



Research article

Biochemical and metagenomic insight into the impact of climate change on an epiphytic fern of the Indian Sunderbans

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Abstract: Sea Level Rise (SLR) as a result of Climate change in combination with anthropogenically altered environment results in alterations in rapid land dynamics in forms of erosion and accretion; along with the associated alteration in species diversity, energy cycling and productivity, significantly in sensitive ecosystems such as river deltas. Geologically, interglacial periods follow bouts of glaciation and presently we are passing through one of those phases wherein, sea level rise (SLR) is being evidenced in case of the Ganga–Brahmaputra basin. It has become more than 100 m higher in the last 18,000 years. Over the years due to the loss of landmass as a rise in sea level, human population has migrated from the different parts of the Sunderban Biosphere Reserve area to specific islands of the Indian Sunderbans. This process of migration has resulted in the removal of forest cover. As a result, extensive loss of epiphytic ferns has occurred. In this work, we have evaluated the distribution of *Drynaria* across the Indian Sunderbans region from an ecological, biochemical and metagenomic perspective. Results indicate that anthropogenic influences results in gradual depletion of *Drynaria* abundance and thus can be used as an important indicator for understanding the effects of migration of human population.

Keywords: Climate Change - Indian Sunderbans - *Drynaria* - Indicator species.

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INTRODUCTION

Drynaria is an important indicator of anthropogenic and industrial pollution and is found in most islands of the unaffected Indian Sunderbans (Debnath *et al.* 2013). It is an epiphytic fern and is found profusely in the islands consisting of the Sunderban Biosphere Reserve. *Drynaria quercifolia* (L.) J. Sm. (*Asvakatri*) belongs to the Family Polypodiaceae has a rich distribution in the plains or valleys in the mountainous regions, on trees or rocks. Apart from India it is also found in regions of South China, Malaysia and Tropical Australia. This has a short thick hard creeping rhizome. In recent years several workers have explored the medicinal importance of the fern in context of total antioxidant potential and anti-inflammatory and analgesic properties (Anuja *et al.* 2010, Prasanna & Chitra 2015). In ethnomedicinal literature, traditionally, the fronds of the plant is reported to be used by tribal communities of Tamil Nadu and Kerala in treatment of diverse ailments including typhoid fever, chronic jaundice. Rhizome decoction has also been explored for antipyretic actions. A considerable number of phytochemical constituents like friedelin, epifriedeinol, β amyryrin, β -sitosterol, 3- β -glucopyranoside and naringin have been isolated from the plant. The local people of the region have also validated the use of the plant in conditions of high fever and administration of leaf extracts in conditions of injury associated with the swelling of body parts. The whole plant body was used for conducting a set of scientific experiments along with metagenomic analyses. The microbiome of a place varies from that of another with the variation in temperature, physiography, humidity, amount of organic matters infused in the soil and other factors. The climatic conditions

also influence the photosynthetic activity, the presence of chlorophyll in the tissues and also the other physiological functions. With the change in the environmental conditions and the steady rise in pollution, the indigenous plant communities of the area are suffering. The rise in temperature and pressure due to toxic effluences caused by the high anthropogenic communities, settled there, the plants are being subjected to adapt to the changing surroundings at a very rapid rate. Hence besides conducting the metagenomic study, measuring the total amount of chlorophyll and other biochemical products present in the ferns under study also seemed significant. This is the first comprehensive report of this tropical plant species from the Indian Sunderbans at the ecological, biochemical and metagenomic level, in context of its distribution and pattern of niche formation in correlation with the effects of climate change and anthropogenic pressure.

