Indian J. Applied & Pure Bio. Vol. 36(1), 161-166 (2021). **ISSN: 0970-2091**
A web of Science Journal

Category: Pteridology Related Fields

Rhizospheric metagenome of the edible water fern *Marsilea minuta* L. from Indian Sunderbans

Sabdar Rahaman

Department of Botany, Bangabasi Evening College, Kolkata-700009 (India)
E-mail address: *drsrahaman@gmail.com*

Abstract

The quest for decoding the diversity of the microbiota prevalent in the rhizosphere of water fern is very paramount for the purpose of identifying bacteria that could provide us valuable insights into the quality of microbial assemblage available in this particular niche and endorse function such as quorum sensing, regulation of microbial gene expression, symbiosis and virulence. Present study is to analyse the rhizospheric microbiome of the edible water fern *Marsilea minuta* Linn. from Indian Sunderbans. Soil samples were collected using standard protocols and 16S rRNA gene V3-V4 region amplicon sequencing was performed using Illumina Hiseq to decipher the microbial communities prevalent in the fern rhizosphere. A total of 6,55,308 quality checked reads were assembled into 2,09,562 contigs.The most enrichedphyla's are Proteobacteria, Actinobacteria, Firmicutes, Bacteroidetes, Cyanobacteria.

Rhizospheres are dynamic biological system that's serves as hotspot for microorganisms which support ecological synergism between plants and soil microbes, processes important for plant growth and health[1]. A huge amount of organic carbon are released by plants root resulting in an repertoire of microbes were they chemotactically attracted to the plant root deciphering vital functions compared to non-rooted bulk soil[11]. Plant roots recruit and influence the structure or function of microbial community of the rhizosphere microbiome by driving processes like quorum sensing, regulation of microbial gene expression, symbiosis, biofilm formation, antibiotic production, motility, conjugation and virulence etc. Unpredictably, the rhizosphere microbiome colonizes <1% of the total surface that is available in soil thus provides a critical link between plant and soil environments[6]. Fern vegetations are distributed in and around the riverine Sundarbans mangrove forest, India. The fern flora of Indian Sundarbans region was once very rich and widespread. However, due to several abiotic factors such as, periodic-cyclones, storms, floods and anthropogenic influence cause very serious threats. Ganguli *et al.*,[3] opined that, ferns found in Indian Sundarbans regions have migrated from their original habitat to area where anthropogenic pressure is much lesser[3]. But some species of ferns are becoming important for their edibility,

medicinal and economic values. *Marsilea miuta* Linn. commonly known as "Sushni-sak" belongs to the family Marsileaceae is a soft, small creeping water fern with erect tetrafoliate long petioled leaves, hairs numerous on rhizome, sporocarp wall, petiole and abaxial surface of the lamina; grows on marshes and at edges of pools, pans, ditches, swamps and lowland rice-fields[4,8]. These water fern are heterosporous in nature contain bean-shaped sporocarps bearing sporangia developing both mega- and microspores and having indusiate sori which are important for distinguishing the species. In ethno-medicinal literature, traditionally the whole plants are used as astringent, coolant and expectorant whereas; the leaves are fried and consumed as vegetables[10]. It also possesses anti-infertility, antibacterial, analgesic and anti-inflammatory, hepatoprotective and antidiabetic like activities has been recently reported explored by several workers[9]. The water fern, *Marsilea minuta* occurs throughout Sundarbans. It, however, cannot produce sporocarps if the salinity of the soil is high[8]. This work preliminarily attempts to analyse the rhizospheric abundance of microorganisms around *Marsilea minuta* Linn. as a measure for assessing the optimum microbial content for proper nourishment of the fern taxon.

Rhizospheric soil collection and metagenomic sequencing :

The 16s rRNA gene consists of 9 hyper variable regions (V1-V9) interspersed between conserved regions, which has been widely used to study and characterize the bacterial community of an environmental sample. In the present study, the microbial community structure would be identified by targeting V3–V4 region, as these regions are highly variable to distinguish bacterial subtypes. The Geographical coordinates of soil sample is 22° 8' 36'' N; 88° 52' 12'' E.

