

**STUDY OF MANGROVE ROOT ARCHITECTURE,  
ANATOMICAL ADAPTATIONS AND MANGROVE  
MICROBE INTERACTIONS IN THE RHIZOSPHERE  
CONTRIBUTING TO THE SOIL ENVIRONMENT  
UNDER DEGRADED AND NON-DEGRADED  
MANGROVE HABITATS.**

**Abstract of the Thesis**

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## **Abstract of the Thesis**

The sites selected were both non-degraded and degraded mangrove environments for my study. Among them, Shiber Ghat (21°50'29.30"N 88°21'23.95"E), Atharogazi (21°50'45.90"N 88°23'18.44"E) and Ramganga (21°47'34.30"N 88°22'53.01"E) are moderately to highly degraded mangrove ecosystems. Whereas Lakhipur (21°49'12.74"N 88°23'19.65"E) and Patharprotima (21°47'34.70"N 88°22'7.18"E) are pristine mangrove forests or non-degraded mangrove ecosystems. The below-ground and above-ground biomass ratio for mangroves is observed to be high, compared to other terrestrial communities suggesting that mangrove trees share a greater proportion of net primary production for their root growth as compared to other terrestrial tree communities. Although mangroves are well adapted to maintain oxygen availability in root cells under waterlogged conditions (negatively geotropic pneumatophores), anaerobicity being predominant in the rhizosphere compels roots to function under stress. My hypothesis was that mangrove roots should harbor endophytic microbes that interact with root cells, and promote growth, facilitating nutrient availability to the cells even under external anoxic ambiance. Endophytes can colonize the internal tissues of their host plant, including symbiotic, mutualistic, and trophobiotic relationships. Most endophytic bacteria originate from the rhizosphere. In this study mangrove species involved are *Avicennia alba*, *Avicennia marina*, *Avicennia officinalis*, *Heritiera fomes*, *Dalbergia* sp., *Bruguiera cylindrica*, *Bruguiera gymnorrhiza*, *Rhizophora mucronate*, *Xylocarpus* sp., *Derris trifoliata*, *Ceriops tagal*, *Excoecaria agallocha*, *Myriostachya wightiana*, *Porteresia coarctata*, from Indian Sundarbans, world's largest mangrove ecosystem. Root and pneumatophore samples from these mangrove species were studied for their anatomical adaptations, pure endophytic bacterial strains were established, genomic DNA was isolated from isolated bacteria, and PCR amplification of 16S rRNA gene from genomic DNA of bacteria was carried out. Partial 16S rRNA gene sequences were submitted to the NCBI database and accession numbers for the same was obtained. I have been successful in the establishment of 78 pure isolates of cultivable endophytic bacteria (NCBI Accession no. MT421976 to MT422053) from roots and pneumatophores of different mangroves and mangrove associate species of Indian Sundarbans. The study was conducted with the following objectives:

**Objective-1: Study of morphological/histological niches, anatomical adaptations,  $\text{Na}^+/\text{K}^+$  ratio in endosphere of roots and pneumatophores of mangrove species from degraded and non-degraded habitats of Sundarbans**

For the study of anatomical adaptations and  $\text{Na}^+/\text{K}^+$  ratio, I have selected *Avicennia alba*, *Avicennia marina*, *Avicennia officinalis*, *Heritiera fomes*, *Dalbergia* sp., *Bruguiera cylindrica*, *Bruguiera gymnorrhiza*, *Rhizophora mucronate*, *Xylocarpus* sp., *Derris trifoliata*, *Ceriops tagal*, *Excoecaria agallocha*, *Myriostachya wightiana*, *Porteresia coarctata*, from Indian Sundarbans. In histological transverse sections of roots and pneumatophores the following anatomical adaptive features are observed such as secondary cork tissues, aerenchymatous cortex, distinct endodermis, well developed vascular xylem and phloem with parenchyma rays, central pith tissues with parenchymatous cells etc.

$\text{Na}^+/\text{K}^+$  ratio of roots, shoots and leaves of the above mangrove species was carried out by flame photometry.  $\text{Na}^+/\text{K}^+$  ratio was estimated in root endosphere niches of the above mangrove species that maintained a low ratio of  $\text{Na}^+/\text{K}^+$  ( $<5$ ) in roots. However, *Bruguiera gymnorrhiza* and *Ceriops* spp. showed higher ( $>5$ )  $\text{Na}^+/\text{K}^+$  ratio in roots.

$\text{Na}^+/\text{K}^+$  ratio was estimated in shoot endosphere niches of the above mangrove species that maintained a low ratio of  $\text{Na}^+/\text{K}^+$  ( $<5$ ) in shoot except *Bruguiera gymnorrhiza* showed higher ( $>5$ )  $\text{Na}^+/\text{K}^+$  ratio in shoot.

