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# Metagenomic Assessment of Fungal Diversity in the Mangrove Sediments from North and Central Kerala, India

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## ABSTRACT

The fungal communities of mangrove sediments have wide ecological significance and are actively involved in nutrient recycling, decomposition of litter and degradation of recalcitrant substances. The present study focused on comparing the abundance, diversity and composition of fungal community in the sediments of two selected mangrove ecosystems of Kannur and Eranakulam districts of Kerala. Metagenomic analysis of the total sediment DNA was performed on an Illumina sequencing platform using fungal ITS primers. The number of Operational Taxonomic Units (OTUs) and the diversity indices showed that the Eranakulam mangrove sediments possessed higher fungal abundance, species richness and diversity than the mangrove sediments of Kannur. Ascomycota was most predominant phyla identified and a total of 11 classes, 20 orders, 34 families and 36 genera were obtained from the mangrove sediments. Talaromyces, which is not a commonly reported genus from Indian mangroves was the most prominent genera found in our study. The diversity heat map showed that 16 genera were exclusive to Eranakulam mangrove sediments while 5 genera were exclusive to Kannur samples. The metagenomics data of the mangrove sediments in our study revealed the presence of many fungal strains with vast biotechnological potential which can be further explored in the technology development.

**Keywords** Fungus, Mangrove sediment, Metagenomics, Diversity.

## INTRODUCTION

Mangrove sediments harbor diverse microbial communities in which fungi are considered as the most diverse and potential organisms. They paly critical role in global carbon cycle, nutrient recycling and availability, decomposition and in soil borne diseases (Thatoi *et al.* 2012, Beng and Corlett 2019). The dynamics of fungal communities in the mangrove sediments depend on various biotic and abiotic factors, sediment characteristics, seasonal variations, mangrove species and anthropogenic activities (Thakur and Geisen 2019). There are many limitations with conventional culture methods in studying the ecological influence on the occurrence and distribution of fungal communities since their ecological and taxonomic diversity is extremely large. More over

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a large percentage of fungi in sediments are unable to be cultured under the laboratory conditions which make them unnoticed. The advent of Next Generation Sequencing (NGS) – metagenomics – that enables the analysis of genetic information from PCR amplicons or direct environmental samples have solved this problem to a large extend in recent years (Bowers *et al.* 2011, Khandeparker *et al.* 2017). It helps to characterize the microbes in the sediments, their genetic diversity, population dynamics and ecological functions (Rastogi and Sani 2011). NGS technique is advantageous over other methods like plate counts, morphometric analyses, real time PCR, FISH and RFLP as they do not require fluorescently labeled primers and clone libraries (Hill *et al.* 2000, Liebner *et al.* 2008). Also, it provides large sequence data, fast detection and good parallelism with procedures that are almost completely automated (Qin *et al.* 2010, Chau *et al.* 2011).

Reports on fungal diversity from mangrove sediments around the globe using metagenomics techniques are meagre (Thompson *et al.* 2013, Simoes *et al.* 2015, Shi *et al.* 2021) and most of the studies were focused on the genes that produce enzymes which are significant in biotechnological and industrial processes (Peng *et al.* 2012, Thompson *et al.* 2013, French 2017). Indian mangrove sediments were least investigated for their fungal diversity using metagenomics and a report on fungal diversity from mangrove ecosystem of Goa using next-generation sequencing (NGS) was found to be the only published work till date (Haldar and Nazareth 2019). Though Kerala which lies along the West coast of India is rich in mangroves, no attempts have been made yet to study in particular the fungal diversity of their sediments. A detailed study on the phylogeny and functional capacities of mangrove sediment fungi is necessary to understand their underlying ecological interactions and adaptations. Therefore, the objective of our study was to investigate and compare the abundance, diversity and composition of fungal communities present in the mangrove sediments of two districts namely Kannur and Ernakulam, Kerala using internal transcribed spacer (ITS) sequencing with the help of an Illumina sequencing platform. The present work can be considered as the first study on fungal diversity of mangrove sediments of Kerala through metagenomic approach.

