

**PINK PIGMENTED FACULTATIVE METHYLOTROPHIC  
(PPFM) BACTERIA ISOLATED FROM THE HAIR SCALP  
AND NASAL CAVITY**

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**ABSTRACT**

A total of 10 PPFM bacteria isolates from the hair scalp and nasal cavity of subject volunteers was described in terms of morphological and biochemical characteristics. Isolates generate pink, small-sized, round, raised, entire and opaque colonies when cultivated in minimal salt medium with 0.5% methanol. Microscopic morphology revealed that the isolates were Gram-negative bacilli. All isolates yielded positive in urease, catalase and oxidase tests. Phenotypic characteristics conform to the features of genus *Methylobacterium*. Phylogenetic analysis using its 16S rDNA sequence revealed that three isolates are identified as *Methylobacterium rhodesianum* while seven are identified to be only *Methylobacterium sp.* Some PPFM bacteria isolates obtained in the study may be potential novel sequences. The presence of these bacteria in the human scalp and nasal cavity may imply that they are part of the resident or transient microbiota. More samples are needed to ascertain their association with the human scalp and nasal cavity.

**KEYWORDS:** *Methylobacterium*, PPFM, nasal cavity, human hair scalp

**INTRODUCTION**

Pink Pigmented Facultative Methylophilic (PPFM) bacteria belonging to the genus *Methylobacterium* are considered to be ubiquitous in nature as isolated from various environmental sources such as plants, soil, water and air (Lee, 2007; Jourand et al., 2004, Gallego et al. 2005, De Marco et al., 2004, Lo and Lee 2007; Weon et al. 2008). It was thought that PPFM bacteria exist and thrive only in environmental sources, however, there are documented reports that have isolated this microorganism in different parts of the human body such as the oral cavity (Anesti et al., 2005; Carvajal et al., 2011) and feet (Anesti et al., 2004; Carvajal et al., 2006). Furthermore, there has been an increase in published case-reports and studies that implicates this microorganism causing infection (Fanci et. al, 2010; de Cal et. al 2009; Hogues et. al, 2008; Abdel-Haq and Asmar,

2008; Anesti et al. 2004 & 2005; Lee et al., 2004; Engler and Norton, 2001; Sanders et. al, 2000; Kaye et al., 1992; Gilchrist et al. 1986; Gilardi and Faur, 1984). These studies underscore the presence of *Methylobacterium* in the human body. Because of such, the existence of this group of microorganisms in the human body may be considered as either transient microflora or opportunistic pathogens (Green 2001; Anesti et al. 2004 & 2005).

With expanding support of the presence of *Methylobacterium* in different parts of human body, it is the best interest of the authors to determine if whether this bacterial group can thrive to other parts of the body such as the nasal cavity and hair scalp. Thus, the primary of the study is isolate and identify PPFM bacteria obtained from the nasal cavity and hair scalp of subject volunteers. The presence of this microorganism may lead to a better understanding of microbiome interaction in the human body.

## **MATERIALS AND METHODS**

***Isolation of PPFM samples from the Nasal Cavity and Scalp.*** Forty (40) 16-19 year old college students participated in taking samples from the nasal cavity and scalp. Samples from the scalp were obtained by swabbing creases of the head and the cowlick of the subject. Meanwhile, samples from nasal cavities were obtained by swabbing the wall of the nasal vestibule. The swabs with the samples were placed in tubes with MMS broth medium and were incubated at room temperature for five to six days. The samples from the broth medium were inoculated in MMS plates with 0.5% methanol and incubated at 37°C for at least one week.

***Morphological and Biochemical Characterization.*** Colonial morphology of the isolate was described after growing in minimal medium with 0.5% methanol for one week at 37°C. Gram staining was utilized for the Microscopic morphology of the isolates. Measurement of the approximate size of the bacterial cell was made using ocular micrometer. Biochemical tests specifically; Oxidase, catalase and urease tests were performed. These biochemical tests are known to be diagnostic to the identification of the genus *Methylobacterium* (Green, 1982).

