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Molecular diversity and phylogenetic analysis of exopolymer producing fungal strains isolated from Krishna river mangrove sediments

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ABSTRACT

Mangroves salt-tolerant coastal woodlands cover 1/4th area of tropical coastlines globally and are a major driver for the accumulation of large deposits of organic matter, offer protection against coastal erosion, and a niche for a broad consortium of microbes. Yet, mangroves and mangrove fungi are the most threatened species understanding their role and distribution is becoming a pressing concern, especially when viewed in the light of rapid climate change. Therefore, the rationale of the study is to describe the fungal communities associated with the Krishna river delta mangroves. A soil investigation found that humans have a large role in the estuarine environment, as well as microbial interactions. Extensively employing a molecular sequencing method that targets the complete 5.8s rRNA [internal transcribed spacer (ITS1–ITS4)], several fungal species were isolated from the mangrove soils. Three diverse fungal populations are present in genera *Penicillium*, *Fusarium*, and *Mucor*, while two diverse fungal populations are present in genera *Trichoderma* and *Paecilomyces*. Fungal species diversity is shown to be modest in sample locations according to the recent research. Using CLUSTAL-W, we found that up to one-third is accounted for by phylogenetic analysis using multiple sequence alignment tool. In this study, numerous fungal species have been discovered in the harsh environment of mangrove soil, and this has helped researchers to get understanding about the variety of species in the Krishna river delta mangroves.

1. INTRODUCTION

Mangroves are unique salt-tolerant coastal woodlands that cover 1/4th area of tropical coastlines globally. The coastal area of India is home to mangrove forests that stretch across nine states and four union territories. The deltaic, estuarine, and insular mangroves are found along the east coast of India (located in Andaman and Nicobar Islands). Mangroves, which is 58% of the east coast, cover 29% of the west coast, and the Andaman and Nicobar Islands, each for 10% of the total area (13%) [1]. The mangrove wetlands of the Krishna estuary are the second largest perennial rivers on the east coast originating at the Western Ghats flowing eastwards and emptying to the Bay of Bengal near Machilipatnam. Krishna delta mangroves' forests cover an area around 582 sq. km with a tropical humid climate (hot summers and moderate winters). Mangroves are one of the threatened habitats witnessing an annual loss of 0.16%–0.39% globally, due to anthropogenic activities, destruction of mangrove forests, and coastal development [2]

In tropical as well as in subtropic areas, mangroves are diverse habitats with high microbial diversity. Fungi are the key components of microbial community in mangrove sediments. The productivity of mangroves is very dependent on the fungal community's metabolic activity, important in mangrove habitats for the cycling of C and N, and degradation of lignocellulose.

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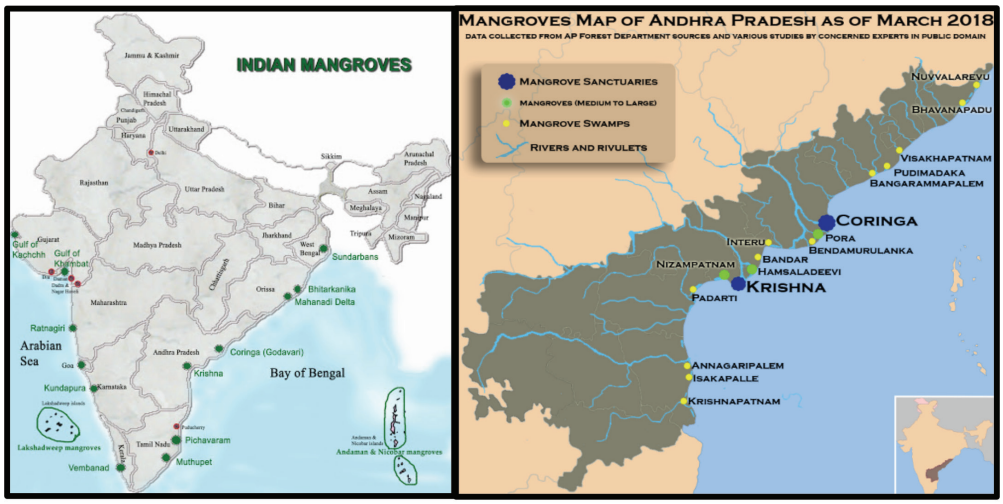
The fungi are involved in bioremediation, biological regulation, and plant growth promotion [3]. However, mangrove fungi are vulnerable to anthropogenic factors such as logging, wastewater contamination, infrastructure projects, and oil spills because of their hygiene, enzyme activity, and their diversity. Overall, there are a total of 414 fungus belonging to 226 genera recorded from the Indian mangroves thus far, and these comprise ascomycetes (325 species in 189 genera), basidiomycetes (35 species in 28 genera), and zygomycetes (which represent over half of all species) (54 species in 10 genera). Ascomycetes have become the primary genus in a marine environment.

