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# Diversity and hydrolytic potentials of manglicolous yeasts associated with mangrove trees *Rhizophora* and *Avicennia* found in Kerala, India

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**ABSTRACT**

Kerala has good natural patches of mangroves, wherein *Avicennia officinalis* and *Rhizophora mucronata* are the dominant species. The microbial communities of these transition ecosystems are mainly composed of bacteria, actinomyces, fungi, and yeasts. Manglicolous yeasts associated with mangrove vegetation from Kerala have got less attention compared to those from sediment and water. In this paper, the yeasts associated with roots, stems, branches, leaves, barks, and flowers from 4 species of mangrove plants, namely *A. officinalis, A.marina, R. mucronata, and R. apiculata,* from the mangroves of Puthuvype and Kumbalam were studied for the first time. It was noted in this study that the population density of yeast was associated with *R. mucronata* (47.1%) was greatest followed by *A. officinalis* (35.3%), *R.apiculata* (9.8%) and least with *A.marina* (7.8%). The majority of the yeast strains were isolated from leaves (35.3%), then roots (25.5%) of mangrove plants, and the least from flowers (1.9%). Among the 51 isolates, the pigmented yeasts comprised of 3.9 % black yeasts and 25.5% red yeasts. Some of the dominant genera isolated in this study were *Rhodotorula* (27.5%), *Debaryomyces* (17.6%), *Cryptococcus* (9.8%), *Candida* (7.8%), *Rhodosporidiobolus* (7.8%), *Kluyveromyces* (5.9%), *Pichia* (3.9%), *Aureobasidium* (3.9%), and *Metchnikowia* (3.9%). Diversity indices of this study were analyzed; which revealed that both Shannon-wiener diversity (H' ($log_2$)) and Peilou's evenness (J') of yeast associated with *R. mucronata* was the maximum and least from *A. marina*. It was interesting to note that all the yeast isolates were lipolytic; apart from that, the manglicolous yeasts showed amylolytic, proteolytic, cellulolytic, aryl sulphatase, pectinolytic, ligninolytic, and ureolytic activity. Very little information is available on the diversity and bioprospecting potentials of yeast associated with the mangrove vegetation of Kerala; the present study provides baseline data in this regard. The results of this study clearly reveals that the mangrove ecosystem harbor diverse groups of yeasts that possess great biotic potential.This information emphasizes the need to preserve these unique ecosystems by reiterating that mangroves are ecological hotspots.

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## 1. Introduction

The state of Kerala is situated on the west coast of India, and the floral diversity of mangroves of the state is represented by 18 species of true mangrove species (Sreelaksmi et al., 2021). *Rhizophora* and *Avicennia* species are the leading constituent trees of the different communities in almost all the mangrove forests in Kerala. The report of ENVIS hub: Kerala (2022) shows that *R. mucrunata* and *A. officinalis* are the dominant species in the dense patches of the Kumbalam and Puthuvype mangrove forests. Both of the stations are privately owned beautiful isles where rare species of mangroves are grown and protected in their natural habitat. This area, with a dense mangrove population, is the natural habitat and also the breeding and nursing ground of many species of freshwater fishes, crabs, and migratory birds, and is unusually rich in microbial species, including yeast (Sievers et al., 2019). The species diversity of mangrove vegetation in Kerala has been reported to be high and diverse, but studies on mangrove microbial diversity are comparatively less, especially on yeasts.

The yeast communities of phyla Ascomycota and Basidiomycota associated with mangrove vegetation depend on plant exudates and inorganic substances. The association of microbial communities with plant compartments fulfills certain functions like nutrient cycling, protection from plant pathogens, and decomposition (Hardoim et al., 2015; Vishwakarma et al., 2020). However, the inter-relationships of manglicolous yeasts in different plant compartments are still poorly understood. The occurrence of yeast from the rhizosphere to the leaf indicates that they can survive in both high and low salinity (Purahong et al., 2019). The manglicous yeast associated with vegetation was studied by culture-dependent approaches and modern techniques, such as high-throughput DNA sequencing techniques, to give a complete picture of yeast communities (Dissanayake et al., 2018). The particular environmental conditions, mainly the salinity of the habitat, influence the percentage of manglicolous yeast. The plant species have been found to influence the endophytic yeast communities more than the epiphytic ones (Yao et al., 2019). The intertidal zone with environmental fluctuations makes yeast cells adapt to extreme conditions by producing certain bioactive compounds like hydrolytic enzymes.

