



A PHYLOGENETIC INSIGHT INTO THE FERN RHIZOSPHERE OF *Acrostichum aureum* Linn.

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ABSTRACT

Abstract:

Fern rhizosphere has not been explored in great detail due to the lack of proper definition of the rhizosphere of the mostly epiphytic group. This work uses metagenomic sequencing, for assessing the rhizosphere of the only reported terrestrial mangrove fern *Acrostichum aureum* Linn. from the Indian Sunderbans. Targeted sequencing of the V3 - V4 region using Illumina Hiseq was performed and the analyses of the sequenced files revealed the presence of a wide variety of microbial members with the highest belonging to Proteobacteria superphyla. We believe that this is the first phylogenetic assessment of the Fern rhizosphere and should provide us with valuable insights into the quality of microbial assemblage available in this particular niche.

KEY WORDS

Fern Metagenomics, Rhizosphere, Phylogenetic Analyses, KRAKEN.

INTRODUCTION:

The soil around the roots of plants was defined as the rhizosphere which has gained importance due to the presence of both growth promoting and pathogenic microbes. Plant phenotypes and traits should now be explored on the basis of the quantum of the rhizospheric microbiome. Foster *et al.* (1983) has opined that soil around plant roots contain significantly large quantities of microbes as compared to non-rooted bulk soil. This has been attributed to the excess organic carbon that is released by the plant roots (Walker *et al.*, 2003; Bais *et al.*, 2006; Hartmann *et al.*, 2009). The signals from the plant roots not only influence the recruitment of the microbiome but also regulate the functions such as quorum sensing, regulation of microbial gene expression and other microbial activities

for example - biofilm production, conjugation and symbiosis and virulence (Miller and Bassler 2001)

Mangrove forests are classified into six distinct categories. The types include over wash mangrove forests which are but small islands that remain covered with mangroves that experience frequent wash by the tides. The dominant species of this area is *Rhizophora mangle* which is also called the red mangrove. The fringing mangrove forests: These are but strips of mangrove found along waterways and are washed off by daily tides. The most prominent species here is *Rhizophora mangle*. The riverine mangrove forests include the luxuriant stands of mangrove along tidal rivers and creeks with a good input of fresh water, often consisting of *Rhizophora*, *Avicennia*, etc. The basin mangrove forests are stunted mangroves located in places such as the interior of swamps. These are often

dominated by *Avicennia*. The Hammock mangrove forests are similar to the basin mangrove forests but are found in more slightly higher areas. The scrub mangrove forests area dwarfed stand of mangroves found on flat coastal fringes. Ferns are essentially found in and around the riverine mangrove forests.

Acrostichum is one of the rare terrestrial erect fern belonging to the family Pteridaceae having woody glabrous stipes which arise from a stout woody rhizome, with unipinnate fronds, which are alternate, linear and oblong. Sori are densely aggregated and are non-indusiate. *Acrostichum* is generally found in elevated

areas of mangrove forests or around agricultural fields. The fronds are used for thatching.

MATERIALS AND METHOD:

Collection of samples:

Profuse growth of *Acrostichum* was located adjoining the Rangabelia High School (Fig. 1A and 1B) located in the Rangabelia island of the Indian Sunderbans and was collected from the site marked by GPS (Fig. 1C) using standard sterile collection techniques, which included proper weighing and sieving of the soil samples in sterile containers (Fig. 1D).

Fig 1



Fig 1: The location of collection and the exact twig, which contributed to the rhizospheric assemblage

Metagenomic Sequencing and Analyses:

Metagenomic sequencing and analysis was performed according to the methods described in Ganguli and Rahaman *et al.* 2017. Literature mining and analyses was performed using the SASSD software.

Phylogenetic Analysis:

RAPSearch was initialised to compare the reads with existing NCBI database using MEGAN (E value ≤ 0.001 , Percent identity ≥ 30). The resultant MEGAN file contained assigned taxon IDs based on the NCBI taxonomy. This file helped for statistical and graphical analysis. Finally, the lowest common ancestor (LCA) algorithm was run to validate assignments, data analysis and to create summaries of the given data. LCA algorithm is a gold standard for identifying the lowest common ancestor of different species.

