBPB-HOD or *Azospirillum brasilense* CW903. The use of these biofertilizers in combination with chemical fertilizers will also provide better plant growth. Biofertilizers make the soil more fertile, plants more resistant to pests, and increase crop yield.

Rhizobium and *Pseudomonas* support plant growth by providing growth limiting nutrients by solubilizing phosphate, fixing nitrogen from the atmosphere to nitrates and nitrites, and by siderophore production which binds to iron. It has been demonstrated that methylotrophs help in increasing plant growth. The phosphate acquisition and nitrogen fixation abilities of methylotrophs allow them to be used as biofertilizers, but these alone are not enough, they must be used alongside with chemical fertilizers and insecticides.

By applying PPFM and Pseudomonas foliar spray in synergy with biofertilizer boosts the population of microorganisms in soil which increases the amount of nutrients that are available to the plants (Jeyajothi et. al. 2014).

Abd El-Gawad et al. 2015 conducted a field experiment that lasted for two years in different seasons and found out the role of PPFM in the enhancement of antioxidant activities, growth, and yield of snap bean crops. These methylotrophs increased the yield of the crop and also the quality of snap beans.

In sugarcane and cotton, yield can be increased by a foliar spray of PPFMs or methanol (Madhaiyan et al. 2005). In strawberry bushes, spraying or irrigating with methanol or ethanol, or both led to a better quality of strawberries and plant growth (Yavarpanah et al. 2015).

Foliar spray of methanol induced the growth of methylotrophs in the phyllosphere, which increased the production of plant growth hormones such as cytokinin and auxins (Ivanova et al. 2001). Along with hormones, it also increased the levels of leaf chlorophylls, carotenoids, fruit sugars, and crop yield. The growth of the red chilli plant can be stimulated by the collaborative effect of phyllospheric spray of methanol and bacterium (*M. oryzae* CBMB20) inoculated in the rhizosphere (Chauhan et al. 2010). Madhaiyan et al. (2006a, b) stated in their report that the application of methanol in the phyllosphere resulted in PPFM-mediated plant growth hormone induction in cotton and sugarcane, leading to better yields. After *M. oryzae* CBMB20 inoculation within the rhizosphere with phyllospheric application of methanol, an increase in plant growth and booming crop yields in red pepper plants were reported.

The amount of fertilizer whether used in low quantity or high had no significant effect on yield, thus, the amount of fertilizer used can be lower than what generally is used without the inoculation of *M. oryzae* (Chauhan et al., 2010).

In an experiment on mung beans, the utility of salt-tolerant *Methylobacterium organophium* was examined as a potential plant growth promoter and biofertilizer (Rekadwad, 2014). These biofertilizers can be employed to protect the plants from excessive heat and under circumstances of drought (ICAR, 2013).

The methylotrophic bacteria make use of methanol that is secreted in the phyllosphere as their carbon source, and they release essential nutrients such as phytohormones, ACC deaminase, and nitrogen, which directly act as plant growth promoters and help in the survival of plants under stressful conditions (ICAR, 2013).

8. CONCLUSION

Methylotrophs live in the soil, water, rhizosphere, and phyllosphere. Plants provide methylotrophs with a habitat to live in and with single carbon compounds. Methylotrophs are used as bio inoculants, and their usage is reducing our dependency on chemical fertilizers. Methylotrophs promote plant growth by phytohormone production, nodulation,

nitrogen fixation, and phosphate solubilization. Methylotrophs also produce ACC deaminase which helps with salt tolerance and these methylotrophic bacteria also act as biocontrol agents. These methylotrophic bacteria can be used as an individual inoculant or they can be used in a consortium of the microbial population which can be formulated in capsules or tablets acting as plant growth promoters and leading to sustainable agricultural systems. More and more use of bio inoculants will reduce the use of chemical fertilizers which pollute the soil and water resources leading to a better ecosystem.

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