Information on manglicolous yeast diversity of Kerala, mainly from vegetation, is scarce, and practically no authentic reports are available. The coverage of the Kumbalam and Puthuvype mangrove areas is on the decline due to anthropogenic activities. The objective of this study was to assess the diversity of yeasts associated with the mangrove vegetation of these stations and generate baseline data.

## 2. Materials and Methods

### 2.1. Study site, experimental design, and sample processing

Healthy, mature, *R. mucrunata, R. apiculata, A. officinalis,* and *A. marina* were selected for study. The mangrove trees

were 140-169 cm tall, and growing in the Kumbalam and Puthuvype wetlands, located on the west coast of Kerala (Fig. 1). Sampling was done from 5 sites each from Kumbalam (KS1, KS2, KS3, KS4, KS5) and Puthuvype (PS1, PS2, PS3, PS4, PS5) stations.

Five compartments (leaves, root, bark, stem, and flowers) of the selected species of *Rhizophora* and *Avicennia* were sampled. Tender and mature dark green leaves from the lower and upper portions of the plant were sampled with sterile scissors and bark with a knife. The stems were collected after removing the bark aseptically. Flowers and flower buds were seasonal. Roots and slit roots were collected from the lower part. Samples from each organ were pooled individually to make a composite sample per compartment. All samples were transported on ice to the laboratory, followed by immediate processing. Each sample was cut into pieces (<2mm long) and ground using sterile mangrove water. Dilutions $10^{-1}$ and $10^{-2}$ were spread plated on Wickerham's agar and incubated for 48 hrs at 28° C.

**2.2. Yeast biome and molecular analysis**

Colony counts were made after the incubation. Morphologically distinct colonies were picked and maintained on Malt extract agar slants for further study. The isolates were identified up to genera as per Barnett *et al*. (1990) and the colony and cell morphology and fermentation tests of the yeasts were performed using the methods described by Kurtzman et al. (2011). DNA was extracted and was identified by sequencing of ITS region. For this, the genomic DNA was extracted per Harju et al.'s protocol (2004). The ITS region was amplified using the primers ITS-1 and ITS-4 (Forward ITS 1-5' TCC GTA GGT GAA CCT GCG G 3' and Reverse ITS 4- 5' TCCTCC GCT TAT TGA TAT GC 3') (White *et al*., 1990). The PCR amplification was carried out and the amplified fragment of approximately 560 bp, containing the ITS 1, 5.8 S, and ITS 4 regions, was used for the sequence similarity search using NCBI BLAST. Nucleotide sequences of yeasts obtained were analyzed using Molecular Evolutionary Genetic Analysis Version X (MEGA X) software and a phylogenetic tree was constructed using the Maximum Likelihood method and nodal support was tested by means of 100 bootstrap pseudo replicates.

**2.3 Data processing by statistical analysis**

The Shannon-Weiner diversity, Peilou's evenness, and Species Richness were analyzed using PRIMER 7 (Clarke and Gorley, 2001). The diversity index provides a good measure of the community composition. The abundance of yeast numbers associated with the mangrove plant part was analyzed for diversity indices along with the whole mangrove plant.

**2.4. Screening for hydrolytic enzymes**

Nutrient agar medium supplemented with casein (2%), gelatin (2%), starch (1%), and tributyrin (1%) were prepared for the detection of caseinase, gelatinase, amylase, and lipase activity respectively. Cellulose agar, Pectin agar, Zobell's agar (supplemented with tripotassium phenolphthalein di sulphate), and Craford's agar ( supplemented with tannic acid) and Urea agar base (supplemented with Urea) were employed for screening for cellulose, pectinase, aryl sulphatase, ligninase and urease enyzymes (Kutty, 2009). The inoculated media were incubated at 28±2° C for 3 to 5 days. The presence of a clear zone indicates positive results except for the Aryl sulphatase test and Urease, where the development of pink colour after incubation indicates a positive result(Kutty, 2009).