***Genotypic Characterization - 16S rDNA sequence analysis.*** Colony PCR was performed to amplify the 16s rDNA of the isolates wherein two to three colonies from two-week-old cultures were added to reaction mixture. The primers used for the 16s rDNA was forward primer 5'CAG GCC TAA CAC ATG CAA GTC 3' and reverse primer 3'GGG CGG WGT GTA CAA GGC (Marchesi et al., 1998). The PCR conditions are as follows: denaturation at 94°C for 1 minute, annealing at 50°C for 5 minutes, extension at 72°C for 1 minute, and final extension at 72° for 5 minutes. This procedure was repeated for 35 cycles. Visualization of amplicons was done by performing electrophoresis at 100 millivolts in 1.6% agarose gel. The gel was stained using ethidium bromide and viewed under UV transilluminator. Syngene Gel documentation system was used to capture the image of the gel. A 100 bp DNA ladder was used to estimate

the amplicon size. The expected amplicon size for 16s rDNA is approximately 1,300 bp. The amplicons corresponding to the expected size were sent to Macrogen Korea for sequencing.

**Identification of PPFM bacterial isolates.** The 16S rDNA sequences were subjected to Nucleotide Basic Local Alignment Search Tool (BLAST). For phylogenetic analysis, *Rhodopseudomonas palustris* DSM 123 was included as an outgroup because it belongs to the same division with *Methylobacterium* (Alpha-proteobacteria group) (Kato et al., 2005). Phylogenetic tree construction was done using the Molecular Evolutionary Genetic Analysis (MEGA) software version 5 using Neighbor-Joining and Kimura-2-parameter methods (Tamura, et al., 2011). All obtained 16S rDNA sequences were deposited in the GenBank with accession numbers JQ904047 - JQ904056.