## MATERIALS AND METHODS

### Study sites

Mangrove ecosystems of Kunhimangalam, Kannur (North Kerala 12.10°N 75.22°E) and Valanthakad, Ernakulam (Central Kerala 9.92°N 76.32°E) were selected and sampled during the 2022 (Fig. 1). The selection of study sites was based on the fact that these are the most luscious mangrove habitats of Kerala with diverse mangrove species along with large number of endemic and rare organisms which calls for the need of conservation and management of these ecosystems. Also, the sites under study belong to North and Central region of Kerala with significant differences in the diversity of their flora and fauna, tidal regime, geographical and sediment characteristics, anthropogenic interactions which can provide a good comparative data between the two habitats.

### Sediment collection

The sub-surface sediment samples (top 10–15cm) were collected from each site and transferred aseptically in to sterile polythene covers. Five sub-samples were collected from each sub sites of Kannur and Ernakulam mangrove habitats, which were then pooled and homogenized to one composite sample per location and immediately stored at $^{-}$20°C. The sediment sample of Kannur mangroves was named as KAN and that of Ernakulam mangroves as EKM.

### Sediment physico-chemical parameters

The pH, temperature and salinity of the sediments were measured during the time of collection using a digital pH meter, thermometer and salinometer respectively. The sediment characteristics and organic matter were analyzed in the laboratory after drying and pulverizing the sediment samples (Trivedi and Goel 1986).

### DNA extraction, PCR amplification and sequencing

The DNA extraction from the sediment samples (0.5 g) were performed using Power soil DNA isolation

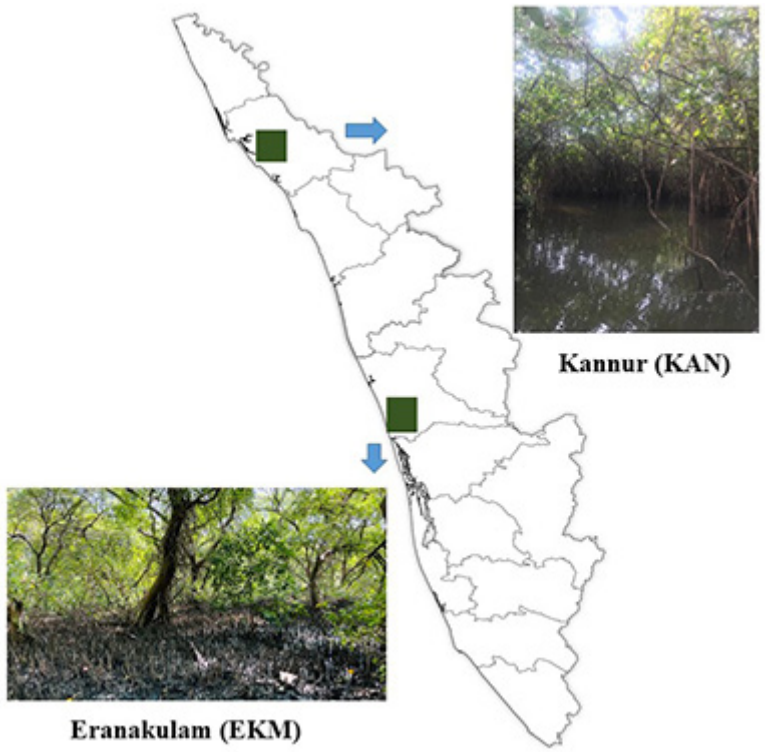


**Fig. 1.** Sites of collection of mangrove sediments during the present study.

kit (MO BIO Laboratories Inc, Carlsbad, CA, USA) in small aliquots according to the manufacturer's instructions. Later they were pooled, purified, and concentrated to achieve sufficient concentration required for PCR amplification and library preparation. The DNA was quantified with the help of a Nanodrop (Nanodrop Technologies, USA) and a concentration of ~30 ng/µl was used for amplification. The ITS regions in the nuclear ribosomal repeat unit ITS1 and ITS2 were amplified using specific primers ITS1: 5′-TTGGTCATTTAGAGGAAGTAA-3′ and ITS2: 5′-GCTGCGTTCTTCATCGATGC-3′ (White *et al.* 1990). The amplified PCR products was checked on a 2% agarose gel and were cleaned-up according to the Illumina HiSeq protocol for amplicon preparation. Library constructions of the amplicons were done using NEB Next Ultra DNA library preparation kit and Agilent 2200 Tape Station was used for the quality analysis and quantification of the libraries. Afterwards, Illumina HiSeq 2500 platform was used to sequence the prepared libraries.