MATERIALS AND METHOD

Field survey and analysis of ecological abundance

Six different locations from islands that have human population both indigenous and migrant and where there is no significant human population was chosen for the study. The locations were - Kumirmari, Satjelia, Rangabelia (having human habitation) and Burirdarbri, Sajnekhali and Netidhopani (Fig. 1). 40 transects (4 m × 10 m) was used to sample terrestrial as well as epiphytic species. The minimum horizontal distance between two transects was greater than 100 m (Ding *et al.* 2015). Each transect was further divided into 2 m × 5 m quadrates. Species that were rooted within the quadrate were included in the analysis. We followed Sandord’s definition of an ‘individual’; a group of rhizomes and leaves belonging to one species, which forms a clearly delimited stand (Zotz & Schultz 2008). Vascular epiphytic species and number of individuals was recorded for each tree in the field using binoculars, sample pole and single rope climbing (Perry 1978). Most epiphytic fern species could be identified and counted from the ground since most of the canopies were easily visible. In cases where binoculars failed to provide proper resolution, 7 trees (16.6%) were climbed. Most of these climbed trees were large, having dbh (diameter at breast height) greater than 40cm. Voucher specimens of the identified species, rhizosphere type specimens and all lectotypes are stored in the herbarium collection at Bangabasi Evening College 19, Rajkumar Chakraborty Sarani, Kolkata, India.

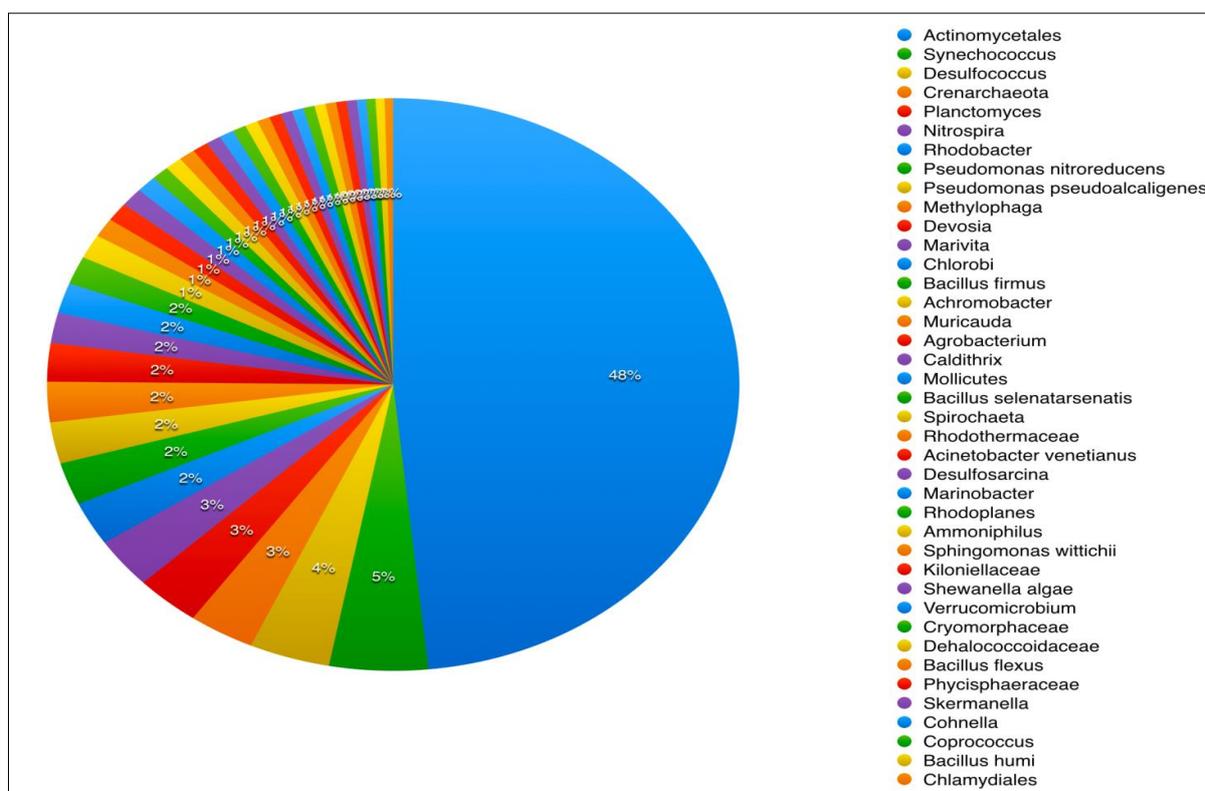


Figure 1. Rhizospheric microbial abundance of *Drynaria* from Satjelia island.

