Plant Phyllo-Sphere Facultative Methylotrophs (PPFM) Kannan Periyan^a, Rengasamy Parthasarathi^{a,b*} and Krishnamoorthi Akash^a

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Abstract

Plant phyllo-sphere facultative methylotrophs (PPFM) are a remarkable group of microorganisms capable of utilizing both multi-carbon compounds and single-carbon (C1) compounds like methanol and methylated amines as sources of carbon and energy. These taxonomically diverse bacteria and fungi, belonging to groups such as Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Ascomycota, and Basidiomycota, colonize the phyllosphere - the aerial parts of plants. Within this niche, they play crucial roles in biogeochemical cycling and promoting plant growth and stress tolerance. PPFM employ metabolic pathways like the ribulose monophosphate cycle to assimilate C1 compounds. As epiphytes, they contribute to nutrient cycling, producing phytohormones (auxins, cytokinins), solubilizing nutrients, fixing atmospheric nitrogen, and inducing plant defense mechanisms, thereby enhancing plant growth and vield. Additionally, PPFM confer tolerance to abiotic stresses through the production of osmoprotectants, antioxidants, and stress-responsive compounds. Genomic studies have revealed the genetic diversity of PPFM, shedding light on genes involved in C1 metabolism, stress response, plant colonization, and secondary metabolite production. This knowledge facilitates genetic engineering approaches to harness their biotechnological potential. PPFM show immense promise as biofertilizers and plant growth promoters in sustainable agriculture, with commercial bioinoculants already available and demonstrating efficacy in field trials, including co-inoculation with beneficial microbes. While substantial progress has been made, further research is needed to fully understand PPFM ecology, elucidate the molecular mechanisms underlying their beneficial effects, and optimize their application strategies. Harnessing the potential of PPFM could contribute to the

development of resilient and productive cropping systems while minimizing reliance on synthetic inputs and promoting environmental sustainability.

Introduction

The plant phyllosphere, a term encompassing the aerial parts of plants including leaves, stems, and reproductive structures, is a dynamic and intricate ecosystem harboring a remarkable diversity of microorganisms. Among this microbial community, a remarkable group known as facultative methylotrophs has garnered significant attention due to their unique metabolic capabilities and ecological significance. Facultative methylotrophs are microorganisms that possess the remarkable ability to utilize both multi-carbon compounds and singlecarbon (C1) compounds, such as methanol and methylated amines, as sources of carbon and energy. This metabolic versatility allows them to thrive in the often resource-limited conditions of the phyllosphere, where they play pivotal roles in various ecological processes.

The phyllosphere provides a suitable habitat for facultative methylotrophs due to the abundance of plant-derived compounds, including sugars, organic acids, and amino acids, as well as the presence of C1 compounds released by plants during growth and development. This unique environment facilitates the coexistence and interactions between facultative methylotrophs and their plant hosts, resulting in a complex and dynamic ecosystem. Facultative methylotrophs in the phyllosphere belong to diverse taxonomic groups, including bacteria and yeasts. Some of the most well-known and extensively studied genera include Methylobacterium, Methylorubrum, Methylobacillus, and Candida. These microorganisms exhibit remarkable diversity in their physiology, metabolism, and ecological roles, reflecting their adaptations to the specific conditions of the phyllosphere.



The distribution of facultative methylotrophs in the phyllosphere is influenced by various factors, plant species, such as developmental stage, environmental conditions, and geographic location. Understanding the patterns of diversity and distribution is crucial for unraveling the ecological significance of these microorganisms and their potential applications in areas such as agriculture, bioremediation, and biotechnology. Within the phyllosphere, facultative methylotrophs contribute to several key ecological processes. One of their most notable roles is their involvement the in biogeochemical cycling of carbon and nitrogen. Through their ability to metabolize methanol and methylated amines, they play a crucial part in the cycling of these compounds, which are released by plants during growth and development. Additionally, some facultative methylotrophs possess the capacity to fix atmospheric nitrogen, enhancing the availability of this essential nutrient for plant growth.