**Bioinformatics analyses**

The quality of the reads from the paired-end sequences generated by the Illumina HiSeq sequencing was checked using FastQC-v0.11.9 software (Andrews 2010) while adapter removal and trimming was performed with Trimmomatic-0.39 (Bolger *et al.* 2014). The raw sequences were then processed with the aid of bioinformatics software package 'Quantitative Insights into Microbial Ecology 2' (QIIME 2) version 2021.11 (Bolyen *et al.* 2019). In this, chimera removal, merging of pair end reads and clustering of sequences (Feature (OTU) table construction) were done using DADA2 plugin (Callahan *et al.* 2016). SILVA OTU database was used to perform taxonomic classification by aligning representative OTUs from clusters against database sequences. Alpha diversity was calculated using Shannon diversity, Peilou's evenness, Total observed amplicon sequence variants (ASVs) and Faith's phylogenetic diversity while beta diversity between the sites were calculated using Jaccard distance, Bray-Curtis distance, Unweighted and

weighted UniFrac distance. All statistical analyses were done using SPSS software, version 21.0 considering values of $p < 0.05$ as statistically significant. PICRUST 2 was used in developing the functional annotation while RStudio and STAMP were used for graphical representations of the metagenomics data (Douglas *et al.* 2020, Parks *et al.* 2014).

## RESULTS AND DISCUSSION

### Sediment physico-chemical parameters

The pH, temperature, salinity and organic matter of the sediments were found to be 6.8, 26°C, 12% and 3.5 respectively in Kannur while 6.3, 28, 19% and 4.3 respectively in Ernakulam mangrove sediments.

### Amplicon analysis by Illumina sequencing

After Illumina sequencing, a total of 1, 40,055 raw reads were obtained from both the samples which remained the same after quality filtering. 74,817 total feature counts/OTUs were detected out of which 44,246 were from EKM while 30,471 were from KAN samples. Sequence data have been deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) under the accession numbers SAMN31239220 for EKM and SAMN31239221for KAN (NCBI Bio Project Accession No: PRJNA889370).

### Taxonomic richness and diversity of the fungal community

It was observed that sediments of EKM showed the highest abundance in fungal population with 97 OTUs than KAN with 56 OTUs. The alpha diversity indices including Shannon diversity, Peilou's evenness, and Faith's phylogenetic diversity also showed that EKM sample possessed higher species richness, abundance, and diversity than KAN samples (Table 1).

**Table 1.** Alpha diversity indices of fungal population from the study sites.

| Diversity parameters | KAN | EKM |
|---|---|---|
| Total paired end reads | 59580 | 80475 |
| Processed reads | 30471 | 44346 |
| Observed features/OTUs | 56.00 | 97.00 |
| Shannon | 2.310 | 2.530 |
| Faith's phylogenetic dfiversity | 9.290 | 17.410 |
| Pielou's evenness | 0.404 | 0.383 |

**Table 2.** Beta diversity indices of fungal population between the sediment samples from the study sites.

| Diversity parameters | Indices |
|---|---|
| Jaccard distance | 0.7355 |
| Bray-Curtis distance | 0.2803 |
| Unweighted UniFrac distance | 0.6426 |
| Weighted UniFrac distance | 0.1668 |