$\text{Na}^+/\text{K}^+$  ratio was estimated in leaf endosphere niches of the above mangrove species that maintained a low ratio of  $\text{Na}^+/\text{K}^+$  ( $<5$ ) in leaf. However, *Bruguiera gymnorrhiza* and *Myriostachya wightiana* showed higher ( $>5$ )  $\text{Na}^+/\text{K}^+$  ratio in leaf.

**Objective-2: Establishment of pure endophytic bacterial strains from mangrove roots and pneumatophores and their molecular identification by sequencing of 16S rRNA gene**

Roots and pneumatophore samples of different mangrove species collected were cut into small pieces. After that surface sterilization was done by 0.1%  $\text{HgCl}_2$  solution. Then the samples were incubated in LB broth medium. From there 50  $\mu\text{l}$  of cultures were spread on LB plate and single colony was isolated after incubation. After that each single colony was identified. All total 78 bacterial colonies were identified as endophytes till date by me. The culturable community of endophytes comprises species of 12 genera viz. *Bacillus*, *Aeromonas*, *Pseudomonas*, *Staphylococcus*, *Vibrio*, *Gallacimonas*, *Serratia*, *Enterobacter*, *Pseudocitrobacter*, *Citrobacter*, *Acinetobacter*, *Mangrovibacter*. Bacterial endophytes were selected repeatedly on differential nutrient cycling media.

### **Objective-3: Plant growth promotion profiling of the established/accessioned endophytic bacterial strains**

All 78 endophytic bacteria were tested for their plant growth-promoting potential under laboratory conditions through assays for indole-3-acetic acid production, phosphate solubilization, siderophore production, free nitrogen fixation (**Acetylene reduction assay for demonstration of nitrogenase activity *in vitro* for free nitrogen fixer endophytes**), 1-aminocyclopropane-1-carboxylate deaminase (ACC) synthesis.

For indole-3-acetic acid production activity the best IAA producer strains are found and their IAA production ranged between 9-17 µg/ml. The best IAA producer strains are as follows, such as *Pseudomonas sp.*, *Aeromonas sp.*, *Bacillus sp.* and *Staphylococcus sp.* The best 15 high IAA producer strains were also tested for their IAA production without adding tryptophan to the assay reaction. The IAA production without adding tryptophan ranged between 4.5-10 µg/ml.

The phosphorus (P) solubilization potential activity among the best P-solubilizer strains ranged between 90-160 µg of plant-available P released/ml of PSM. The best P-solubilizer strains found were *Aeromonas sp.*, *Pseudomonas sp.*, *Staphylococcus sp.*, *Gallacimonas sp.*, *Vibrio sp.*, and *Serratia sp.*

In case of siderophore production activity, the best siderophore-producing strains demonstrated siderophore production between 80%-95% in iron-free media. The best siderophore-producing strains observed are *Aeromonas sp.*, *Pseudomonas sp.*, *Staphylococcus sp.*, *Bacillus sp.*, *Mangrovibacter sp.*, and *Citrobacter sp.*

All 78 endophytic bacterial strains were tested for their ACC deaminase production potential. The best ACC deaminase-producing strains demonstrated ACC deaminase production units between 1.8-9. The best ACC deaminase-producing strains are as follows, such as *Aeromonas sp.*, *Pseudomonas sp.*, *Staphylococcus sp.*, *Bacillus sp.*, and *Citrobacter sp.*

Regarding the free nitrogen fixing potential of 78 endophytes, the best free nitrogen-fixing strains demonstrated reduced ethylene synthesis in the range of 0.4-14 nmole/100 µl/24 hour. The best reduced ethylene synthesis demonstrating strains are *Aeromonas sp.*, *Pseudomonas sp.*, *Staphylococcus sp.*, *Bacillus sp.*, *Pseudocitrobacter sp.* and *Citrobacter sp.*

**Objective-4: Selection of PGP endophytic bacterial consortia on the basis of growth promotion criteria and growth curves for nursery/greenhouse/field applications**

After analysing the plant growth promotion activity and salt tolerance profile via quantifying growth by measuring absorbance at 600 nm for some promising endophytic free nitrogen fixers, cellulose degraders and P-solubilizers on FNFM/CDM/PSM normal broth (3.99-5.44 dS/m), and also normal FNFM/CDM/PSM with additional 0.3M NaCl (25.23-31.94 dS/m), with additional 0.5 M NaCl (36.15-45.72 dS/m), and 0.7 M (45.09-56.73 dS/m), for 72 hrs starting at 0.1 OD at 0 hr. However, in most of the cases, the growth on FNFM/CDM/PSM broth without or with NaCl decreased in absorbance rather than increase after 72 hrs. On the basis of PGP activity and salt tolerance profile, 18 bacterial strains were selected for making 3 combinations. The combinations are as follows:

**A. Consortium BC1:** *Aeromonas dhakensis* strain HPR7 (MT422007), *Mangrovibacter plantisponcer* strain BCRP5 (MT422011), *Pseudomonas stutzeri* strain BCY5 (MT422041), *Pseudomonas stutzeri* strain BCY7 (MT422042), *Bacillus subtilis* strain AOR5 (MT421976) and *Serratia marcescens* strain AOR4 (MT422009).