Taxonomic labels were assigned to metagenomic DNA sequences using Kraken. Kraken's classification algorithm performs K-mer mapping to taxa by querying a pre-computed database - a multistep process which begins with the selection of a library consisting of genomic sequences. The default Kraken library is based

on completed microbial genomes in the National Center for Biotechnology Information's (NCBI) RefSeq database.

Results and Discussion:

The classification suggested that 99% of the sequences belonged to Bacteria, while 0.4% belonged to Archaea (as shown in Figure 2). In Bacterial sequences, the major three groups included *Proteobacteria* (44%), *Terrabacteria* (27%) and PVC (12%) (*Planctomycetes*, *Verthrucomicrobia* and *Chlamydiae*) superphyla.

The major superphyla *Proteobacteria*, comprised of *Delta/Epsilon Proteobacteria* (17%), *Alpha Proteobacteria* (12%), *Gamma Proteobacteria* (7%) and *Beta Proteobacteria* (6%) in that order of abundance (Figure 3). The majority of the sequences analyzed by all the methods, belonged to the phylum *Proteobacteria*, which includes a wide variety of pathogens, such as *Escherichia*, *Salmonella*, *Vibrio*, *Helicobacter*, *Yersinia* and many other notable genera. Others are free-living (nonparasitic) and include many of the bacteria which are responsible for nitrogen fixation.