Over the last decade, advancements in molecular technique led to numerous new results in microbial ecology studies. An important approach to explore microbial populations reported were polymerase chain reaction (PCR) amplification and sequencing of PCR 5.8S rRNA gene products metagenomics, heterologous cloning, expression of metagenomic samples used for functional/sequence-based screening. Before developing massively parallel sequencing techniques, mangrove fungi studies focused on identifying cultivable fungi, characterizing extracellular enzymes, polysaccharides and their function in biodegradation, emulsification, flocculation, rheology, oil recovery, biosorption. These communities have been molecularly characterized primarily by regions [internal transcribed spacer (ITS1–ITS4)]. The outcome from many sequencing projects comprehended that the population of mangrove fungi was affected by different factors such as soil chemistry, sample sources such as the roots, pneumatophores or soils of aerial or submerged, rhizosphere, and non-rhizospheric mangroves. The most abundant phyla with functional groups were detected in Ascomycota, Basidiomycota (saprotrophic fungi) [1]. Furthermore, most of the mangrove fungi are untapped bioresources



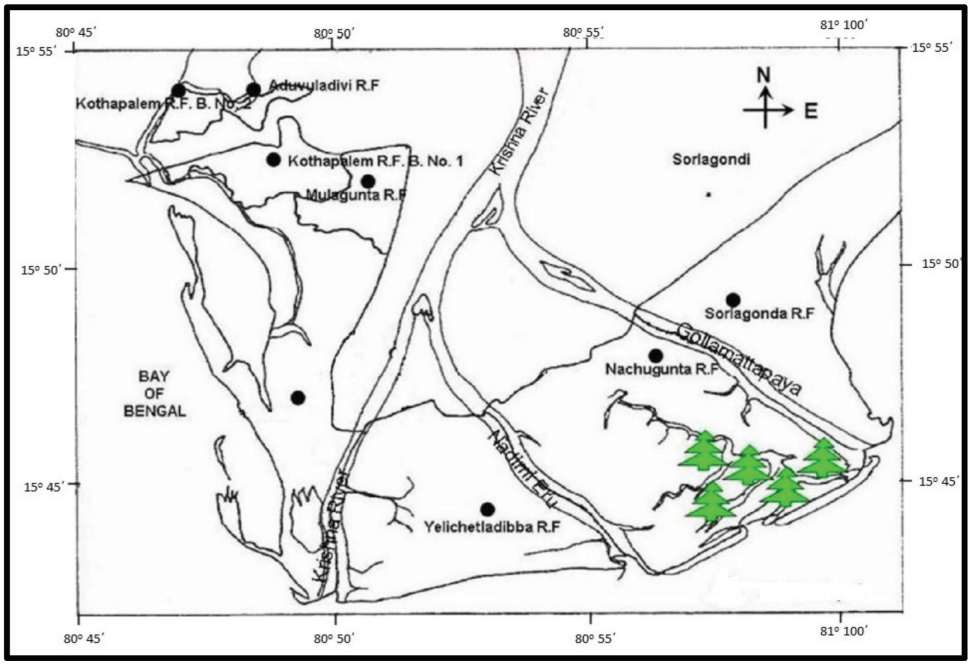


Figure 1: Map showing sampling areas in Krishna delta mangrove wetland ecosystem (source: M.S. Swaminathan Research Foundation).

Table 1: Salinity conversion factors (adopted from Hardie and Doyle [9]).

Soil texture	Multiplication factor
Sands	17
Sandy loams soils	13.8
Loam soils	9.5
Clay loams and light Clay soils	8.6
Medium and heavy clay soils	7

for novel natural products, yet scanty literature is available regarding their biodiversity and global distribution [4,5].

We attempted to study how fungal community composition, shaping factors, assembly, and co-occurrence patterns varied due to molecular identification and phylogenetic analyses. We predicted that: (1) There are a lot of fungi living in mangrove sediment, and many of these fungi are part of the ancient fungus lineage; (2) Specific locations and sediment depth impact on the types of fungi found in mangrove ecosystems. The soil under the mangroves also has numerous other characteristics evaluated.

2. MATERIALS AND METHODS

2.1. Study Area

The mangrove wetlands of Krishna estuarine, Andhra Pradesh falls under geographical location i.e; 16°0′ to 16°15′N latitudes and 81°10′ to 81°15′E longitudes. Krishna delta mangrove wetlands includes 1. Sorlagondi Reserve Forest (RF) 2. Nachugunta RF 3.Yelichetladibba RF 4. Kottapalem, RF 5. Molagunta RF 5. Adavuladivi RF 6. Lankivanidibba RF. The other field stations viz. Gilakaladindi, Pedapatnam, Avanigadda, and Nagayalanka gained significance in mangrove study as they receive seawater by the tidal effect. Figure 1 shows the mangrove vegetation and sampling area of the Krishna delta.

2.2. Sample Collection

Mangrove detritus and sediment samples were collected randomly from six sampling sites of Krishna delta, namely, 1. Machilipatnam, 2. Avanigadda, 3. Nagayalanka, 4. Gilakaladindi, 5. Pedapatnam, and 6. Sorlagondi. We have taken the help of Global Positioning System to determine the sampling area. We planned the study from July to November 2015. Mangrove sediment sample was collected at 2–3 m depth using a soil corer during low tides in precleaned polycarbonate glass bottles and stored at −20°C before further analysis. Sediment samples were used to carry out a physiochemical and fungal analysis within 24 hours of collection. Samples were collected in three replicates at each station [6].

Table 2: Geographical locations of EPS producing fungal population.