Previous studies on mangrove sediment fungi showed similar results in which their abundance levels were considerably low (Kuramae *et al.* 2012, Simoes *et al.* 2015). The lower abundance of fungi in mangrove sediments can be accounted to various factors including frequent water logging, low contents of total nitrogen (N) and phosphorus (P), and reduced nutrient availability (Hyde and Lee 1995). Moreover, reports say that the fungal abundance were seen low in mangrove sediments which were characterized by trees with smaller tree size and stands and also with low diversity of mangrove flora (Reef *et al.* 2010). In our study areas, the mangrove ecosystem showed these above features where they appeared in patchy arrangement with shorter trees and stands (Mohandas *et al.* 2014). They also showed poor nutritional conditions and substratum availability with low concentrations of total nitrogen and phosphorus (Aslam *et al.* 2020). The combined effect of all these factors might have resulted in the low abundance of fungal community in the mangrove sediments studied. Also, the variations in these factors between the two stations would have caused for the difference in their abundance in the sediments. The beta diversity of fungal community in the mangrove sediments between two sites were calculated using indices like Jaccard distance, Bray-Curtis distance, Weighted and Unweighted UniFracdistance (Table 2).

Core-group fungi which have more than 10% frequency of occurrence and high competence in terms of growth and substrate utilization are responsible for the maintenance of an ecosystem (Sridhar 2009). Approximately 35 fungi have been reported as core group in the mangroves of Indian peninsula since

they were commonly and abundantly found in most of the India mangrove ecosystems (Sarma and Hyde 2001). In our study, more than half of the observed taxa were shared by the two stations which were due to the presence of core group fungi common to mangrove sediments.

**Taxonomic composition of the fungal community**

A total of 121 OTUs were obtained from the processed reads in which 97 were from EKM and 56 were from KAN samples. Ascomycota was identified as the most predominant phyla (91.9% EKM and 92.2% KAN), while Basiodiomycota was observed as a minor phylum (1.6% EKM and 1.7% KAN) in the sediment samples. The dominance of Ascomycota in marine and mangrove sediments have been reported in previous studies (Alias *et al*. 2010, Abdel-Azeem and Salem 2012, Abdel-Wahab *et al.* 2014). Those sequences which were not able to resolve taxonomically were represented as unassigned phyla which accounted to 5.4% in EKM and 4.3% in KAN samples. Previous studies on the mangrove marine fungi of Indian peninsula and of Kerala in particular have shown the dominance of Ascomycetes in comparison with Basidiomycetes and other phyla, similar to our findings (Sridhar 2009, Nambiar and Raveendran 2009). Ascomycetes from marine environments are considered to be of high ecological importance as they involve in various biogeochemical processes (Simoes *et al.* 2015). Also, the fungi belonging to phylum Ascomycota isolated from mangrove sediments of Kerala were reported to have significant bio remedial properties and are considered as potential candidates for various industrial and biotechnological processes due to their hydrolytic enzyme activity and secondary metabolite production (Anilkumar and Reshma 2017, Vidya and Sebastian 2020, 2022).

A total of 11 classes were identified from the sediment samples in which 5 classes were exclusively present in EKM sample. The classes were then divided in to families, orders, genera and species and those sequences which were not able to resolve taxonomically were considered as unassigned class, family, order, genus, and species (Fig. 2). Eurotiomycetes (60% EKM and 58% KAN), Dothideomycetes (11% EKM and 26% KAN), Sordariomycetes (18% EKM and 5.9% KAN), Saccharomycetes (1.9% EKM and 1.9% KAN), and Agaricomycetes (0.4% EKM and 1.5% KAN) were the top five classes obtained from the samples. Similar trend in the fungal abundance was observed in a metagenomics study by Simoes *et al.* (2015) in soil and rhizosphere associated fungi in grey mangrove, *Avicennia marina*, from the Red Sea. In their study, Eurotiomycetes was the most abundant class observed followed by Dothediomycetes,

