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Short Communication

16S rRNA gene taxonomic profiling of endophytic bacteria associated with *phylaenopsis* roots

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ABSTRACT

Orchids are one of the main groups of ornamental plants commercially exploited. In the present study, we analyzed the diversity of bacterial community in *Phalaenopsis* root using metagenomic approach. The diversity of bacterial taxonomic category was assessed at different Operational Taxonomic Unit (OTU) levels using Ribosomal Database Project (RDP) pipeline and MG-RAST. At phylum level, Proteobacteria (61.34%) was the most dominant group followed by unclassified derived from bacteria (24.74%) and Actinobacteria (12.52%). Genus level analysis revealed the abundance of *Rubrobacter*, *Pseudomonas* and *Acinetobacter*. The study revealed that of the total species detected 50.83 per cent were unclassified, stressing the importance of metagenomics to assess the diversity of endophytes associated with orchid roots.

Keywords: Endophyte, orchid, diversity

INTRODUCTION

Orchidaceae is one of the largest plant families, including almost 10% of all flowering plant species. Among these, the monopodial epiphytic *Phalaenopsis* or 'Moth Orchid is one of the most popular orchids due to its ease of production and blooming year-round. The orchid roots are associated with various fungi and endotrophic bacteria (Teixeira *et al*., 2015). Apart from mycorrhizal fungi, previous reports revealed the abundance of endophytic bacteria on the roots of the cultivated tropical orchids of genera *Calanthe*, *Acampe* and *Dendrobium* (Tsavkelova *et al*., 2003). Orchids are characterized by low survival rate in the green house due to the germination under asymbiotic conditions *in vitro*. Generally, endophytes play an important role in promoting plant growth and yield, suppress pathogens, aid in removing heavy metal contaminants, solubilize phosphate or contribute to nitrogen assimilation for plants (Hallmann *et al*., 2006). Over the past decade, our understanding of microbial diversity and function in complex environments has increased significantly, primarily because of the introduction of next generation sequencing (NGS) (Lozupone and Knight, 2007). The culture-independent, high-throughput sequencing-based community analysis allows us to observe the microbiome associated with the plants. Since the endophytes have a strong impact on orchids growth, it is very important to study their relationships with plant for developing new strategies for orchid conservation and better exploitation of their medicinal principles.Therefore, in the present study, we employed NGS technology to unveil the culturable and unculturable endophytic bacteria in *Phalaenopsis* root to elucidate the microbial plant colonisation pattern and evaluate its microbial diversity.

The *Phalaenopsis* plants grown in Sphagnum moss under green house conditions were collected from the Department of Pomology and Floriculture, College of Horticulture, Vellanikkara. Samples were immediately transferred and processed for further studies. The roots were detached with sterile knife and washed with sterile distilled water plus a few drops of Tween-20 and left for 10–15 min to drain. These were then cut into 4–5 pieces (2–3 cm in size). Surface sterilization was performed by immersing separately in 90% ethanol (5 min), followed by sodium hypochlorite (3%) solution (2 min), and 75% ethanol (3 min). The disinfected roots were rinsed three times in sterile distilled water. Total genomic DNA was extracted from the surface sterilized root tissues using QIAGEN DNeasy plant kit following the manufacturer's protocol. Extracted DNA was

suspended in QIAGN elution buffer and stored at -20^{0}C. PCR amplification was carried out to amplify V3 conserved region of 16S rRNA gene sequences using the 16S rRNA gene primers (forward primer 5¹-CCTACGGGNGGCWGCAG-3¹ and reverse 5¹-GACT ACHVGGGTATCTA-3¹). The amplicons were purified and sequenced on the Illumina Miseq platform at Scigenom Pvt. Ltd. Cochin. The FASTQ sequences were filtered to remove chimeric sequences and singletons to obtain preprocessed reads, which was then clustered to obtain OTUs. The chloroplast sequences that comprised of almost 97.5 per cent of the total reads were removed using QIIME analysis. Further taxonomic annotation of the 301 OTUs obtained were done using QIIME and MG-RAST tools.

