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A comparative study on the diversity of marine yeasts associated with sediment and water samples of two mangroves in Kochi, Kerala, India

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ABSTRACT

The knowledge of marine yeast associated with mangroves is limited as most studies are focused on species of clinical importance or those from terrestrial habitats. Mangrove sediment and water are hotspots for fungal diversity that include yeasts, which possess industrial and bioremediation potential. The study was aimed at comparing the diversity of yeasts from the mangroves of Puthuvype, which faces a great deal of anthropogenic stress, with that of Valanthakad, which is a more pristine mangrove. Sediment and water samples from the two stations were aseptically collected, and the yeast was isolated on Wickerham's agar. The strains were then identified. The average yeast counts were found to be greatest in sediments of Puthuvype (23 x 10^2 CFU/gm). The isolates (228) belonged to 7 genera, namely *Candida* sp. (63.2%), *Cryptococcus* sp. (14.5%), *Hortea* sp.(1.7%), *Kodameae* sp.(1.7%), *Trichosporon* sp.(4.4%), *Rhodotorula* sp.(12.7%), and *Rhodosporidiobolus* sp.(0.4%). Non-pigmented yeasts (85.5%) were found to be more abundant in the mangroves than pigmented ones (14.5%). The pigmented yeasts comprised both black yeasts and red yeasts. The maximum number of pigmented strains was isolated from Valanthakad (52 %). It was found that the yeasts isolated in this study mainly belonged to Ascomycetes, and *Candida tropicalis* was the most dominant species. Spatio-temporal variation in the generic composition of yeasts was noted in this investigation. This study also revealed that an increase in the number of *C. tropicalis* and a reduction in *Rhodotorula* sp. could probably act as an indicator of mangrove pollution. The yeast diversity was found to be greater in Valanthakad mangroves than in Puthuvype. The hydrolytic potentials of the yeast isolates were also found to vary between the two stations. The bioactive potentials of the yeast isolates clearly point towards the need to conserve this fast-declining treasure trove of nature.

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Marine yeasts, Mangroves, *Candida tropicalis*, *Rhodotorula* sp., Puthuvype, Valanthakad

1. Introduction

Mangroves are transition ecosystems located at interfaces between terrestrial and marine environments (Wen-jiao et al., 1997). These ecosystems act as spawning and breeding grounds, nurseries, and habitats for many aquatic as well as terrestrial animals. They provide natural barriers that lessen the impacts of storms, cyclones and coastal erosion (Salem and Mercer, 2012). These mangrove forests also encompass genetically diverse microbial communities (Wen-jiao et al., 1997). As mangroves are mainly detritus-based, microbes, especially yeasts, play an important role in the functioning of these ecosystems and the services they provide.

Yeasts have been defined as unicellular fungi occurring in various lineages (Boekhout et al., 2021) and characterized by budding or fission as a means of asexual reproduction and their sexual states are not enclosed within fruiting bodies (Kurtzman et al., 2011).The knowledge of marine yeast associated with mangroves, especially those of Kerala, is limited, as most studies are focused on species of clinical importance or those from terrestrial habitats. To improve our understanding of the diversity of marine yeasts in mangroves of Kerala, in this study, we compared the diversity and hydrolytic potentials of yeasts from two very different mangroves, namely Puthuvype and Valanthakad located in Kochi, Kerala, India. The mangroves of Puthuvype are the ones that have been facing a great deal of anthropogenic stress, while that of Valanthakad is more pristine in nature.

2. Materials and Methods

2.1 Study Area

Samples (water and sediment) for the study were collected from Mangroves of Puthuvype (9^O 59' 42 ' 'N, 76^O 13' 26'' E) and Valanthakad (9^O 55' 12'' N 76^O 19' 12'' E).

2.2 Sample Collection

Samples were aseptically collected 3 times between April 2018 and March 2019 from both stations. Sampling was done in 1) Apr-May 2018, 2) Sep-Oct 2018 and 3) March 2019. Samples were transported to the lab on ice and processed immediately. Sep-Oct 2018 was conducted immediately after the 2018 Kerala floods.

2.3 Isolation and Enumeration

For the isolation and enumeration of yeasts in the water and sediment samples, they were serially diluted and spread plated on to Wickerham's agar /Malt-yeast-glucose-peptone agar (Malt extract -3 g, Yeast extract - 3g, Peptone - 5 g, Glucose - 10 g, Agar - 20g, Mangrove water – 1000 ml) supplemented with 200 mg/l chloramphenicol (Wickerham, 1951, Kutty and Philip, 2008). The inoculated plates were incubated at 28^O C for up to 2 days. The colony count was done and tabulated. Morphologically distinct colonies were sub-cultured and maintained on Malt extract agar (Malt extract - 15 g, Peptone - 5 g, Agar - 20 g, Mangrove water – 1000 ml, pH – 7) slants for further studies

2.4 Identification of Isolates

For the identification of the microscopic appearance, mode of reproduction and biochemical characteristics like

the ability to answer the Diazonium Blue B (DBB) test, use nitrate as a sole source of nitrogen, utilize dextrose aerobically (oxidative) or anaerobically (fermentative) were considered (Barnett et al., 1990). Isolates with similar reactions were clustered using PRIMER 5 software. A representative strain from each cluster was further identified using API 20 C AUX strips and/ or molecular identification.

