Review

PINK PIGMENTED FACULTATIVE METHYLOTROPHIC BACTERIA: COMMON YET UNEXPLORED LOCALLY

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Ubiquity seems to be the appropriate adjective to describe pink-pigmented facultative methylo trophic (PPFM) bacteria. Their cosmopolitan distribution could be explained by two properties of this group, namely, pigmentation and facultative methylo trophy. PPFM bacteria when cultivated in the laboratory mostly produce pink to orange small sized colonies in solid media or appear as pinkish pellicle in a stationary liquid media.

PPFM bacteria yield a gram negative to gram variable reaction. Gram variability may be due to the observation that they produce sudanophilic cysts, which are believed to be stores of polyhydroxybutyrate. Moreover, PPFM bacterial strains may yield rod-shaped cells, but often times forming branching morphology. Ambiguity in the gram reaction as well as cell morphology stirred difficulty and confusion in the taxonomy of PPFM bacteria. Green (2001) provided a comprehensive historical review narrating how PPFM bacteria are shifted from one genus to another. Initially, PPFM bacteria were assigned to the genus Bacillus when Bassalik reported the first strain in 1916. Since then, further studies of the reported strain, as well as discovery of new PPFM bacteria led investigators to assign PPFM bacteria to several genera such as Vibrio, Pseudomonas, Flavobacterium, Protaminobacter, Mycoplana and Protomonas. Through the taxonomic work of Green and Bousfield in 1983, all known pink pigmented facultative methylo trophic bacteria are assigned to the genus Methylobacterium. Phenotypic properties such as strictly aerobic rod-shaped cells yielding positive catalase and urease tests, and weakly positive oxidase test, as well as capability to utilize or assimilate C1 compounds can be assigned to the genus Methylobacterium. Subsequent studies involving DNA-DNA similarity, electrophoretic comparison of total soluble proteins, analysis of fatty acid composition of cell membrane among reported strains recognizes Methylobacterium as a single, but quite heterogenous taxon, which can be clearly distinguished from other methylo trophic and non-methylo trophic genera.

Anesti et al. (2004) recognize that there are fifteen known species under the genus Methylobacterium, which can be differentiated by their ability to use various compounds as sole sources of carbon and energy. Table 2 summarizes the characteristics of the fifteen known species.
Table 1: Carbon assimilation patterns of the fifteen validated species of Methyloabacterium (Anesti et al., 2004)

<table>
<thead>
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<th>Compound</th>
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</tr>
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1 M. podarum, 2 M. thiocyanatum and M. rhodinum, 3 M. dichloromethanicum, 4 M. extorquens, 5 M. suomiense, 6 M. lusitanum, M. rhodesianum and M. zaitmanii, 7 M. aminovorans, 8 M. chlormethanicum, 9 M. organophilum, 10 M. mesophilicum, 11 M. radiotolerans and M. fujisawaense

PHYLOGENETIC STUDIES ON PPFM BACTERIA

Several studies were done to establish the genetic relatedness among PPFM bacteria as well as with other known bacteria. Bulygina et al. (1990) compared 5S rRNA sequences of 19 strains of gram-negative methylotrophic bacteria. Their analysis revealed that these bacteria are genetically related to the Proteobacteria and fell into several clusters, including obligate methanotrophs, obligate methylotrophs and several groups of facultative methylotrophs. Their results suggest that PPFM bacteria could be a heterogenous group.

The study by Bratina et al. (1992) compared the 16S rRNA sequences of PPFM bacteria with other gram-negative bacteria. Results of their analysis showed that PPFM bacteria are closely related to members of the alpha (α) subdivision of the class Proteobacteria. Furthermore, the unique sequences within the 16S rRNA can distinguish two major physiological groups of methylotrophs namely, those that employ the serine pathway methanotrophs and methylotrophs, as well as those that utilize the ribulose monophosphate pathway.

Hiriashi et al. (1995) compared the 16S rDNA sequences of 6 strains of PPFM bacteria with known Methyloabacterium species. Their study revealed that their isolates fell into several clusters with other known species. Such results mirrored the diversity of PPFM bacteria.