Total DNA was isolated from the roots of *Phalaenopsis* plants and the presence of 16S rRNA gene was confirmed by amplification with universal primers. Total raw sequencing reads (paired end) of 1,96,595 with average sequence length of 151 bp each was obtained from Illumina MiSeq™ sequencer.

The abundance of major bacterial groups in each taxonomic category is given in **Table 1**. Altogether, 10 bacterial phyla were detected and among these, Proteobacteria (61.34%) was the most dominant group followed by unclassified derived from bacteria (24.74%) and Actinobacteria (12.52%). Reads belonging to Acidobacteria, Bacteriodetes, Chloroflexi, Cyanobacteria, Spirochaetes, Tenericutes, Firmicutes and Bacteriodetes were found to be the other phyla with less than 1 per cent **(Fig 1).** The higher abundance of Proteobacteria in the roots of orchids suggests that members of this phylum are particularly well adapted to colonize inner plant tissues and establish as root endophytes. The phylum Proteobacteria comprises several species that promote plant growth and act as biological control agents of different diseases (Bulgarelli *et al.*, 2013). Actinobacteria play specific roles, for instance, protecting the host plants against insects and diseases especially by the production of bioactive compounds. Firmicutes were found to be metabolically the most versatile group with production of multiple enzyme activities. Cyanobacteria are photosynthetic; some are capable of fixing nitrogen and others improve soil-aggregation stability (Issa *et al*., 2007), a key aspect of soil conservation. Results of the present investigation are in agreement with the earlier reports on Proteobacteria, Actinobacteria, Firmicutes and Bacteriodes being the prominent phyla in the roots of tree peony (Yang *et al*., 2017).

A total of 7 bacterial classes were identified and among them Gammaproteobacteria was the most dominant group (41.90%) followed by unclassified (derived from bacteria) (31.35%) Actinobacteria (25.11%) and Bacilli (1.37%) (**Fig 2).** A total of 17 bacterial orders were detected. The most dominant group was unclassified derived from bacteria (35.99%) followed by Pseudomonadales (30.69%) and Rubrobacterales

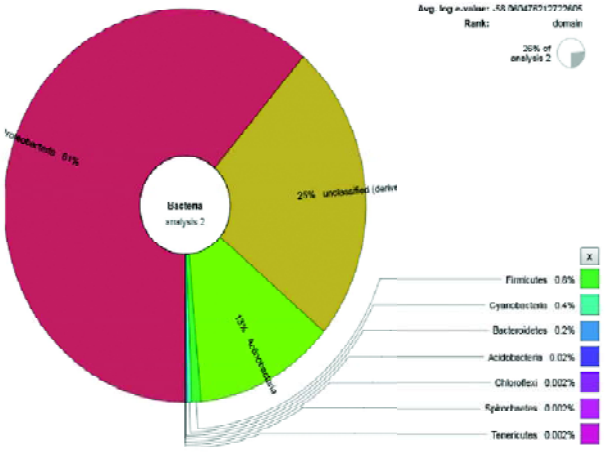

Fig. 1. Abundance of endophytic bacteria at phylum level constructed in MG-RAST with illumina sequencing data set

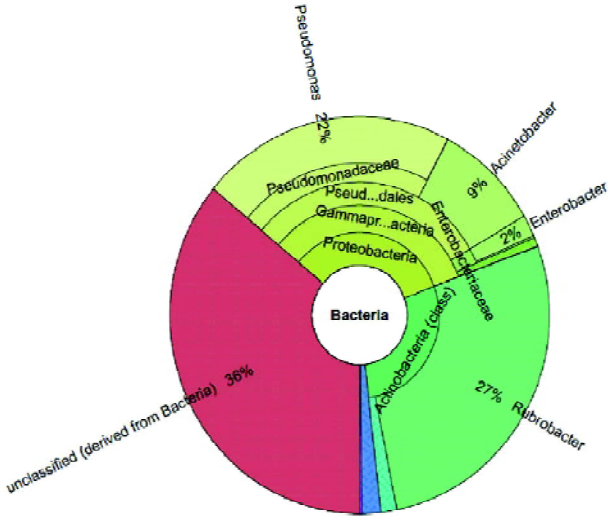

Fig. 2. Abundance of endophyticbacteria at genus level constructed in MG-RAST with illumina sequencing data set