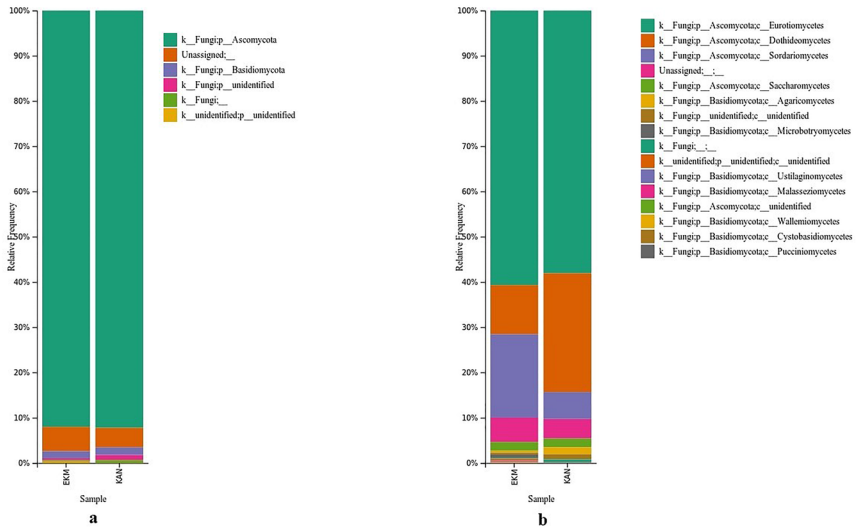


**Fig. 2.** Phylum level (a) class level (b) abundance of fungi from mangrove sediments.

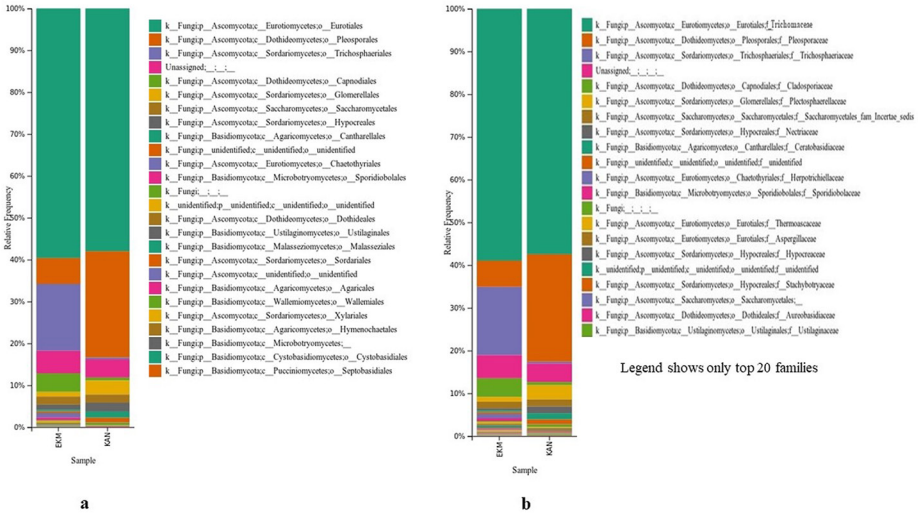


**Fig. 3.** Order level (a) family level (b) abundance of fungi from mangrove sediments.

Soradariomycetes and Saccharomycetes. Classes including Malasseziomycetes, Wallemiomycetes, Cystobasidiomycetes and Pucciniomycetes were found to be exclusive to EKM sediment sample.