Biochemical analysis

DPPH based free radical scavenging activity: The antioxidant activity of the plant extracts was estimated using DPPH radical scavenging protocol (Blois 1958) and compared against the activity of ascorbic acid which was taken as standard. DPPH solution (0.004% w/v) was prepared in 95% ethanol. The stock solution of

ethanolic extract was prepared in the concentration of 10 µg ml⁻¹. From stock solution 2 ml, 4 ml, 6 ml, 8 ml and 10 ml were taken in five test tubes respectively. With same solvent made the final volume of each test tube up to 10ml whose concentration was then 20 µg ml⁻¹, 40 µg ml⁻¹, 60 µg ml⁻¹, 80 µg ml⁻¹ and 100 µg ml⁻¹ respectively. 2 ml of freshly prepared DPPH solution (0.004% w/v) was added in each of these test tubes. The reaction mixture was incubated in the dark for 30 minutes and thereafter the optical density was recorded at 523 nm against the blank. For the control, 2 ml of DPPH solution in ethanol was mixed with 10 ml of ethanol and the optical density of the solution was recorded after 30 minutes. The assay was carried out in triplicate. The percentage inhibition of DPPH radical was measured by the alteration in optical density (an indicator of percentage inhibition) of DPPH solution in addition of test samples in relation to the control that was used to calculate the anti-oxidant activity. The capability of scavenging DPPH radical was calculated using the following equation:

$$\text{DPPH Scavenged (\%)} = \frac{A \text{ control} - A \text{ test}}{A \text{ control}} \times 100$$

Where, “A control” is the absorbance of the control reaction and “A test” is the absorbance of the sample containing plant extracts.

Determination of Total Phenolics: Folin-Ciocalteu method (Lowry 1958) has been employed for estimation of total phenolics in the aqueous plant extracts. An aliquot (100 µl) of the extracts have been mixed with 2.5 ml Folin-Ciocalteu reagent (previously diluted with water; 1 : 10 v/v) and 2 mL (75 g l⁻¹) of sodium carbonate. The tubes would be vortexed for 15 s and allowed to stand for 30 min at 40°C for color development. The optical absorbance was recorded against reagent blank at 765 nm wavelength using the double beam spectrophotometer. The concentration of each plant extract was found to be 0.1 g ml⁻¹ and thus total phenolic contents would be expressed as mg/g n-propyl gallate equivalent.

Total Protein Estimation: The total protein estimation was performed using the method proposed by Lowry (1951). Folin-Ciocalteu reagent was used to detect the presence of total protein in the sample at an absorbance of 660nm.

Rhizosphere Metagenomic Analyses: Rhizosphere metagenome would be analysed using the method proposed by Ganguli *et.al.* (2017).

Rhizospheric soil collection and metagenomic sequencing

The 16s rRNA gene consists of nine hypervariable regions 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.

Sample preparation

Genomic DNA had been isolated from a rhizosphere sample using an in-house standardized protocol. 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 the 16S 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 MiSeq using 300 × 2 PE chemistry.

Bioinformatic analysis

The quality control of raw reads were carried out using FASTQC toolkit (<http://www.bioinformatics.babraham.ac.uk/projects/fastqc>). The quality controlled sequences were processed. The paired end reads were clustered into OTU's (Operational Taxonomic Units) by using QIIME software (qiime.org), to identify the microbial communities. These OTU's were then, further used for taxonomic assignment (Greengenes database), phylogenetic and diversity analysis. During initial bioinformatics analysis, the processed reads were assembled into contigs by QIIME (Quantitative Insights Into Microbial Ecology).

RESULTS

Ecological abundance studies revealed that the highest abundance of *Drynaria* was identified in Satjelia Island despite having significant anthropogenic influence. Interestingly the abundances were insignificantly low in the islands of Burirdabri, Netidhopani (absent) and Sajnekhali indicating that this species grows profusely in the presence of anthropogenic influence for its survival (Table 1). Biochemical parameters studied revealed that

the free radical scavenging activity of the plant was very low in most of the specimens except for the one at Satjelia (Table 2). Total protein and phenolic contents were found to be uniform across the samples indicating that they are ubiquitously metabolised in the plant system irrespective of the prevalent associations (Table 3). Metagenomic analysis from the most abundant site was performed and the results obtained enabled the identification of core microbiomes such as *Actinomycetales*, *Synechococcus*, *Plactomyces* and *Crenarcheota* members which were markedly abundant than their counterparts (Fig. 1).