Table 1. Abundance of major taxonomic category from phyla to species level of endophyticbacteriain *Phalaenopsis*root

Sl No	Phylum	Class	Order	Family	Genus
1	Proteobacteria (61.34 %)	Gammaproteobacteria (41.90%) Betaproteobacteria (0.04%)	Pseudomonadales (30.68%) Enterobacteriales (1.96%) Xanthomonadales (0.71%) Burkholderiales (0.03 %)	Pseudomonadaceae (21.67%) Moraxellaceae (9.00%) Enterobacteriaceae (1.96%) Xanthomonadaceae (0.71%)	Pseudomonas (21.67%) Acinetobacter (9.00%) Enterobacter (1.78%) Achromobacter (0.03 %)
2	unclassified (derived from Bacteria) (24.74%)	unclassified (derived from Bacteria) (31.35%)	unclassified (derived from Bacteria) (35.99%)	unclassified (derived from Bacteria) (35.99%)	unclassified (derived from Bacteria) (35.99%)
3	Actinobacteria (12.52%)	Actinobacteria (25.11%)	Actinomycetales (0.5 %) Rubrobacterales (27.47%)	Rubrobacteraceae (27.47%) Nocardioidaceae (0.69 %) Pseudonocardiaceae (0.66 %)	Rubrobacter (27.47%) Pseudonocardia (0.66%) Kribbella (0.44 %)
4	Firmicutes (0.83%)	Bacilli (1.57 %) Clostridia (0.002 %)	Bacillales (1.57%)	Paenibacillaceae (1.27%)	Aneurinibacillus (1.27%) Bacillus (0.14%)
5	Cyanobacteria (0.38%)	Unclassified (derived from cyanobacteria) (0.001 %)			
6	Bacteroidetes (0.17%)	Bacteroidia (0.14%) Flavobacteria (0.03%) Cytophagia (0.002 %)	Bacteroidales (0.14 %) Flavobacteriales (0.03 %)	Rikenellaceae (0.09 %)	
7	Acidobacteria (0.02%)				
8	Chloroflexi (0.002%)				
9	Spirochaetes (0.002 %)				
10	Tenericutes (0.002 %)				

(27.47%). Orders Actinomy etales, Bacillales and Enterobacteriales were also present more than one per cent. Analyses at family level revealed a total of 22 bacterial families were present in the sample. Major bacterial families present in the sample were unclassified derived from bacteria (36%), followed by Rubrobacteraceae (27.48%), Pseudomonadaceae (21.68%) and Moraxellaceae (9.01%). The Gamma Proteobacteria included *Pseudomonas*, *Pantoea*, *Acinetobacter*, *Stenotrophomonas* and *Xanthomonas* making it phylogenetically the most diverse group in the current study. Altogether 31 bacterial genera were present. Unclassified derived from bacteria (35.99%) was the most dominant group in the present study followed by *Rubrobacter* (27.47%), *Pseudomonas* (21.67%) and *Acinetobacter* (9%). Genus *Enterobacter* and *Aneurinibacillus* were also present at more than one per cent abundance. Genus *Rubrobacter* is well known to be a radiation resistant bacterium. Genus *Pseudomonas* can utilize more than 200 compounds as carbon source, can fix atmospheric N and solubilize P. Several of the genera isolated in the current study, including *Pantoea*, *Pseudomonas*, *Bacillus* and *Acinetobacter* have been isolated from different plants and shown to possess plant growth promoting activities (Trivedi *et al.*, 2011). It has been previously observed that in many cases, *Pseudomonas* and members of *Enterobacteriaceae* are abundant in both the soil environment and the plant interior (Spiers *et al.*, 2000). The prevalence of *Pseudomonas* and *Bacillus* endosymbionts was also reported in Australian terrestrial orchids (Wilkinson *et al.*, 1994). The study emphasizes on the importance of metagenomics to assess the diversity and role of endophytic microbes in plants.

This study extends the knowledge on the composition and diversity in the orchid microbial populations. Moreover, most of the endophytes observed in the present study are perhaps good producers of bioactive compounds, which can promote the growth of orchids in seedling stage and also in *ex vitro* acclimatization.

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