**B. Consortium BC2:** *Aeromonas allosaccharophila* strain DAL2 (MT422027), *Pseudomonas* sp. strain DER1 (MT422045), *Pseudomonas putida* strain DER3 (MT422020), *Pseudomonas fulva* strain DER9 (MT422023), *Aeromonas veronii* strain POT3 (MT422035), *Aeromonas veronii* strain POT7 (MT422025) and *Serratia marcescens* strain HPR4 (MT422006).

**C. Consortium BC3:** *Bacillus subtilis* strain AMR4 (MT421979), *Aeromonas hydrophila* strain HER3 (MT422047), *Bacillus altitudinis* strain XYL1 (MT422037), *Pseudocitrobacter faecalis* strain HRR5 (MT422004) *Serratia marcescens* strain HPR4 (MT422006) and *Enterobacter kobei* strain HRR1 (MT421989).

**Objective-5: Application of selected PGP endophytic bacterial consortia on *Avicennia officinalis* saplings in mangrove nursery at Sundarbans for observing their effect on mangrove growth modulation.**

The 3 selected consortia were applied twice on 23 days-old *Avicennia officinalis* saplings, 80 saplings for each consortium, and 80 saplings were used as control where no bacteria were applied in the mangrove nursery at Ramganga village of Sundarbans. Bacterial cultures were applied twice at 30 days intervals. Final shoot height, leaf width, and number of leaves per plant were measured from 95 days-old saplings and their effect on mangrove growth modulation was observed. Among 3 combinations consortium BC3 showed significant shoot

height improvement of *Avicennia officinalis* saplings. No significant difference in no. of leaves per sapling was observed among all the categories. BC1 and BC2 consortia-applied saplings showed a significant increase in leaf length. No significant difference in leaf width per sapling was observed among all the categories.

**Objective-6: Application of selected PGP endophytic bacterial consortia on cultivated rice of Sundarbans at greenhouse for observing their effect on rice growth modulation.**

Two selected rice varieties, Lunishree (N) and Dudheswar (D), were used for plant growth promotion studies at greenhouse pot experiments with control set where no bacterial culture was applied and also with conventional fertilizer NPK (20:20:20) applied in conventional doses for approximately 0.8 Kg of soil per pot used in the experiment.

A total of 12 bacterial consortia were used for the pot experiment; 5 pots for NPK with 3-4 seedlings per pot and 5 pots for control with 3-4 seedlings per pot were used for both the rice varieties. Among 12 bacterial consortia applied, 3 consortia belonged to only endophytic bacterial consortia BC1, BC2, and BC3. The remaining 9 consortia were made combining 6 rhizospheric consortia from halophytic native grasses from Sundarbans developed in another study. The greenhouse experiments were performed twice and the consortia were applied two times in both experiments. From both experiments we could obtain some significant results in shoot heights and leaf lengths of the two selected rice varieties, Lunishree (N) and Dudheswar (D). In addition, significant improvement was observed in terms of N and P enrichment in the consortia-added pot rhizosphere soil in comparison to the control pots where no bacterial consortia were added and also the NPK-added pot soil.

The same 3 root endophytic consortia were also applied in combination with PGP native halotolerant grass rhizobacteria in two consecutive greenhouse experiments. Tracking of P-solubilization profile at rhizospheres after addition of bacteria revealed enrichment of added consortia validated through increase of soluble-P release at rhizosphere even at the time of maximum utilization by the plants.

**Objective-7: Application of selected PGP endophytic bacterial consortia on cultivated rice of Sundarbans at the field at Sundarbans for observing their effect on rice growth and yield modulation.**

After the greenhouse experiments, 3 combinations of 18 selected growth-promoting bacteria out of these 78 accessions were applied to the rice field of Dudheswar landrace (IC No. 593998), widely cultivated at Sundarbans and also known to be an ideal salt tolerant variety

for growing at low lands. Field experiments were performed for twice to date, indicating significantly increased yield for the consortia-added plots in comparison to the control plots where bacterial consortia were not added and also compared to the rice grain yield data collected from several other neighbouring rice fields grown by local farmers where rice was cultivated with conventional fertilizers.