Sampling site	Sampling date	Geographical positions		Viable count (sCFU × 10^2 cells/g)
		Latitude	Longitude	
Machilipatnam	10/07/2015	16°11′25.96″N	81°8′10.1544″E	15.5 ± 1.2 × 10^2
Gilakaladindi	12/07/2015	16°8′57.78″N	81°9′44.44″E	8.6 ± 0.7 × 10^2
Avanigadda	16/08/2015	16°1′17.13″N	80°54′57.39″E	11.1 ± 1.5 × 10^2
Nagayalanka	18/08/2015	15°56′43.97″N	80°55′4.83″E	12.6 ± 1.5 × 10^2
Pedapatnam	24/11/2015	15°47′16.83″N	80°53′17.80″E	10.5 ± 0.8 × 10^2
Sorlagondi	26/11/2015	15o51′50.36″N	80°58′1.46″E	13.5 ± 0.9 × 10^2

2.3. Physicochemical Analysis of Soil Samples

The physicochemical parameters of soil samples, such as temperature, pH, salinity, total nitrogen, phosphates, organic matter, soil nature, were estimated as per the standard procedures [7]. The study adopted both field and lab procedures for soil sample analysis. The sediment and detritus samples were air-dried before analysis where pH and conductivity were analyzed using wet samples. pH was calculated using a pH meter and digital thermometer respectively as per the method described by Grasshoff *et al.* [8]. Salinity was measured by a Salinity meter. Obtained readings ($EC_{1:5}$) were converted to soil salinity (EC_e) by multiplication of the value with conversion factor based on texture of soil sample (Table 1) [9]. We grounded the sediment samples into fine powder after drying in the oven at 110°C and used them for the analysis of other parameters. The total nitrogen content of the sediment samples was calculated using the Kjeldahl method [10]. While available phosphorus in sediment was determined by Bray and Kurt method. Total organic carbon in sediment was

Table 3: Physical and chemical properties of mangrove sediments.

Sampling site	pH	Temperature °C	Salinity %	Organic matter (g kg^{-1})	Total *N* (g kg^{-1})	Total *P* %	Sediment nature	Sand (g kg^{-1})	Silt (g kg^{-1})	Clay (g kg^{-1})
Machilipatnam	7.9	30	28	54.9	2.38	0.92	Sandy loam	570	290	70
Gilakaladindi	7.8	30	29	47.2	2.05	0.88	Sandy loam	545	205	66
Avanigadda	7.6	29	25	35.8	1.06	0.74	Sandy loam	450	195	63
Nagayalanka	7.6	28	26	39.3	1.23	0.81	Sandy loam	520	230	54
Pedapatnam	7.5	29	27	48.5	2.11	0.79	Sandy loam	552	243	65
Sorlagondi	7.7	27	26	50.2	1.95	0.85	Sandy loam	529	278	58

Total *N* = Kjeldhal N; Total *P* = Phosphates.

Table 4: Identification of selected EPS-producing fungal isolates by 5.8SrRNA gene sequence.

S.no.	Isolate no.	Species as close relatives	Accession number	Similarity (%)
1.	ANP1	*Penicillium oxalicum*	KY560310	99.47
2.	ANP2	*Fusarium equiseti*	KY560311	99.39
3.	ANP3	*Penicillium oxalicum*	KY560312	95.97
4.	ANP4	*Fusarium equiseti*	KY560313	98.39
5.	ANP5	*Penicillium oxalicum*	KY560314	99.26
6.	ANP6	*Mucor fusiformis*	KY560315	89.40
7.	ANP7	*Fusarium equiseti*	KY560316	98.80
8.	ANP9	*Fusarium equiseti*	KY560317	98.03
9.	ANP10	*Penicillium oxalicum*	KY560318	99.08
10.	ANP10	*Trichoderma arundinaceum*	MT496887	98.63
11.	ANP11	*Paecilomyces sp.*	MT496888	97.58
12.	ANP12	*Aspergillus terreus*	MT496889	99.16
13.	ANP13	*Aspergillus aculeatus*	MT496890	99.13
14.	ANP14	*Penicillium goetzii*	MT496891	99.15
15.	ANP15	*Aspergillus niger*	MT563164	96.06
16.	ANP16	*Aspergillus flavus*	MT563165	98.45
17.	ANP17	*Fusarium solani*	MT563166	95.14
18.	ANP18	*Trichoderma pubescens*	MT563167	99.36
19.	ANP20	*Fusarium oxysporum*	MT641197	98.22
20.	ANP20	*Fusarium oxysporum*	MT641198	95.67
21.	ANP21	*Fusarium solani*	MT641199	97.24
22.	ANP22	*Fusarium solani*	MT641200	95.16
23.	ANP23	*Aspergillus aculeatus*	MT641201	98.60
24.	ANP24	*Aspergillus terreus*	MT641202	99.16
25.	ANP25	*Penicillium solitum*	MT703780	99.02
26.	ANP26	*Fusarium equiseti*	MT703781	91.21
27.	ANP27	*Penicillium solitum*	MT703782	98.34
28.	ANP28	*Penicillium expansum*	MT703783	96.74
29.	ANP29	*Mucor fusiformis*	MT703784	99.16
30.	ANP30	*Mucor sp.*	MT703785	96.58
31.	ANP31	*Mucor hiemalis*	MT703786	99.17
32.	ANP32	*Penicillium oxalicum*	MT703787	98.75
33.	ANP33	*Penicillium oxalicum*	MT703788	98.75

analyzed using the method described by Byers *et al.* [11]. Soil texture analysis (silt, clay, and sand) were measured according to the US Department of Agriculture soil classification.