## 3. Results and Discussion

The yeast strains were isolated from the roots, stems, leaves, barks, and flowers of 4 species of mangrove plants from the two dominant genera in Puthuvype and Kumabalam from 5 different locations of each station. Therefore, the yeast strains isolated from these dominant mangrove species invariably represent the main yeast communities from the study area.

The results in Table 1 indicated that the sampling sites had the characteristics of tropical marine environments such as the high temperature, pH, and salt. Both of the sampling sites had minimum and maximum temperatures ranging

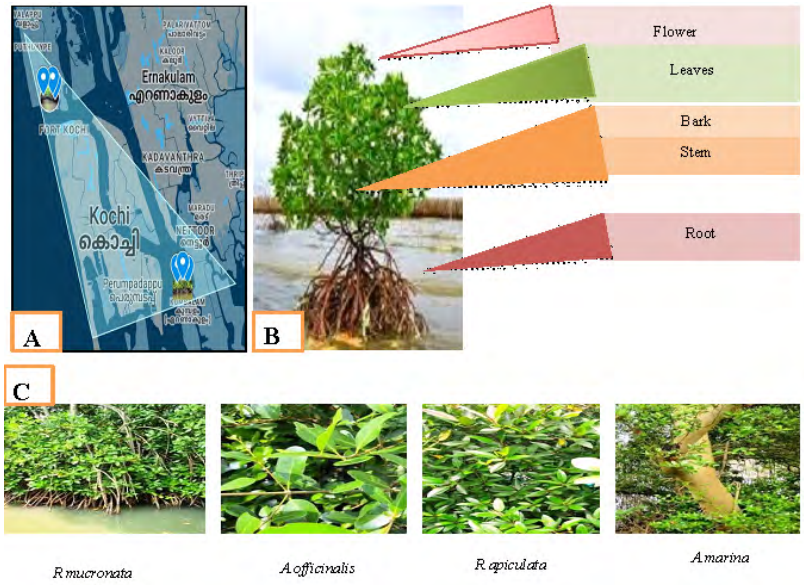


**Fig. 1.** Experimental design of this study **A.** Sampling sites, **B.** The five samples analyzed from each plant and **C.** Plant species

from 28-30˚C, pH of 6-8, and salinity of 15-25ppt. Colony Forming Units per milligram (CFU/gm) of the sample of the cultivable yeasts varied between the samples 18-23 x $10^1$ CFU/gm. The colony counts were greatest at sites KS1, and PS3 at the Kumbalam and Puthuvype stations, respectively. It was interesting that both KS1 and PS3 have similar conditions (temperature 28°C, pH7, and salinity 25 ppt) (Table 1).

After isolation and purification, a total of 51 morphologically and biochemically different yeast strains were obtained from the samples. The plant exudates, organic compounds, and secondary metabolites can influence the diversity of yeasts (Limtong and Nasanit, 2017). They may be endophytes, pathogens, or parasites. However, their roles in different plant parts or the basis of their association are not well known till now.

Out of 51 isolates, the pigmented yeasts comprised both black yeasts (3.9 %) and red yeasts (25.5%). According to the molecular identification, it was found that the isolates belonged to 17 genera and 18 species. Out of 17 genera 4 belonged to basidiomycetes while the rest were ascomycetes. Among the isolated yeasts, 2 strains could not be identified at the species level because their best-matched references from the BLAST search were lower than 95% of similarities. It is likely they are new species. In the phylogenetic tree based on ITS, most species were grouped in their own clade (Fig. 2). The internal transcribed spacer region (ITS 1 and 4) with the flanking 5.8S genes showed that the yeasts belong to the genera *Rhodotorula* (27.5%), *Debaryomyces* (17.6%), *Kluyveromyces* (5.9%), *Cryptococcus* (9.8%), *Candida* (7.8%), *Rhodosporidiobolus* (7.8%), *Pichia* (3.9%), *Aureobasidium* (3.9%), and *Metchnikowia* (3.9%). *Geotrichum* (1.9%), *Lodderomyces*(1.9%), *Ogataea* (1.9%), *Yarrowia* (1.9%), *Clavispora* (1.9%), and *Saitozyma* (1.9%) (Fig.3). The sequences of the marine yeast isolates, from Kumbalam and Puthuvype, were compared with other sequences found in the NCBI GenBank. The sequence of all the isolates has been deposited at the NCBI GenBank, and details are given in Table 2.