Sample preparation :

The genomic DNA of marshy habitat had been isolated from a rhizosphere sample using the method proposed by Ganguli *et.al*., protocol[3]. DNA quality was then assessed by using Nanodrop technology on agarose gel, which was then quantified using QUBIT. The library preparation was carried out using Illumina standardized V3-V4 regions of the16S rRNA library protocol. The enriched library was quantified and validated using qPCR and Agilent Bioanalyzer (DNA 1000 chip). The library generated containing V3-V4 amplicons would be sequenced on Illumina HiSeq 2000 using 300 × 2 PE chemistry.

Bioinformatic analysis :

The quality control of raw fastq reads were carried out using FASTQC toolkit (http://www.bioinformatics. babraham.ac.uk/projects/fastqc), the processed reads were than assembled into contigs. The contigs were clustered into OTU's (Operational Taxonomic Units) by using MG-RAST web-server (https://www.mg-rast.org/), to identify the microbial communities[5]. These OTU's were then, further used for taxonomic assignment (RDP database[2]), phylogenetic and diversity analysis. The taxonomic composition of metagenomic sample at all taxonomic levels was visualized using the Krona map. The data is available at the NCBI- Sequence Read Archive with accession number: SRX6849503.

Table-1. Summary statistics table.

Reads count	Pre-QC	Post-QC	No. of OTUs
Sequences	655308	209562	3638
bp count	196,417,656 bp	62,938,579 bp	
Mean sequence length	300 ± 14 bp	300 ± 2 bp	
Mean GC percent	56 ± 3 %	56 ± 4 %	
Failed reads:	445746		

The *Marsilea* soil rhizosphere dataset has 6,55,308 raw paired sequences of totalling 1,96, 417,656 bp with average length of 300 ± 14 bp and mean GC 56 ± 3%. Of the sequences tested about 68.02% sequences failed to pass the QC pipeline of MG-RAST web-server. However, of the sequences that passes the QC is about 31.98% *i.e.*, 2,09,562 of totalling 62,938,579 bp with average length of 300 ± 2 bp and mean GC 56 ± 4%, (Table 1). During initial bioinformatics analysis, processed readswere assembled into contigs which are clustered into OTUs (Operational Taxonomic Units). Total count of contigs assembled in the *Marsilea* rhizosphere dataset is 2,09,562. Of the contigs sequence that matched RDP reference database, 65.35%, 0.1%, 2.3% were affiliated with bacteria, archaea and eukaryotes respectively, whereas 34.69% bacteria remain unclassified.

The phylogenetic analysis of the metagenomic libraries classified into 26 phylum, 49 class, 115 order, 275 family, 938 genus, 3464 species of bacteria. And, the archaea were classified into 05 phylum, 10 class, 14 order, 19 family, 46 genus, 71 species (Fig. 1). A huge number of microbial communities were identified in the bacterial sequences. Proteobacteria was observed as the most abundant phylum followed by Actinobacteria, Firmicutes Bacteroidetes, Cyanobacteria, Acidobacteria (Fig. 2). Surprisingly, the most abundant class is Actinobacteria was identified which make *Marsilea* one of the most researched fern for ethnomedicinal and ayurveda studies followed by Gammaproteobacteria, Deltaproteobacteria, Betaproteobacteria, Bacilli and Alphaproteobacteria, (Fig. 3). Further analysis at species level revealed *Marinobacter lipolyticus,* gram negative, aerobic, rod-shaped, halophilic bacteria with lipolytic activity and produce the halophilic enzyme lipase LipBL, with potential biotechnological applications due to its high regioselectivity to be the most abundant species[7] followed by nitrogen-fixing species *Azoarcus tolulyticus,* Gram negative, motile rod-shaped, mesophilic bacteria used as a bioremediation of toluene[12] (Fig. 4). The comparative analyses from the *Marsilea* metagenome dataset reveals similar microbial population as reported in previous metagenomic assemblies from the Sunderbans with the exception in the abundance of *Marinobacter* species. In our previous studies of *Acrostichum* (Tiger fern) soil metagenome, we found Proteobacteria followed by Acidobacteria were most abundant phyla[3]. But the *Marsilea* soil metagenome exhibited very distinct results; which highlighted the highest abundance of Proteobacteria followed by Actinobacteria. This is may be occurred due to the variation caused by the rhizospheric environment.