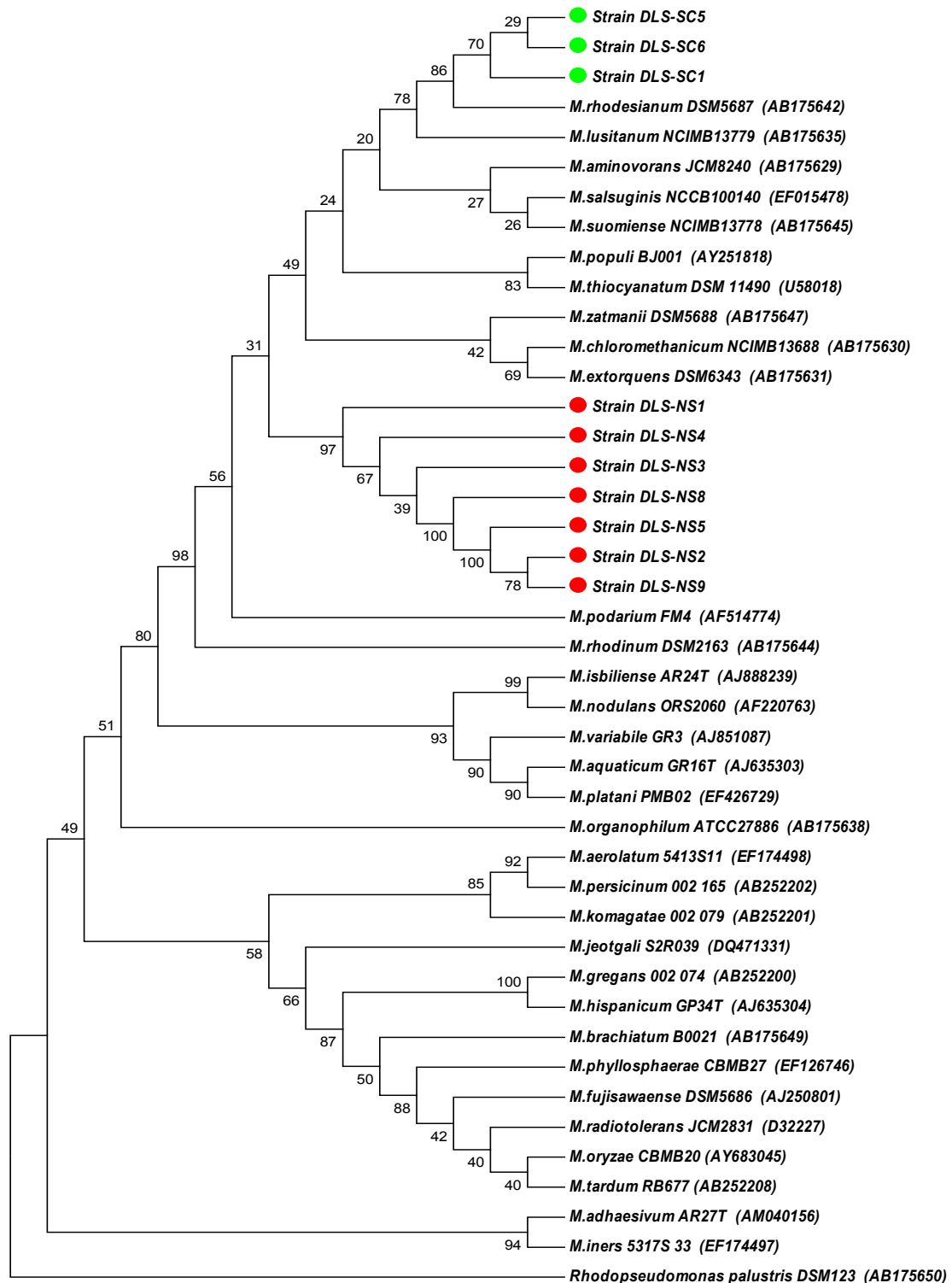
## **RESULTS AND DISCUSSION**

A total of 10 PPFM bacterial isolates were isolated from hair scalp and nasal cavity of the subject volunteers. Out of the 10 isolates, seven were isolated from the nasal cavity while three were isolated from the human scalp. The colonial morphology of all PPFM bacterial isolates displayed reddish-pink, approximately 1 mm in diameter in size, entire, convex, butyrous consistency, and raised opaque colonies when cultured on Glycerol Peptone Agar (GPA) medium. Microscopic examination revealed that these bacterial isolates are Gram-negative bacilli to cocco-bacilli. The bacterial cells was measured approximately 1.0 um long by 0.5 um wide. The description of the colonial and microscopic morphology of the PPFM bacterial isolates is in agreement with the descriptions made by Holt et al. (1994) and Green (2006) for genus *Methylobacterium*. The results of the biochemical tests showed that the isolates are oxidase-, catalase- and urease-positive. This implies that the PPFM bacterial isolates conform to genus *Methylobacterium* (Kato et al., 2005; Gallego et al., 2005a; Gallego et al., 2005b; Green and Bousfield, 1983).

To ascertain the bacterial isolates to belong to genus *Methylobacterium*, genotypic characterization was employed using 16S rDNA wherein the study generated approximately a total of 1,119 base pairs. BLAST analysis showed that all bacterial isolates belonged to genus *Methylobacterium*. The percent similarity of the sequences of the PPFM bacterial isolates to the sequences from the NCBI showed a range of between 98% - 99%. However species identification using BLAST Analysis was shown to be inconclusive due to high number of deposited 16S rDNA sequences from different strains. Also, most of them were not properly identified in the species level. Figure 1 depicts the phylogenetic tree showing the relationship of all bacterial isolates with 32 valid species and type strains of *Methylobacterium*. All PPFM bacterial isolates from the human hair scalp (DLS-SC1, DLS-SC6 and DLS-SC5) were 99.9% similar with *Methylobacterium rhodesianum* (JQ904048, JQ904052, JQ904053) using pairwise analysis. The seven PPFM bacterial isolates from the nasal cavity were observed to form a distinct group. Pair-wise analysis of the 16S rDNA

sequences of these isolates showed that there is 92%-99% similarity among all 32 type species of genus *Methylobacterium*, thus, suggesting to be of novel sequences.

This study demonstrates the presence of *Methylobacterium* on the human scalp and nasal cavity. One plausible reason why *Methylobacterium* isolates were able to thrive in the surfaces of human body is because of its facultative methylotrophic nature. Anesti et al. (2004) reported that *Methylobacterium*



**Figure 1.** Phylogenetic tree showing the relationship of PPFM isolates of the nasal cavity (DLS-NS, ●) and hair scalp (DLS-SC, ●) with 32 valid species and type strains of genus *Methylobacterium* using Neighbor-Joining GTR method. The bootstrap consensus tree inferred from 1000 replicates. There were a total of

1,119 positions in the final dataset. Phylogenetic analyses were conducted in MEGA 5.

*podarium* was different from other *Methylobacterium* species because they were able to grow on a wider range of organic and one-carbon compounds such as with dimethylsulphone. Nowadays, shampoos and hair treatment formulas contain Methylsulfonylmethane (MSM) (Saute and Saute, 2010). With this, dimethylsulfone compound found in these hair products can be utilized as an energy source for PPFM bacteria. Not only that, Berresheim et al. (1998) and Harvey and Lang (1986) reported that dimethylsulfone could be found in air. These reports may explain the possible occurrence of *Methylobacterium* sp. in the hair scalp and nasal cavity. Furthermore, the results of the study underscores that this group of microorganisms could be a part of the normal microbiota or may be considered as transient microflora. More research has to be done to ascertain their interaction with the human body.

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