The dominant genera in this study were *Rhodotorula, Debaryomyces Cryptococcus, Candida, Rhodosporidiobolus, Kluyveromyces, Pichia, Aureobasidium* and *Metchnikowia.* At the same time, the other genera represented by single isolates were *Geotrichum, Lodderomyces*, *Ogataea*, *Yarrowia*, *Clavispora* and *Saitozyma*. *Debaryomyces hansenii* has been found to be a common member in the marine environment as it can tolerate high salt concentrations (Prista et al., 2005). We too found that a large number of yeast strains (17.6 %) obtained from the mangrove vegetation belonged to *D. hansenii*.

In this study, the maximum number of yeasts were associated with *R. mucronata* (47.1%), followed by *A. officinalis* (35.3%), *R. apiculata* (9.8%) and the least with *A. marina* (7.8%). The majority of the yeast strains were isolated from leaves (35.3%) and roots (25.5%), least from flowers (1.9%) of mangrove plants (Fig. 3).

We found that *Candida* spp. are widely distributed in all parts of mangrove vegetation. *Kluyveromyces aestuarii* has been frequently isolated from marine environments (Nagahama, 2006), and we found that it is also associated with mangrove vegetation. Reports indicated that *Pichia* spp. had been quite frequently isolated from marine environments like the surface of seaweed and the guts of marine invertebrates and fishes (Li et al., 2008), and we found they were also associated with mangrove vegetation. *Aureobasidium pullulans* are popularly known as black yeast and are widely distributed in the estuarine, marine sediments, hypersaline habitats, seawater, and deep-sea (Chi et al., 2009). However, this is the first report from Kerala of the isolation of *Aureobasidium pullulan* (3.9%) from the mangrove phylloplane.

*Candida* spp., *D. hansenii, Rhodotorula* sp. and *Cryptococcus* sp. were found to have a strong association with mangrove vegetation as they were isolated irrespective of plant part or mangrove species. Whereas other yeast species were either plant species or plant part or habitat-specific in their distribution. Diversity indices of this study were analyzed; this revealed that both Shannon-wiener diversity (H'(log2)) and Peilou's evenness (J') of yeasts isolated from *R. mucronata* were found to be maximum and least from *A. marina* (Fig.4).

It was interesting to note that all the yeast isolates were lipolytic (100%). Apart from that, the manglicolous yeasts showed amylolytic (60.8%), proteolytic (37.3%), cellulolytic (27.5%), aryl sulphatase (41.2%), pectinolytic (33.3%), ligninolytic (25.5%), and ureolytic (31.4%) activity. Isolates from *R. mucronata* and *R. apiculata* showed maximum amylolytic activity, whereas they exhibited no pectinase and ligninase activity. Maximum pectinase activity was shown by strains isolated from *Avicennia* species (Fig.5). Most isolates showed proteolytic, cellulolytic, and chitinolytic activity; this indicated that they have the potential to be explored as biocontrol agents against plant pathogens. The pectinase,

**Table 1.** Geographic location, Temperature, pH, and salinity of the study area

| | | Sites | Latitude and longitude | Temperature (°C) | pH | Salinity(ppt) |
|---|---|---|---|---|---|---|
| STATIONS | Kumbalam | KS1 | 9°54'44.0"N 76°18'51.9"E | 28 | 7.0 | 25 |
| | | KS2 | 9°54'42.7"N 76°18'52.4"E | 30 | 7.3 | 24 |
| | | KS3 | 9°54'41.1"N 76°18'52.6"E | 30 | 8.0 | 23 |
| | | KS4 | 9°54'33.6"N 76°18'54.5"E | 29 | 7.9 | 21 |
| | | KS5 | 9°54'31.5"N 76°18'54.5"E | 30 | 8.1 | 20 |
| | Puthuvype | PS1 | 9°59'10.0"N 76°13'58.0"E | 30 | 7.0 | 20 |
| | | PS2 | 9°59'08.3"N 76°13'56.6"E | 30 | 7.1 | 25 |
| | | PS3 | 9°59'08.5"N 76°13'56.3"E | 28 | 7.0 | 25 |
| | | PS4 | 9°59'12.2"N 76°13'47.6"E | 29 | 8.0 | 24 |
| | | PS5 | 9°59'11.7"N 76°13'47.6"E | 30 | 7.5 | 23 |