2.4. Isolation and Molecular Identification of Isolated Fungal Strains

Mangrove sediments and detritus samples were serially diluted using 0.9% NaCl and 0.1 ml of each sample was surface speeded onto potato dextrose agar (PDA) media supplemented with Penicillin G. A control plate was prepared by spreading 0.9% NaCl. All the inoculated plates were incubated at 27°C and were monitored regularly until the development of colonies. Mycelium was sub-cultured onto a new plate until pure cultures were obtained. Morphologically distinct individual fungal colonies were picked and to obtain pure spores were streaked on PDA agars which were used as inoculums for further experiments [12]. The fungal strains were identified by the sequencing of 5.8S rDNA. The genomic DNA was obtained from pure cultures and PCR for 5.8S rRNA gene amplification was done with universal primers ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′), ITS4 (5′ TCCTCCGCTTATTGATATGC-3′). The amplified sequences were aligned in National Center for Biotechnology Information -Basic Local Alignment Search Tool.. Obtained sequence data was analyzed to identify the nearest phylogenetic homologs of all the isolated fungal strains and submitted in GenBank with accession numbers.

2.5. Diversity and Phylogenetic Analysis of Isolated Fungal Strains

Diversity relationships among EPS-producing fungal species isolated in this study were statistically studied. Univariate (Simpson's index *D*, species richness–Margalef index *M*, Simpson's index of diversity 1−*D*; Simpson's reciprocal index 1/*D*; Evenness *E*; Shannon-Weaver index *H*) and phylogenetic analysis were employed. Sequences of isolated strains were aligned using CLUSTAL-W. Evolutionary analysis and the phylogenetic tree was constructed in MEGA7 software by unweighted pair group method with arithmetic mean, neighbor-Joining method with 1,000-bootstrap replicates [13,14]. Furthermore, Tajima's [15] neutrality test was used to determine size and power properties based on molecular polymorphism data. Maximum likelihood estimate of γ-parameter for site rates, substitution matrix, and transition/transversion bias were calculated. The rate variation among sites was modeled with a γ distribution (shape parameter = 1) [16]. Alpha, beta, and γ-biodiversity indices were calculated using a biodiversity online calculator which computes multiple indices for the input data (https://www.alyoung.com/labs/biodiversity_calculator.html).

3. RESULTS AND DISCUSSION

3.1. Diversity of Microbial Communities Regarding Environmental Parameters

In the present study, six sampling sites were selected for the sampling of mangrove sediments, viz., Machilipatnam, Avanigadda, Pedapatnam, Nagayalanka, Pedapatnam, and Sorlagondi. The

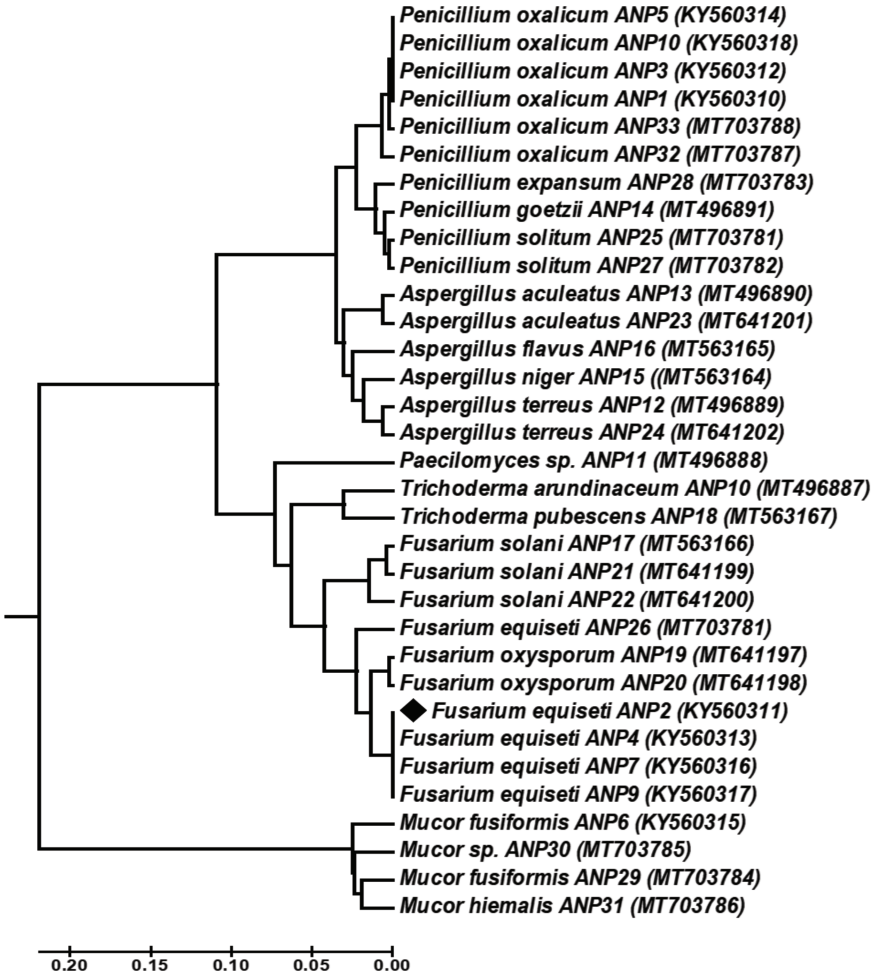


Figure 2: Phylogenetic tree based on 5.8S rRNA gene comparison among the isolated fungal strains from mangrove delta. Accession number were given in parenthesis.