Furthermore, facultative methylotrophs in the phyllosphere have been shown to promote plant growth through various mechanisms, such as the production of phytohormones, siderophores, and other beneficial compounds. They can also help protect plants against pathogens by producing antimicrobial compounds or inducing systemic resistance mechanisms in the host plant, thereby contributing to plant health and productivity. The unique metabolic capabilities and ecological roles of facultative methylotrophs in the phyllosphere have sparked significant interest in their potential applications. These microorganisms have been explored for their use in bioremediation, biodegradation of pollutants, and the production of value-added compounds like enzymes, biosurfactants, biopolymers. Additionally, the study and of facultative methylotrophs in the phyllosphere has implications for understanding plant-microbe interactions, developing sustainable agricultural practices, and mitigating the effects of climate change.

Methylotrophs are a diverse group of microorganisms capable of utilizing one-carbon (C1) compounds such as methanol, methane, and methylamine as their sole source of carbon and energy. Among these, facultative methylotrophs, particularly those residing in the plant phyllosphere, have garnered significant attention due to their potential applications in agriculture and environmental management. The plant phyllosphere, which comprises the above-ground portions of plants, is a unique ecological niche that harbors a diverse community of microorganisms, including plant phyllo-sphere facultative methylotrophs (PPFM).

The Metabolism and Diversity of Methylotrophs

Methylotrophs are taxonomically diverse, spanning various bacterial and fungal phyla. Bacterial methylotrophs are primarily found within the Alpha-, Beta-, and Gammaproteobacteria classes, with representatives from genera such as Methylobacterium, Methylocystis, and Methylomonas. Fungal methylotrophs, on the other hand, belong to the phyla Ascomycota and Basidiomycota, with prominent genera including Pichia and Candida.

Methylotrophs employ various metabolic pathways to assimilate C1 compounds, with the ribulose monophosphate (RuMP) and serine cycles being the most prevalent. The RuMP cycle is predominant in facultative methylotrophs, while the serine cycle is more common in obligate methylotrophs. These pathways involve a series of enzymatic reactions that ultimately incorporate C1 units into central metabolic pathways for energy generation and biomass production.

Methylotrophic Communities in the Phyllosphere

The phyllosphere, which includes the leaf surface, is a nutrient-rich environment that supports a diverse microbial community. Methylotrophs, particularly PPFM, are well-adapted to thrive in this niche due to the presence of various C1 compounds derived from plant metabolism and atmospheric sources. Methylobacterium, Methylorubrum, and Methylovorus are among the prominent genera of PPFM found in the phyllosphere.

Epiphytic PPFM in the Phyllosphere

Epiphytic methylotrophs are those that colonize the surfaces of plant leaves and stems. These microorganisms play crucial roles in the phyllosphere, contributing to nutrient cycling, plant growth



promotion, and stress tolerance. Epiphytic PPFM methylotrophs, such as Methylobacterium spp., are well-adapted to the phyllosphere environment, exhibiting traits like pigment production, biofilm formation, and the ability to utilize various plant-derived C1 compounds.

Genomics of PPFM

Advances in genomics have provided valuable insights into the genetic diversity and metabolic capabilities of PPFM bacteria. Comparative genomic studies have revealed the presence of genes and gene clusters involved in C1 metabolism, stress response, plant colonization, and the production of secondary metabolites. Furthermore, the availability of genome sequences has facilitated the development of genetic engineering tools for PPFM, enabling the exploration of their biotechnological potential.

Genetic Diversity of Methylotrophs

Methylotrophs exhibit remarkable genetic diversity, reflecting their ability to adapt to diverse environments and utilize a wide range of C1 compounds. This diversity is evident in the diversity of functional genes involved in C1 metabolism, as well as in the presence of mobile genetic elements, such as plasmids and genomic islands. This genetic diversity contributes to the versatility and resilience of methylotrophic communities, enabling them to thrive various ecological including in niches, the phyllosphere.