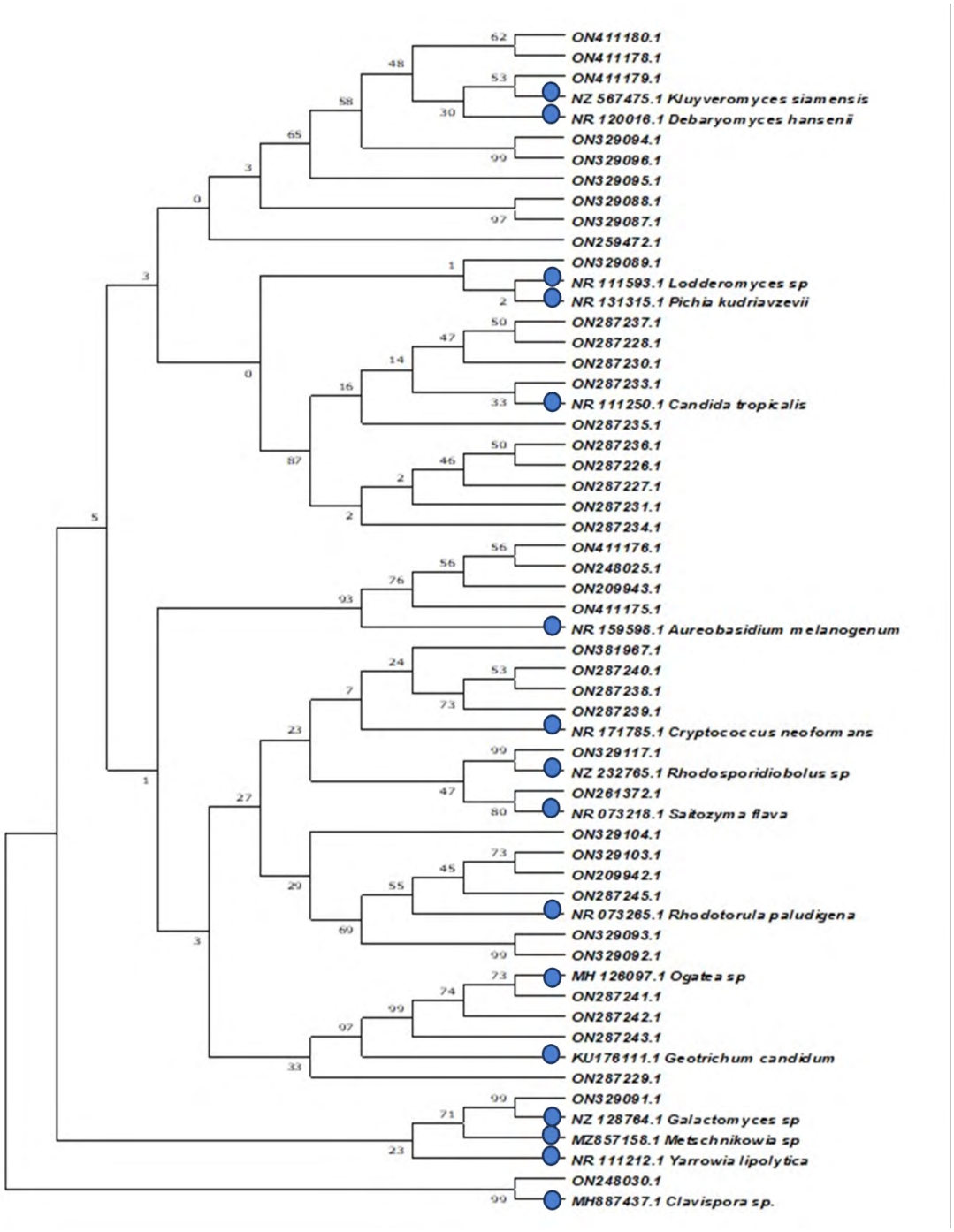


**Fig. 2.** Phylogenetic tree showing the yeast isolates from *Rhizophora* spp. and *Avicennia* spp.

lipase, and ligninase activity of the isolates pointed towards their role in biodegradation in the mangrove environment. These enzymatic activities were mainly exhibited by yeast associated with the roots.