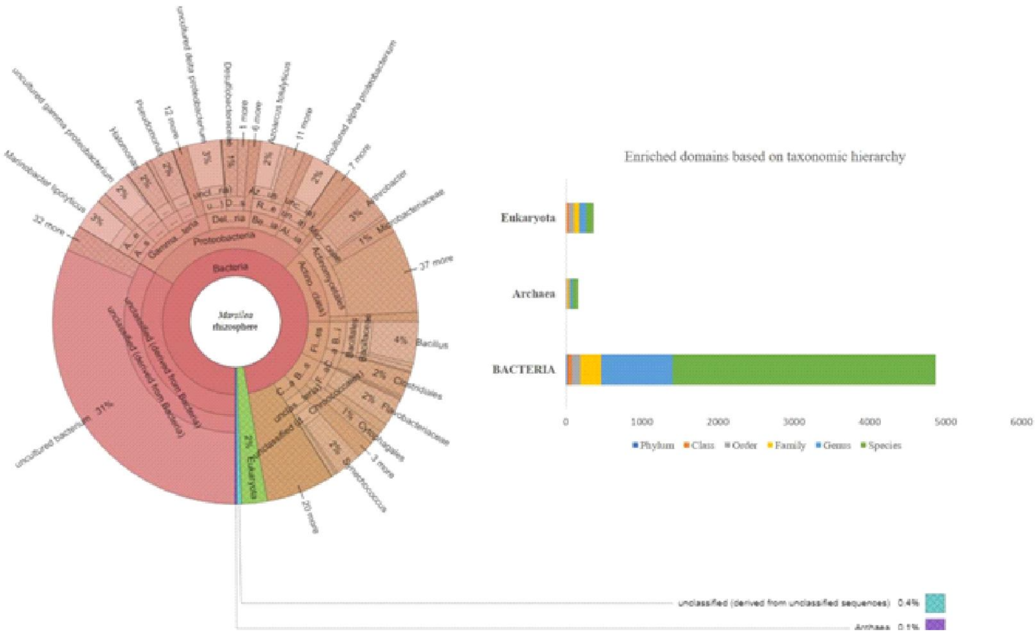

Figure 1. Total Phylogenetic Abundance of the members: Inner circles represent higher taxonomic ranks, and more detailed ranks (up to species level) are presented in the outer circles.