geographical locations of the sampling sites along with viable count were shown in Table 2. The physical and chemical properties of the sediments from six sites are depicted in Table 3. All the sediments are sandy loams having temperature (27°C–30°C); pH (7.5–7.9); salinity (25%–29%); phosphates (0.74%–0.92%); nitrogen (1.06%–2.38%). Organic matter and percent total nitrogen and phosphate contents were higher in the Machilipatnam sediments when compared to other sampling sites enlisted. In the present investigation, Machilipatnam sampling location showed a high viable count of $15.5 \pm 1.2 \times 10^2$ cells/g where other sampling sites showed comparatively less viable counts $8.6 \pm 0.7 \times 10^2$, $11.1 \pm 1.5 \times 10^2$, $12.6 \pm 1.5 \times 10^2$, $10.5 \pm 0.8 \times 10^2$, and $13.5 \pm 0.9 \times 10^2$ cells/g. The total viable count of fungi from all the sediment samples ranged between 8.6 and 15.5×10^2 CFU/g when sediment samples were plated on PDA plates.

Environmental factors like temperature, pH, salinity, and availability of organic carbon control the load of microbial activities. Variation in salinity is a predominant factor in mangroves and depends on the behavior of tides, the reason for changes in salinity levels (25%–29%) in sampling sites. In addition, high levels of salinity in Krishna mangroves are related to high evaporation percentage

Table 5: Comparison of species diversity indices among isolated EPS producing fungi.

Environment	Method	*I*	*S*	*D*	1−*D*	1/*D*	*H*	*E*	*M*
Site 1	5.8S rRNA	8	6	0.188	0.812	5.319	1.733	0.967	2.4045
Site 2	5.8S rRNA	6	4	0.278	0.722	3.597	1.33	0.959	1.6743
Site 3	5.8S rRNA	7	3	0.388	0.612	2.577	1.004	0.914	1.0278
Site 4	5.8S rRNA	3	2	0.556	0.444	1.799	0.637	0.918	0.9102
Site 5	5.8S rRNA	4	3	0.375	0.625	2.667	1.04	0.946	1.4427
Site 6	5.8S rRNA	5	4	0.28	0.72	3.571	1.332	0.961	1.864

Table 6: Biodiversity indices of among communities of producing fungi isolated from mangrove sediment.

Dataset totals			
Total number of organisms:	33	Total number of species:	23
Average population size:	1.435	Decimal accuracy:	4
Total number of regions:	6	Total number of region sets:	6
Alpha biodiversity (α)			
Simpson index $\frac{\sum_i n_i(n_i-1)}{N(N-1)}$	0.0303	Simpson index approximation $\frac{\sum_i n_i^2}{N^2}$	0.05969
Dominance index $1-\left(\frac{\sum_i n_i(n_i-1)}{N(N-1)}\right)$	0.9697	Dominance index approximation $1-\left(\frac{\sum_i n_i^2}{N^2}\right)$	0.9403
Reciprocal Simpson index $\frac{1}{\left(\frac{\sum_i n_i^2}{N^2}\right)}$	33	Alternate reciprocal Simpson index $\frac{1}{\left(\frac{\sum_i n_i(n_i-1)}{N(N-1)}\right)}$	16.75
Shannon index $-\sum_i\left(\frac{n_i}{N}\cdot\log_2\left(\frac{n_i}{N}\right)\right)$	4.317	Berger-Parker Dominance index $\frac{n_{max}}{N}$	0.1212
Shannon index $-\sum_i\left(\frac{n_i}{N}\cdot\ln\left(\frac{n_i}{N}\right)\right)$	2.992	Inverted Berger-Parker Dominance index $\frac{N}{n_{max}}$	8.25
Shannon index $\sum_i\left(\frac{n_i}{N}\cdot\log_{10}\left(\frac{n_i}{N}\right)\right)$	-1.3	Margalef Richness index $\frac{S-1}{\ln N}$	6.292
Menhinick index $\frac{S}{\sqrt{\sum_i n_i}}$	4.004	Rényi Entropy/Hill numbers (r = 0, 1, 2, ∞) $\frac{1}{1-r}\cdot\ln\left(\sum_i p_i^r\right)$	23, 19.93, 16.75, ≈∞
Buzas and Gibson's index $\frac{e^{-\sum_i\left(\frac{n_i}{N}\cdot\ln\left(\frac{n_i}{N}\right)\right)}}{S}$	0.8667	Gini coeffificient $\frac{2\sum_i in_i}{n\sum_i n_i}-\frac{N+1}{N}$	0.2451
Equitability index $=\frac{\sum_i\left(\frac{n_i}{N}\cdot\ln\left(\frac{n_i}{N}\right)\right)}{\ln N}$	0.9544	Ln () of Hill numbers (0, 1, 2, ∞):	3.135, 2.992, 2.819, ≈∞
Beta biodiversity (β)			
Comparing two sample areas			
Absolute beta value ((S_0-c)-(S_1-c)...):	-13	Whittaker's index (S/alpha):	6
Sørensen's similarity index:	0	Alternate Whittaker's index (S/alpha1):	5
Sørensen's similarity index (%):	0%	Jaccard index:	0
Routledge beta-RiIndex:	23	Jaccard index (%):	0%
Mountford index:	0	Number of common species:	0
Mountford index (%):	0%	Bray Curtis dissimilarity	1
Gamma biodiversity (γ)			
Comparing many sample areas			
Absolute gamma (S_0 + S_1...-c):	23		