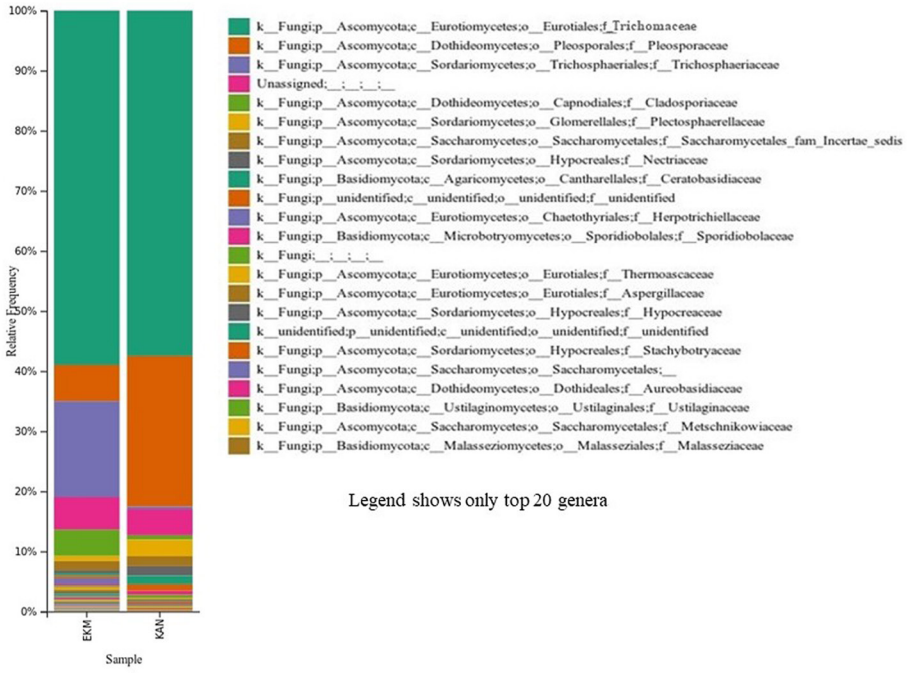


**Fig. 4.** Genus level abundance of fungi from mangrove sediments.

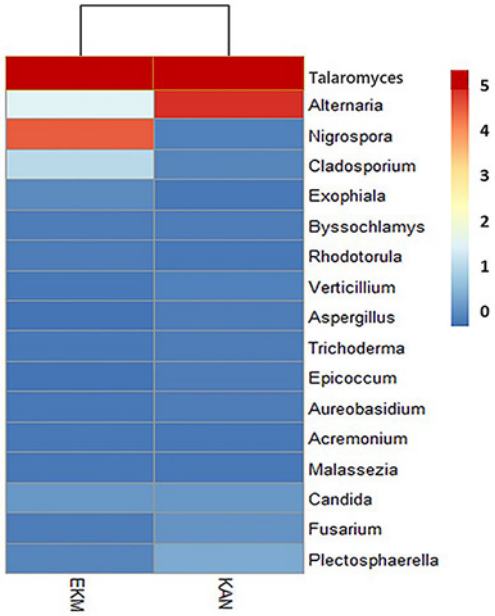


**Fig. 5.** Diversity heat map and cluster analysis at the genus level of fungi from mangrove sediments.

The classes were subdivided in to 20 assigned orders in which Eurotiales (59.6% EKM and 57.9% KAN), Pleosporales (6.2% EKM and 25.4% KAN), Trichosphaeriales (15.9% EKM and 0.4% KAN), Capnodiales (4.4% EKM and 0.8% KAN),Glomerellales (1.1% EKM and 3% KAN), Saccharomycetales (0.4% EKM and 1.4% KAN) and Cantharellales were the dominant orders identified. Orders Malasseziales, Sordariales, Wallemiales, Agaricales, Xylariales, Hymenochaetales, Cystobasidiales and Septobasidiales were found to be exclusive to EKM sediment sample and were absent in KAN sample. 34 families were identified after finer resolution and the topmost families present were Trichomaceae (58.9% EKM and 57.4% KAN), Pleosporaceae (6.1% EKM and 22.9% KAN), Trichosphaeriaceae (15.9% EKM and 0.4% KAN), Cladosporiaceae (4.4% EKM and 0.8% KAN) and Plectosphaerellaceae (1.1% EKM and 3.3% KAN). 13 out of 34 assigned families were exclusively found in EKM sample while 4 families were exclusive to KAN sediment sample (Fig. 3). Our findings are in accordance with the observations of a previous study on mangrove sediments of Goa using metagenomics approach except the fact that the fungi of class Agaricomycetes was the most abundant ones than Eurotiomycets, which was the dominant class in our study (Haldar and Nazareth 2019).