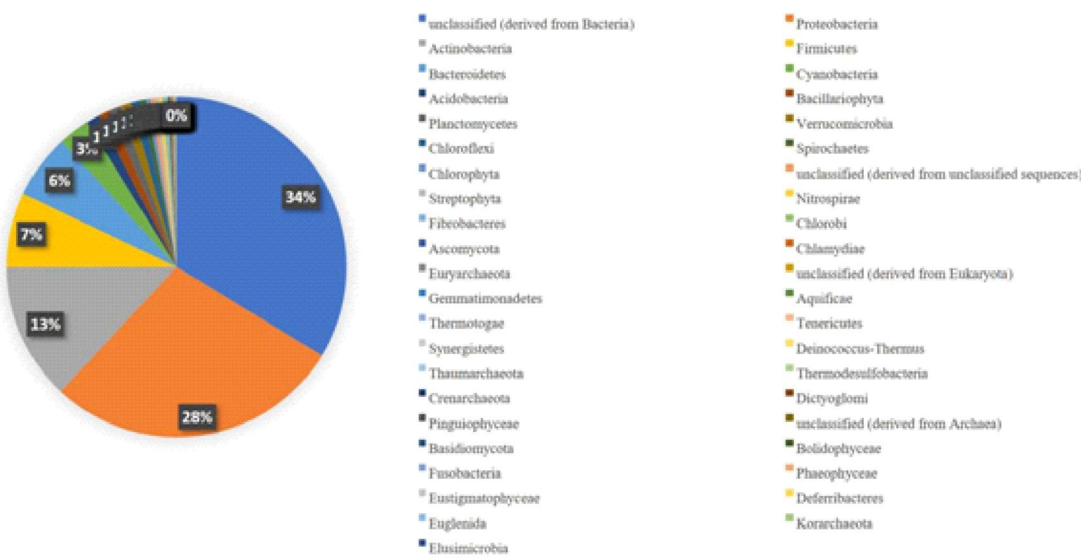

Figure 2**.** Distribution of various microbial Phyla in the rhizospheric sample

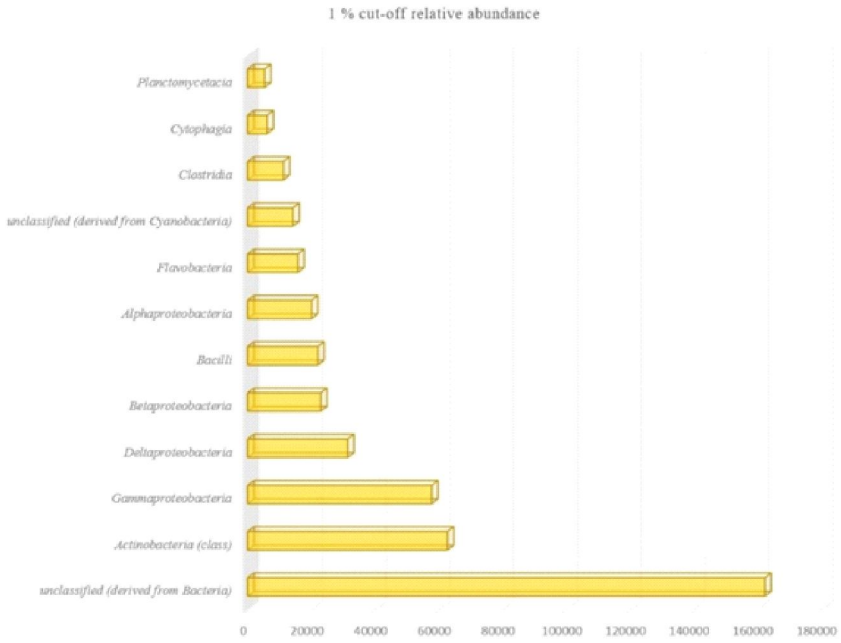

Figure 3. Most abundant Class identified in the rhizospheric sample

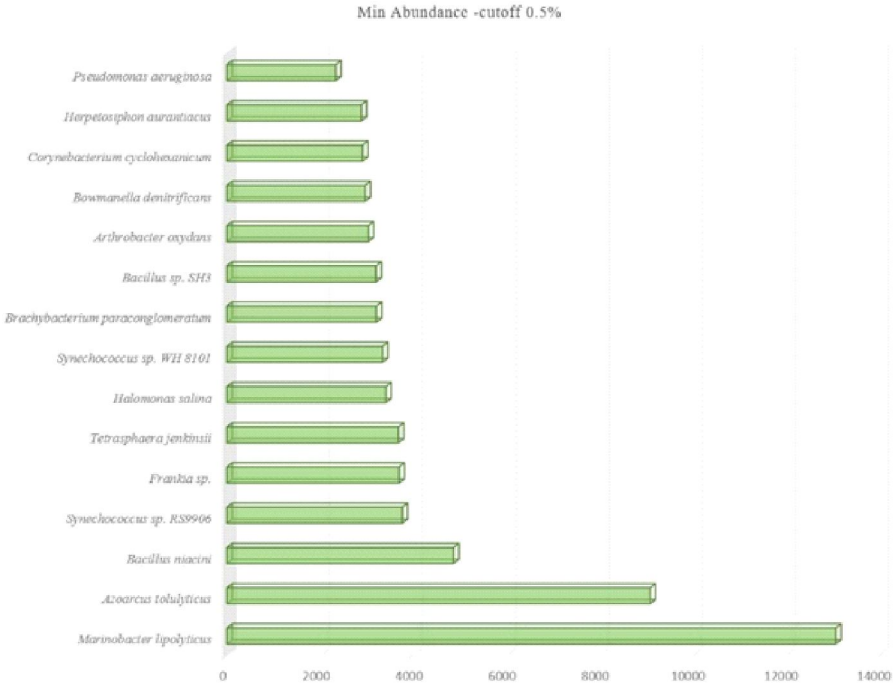

Figure 4. Most abundant Species identified in the rhizospheric sample.

Thus, the soil metagenomic analyses of the rhizospheric community of *Marsilea* revealed a distinct and unique assemblage of both nitrogen fixing and non-nitrogen fixing bacteria, actinobacteria and others prokaryotic affiliates with a high percentage of unassigned taxa suggestive of our scarce knowledge in the diversity of rhizospheric communities.

Declaration on conflict of interest :

The author declares no conflict of interest.

The author is thankful to Dr.Sayak Ganguli for constructive support and Pinaki for his effort during sample collection.

Funding :

The author acknowledges the funds provided by West Bengal Department of Science and Technology sanction number: 933(Sanc.)/ST/P/S&T/1G - 17/2016.

References :

1. Buscot, F. (2005) Microorganisms in soils: roles in genesis and functions (pp. 139-153). A. Varma (Ed.). Germany: Springer.
2. Cole, J. R., Q. Wang, J. A. Fish, B. Chai, D. M. McGarrell, Y. Sun and J.M. Tiedje, (2014) *Nucleic acids research, 42*(D1): D633-D642.
