Agri Articles
(e-Magazine for Agricultural Articles)
Volume: 03, Issue: 02 (MAR-APR, 2023)
Available online at http://www.agriarticles.com
©Agri Articles, ISSN: 2582-9882

Pink Pigmented Facultative Methylotrophs (PPFMS)

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The living space on the leaf surface is called phyllosphere, is home to a diverse range of organisms that have beneficial, harmful, or neutral effects on the plant. The interaction of such microorganisms with higher plants has an impact on the plant's physiological activities. Pink pigmented facultative methylotrophs (PPFMs) of the genus *Methylobacterium* are frequently found in plant communities. They are thought to potentially dominate the phyllosphere bacterial population. The plant *Methylobacterium* association ranges from strong or symbiotic to loose or epiphytic, including the intermediate endophytic association. *Methylobacterium* spp. is distinguished by a distinct pink pigmentation caused by the presence of carotenoid pigment.

Plant leaf surfaces release diverse carbon sources, mainly sugars and organic acids, at low amounts (µM range), and these sources are heterogeneously located and result of leaching through the cuticle. In addition to these substrates, volatile carbon substrates, particularly the plant cell wall metabolism byproduct methanol, are released via the stomata. Pink-pigmented facultative methylotrophic bacteria (PPFMs) consume volatile C1 compounds produced by growing plants during cell division. In return, they can positively affect plant growth and survival by crop seed germination, yield, pathogen resistance, and drought stress tolerance, PPFMs have received a lot of attention in agricultural systems. They are aerobic, Gram-negative, methylotrophic rod-shaped bacteria that can grow on a variety of multicarbon substrates like formate, formaldehyde, and methanol as well as also on a variety of C2 C3 and C4 compounds. Several *Methylobacterium* species and strains have been isolated from plants including liverworts, mosses, angiosperms, and gymnosperms. They are isolated using the leaf impression method on a methanol-based mineral medium, supplemented with 0.5% methanol and cycloheximide at 100 mg L^{-1} (to inhibit fungal growth). According to many reports, PPFMs can act as potential plant growth promoters and also aid in plant survival from pathogenic attack. Plant growth regulators such as zeatin and related cytokinins and auxins have been reported to be produced by them, and they have a significant effect on seed germination and seedling growth. *Methylobacteria* have previously been reported to produce gibberellic acid (GA) Furthermore, *Methylobacterium* has been reported for nitrogen fixation, nodule formation, phosphate solubilization, siderophores synthesis, urease enzyme, vitamin B12, and the prevalence of ACC deaminase enzyme.

Drought mechanisms by PPFM

Drought causes plants to produce more ethylene and reactive oxygen species, as well as increase the frequency of stomata closure and membrane damage, resulting in yield loss. Methylotrophic bacteria produce 1-aminocyclopropane-1-carboxylate (ACC) deaminase that stimulate the production of antioxidant enzymes and osmolytes in plants, which aids in the prevention of drought stress.

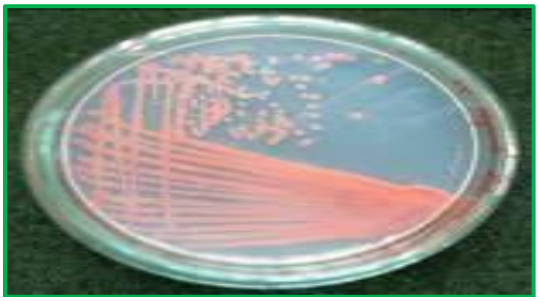
ACC Deaminase and hormone production: In plants, ethylene disrupts a number of processes involved in plant growth and yield and plays an important role in plant biotic and abiotic responses. Increased ethylene concentrations in plants have negative consequences. Ethylene influences photosynthesis and stomatal conductance, as well as interacting with other plant hormones such as auxin and abscisic acid. Plants treated with PPFMs produce the enzyme 1-aminocyclopropane-1-carboxylate (ACC deaminase), which converts ACC to ammonia and alpha ketobutyrate. Under stress conditions, ethylene production in plants is reduced due to the bacterial ACCdeaminase enzyme. Furthermore, it is capable of producing phytohormones such as auxins, cytokinins, and gibberellic acid, which promote cell division in shoots and roots.

Stomatal conductance: Drought stress causes abscisic acid (ABA) production, which causes stomatal closure and the accumulation of compatible solutes. Stomata closure is controlled by PPFMs in both direct and indirect ways. Volatile compounds, or microbial-associated molecular pattern (MAMP), activate plant defense-related plant hormones like salicylic acid (SA) and jasmonic acid (JA), and cause ABA-independent stomatal closure via the nitric oxide (NO) and open stomata 1 (OST1) signal cascade. The indirect method reduces the negative effect of ethylene on ABA in drought stress by producing PPFM from ACC deaminase.

Prevention of ROS accumulation: Under stress, plants produce reactive oxygen species (ROS), which react with proteins, lipids, and deoxyribonucleic acid (DNA), causing oxidative damage and impairing plant function. Plants develop antioxidant defence systems to counteract the negative effects of ROS, which include both enzymatic and non-enzymatic components that serve to prevent ROS accumulation and mitigate oxidative damage caused by drought stress. PPFM spray stimulates the synthesis of various antioxidants (superoxide dismutase, catalase, ascorbate peroxidase, and glutathione reductase) and non-enzymatic (cysteine, glutathione, and ascorbic acid) components in plants that protect against ROS and thus relieves drought stress.

Isolation of pink pigmented facultative methylotrophs (PPFMs): Ammonium Mineral Salts (AMS) medium is a selective medium for isolation of methylotrophs. The AMS medium was sterilized by autoclaving at 121°C for 15 min and cooled to 45°C. Filter sterilized vitamin solution along with 0.5 per cent (v/v) methanol was added after sterilization and before pouring media on to petriplates. The pH of the medium was adjusted to pH 7.0. On the solidified AMS agar medium upper and lower surface of leaf samples were placed separately, in such a way as to make impression of it. Then the leaves were lifted away and plates were incubated at 30^0C for 7 days. Based on characteristic pink pigmentation of colonies they were tentatively identified as PPFMs.

Purification of Pink Pigmented Facultative Methylotrophs (PPFMs): PPFMs obtained by leaf imprint technique were purified by the streak plate method and well isolated colonies on the plates were preserved on Peptone Glycerol Agar (enrichment medium) slants at 4°C in a refrigerator for further use.



Beneficial effects on plant

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

Method of application

- Seed treatment 1%
- Foliar spray 1%PPFM
- Spray during morning or evening
- Recommended for all crops
- Spray at critical stages of crop growth
- Foliar application 1000ml/acre