Plant Growth Promoting Methylotrophs

PPFM have been extensively studied for their plant growth-promoting abilities, which are mediated through various mechanisms. These include the production of phytohormones, such as auxins and cytokinins, nutrient solubilization, biological nitrogen fixation, and the modulation of plant stress responses. Several PPFM strains have been reported to enhance plant growth, yield, and biomass under both controlled and field conditions, making them promising candidates for agricultural applications.

Phytohormones Production by PPFM

Phytohormones are signaling molecules that regulate various aspects of plant growth, development, and stress responses. Many PPFM strains have been shown to produce phytohormones, such as indole-3-acetic acid (IAA), cytokinins, and gibberellin. The ability to produce these phytohormones contributes to the plant growthpromoting effects of PPFM, as they can positively influence processes like cell division, root elongation, and shoot development.

PPFM as Bioinoculant

The application of PPFM as biofertilizers has gained considerable interest due to their potential to enhance nutrient availability and uptake for plants. PPFM strains have been reported to solubilize phosphates, fix atmospheric nitrogen, and produce siderophores, which are iron-chelating compounds that facilitate iron acquisition by plants. Additionally, PPFM can promote nutrient cycling through the breakdown of organic matter, contributing to the overall fertility and productivity of agricultural systems.

PPFM in the Nitrogen Metabolism

Nitrogen is an essential nutrient for plant growth and development, and PPFM play a crucial role in nitrogen metabolism within the phyllosphere. Some PPFM strains possess the ability to fix atmospheric nitrogen through the action of the nitrogenase enzyme complex, while others are involved in the cycling of nitrogen through processes like denitrification and ammonification. Additionally, PPFM can influence plant nitrogen metabolism by producing phytohormones and other signaling molecules that modulate nitrogen uptake and assimilation pathways.

PPFM as Co-inoculants

The application of PPFM as bio-inoculants has shown promising results in promoting plant growth, yield, and stress tolerance. Several commercial PPFMbased bio-inoculants are available for various crop species, and their efficacy has been demonstrated in field trials. Furthermore, PPFM can be co-inoculated with other beneficial microorganisms, such as rhizobia and arbuscular mycorrhizal fungi, to enhance their combined plant growth-promoting effects through synergistic interactions.



Role of PPFM in Abiotic Stress Tolerance

Abiotic stresses, such as drought, salinity, and temperature extremes, pose significant challenges to crop productivity worldwide. PPFM have been explored as potential bio-inoculants to enhance plant tolerance to these stresses. Several mechanisms are production involved, including the of osmoprotectants, antioxidants, and stress-responsive phytohormones, as well as the modulation of plant stress signaling pathways. PPFM strains have been shown to improve plant performance under various abiotic stress conditions, making them valuable tools for sustainable agriculture in the face of climate change and environmental challenges.

Conclusion and Future Prospects

Plant phyllo-sphere facultative methylotrophs (PPFM) represent a diverse and fascinating group of microorganisms with immense potential for applications in agriculture and environmental management. Their ability to utilize C1 compounds, promote plant growth, enhance nutrient availability, and confer stress tolerance makes them attractive candidates for the development of bio-inoculants and biofertilizers. However, further research is needed to fully understand the complex interactions between PPFM, plants, and the phyllosphere environment, as well as to optimize their application strategies.

Future directions in PPFM research may include exploring the genetic and metabolic diversity of these microorganisms through advanced genomic and metabolomic approaches, elucidating the molecular mechanisms underlying their plant growthpromoting and stress tolerance effects, and developing efficient formulations and delivery systems for field applications. Additionally, integrating PPFM with other beneficial microorganisms, such as rhizobia and mycorrhizal fungi, could lead to synergistic effects and enhanced agricultural productivity.

As the world faces increasing challenges related to food security, environmental sustainability, and climate change, the exploration and utilization of PPFM hold great promise for developing eco-friendly and sustainable agricultural practices. By harnessing the potential of these remarkable microorganisms, we can contribute to the development of resilient and productive cropping systems, while minimizing the reliance on synthetic inputs and promoting environmental stewardship.

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