## 4. Conclusion

With the increase in population and related developmental activities, the coverage of mangrove forests is declining at an alarming rate. Many potent tapped and untapped yeasts inhabit this mangrove vegetation that is suited for industrial, medical and agricultural use. The destruction of mangroves will lead to the loss of these invaluable species. This is the first study on mangrove associated yeasts from Kerala which was undertaken to make a record of these yeast species. The study revealed that the highest diversity of yeast was associated with *R. mucronata* and the least with *A. marina*. Some species, like *Candida* spp., *D. hansenii, Rhodotorula* sp. and *Cryptococcus* sp., were cosmopolitan in distribution; others were specific. Environmental factors were found to play a major role in driving yeast composition. The hydrolytic potentials of manglicolous yeasts clearly revealed their potential to be used as agents of biocontrol and bioremediation. This study was successful in building a baseline data on the diversity of manglicolous yeast associated with mangrove vegetation.

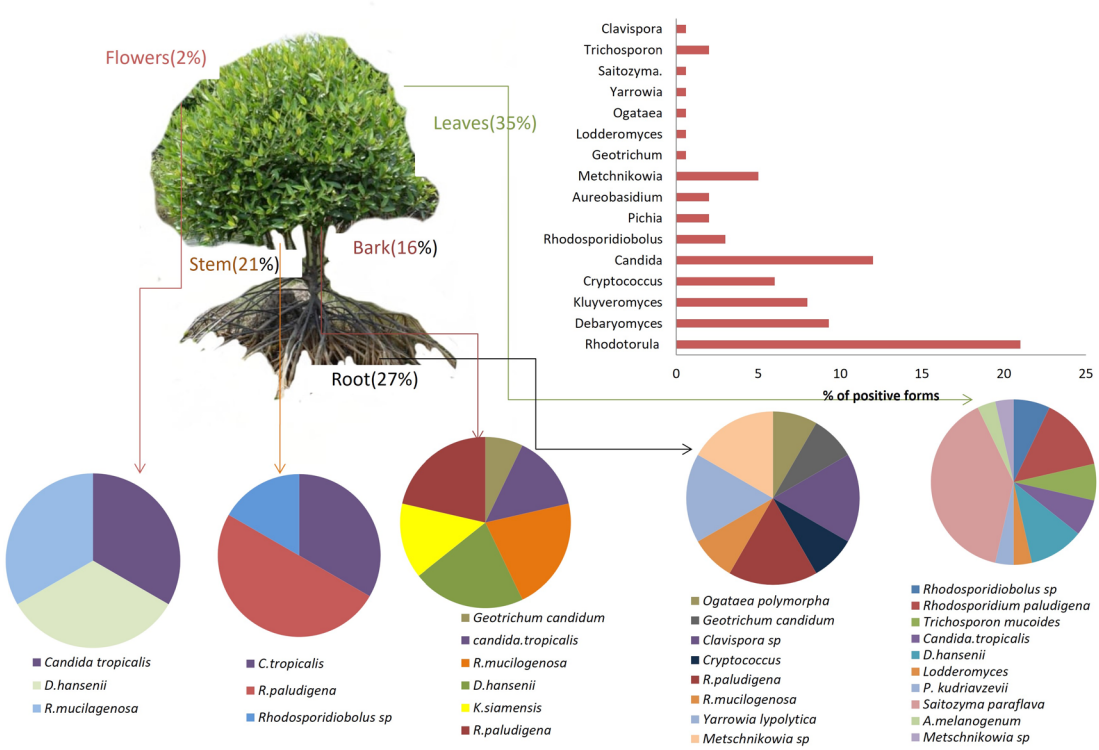


**Fig. 3.** Yeast genera found in different parts of mangrove vegetation

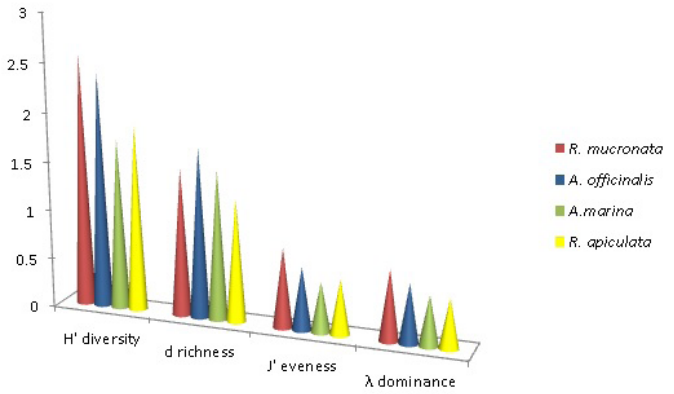


**Fig. 4.** Diversity indices

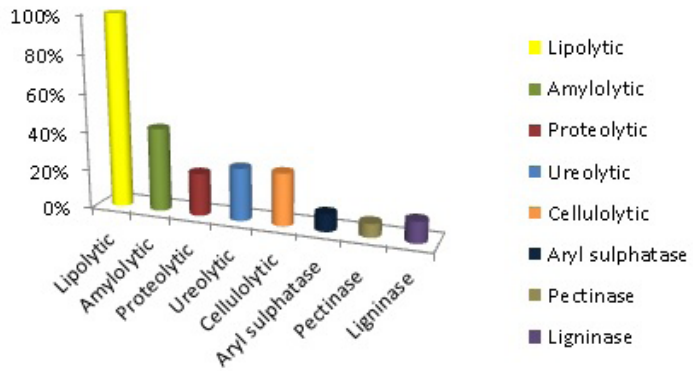


**Fig. 5.** Hydrolytic profile of manglicolous yeasts

**Table 2.** Gen bank details of yeast strains isolates from mangrove vegetation

| Sl. No. | Isolates | Organism | Gen Bank Accession No |
|---|---|---|---|
| 1 | KV35 | *Aureobasidium melanogenum* | ON411175 |
| | KV36 | | ON411176 |
| 2 | KV47 | *Debaryomyces hansenii* | ON411177 |