(15%) [17]. A slight fall in temperature (1°C–3°C) was noticed in samples due to warmer coastal atmospheric temperatures and comparatively cooler conditions inland regions. Additionally, the presence of anthropogenic agrochemical constituents and the influence of ionic strength seem to increase the heat retention capacity of marine sediments of Krishna delta. The results for the analysis of environmental factors were corroborated with the finding of Prabhakar Rao and Bramhaji Rao [18,19].

The availability of carbon and nutrients, as well as the solubility of metals, are greatly influenced by the soil's pH. Fungi growth exhibited a negligible variation in pH throughout the locations tested. The pH of sediment samples in this study *w*as slightly alkaline (7.5–7.9). Photosynthesis, which removes carbon dioxide via bicarbonate degradation, is a major driver of changes in pH in the research region. Water intake, which promotes dilution of seawater and reduction in salinity, temperature, and breakdown of organic matter, also plays a role. Hoq *et al.* [20] reported that the pH of the sediments in Sundarbans mangrove estuaries was slightly alkaline, changing from neutral to slightly alkaline (pH of 7.4–8.1) each year.

Mangrove soils are generally composed of a mixture of sand, silt, clay, and organic matter [21]. In this study, sand content was found higher (450–570 g/kg) than silt (195–290 g/kg) and clay (58–70 g/kg) similar to the study reported by Sarin *et al.* [17]. Moderate levels of nitrogen concentrations (1.06–2.38 g/kg) were observed in this study, which was attributed to high human

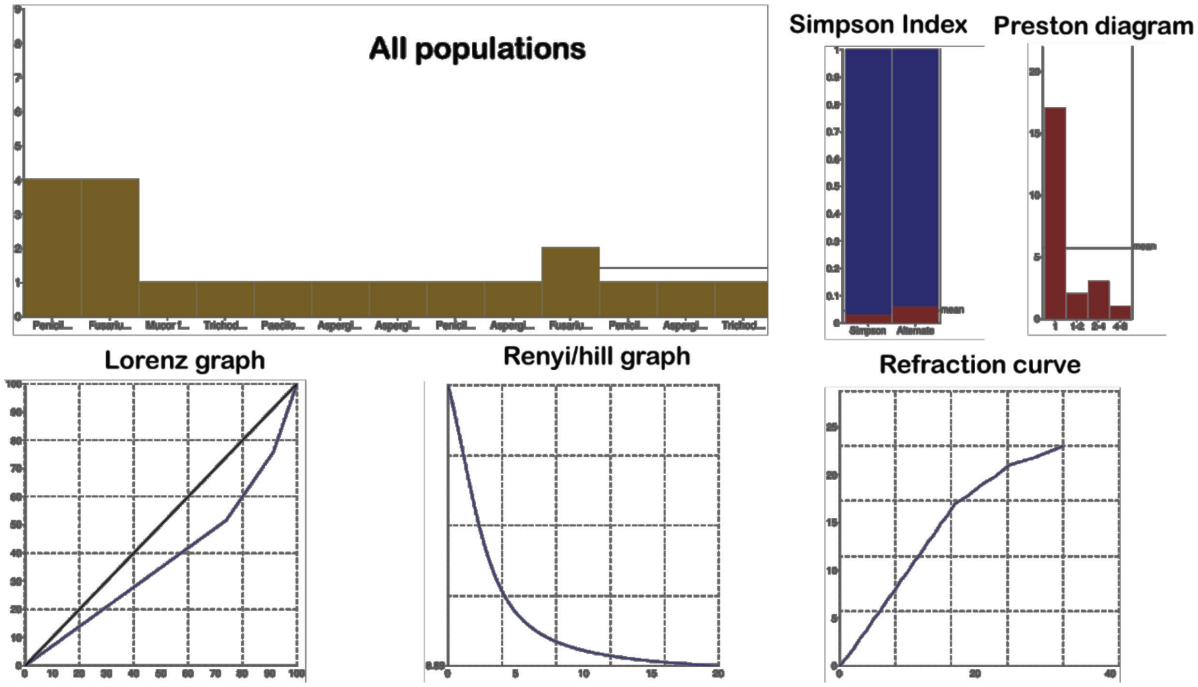


Figure 3: Static charts disphering biodiversity of fungal isolates from mangrove sediments

intervention in the estuarine ecosystem. Moreover, increased ambient N_2 concentration and its frequent mixing with the water through contacts between air and surface waters may be the relevant cause for unusual fluctuations. All of the mangrove soil samples had the same nitrogen level. Higher fungal spore and hyphal development in the mangrove soils are conceivable, since higher levels of nitrogen in the soil result in increased fungal sporulation and hyphal growth. The recorded total phosphorous values were comparatively similar to the findings of Wahid *et al.* [22]. It has been hypothesized that the minimal freshwater input in the estuarine environment could have led to the lower levels of the element Phosphorous contained in the sample region. Microbial interactions are also responsible for most of the carbon flux in mangrove sediments, which act as a carbon drain [23]. The mangrove organic matter and litter undisturbed by tides will be settled down in sediments and subsequently degraded or chemically modified by microbial activities [24]. In the present investigation, the Machilipatnam sampling location showed a high viable count compared to other sampling sites this can be attributed to the migration of the fungal population due to the depletion of petroleum hydrocarbons which act as substrates for their metabolic activities.