Fig 2

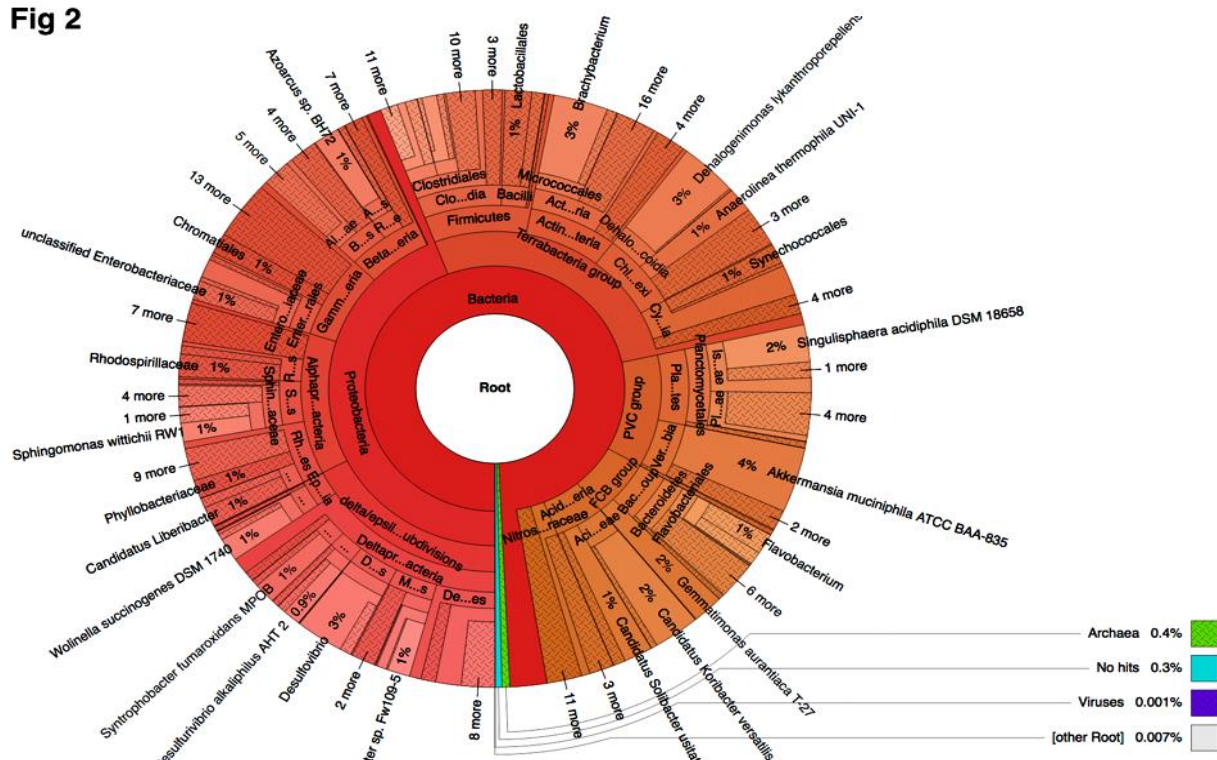


Fig 2: Total Phylogenetic Abundance of the members

Fig 3

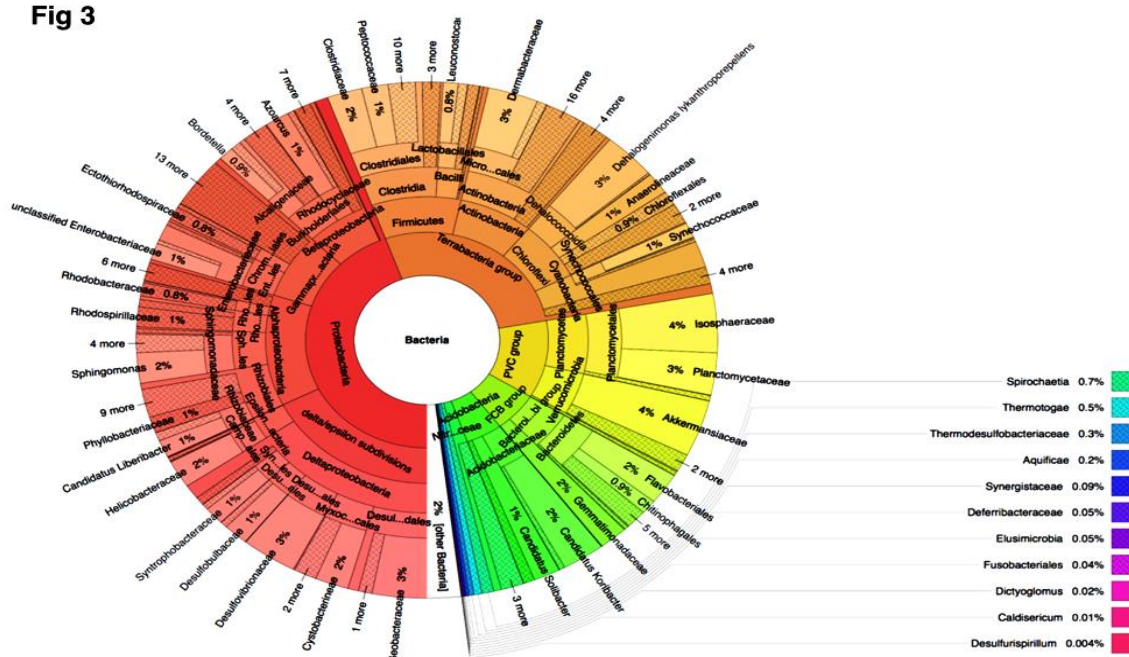


Fig 3: Phylogenetic abundance of the bacterial members of the rhizosphere

Most abundant class of *Proteobacteria* turns out to be *Alphaproteobacteria*, which include agriculturally important bacteria capable of inducing nitrogen fixation in symbiosis with plants. The rhizosphere soil depicted the high

abundance of nitrogen-fixing bacteria. *Heliobacterium modesticaldum* Ice1, according to MEGAN analysis which is a well-known nitrogen-fixing bacterium.

The sequence based phylogenetic analyses revealed further interesting results - the presence of few eukaryotic sequences including a few fungi and cyanobacteria members were identified (Figure 4).