2.4.1 Metabolic Identification using API 20 C AUX system of selected isolates

For species level identification, this test was performed as per the manufacturer's protocol (bioMérieux® SA). The reactions were read, and identification was done by referring to the Analytical Profile Index

2.4.2Molecular identification of the selected marine yeast isolates

For the molecular identification of the representative isolates, the DNA was extracted using NucleoSpin® Plant II Kit (Macherey-Nagel) as per the manufacturer's protocol. The ITS region was amplified using the primers ITS-1(Forward ITS 1-5' TCC GTA GGT GAA CCT GCG G 3' and ITS-4 and Reverse (ITS 4- 5' TCCTCC GCT TAT TGA TAT GC 3') as per White et al. (1990). The amplicon obtained was sequenced at Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram, using the BigDye Terminator v3.1 Cycle sequencing Kit (Applied Biosystems, USA). To determine the degree of similarity with the closest phylogenetic group, the sequence obtained was analysed using the Basic Logical Alignment Search Tool (BLAST) online (https://blast.ncbi.nlm.nih.gov/) in the GenBank database.

2.5 Statistical Analysis

PRIMER 5 was used to determine diversity indices, namely the Shannon-Weiner diversity, Peilous evenness and Species richness (Clarke and Gorley, 2001). Values obtained were used to compare the diversity at the 2 stations in this study

2.6 Determination of Hydrolytic Profile of Isolates

In order to screen for the amylase, caseinase, gelatinase, and lipase activity; Nutrient agar basal medium was supplemented with the varied substrates namely starch (1%), casein (2%), gelatin (2%), and tributyrin (1%), respectively. The inoculated plates were incubated for 2 - 3 days at 28±2ºC. After incubation starch agar plates were flooded with iodine solution to detect amylase activity, and gelatin agar plates overlayed with mercuric chloride (15 %) solution for gelatinase activity. The clearance zones were indicative of enzymatic activity.

3. Results and Discussion

3.1Enumeration

The yeast population density was greater in the sediment samples than in water from both Puthuvype and Valanthakad mangroves. The yeast cell counts ranged between 23 x 10^2 – 18.6 x 10^2 CFU/ gm in the sediment sample, whereas in water, it ranged between 7.6 x 10^2 – 7.0 x 10^2 CFU/ ml. The yeast counts dropped in the Puthuvype station from the first sampling i.e. April-May 2018 to the March 2019 sampling; the trend was, however, the reverse in Valanthakad (Fig.1). The drop in the yeast counts from Sept-Oct 2018 sampling to March 2019 in the Puthuvype station is in agreement with the observation of Pothayi and Devasai (2020), who also noted a drastic decrease in cultivable yeast colonies from the mangrove sediments during post-monsoon (Dec-March 2019) compared to monsoon period (June –Sept 2018). Pothayi and Devasai (2020) have attributed this change to the decrease in the organic matter and the moisture content. However, the observations made at Valanthakad in this study are not in agreement with this. We feel the location of stations could well be a factor; Puthuvype being located nearer to the coast and Valanthakad being more interior in location. Moreover, it has been reported yeast populations mainly depend on the type and concentration of organic materials present (Kutty and Philip, 2008).

A total of 228 morphological distinct isolates were obtained during this study, of which 139 isolates were from Puthuvype and the rest (89) from Valanthakad. Non-pigmented yeasts were found to be more abundant (85.5%) than pigmented forms (14.5%) (Fig.2a). A greater number of pigmented forms were isolated from Valanthakad (Fig. 2b). Pigmented yeast comprised of those with carotenoid pigments (red yeasts) and those with melanin (black yeasts). On microscopic observation, the yeast cells appeared ovoid to elliptical in shape. They exhibited budding. Some isolates were also found to produce pseudohyphae (Fig. 3).