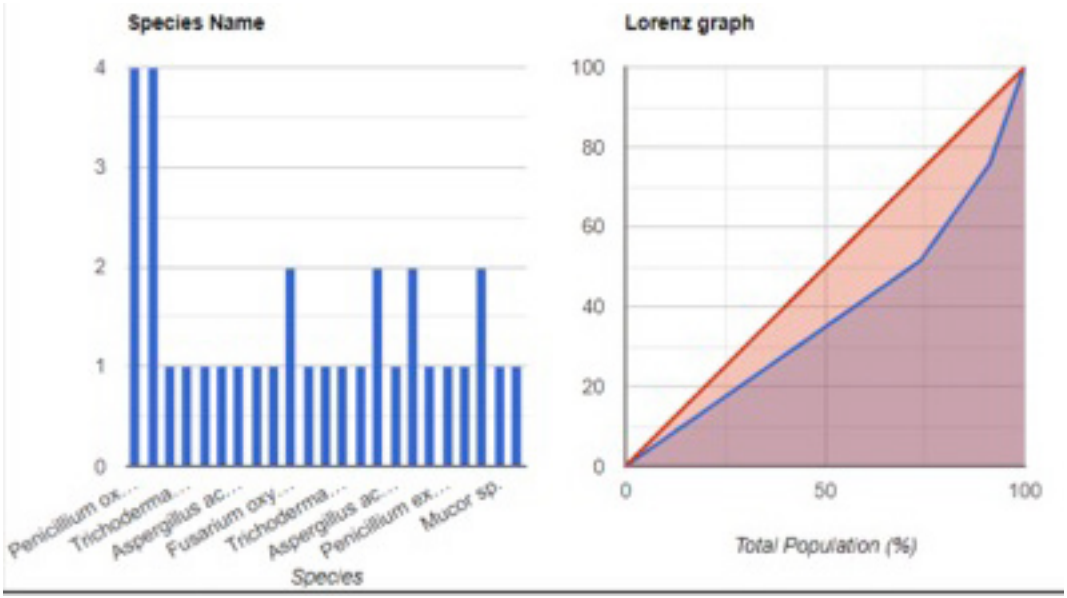


Figure 4: Dynamic charts of biodiversity of fungal isolates from mangrove sediments

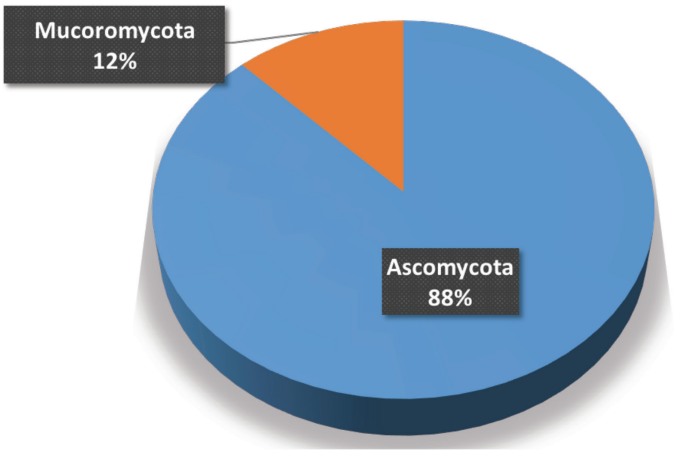


Figure 5: Taxonomic distribution of mangrove sediment bacteria.

3.2. Molecular Identification and Phylogenetic Analysis of Selected Strains

All the screened EPS producing fungal isolates were identified and gene sequences were submitted in GenBank with an accession number (Table 4). The phylogenetic tree was plotted based on multiple sequence alignments of 5.8S rDNA, which showed wide heterogeneity. Total fungal isolates were affiliated to seven genera. Figure 2 shows the phylogenetic tree constructed based on 5.8S rDNA sequences of potent EPS producer strains in this study. The unweighted pair group method with arithmetic mean tree revealed a large range of phylogenetic isolates, distributed among the Ascomycota and Mucoromycota divisions. Most of the strains isolated from mangrove sediments fall in the cluster Ascomycota; however, few isolates belonged to the division Mucoromycota.

Microorganisms coevolve together to create intricate interaction networks. The dynamics of the microbial community are profoundly influenced by interactions such as cooperation and competition amongst microorganisms. Fungal network in deep sediment is made up of strongly linked taxa (nodes) and is more complicated than that in the top sediment, according to the findings in the current study. Biotic variables, such as animal activity, or abiotic ones, such as daily rising tides, have been shown to help maintain complex microbial communities on the seafloor, known as the seafloor sediment microbial community. Co-occurrence networks of low complexity might form due to such frequent contacts and the presence of microorganisms from different environments. It is additionally important to bear in mind that above-ground environmental factors, like biotic and abiotic factors, as well as various hydrological variables, significantly influence the microenvironmental conditions in the upper sediment, and have much more of an impact on the composition and complexity of the fungal ecological network in the upper sediment than the deep sediment.