Fig 4

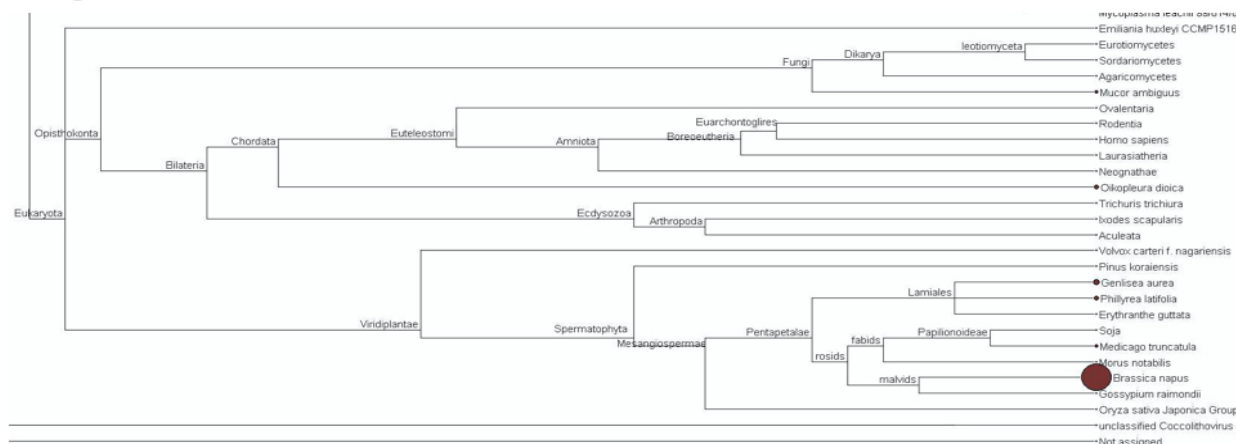


Fig 4: Few eukaryotic members having representative units in the rhizosphere

Apart from this fragment-based sequencing revealed the presence of few higher plant species such as *Oryza*, *Medicago* and *Gossypium* probably originating from adjoining deposition of reproductive propagules from adding cultivation fields. The bacterial phylogeny (Figs. 5 A and B) revealed similar results as those obtained in the KRAKEN analyses.

Fig 5

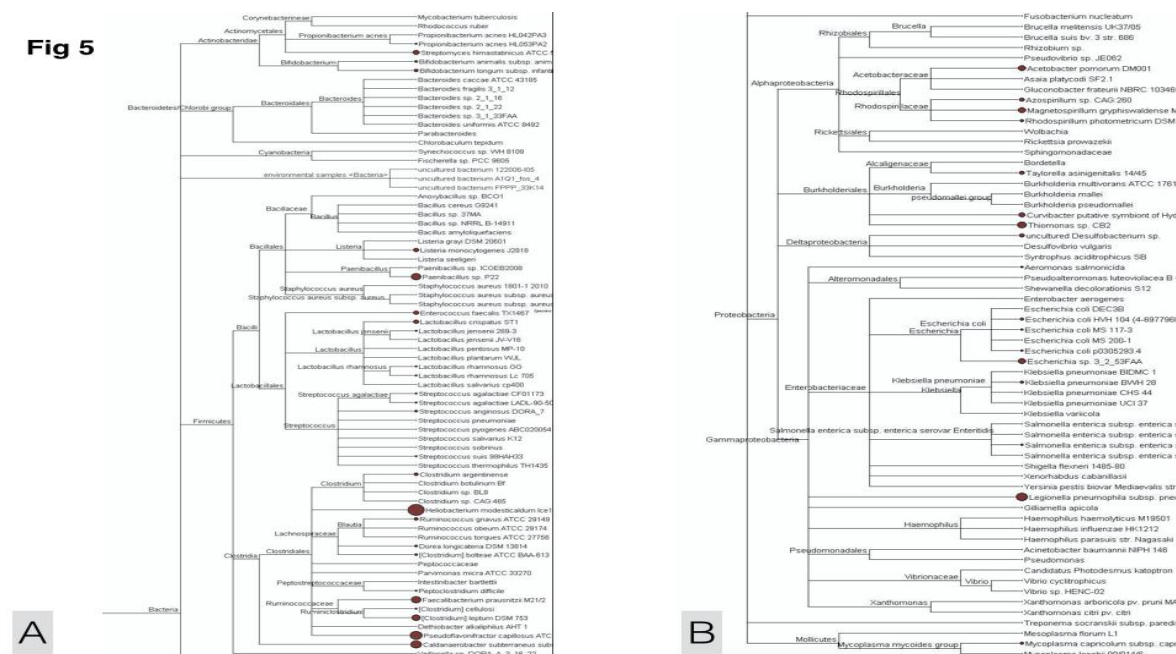


Fig 5: Phylogenetic Relationship among the bacterial members

CONCLUSION:

Thus, the metagenomic analyses of the rhizospheric community of *Acrostichum* revealed a unique

assemblage of both nitrogen fixing and non-nitrogen fixing prokaryotic members with a high percentage of unassigned taxa indicative of our sparse knowledge in the diversity of rhizospheric communities.

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