3. Ganguli, S., S. Rahaman, A. R. Bera, V. Vishal, S. Malik, K. Roopalakshmi and P.K. Singh (2017) *Genomics Data, 14:* 53-55.
4. Gupta, K. M. and T. N. Bhardwaja (1958) *Journal of the Bombay Natural History Society, 55:* 287-296.
5. Meyer, F., S. Bagchi, S. Chaterji, W. Gerlach A. Grama, T. Harrison and A. Wilke (2019) *Briefings in bioinformatics, 20*(4): 1151-1159.
6. Miller, M. B., and B. L. Bassler (2001) *Annual Reviews in Microbiology, 55*(1): 165-199.
7. Papke, R. T., R. Rafael, C. Infante-Domínguez, D. Pérez, C. Sánchez-Porro, P. Lapierre and A. Ventosa, (2013) *Genome announcements, 1*(4).
8. Sen, T., K. Chakraborti, S. Rahaman, M. Bandyopadhyay and U. Sen, (2010) *Proceedings of the National Academy of Sciences India. Section B, Biological Sciences, 80*(3): 201-220.
9. Shirolkar, A. R., S. Wadurkar, G. A. Rao, S. N. Murthy, and S. D. Pawar, (2014) *International Journal of Pharmacy and Pharmaceutical Sciences, 6*(2): 78-80.
10. Udayaprakash, N. K., N. Ashwinkarthick, D. Poomagal, M. Susithra, M. Chandran and S. Bhuvaneswari, (2018) *Current Research in Environmental and Applied Mycology, 8:* 86-95.
11. Van Elsas, J.D., J.T. Trevors, A.S. Rosado and P. Nannipieri (Eds.). (2019) Modern soil microbiology. CRC press.
12. Zhou, J., M. R. Fries, J. C. Chee-Sanford and J. M. Tiedje (1995) *International Journal of Systematic and Evolutionary Microbiology, 45*(3): 500-506.