| | KV46 | | ON411178 |
| | KV45 | | ON411179 |
| | KV48 | | ON411180 |
| | PV22 | | ON287210 |
| | PV23 | | ON287209 |
| | PV27 | | ON287211 |
| 3 | KV18 | *Rhodotorula mucilaginosa* | ON411181 |
| | KV49 | | ON411182 |
| | KV50 | | ON411183 |
| 4 | KV8 | *Cryptococcus marina* | ON381967 |
| 5 | PV91 | *Trichosporon mucoides* | ON381968 |
| | PV94 | | ON381969 |
| 6 | PV81 | *Cryptococcus neoformans* | ON329117 |
| | PV82 | | ON329118 |
| | PV85 | | ON329119 |
| 8 | PV96 | *Candida tropicalis* | ON329129 |
| | PV97 | | ON329130 |
| | PV92 | | ON329131 |
| | PV95 | | ON329132 |
| | PV38 | | ON259470 |
| | PV3 | | ON259473 |
| | KV2 | | ON248020 |
| | KV1 | | ON248019 |
| 9 | PV11 | *Kluyveromyces siamensis* | ON287195 |
| | PV12 | | ON287196 |
| 11 | PV19 | *Pichia kudriavzevii* | ON287203 |
| | PV20 | | ON287204 |
| 13 | PV29 | *Rhodotorula paludigena* | ON287207 |
| | PV55 | | ON263468 |
| | PV54 | | ON263469 |
| | PV47 | | ON263470 |
| | PV41 | | ON262897 |
| 15 | PV58 | *Ogataea polymorpha* | ON263467 |
| 16 | PV43 | *Pichia kluyveri* | ON262945 |
| | PV44 | | ON262946 |
| 17 | PV45 | *Lodderomyces elongisporus* | ON262898 |
| 18 | PV51 | *Metschnikowia reukaufii* | ON262902 |
| | PV52 | | ON262903 |
| | PV53 | | ON262904 |
| 19 | PV56 | *Rhodosporidiobolus sp* | ON262906 |
| | KV31 | | ON329091 |
| 20 | PV39 | *Geotrichum candidum* | ON261369 |
| 21 | PV30 | *Saitozyma paraflava* | ON261372 |
| 22 | KV7 | *Yarrowia lipolytica* | ON248025 |
| 23 | KV19 | *Clavispora lusitaniae* | ON248030 |

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## Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

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