METABOLISM OF PPFM BACTERIA

The pigmentation of PPFM bacteria is due to the carotenoids that play some protective role against highly destructive radicals when exposed to radiation or
illumination from the sun. The ability of PPFM bacteria to withstand illumination allows them to be phototrophic under certain conditions (Hiriashi et al., 1995). However, in most situations, PPFM bacteria employ chemical oxidation, rather than light as energy source. Hence, they are chemotrophic most of the time. The chemical compounds to be oxidized tapped by PPFM bacteria to yield energy may be one-carbon compounds such as formate, methanol, methylamine, methylsulphate. The utilization of C1 compounds as energy source are referred to as methylothrophy. The ability of PPFM bacteria to utilize methanol and other related C1 compounds is due to their innate capacity to convert methanol into formaldehyde. Formaldehyde serves as a central intermediate that can either be further oxidized to formic acid and eventually, carbon dioxide (CO₂) to yield energy, or channeled into the serine-glycine pathway or ribulose monophosphate pathway for carbon assimilation and energy production.

In the serine-glycine pathway, formaldehyde reacts with glycine to yield serine. The resultant amino acid undergoes a series of chemical reactions that would lead to the formation of acetyl-Coenzyme A. Such substance served as an intermediate for the Krebs or citric acid cycle for energy generation or for the synthesis of cellular material. Most of the known PPFM bacteria employ the serine-glycine pathway for formaldehyde assimilation (Brusseau et al., 1994).

In the ribulose monophosphate pathway, formaldehyde reacts with ribulose 5-phosphate that leads to the formation of sugars that serve as precursor material for cellular synthesis. Some amounts of ATP are also produced in this pathway. Such pathway is more conserved, as all carbons for cellular material synthesis are totally derived from methanol. A total of three molecules of methanol are required to complete each cycle of the pathway. It is worth noting that the substrate for the initial reaction in this pathway, ribulose 5-phosphate is almost identical to the C1 acceptor of the Calvin cycle found in photosynthetic, autotrophic microorganisms. Such observation poses some evidence of phylogenetic relatedness of methylothrophic bacteria with the autotrophs (Madigan et al., 2000).

It was discovered by Vorholt (2001) and Choncharoee et al., (2005) that PPFM bacteria have several enzymes that detoxify formaldehyde, which is a toxic central intermediate of the pathway.

**PPFM BACTERIA ASSOCIATED WITH PLANTS**

Interest in PPFM bacteria among researchers and scientist stems from the potential application of these bacteria in agriculture to increase crop yield. Most of the reported PPFM strains were derived from leaf surfaces of plants. These strains have been shown to produce phytohormones, such as cytokinins and auxins (Long et al., 2000; Ivanova et al. 2000; 2001; Lidstrom and Chistoserdova, 2002). Hence, they can stimulate seed germination and enhance plant growth. Scientific investigations focused on elucidating the physiology and genetics behind the production of these plant hormones in these bacteria. Koenig et al. (2002) demonstrated that cytokinin production in PPFM bacteria is through the
modification of selected tRNA via isopentyl transferase. Such ability is due to the presence of miaA gene, which encodes for the enzyme isopentyl transferase. It is worthwhile to note that higher plants generate cytokinin in a similar mechanism. The mechanism and form of cytokinin produced by PPFM bacteria are different from the ones employed by plant pathogens such as Agrobacterium tumefaciens. The form of cytokinin by PPFM bacteria is in the trans configuration, such as trans-zeatin riboside, through isopentylation of selected tRNA. Whereas, plant pathogens produced cytokinin in the cis conformation, through the isopentylation of AMP catalyzed by the enzyme, dimethylallyl transferase (DMAT).

It is worthwhile to note that the cytokinin produced by PPFM bacteria is not plant species specific. Holland and Pollaco (1994) showed that PPFM bacteria isolated from Arabidopsis, maize and barley enhanced seed germination and growth of heat-treated soybeans. Moreover, several investigators demonstrated that the cytokinin from Methylobacterium extorquens, isolated from soil, promoted the growth of heat treated soybeans (Koenig et al., 2002) as well as other plant tissue culture system (Long et al., 2000).

The mechanism of auxin production in PPFM bacteria may involve breakdown of the amino acid tryptophan, and modification of metabolites such as pyruvic acid, butyric acid and acetic acid (Ivanov et al., 2001).