Table 7: Maximum likelihood estimates of substitution matrix.

	A	*T/U*	*C*	*G*
A	-	*7.00*	*7.57*	**8.54**
T/U	*7.11*	-	**13.25**	*6.85*
C	*7.11*	**12.25**	-	*6.85*
G	**8.87**	*7.00*	*7.57*	-

3.3. Diversity Studies of Fungal Strains Isolated from Mangrove Sediments

Samples of mangrove silt were collected and subjected to mathematical diversity indices in order to quantify biodiversity and explain the connections between many species that exist within the community of EPS-producing fungus (Table 5). Based on Simpson's index (1−*D*), and Shannon-Weaver index (*Z*−*X*), isolates from sampling site 4 were found to have a higher level of variety (*H*). Evenness shows that no dominating population is present, while simultaneously conveying that all individuals are on an equal footing. Higher Shannon-Weaver index (*H*) value and lower Simpson's index (1−*D*) value indicate higher diversity of species. However remaining sampling sites showed diverse populations as predicted by the diversity indices (Tables 6 and 7). All isolates were classified under domain Fungi, Ascomycota represents 88% of total isolates and Mucoromycota represents 12% of the total isolates (Fig. 5). Static and dynamic charts deciphering biodiversity of fungal isolates from mangrove sediments were shown in Figures 3 and 4. Alpha indices are for a single sample of a single region. This is the most typical way to study and measure biodiversity. These indices are calculated with input provided to the calculator as a single sample, if you are unsure which indices to use, start with Alpha values. Beta diversity indices compare two sample regions for "similarity" and other correlations of biodiversity between two different areas/regions. γ-diversity indices calculator for large or global areas, where many samples are being compared, and are the rarest to use in published studies and articles (Table 6).

3.4. Maximum Likelihood Estimate of γ-Parameter for Site Rates

The predictable value of the shape parameter for discrete γ-Distribution is 0.5212. Substitution patterns and rates were approximated using Tamura and Nei [25] model (+*G*). Mean evolutionary rates in these categories were 0.02, 0.17, 0.48, 1.12, 3.21 substitutions per site. The nucleotide frequencies are *A* = 24.92%, *T*/*U* = 24.53%, *C* = 26.53%, and *G* = 24.02%. The maximum likelihood value for this computation was −1,582.680. The analysis involved 33 nucleotide sequences. Codon positions included were first + second + third + Noncoding. All the positions with gaps and missing data were removed. There was a total of 242 positions in the final dataset. Evolutionary analysis was conducted in MEGA7. Tajima's [15] neutrality test (Table 8) revealed the 33 sequences, 125 segregating sites were present. Nucleotide diversity was found to be 0.169969 and the Tajima [15] statistical test was 1.269643.

The mathematical indices that predict species diversity in microbial populations were applied to observed data. In the present study, some fungal species were reported to be less in number which may reflect the narrow adaptability of that particular species. In contrast, fungi belong to the genus *Penicillium, Aspergillus*, and *Fusarium* was reported to be more in numbers indicating their adaptability. The information present study indicates that *Penicillium* sps. were far

Table 8: Tajima's [15] neutrality test.

m	*S*	ps	*θ*	*π*	*D*
33	125	0.516529	0.127271	0.169969	1.269643

more frequent than other genera. The diversity of species was more in the Machilipatnam sampling site. Molecular genotype analysis is crucial for reconstructing evolutionary relationships of species and inferring selective forces on genes and species [26]. Although Tajima's [15] test could indicate a selective sweep or bottleneck if it occurs in a certain time or population subdivision, or has lasted for a long time, the test is less reliable if used in other populations and in other time periods. When Tajima's *D* [15] is negative, it indicates that there are more low-frequency polymorphisms than expected.

I = number of isolates; *S* = species or genus number; *D* = Simpson's index; 1−*D* = Simpson's index of diversity; 1/*D* = Simpson's reciprocal index; *H* = Shannon-Weaver index; *E* = Evenness; *M* = Margalef Richness Index in Biodiversity.

Rates of different transitional substitutions are shown in bold and those of transversional substitutions are shown in italics.

m = number of sequences, *n* = total number of sites, *S* = Number of segregating sites, ps = *S*/*n*, $\theta = p_s/a_1$, π = nucleotide diversity, *D* = Tajima test statistic.

4. CONCLUSION

There are numerous fungus species found in the mangrove soil of the Krishna River Delta in the current study. The several sample locations in the Krishna delta showed great diversity in nitrogen concentration, organic matter, but not in pH, temperature, phosphorus, and salinity. The present study revealed that several types of fungal spores were identified in mangrove soils. These spores belong to the Ascomycota cluster. Since mangrove soils include several species of Ascomycota that are probable saprophytes, they might potentially affect plants and people indirectly. The investigation on the mangrove soils of the Krishna River delta suggests that these materials may have the ability to produce a range of biotechnologically significant chemicals owing to the unique maritime environment. Other useful data was found in mangrove soil, which also included the varied fungus species found within.

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6. CONFLICT OF INTEREST

The authors declared that they have no conflict of interest.

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