Table 1. Ecological indices to understand abundance of *Drynaria* from Indian Sunderbans.

S.N.	Location	Simpson's Index for <i>Drynaria</i> alone	Shannon's Biodiversity Index of the Collection areas	Gamma Diversity (Overall Measure)
1	Rangabelia	0.1336	1.460	-
2	Satjelia	0.2234	2.502	-
3	Kumirmari	0.1468	2.445	8
4	Burirdabri	-	2.870	-
5	Sajnekhali	0.0460	2.354	-
6	Netidhopani	0	2.560	-

Table 2. Results of DPPH assay to assess the free radical scavenging activity of *Drynaria*.

S.N.	Location	% Inhibition
1	Rangabelia	27.46
2	Satjelia	35.318
3	Kumirmari	28.75
4	Burirdabri	No inhibition
5	Sajnekhali	23.56
6	Netidhopani	No inhibition

Table 3. Analysis of total protein and total phenolic content of *Drynaria* from the different locations.

S.N.	Location	Total Protein (per gm)	Total Phenolics (gm)
1	Rangabelia	2.307	0.236
2	Satjelia	2.302	0.325
3	Kumirmari	2.225	0.348
4	Burirdabri	0.006	0.012
5	Sajnekhali	2.340	0.345
6	Netidhopani	0.002	0.041

DISCUSSION

Over the years there has been several reports on the soil characters of the Sunderbans area both from the chemical as well as from the microbial content. This is as a result of the fact that the sunderban delta presents an unique transitional zone which also harbors anthropogenic influences. Due to the gradual erosion of the banks of the islands and landmasses of the Indian sunderbans (Goodbred & Kuehl 2000), more and more deforestation has taken place as the settlers have gradually moved inwards and have cut down large areas of the forest cover. This represents two significant ecological pressures, - first since these mangrove forests offers the first barrier for tsunamis and other cyclones - their erosion has resulted in the loss of more and more property. Secondly the habitat of important fauna is also lost as a result of the illegitimate felling of trees. Following the declaration of the Indian Sunderbans as the world heritage site there has been concerted efforts from both the government and local dwellers towards a forestation by planting of true mangroves. Unfortunately the expected success rate has not been achieved as a majority of the saplings have died at the initial stages. Numerous workers (Ganguli *et al.* 2017) have attributed this observation to the lack of acclimatization of these newly planted saplings to the environment. The biochemical analysis results present important insights into the potential role of the microbial members abundant in the rhizosphere. The profiles of the antioxidant assays from the standardized extract indicate that free radical scavenging activity is present ubiquitously and in considerable amount. However, current ideas regarding the plant rhizosphere associations indicate that each plant possesses a core microbiome that is constant for a particular plant. Thus it is important that these core microbiomes be identified and used as possible standardized supplements wherever that plant is being replanted (Mendes *et al.* 2011, Toju *et al.* 2018). In our results we have been able to understand the biochemical functioning of the plant using standard content analysis of phenol and protein both important by-products of metabolism. Further, the antioxidant potentials of the leaf extracts were also tested using standard protocols of DPPH assay. This leads us to conclude, that the

plant under study harbours reservoirs of biologically active compounds that can be explored further. Bodenhausen *et al.* (2013) have reported that surface microorganisms of plants are rich in Burkholderiales and Actinomycetales on the phyllosphere. Bringel & Couee (2015) further state that phyllospheremicrobiota are related to original and specific processes at the interface between plants, microorganisms and the atmosphere and thus the abundance of Actinomycetales in the rhizosphere of the epiphyte *Drynaria*, further signifies its role in the atmospheric continuum. Hence from the data obtained in this study it is evident that the impact of climate change and anthropogenic influences have not affected this particular epiphyte from the Indian Sunderbans and its population is found to thrive significantly in the areas where there is human settlement. Hence it can be safely concluded that *Drynaria* may serve as a direct ecological indicator of human settlement in the Indian Sunderbans.

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