Aside from phytohormones, PPFM bacteria also produce vitamin B12 that can promote plant growth. In the review by Hirano and Upper (2000), they mentioned that the growth of liverwort, Scapania nemorosa and Streptocarpus prolixus, a flowering plant, were enhanced when these plants were contaminated with PPFM in tissue culture system. Further experimentation revealed that PPFM bacteria yielded vitamin B12, which promotes the growth of these plants.

Methylotrophic bacteria are not restricted to leaf surfaces of plants. Whipple et al. (2005) reported that Methylobacterium strains have been localized as endosymbionts within cells in the buds of the Scotch pine Pinus sylvestris. Van Aken (2004) reported isolating a PPFM bacterium, Methylobacterium populi, from the internal tissues of Populus deltoides nigra DN34. Journad et al. (2004) isolated a non-pigmented facultative methylotrophic strain that can form root nodules in Crotalaria legume species. Their study also provided evidences that such strain is capable of nitrogen fixation.

In addition to promoting plant growth, PPFM bacteria may help prevent diseases among crops by inducing systemic resistance to plant pathogens. In the study by Madhaiyan et al. (2004), it was demonstrated that rice seeds inoculated with Methylobacterium sp. strain PPFM-Os07 yielded increased plant height, number of tillers, plant biomass and grain yield. Furthermore, Methylobacterium inoculation of rice seeds tends to alter the plant susceptibility to Rhizoctonia solani, a fungal pathogen of rice. The inoculation of PPFM bacterial strain to rice seeds induced the production of pathogenesis-related proteins (PR-proteins) such as phenylalanine lyase, peroxidase, chitinase, β-1,3 glucanase and phenolics. These
substances are considered defense enzymes that reduced the susceptibility to *Rhizoctonia* infection.

PPFM bacteria can also improve the quality of crops. According to Lidstrom and Chistoserdova (2002), the presence of specific PPFM strain of *Methyllobacterium* spp. tends to add flavor to strawberries.

The predominance of PPFM bacteria in leaves of plants may be due to their ability to utilize methanol emitted from the stomata of leaves of plants as carbon source. (Holland *et al*., 2000). Methanol emission occurs during the dissolution of the cell wall of plants during plant growth. The ability of PPFM bacteria to utilize methanol is due to methanol dehydrogenase, enzyme involved in the oxidation of methanol. Furthermore, PPFM bacteria also utilize urea released during protein degradation during plant growth as nitrogen source. PPFM bacteria were observed to generate the enzyme, urease that facilitates them to utilize urea as nitrogen source. The carotenoid pigments responsible for the pink color of PPFM bacteria serve as photoprotective agents (Fasim *et al*., 2003), as these bacteria thrive on the leaf surfaces, which may be exposed to the sun. The ability of PPFM bacteria to be exposed in illuminated areas enables them to exhibit phototrophy. Furthermore, Hiraiashi *et al.* (1995) demonstrated that some strains yield chlorophyll *a* under aerobic condition, and have photochemical reaction center similar to that found in purple phototrophic bacteria.

**PPFM BACTERIA FROM NON-PLANT SOURCES**

PPFM bacteria are not only associated with plants. They can be found in almost anywhere. Their ability to utilize several C1 compounds as well as other organic compounds makes them cosmopolitan in distribution. Moreover, most PPFM bacterial strains can tolerate certain degree of desiccation and to scavenge trace amounts of nitrogen and carbon. These properties enable them to thrive in a wide range of habitat. In fact, the very first reported PPFM bacterial isolate was derived from contents of earthworm and was named *Bacillus extorquens* by Bassalik in 1913 (Green, 2001). Despite the ubiquitous nature of PPFM bacteria, extensive isolation and studies on this group of bacteria were not done until the 1960’s and 1970’s, when scientists realized their potential commercial importance.

**Soil as Source of PPFM Bacteria**

Soil serves as haven for various kinds of microorganisms, and PPFM bacteria are no exceptions. In fact, Doronina *et al.* (2002) reported two novel species of PPFM bacteria, namely *Methyllobacterium suomiense* and *Methyllobacterium lusitanum* isolated from soil in Finland and sewage samples in Portugal, respectively.

There are several plausible explanations why PPFM bacteria are present in the soil. PPFM bacteria may be derived from the surrounding plants or are inher-
ent to the soil. Some researchers, however, contend that PPFM bacteria isolated from plant surfaces may be due to dust or soil particles deposited on them by the wind. Thus, some PPFM bacteria isolates from leaf surfaces reflect the bacterial population in the surrounding soil (Green, 2001). Soil and leaf litters exposed to vehicular emissions in urban areas may also contain PPFM bacteria. These bacteria may have the ability to utilize aliphatic hydrocarbons, and may serve as good pollution indicators (Green, 2001).