36 assigned genera of fungus were identified from the sediments in which genus *Talaromyces* dominated in both the samples (58.9% EKM and 57.4% KAN) (Fig. 4). The diversity heat map was drawn at the genus level and it gives a comparative abundance data of fungus in the mangrove sediment (Fig. 5). *Nigrospora* was the next most dominant genus found in EKM (16%) and *Alternaria* in KAN sediments (25%). 16 genera were exclusive to EKM which included genus *Rhodotorula*, *Acremonium*, *Malassezia*, *Wallemia* while 5 were exclusive to KAN samples that included genus *Epicoccum*, *Schizophyllum, Humicola, Pichia* and *Bipolaris*. Our findings support earlier research on the fungi of Kerala's mangrove sediments using conventional studies and most of these genera were reported (Sridhar 2005, Nambiar and Raveendran 2009, Manimohan *et al.* 2011). It is noteworthy that *Talaromyces* which is the most predominant genus in our metagenomic study is not a commonly reported genus from Indian mangroves. *Talaromyces* is a dimorphic fungus which exists as a mold in environment and as yeast in tissues which divides by septation (Kauffman 2017). Taxonomic classification and identification of *Talaromyces* was ambiguous which was later resolved by the advent of molecular and genomic approach. This might be the reason for not having enough representation of this genus in previous studies. The identification of fungi belonging to *Talaromyces* species increased recently due to the presence of complete sequence data from all over the world in the genomic database (Sun *et al.* 2020). *Talaromyces* is considered to have major role in food spoilage due to the presence of their heat resistant ascospores and produce unique mycotoxins which are carcinogenic to liver and kidneys. They are also reported to be pathogenic and causes serious infections in HIV patients and other animals (Yilmaz *et al.* 2014, 2016). On contrary, *Talaromyces* is of great biotechnological potential as they are able to produce potent anticancer, antibacterial, antifungal, antioxidative and antiproliferative compounds, natural colorants, enzymes and biocontrol agents (Guevara-Suarez *et al.* 2020). Other groups of fungi identified in our study have reported to be of great ecological significance due to their involvement in

various bioremediation processes. Many of them are found to be potent candidates for biotechnological and industrial purposes (Thatoi *et al*. 2013, Bicholkar and Nazareth 2015). *Alternaria, Nigrospora, Aspergillus*, *Cladosporium, Fusarium, Penicillium* and *Trichoderma* are root endophytes which helps the mangroves to resist adverse environmental conditions by producing bioactive secondary metabolites and they also compete against root decomposing saprophytes (Liu *et al.* 2007). *Penicillium* and *Aspergillus*, which are commonly reported in mangrove sediments are great phosphate solubilizers and also found to have therapeutic activities (Turan *et al.* 2006).

Most of the genus could not be taxonomically resolved in to their corresponding species and were represented as unidentified. *Nigrospora oryzae, Plectosphaerella oligotrophica, Candida tropicalis, C. parapsilosis, C. ethanolica, Fusarium solani, Verticillium dahlia, Rhodotorula mucilaginosa, R. babjevae, Kodamaea ohmeri, Aspergillus penicillioides, Knufia tsunedae, Schizophyllum commune, Fuscoporia torulosa, Humicola nigrescens, Clavispora lusitaniae, Pichia mandshurica, Bipolaris sorokiniana, Cystobasidium pinicola, Paraphaeosphaeria viciae* and *Malassezia obtuse* were the species identified.

## CONCLUSION

Mangrove fungi which occupy second largest percentage of earth's marine fungi are of great scientific interest. This due to their adaptability to extreme environmental conditions and ability to produce novel bioactive compounds and hydrolytic enzymes those are biotechnologically significant. Despite this, little studies have been conducted on mangrove fungi and hence not much is known about their diversity and potential applications. Since traditional culture techniques fails to identify 90% of their diversity, inculcation of culture independent approach like metagenomics have become quintessential in studying the complete diversity and ecological functions of fungi in mangrove ecosystem. Taken together, our study on the fungi present in mangrove sediments using NGS technique identified Ascomycota as the major phylum compared to Basidiomycota and these phyla were further classified and assigned in to 11 classes, 20 orders, 34 families and 36 genera. The diversity analysis of the observed fungal genera showed that the sediments of Ernakulam mangroves are richer in terms of abundance and diversity than Kannur mangrove sediments. Most of the fungi identified in our study were reported to be ideal candidates for biotechnological applications and bioremediation processes. Moreover, this work on the metagenomics of the mangrove sediments revealed certain fungal species including that of genus *Talaromyces* which were otherwise not so commonly reported in conventional studies. The present work is the first report on the metagenomic assessment of fungal diversity from the mangrove sediments of Kerala.

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