1<sup>st</sup> Field application of Consortium BC1 showed a yield of an average of 9.58 quintal/bigha, Consortium BC2 showed a yield of an average of 11.2 quintal/bigha, and Consortium BC3 showed a yield of an average of 11.3 quintal/bigha in comparison to control fields (without bacteria) yielding average of 7.52 quintal/bigha. The data collected from local farmers' field in the Sundarban western part in Patharpratima block exhibited only a yield of average of 3.7 quintal/bigha. The results of the study is found to be significant with respect to the control.

From the observation of 1<sup>st</sup> rice field experiment significant results, 2<sup>nd</sup> rice field experiment was conducted with the selected bacterial consortia where one endophytic consortium as BC3/BEC3 and two mixed combinations such as BEC2+SRC2 and BEC3+SRC3 were applied. 2<sup>nd</sup> rice field experiment conducted in two rice field plots such as field 1 and field 2.

In field 1 of 2<sup>nd</sup> field application, Consortium BC3 showed an yield of average 3.57 quintal/bigha, Consortium BEC2+SRC2 showed a yield of average 28.97 quintal/bigha and BEC3+SRC3 showed a yield of average 4.18 quintal/bigha in comparison to control fields (without bacteria) yielding average 0.66 quintal/bigha.

In field 2 of 2<sup>nd</sup> field application of Consortium BC3 showed a yield of average 18.94 quintal/bigha, Consortium BEC2+SRC2 showed a yield of average 16.14 quintal/bigha and BEC3+SRC3 showed a yield of average 13.96 quintal/bigha in comparison to control fields (without bacteria) yielding average 9.27 quintal/bigha. In this case the yield demonstrated significant increase in quintal/bigha as compared to the control both in field 1 and field 2.

Tracking of P-solubilization profile at rhizospheres in both the field experiments at Sundarbans after addition of bacteria revealed enrichment of added consortia validated through increase of soluble-P release at rhizosphere even at the time of maximum utilization by the plants. N-enrichment was also observed at rice rhizospheres in field where bacterial consortia were applied.

### **Objective-8: Observation of bacterial community abundances by Next Generation Sequencing (NGS) from rice field before and after rice cultivation with PGP endophytic bacterial consortia**

The next generation metagenomic sequencing of rhizobacterial community from the collected rhizospheric soil from the 1<sup>st</sup> field experiment of Indian Sundarbnas, established noticeable enrichment of the applied bacterial consortia through analysing the relative abundance of the constituent bacterial members by amplicon based V3-V4 16S rRNA metagenomic sequencing through Illumina MiSeq platform. When the data was compared on the basis of phylum level, it showed enrichment of Firmicutes at BC1, BC2, BC3 consortia added soil as well as when all root endophyte consortia added soil was sampled in a mixed way. The constituent genus of root endophyte consortia *Bacillus* spp. belongs to the phylum Firmicutes. When the data was compared on the basis of class level it showed enrichment of Bacilli at BC1, BC2, BC3 consortia added soil as well as enrichment of Gammaproteobacteria at BC1, BC2, BC3 consortia added soil and when all root endophyte consortia added soil was sampled in a mixed way. The constituent genus of root endophyte consortia *Bacillus* spp. belongs to the class Bacilli, whereas *Aeromonas*, *Mangrovibacter*, *Pseudomonas*, *Serratia*, *Pseudocitrobacter*, *Enterobacter* spp. all belong to the class Gammaproteobacteria. When we the data was compared on the basis of family level it showed enrichment of Bacillaceae at BC1, BC2, BC3 consortia added soil. The family Prevotellaceae possesses the genus *Prevotella\_9* and *Prevotella* which are signature sequences of soil manured with cow-dung as *Prevotella\_9/Prevotella* has been reported to be present in gut microbiome of these animals. Relative abundance of Prevotellaceae was observed to be higher in control rhizosphere as compared to BC1, BC2, BC3 consortia added soil. However, the referred abundance is observed to be very high when all root endophyte consortia added soil was sampled in a mixed way (might be due to sampling error).

Comparative account of relative abundances at genus level shows the following features:

- Abundance enrichment of *Aeromonas* at BC2, BC3 consortia added soil
- Noticeable abundance enrichment of *Bacillus* at BC1, BC2, BC3 consortia added soil
- Consistent relative abundance of *Pseudomonas* observed in all the samples with highest abundance observed when all root endophyte consortia added soil was sampled in a mixed way



- Decrease in abundance of *Prevotella\_9* at BC1, BC2, BC3 consortia added soil. However, the referred abundance is observed to be very high when all root endophyte consortia added soil was sampled in a mixed way (might be a cause of sampling error).
- Noticeable abundance of *Mangrovibacterium* at BC2, BC3 consortia added soil, all root endophyte consortia added soil as well as all halo-grass rhizosphere bacterial consortia added soil.
- The constituent genera of root endophyte consortia are *Aeromonas* spp., *Bacillus* spp. *Pseudomonas* spp., *Mangrovibacter* spp.