Eller and Frenzel (2001) described the microbial structure of wetland soil in rice cultivation. It was observed in their study that there was a dramatic increase in methane emission due to increase in rice cultivation globally. Such increase could favor activities of methanotrophic and methylotrophic bacteria, which reduce the amount of methane in the global atmosphere.

Water as Source of PPFM Bacteria

Practically any environment that contains water may harbor PPFM bacteria. They may be encountered in any body of fresh water environment containing dissolved oxygen, including aquatic sediments, potable water supplies, bathrooms and washstands, where they appear as pink ropy masses of growth. Hiriashi et al. (1995) were able to isolate and characterize various PPFM bacterial strains isolated from drinking water that were chlorine resistant. Furuhata et al. (2006) isolated 58 PPFM bacteria from tap water in Japanese hospitals and were found to be multiple drug resistant. Gallego et al. (2005) reported two novel species of PPFM bacteria namely, Methylobacterium hispanicum and Methylobacterium aquaticum from drinking water of a municipal water supply in Spain. PPFM bacteria may also be isolated from the interfaces between fuel and water, such as those that might occur in storage tanks for middle distillate, fuel-oils for heating or diesel engines. These bacteria may form part of the biofilm-or cohesive mats, which may present potential problems for filters and injectors, that may lead to engine failure (Whipple et al., 2005).

PPFM Bacteria as Part of the Human Flora

The works of Anesti et al. (2004, 2005) demonstrated that PPFM bacteria could be part of the normal human flora, specifically in the feet and mouth. The human feet may contain bacteria such as Brevibacterium linens, which have been shown to yield a variety of methylated sulphur compounds from the breakdown of amino acids (such as methanethiol from methionine). These compounds serve as carbon and energy source for methylotrophic bacteria. Anesti et al. (2004) reported several methylotrophic bacteria from the feet of subject volunteers, and were able to isolate a novel species with the proposed name Methylobacterium podarium.

The human buccal cavity has been found to be a rich source of methylotrophic bacteria. This is due to the observation that the human mouth is a constant source of volatile one-carbon compounds, including malodorous methylated sulfides such
as methanethiol. Bacteria found in the interdental areas and the tongue, liberate these compounds that are acted upon by methylotrophs. Anesti et al. (2005) were able to isolate and identify through PCR seven *Methylobacterium* strains as well as other methylotrophic bacteria.

Although PPFM bacteria may be part of the normal flora, they can also be opportunistic pathogens. Gilardi and Faur (1984) and Gilchrist et al. (1986) were able to isolate PPFM bacteria from clinical specimens, including blood from AIDS patient. Lee et al. (2004) also demonstrated PPFM bacteria can cause urinary tract infections among immunocompetent patients.

**POTENTIAL BIOTECHNOLOGICAL APPLICATIONS OF PPFM BACTERIA**

Considering the diverse metabolic features of reported PPFM, there are several biotechnological applications that can be harnessed from them. The phytohormones such as cytokinins and auxins (Long et al., 2000; Ivanova et al. 2000; 2001), derived from PPFM associated with plants, can be harnessed to improve crop yield in agriculture. The carotenoid pigments produced by these PPFM are potential colorant in food industries (Green, 2001). Furthermore, the ability of some strains to metabolize long chain aliphatic hydrocarbons and thrive in polluted environment, make them potential pollution indicators (Green, 2001). PPFM bacterial strains in polluted waters may be employed for bioremediation (McDonald et al., 2001). The sudanophilic cysts observed in some PPFM strains are stores of polyhydroxybutyrate, a raw material for the synthesis of biodegradable plastics.

Despite the economic and clinical importance of these eubacteria, there are limited studies on this bacterial group in the country. Moreover, there could be novel species of PPFM in our local environment waiting to be explored. Thus, studies are needed to answer the dearth of information regarding local PPFM strains.

**LITERATURE CITED**


Van Aken, B., C.M. Peres, S.L. Doty, J.M. Yoon and J.L. Schnoor. 2004. *Methylobacterium populi* sp. nov., a novel aerobic, pink pigmented, Facul-