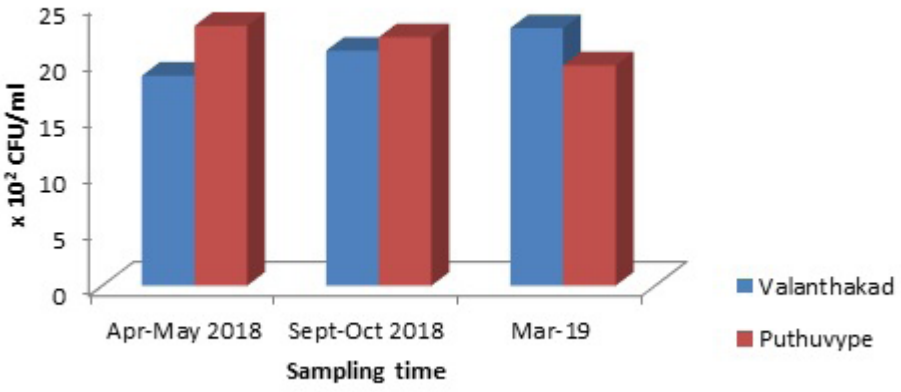

Fig. 1. Population density of yeast in sediments of Puthuvype and Valanthakadu

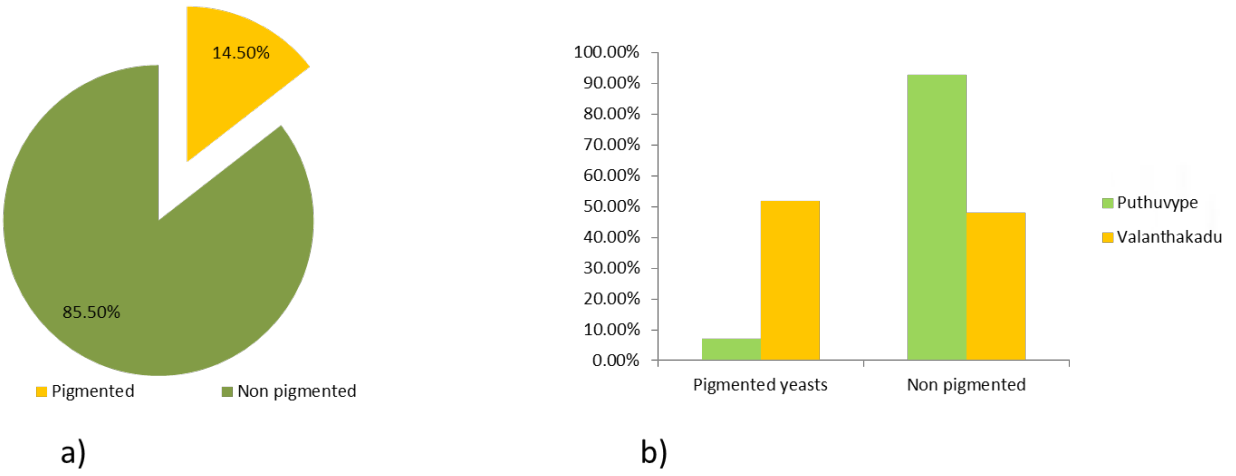

Fig. 2. Pigmented yeasts: a) Percentage of pigmented yeasts b) Distribution of pigmented yeasts in the Puthuvype and Valanthakadu stations

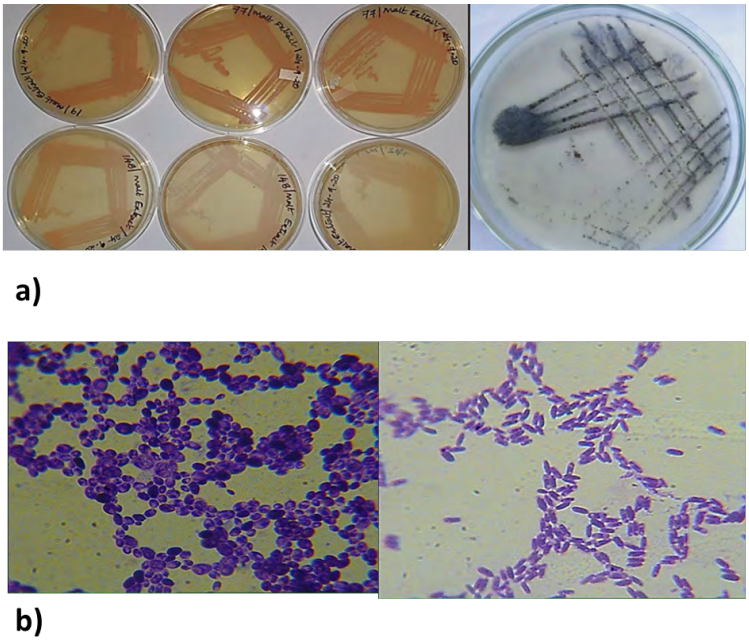

Fig. 3. Appearance of yeast isolates a) Macroscopic b) Microscopic

3.2 Generic composition

The isolates (228) belonged to 7 genera, namely *Candida* spp. (63.2%), *Cryptococcus* spp. (14.5%), *Hortea* sp. (1.7%), *Kodameae* sp. (1.7%), *Trichosporon* sp. (4.4%), *Rhodotorula* spp. (12.7%) and *Rhodosporidiobolus* sp. (0.4%), with Candida being predominant (Fig. 4). Several other workers too have reported that *Candida* sp. is a dominant genus in mangrove water and sediments (Da Costa and D'Souza, 1979; De Almeida, 2005; Statzell-Tallman et al., 2010; Fell et al., 2011; Kathiresan and Saravanakumar, 2011; Patel, 2020, Nefla et al., 2022).

It was noted that the generic composition of yeasts clearly varied between the stations (Fig.5). The genera *Kodamaea* sp., which was reported from Puthuvype, was absent in the Valanthakadu station whereas *Hortea werneckii* found in Valanthakad was not isolated from Puthuvype, indicating a difference in species distribution. In Puthuvype, *Cryptococcus gatti* was the second most dominant species, whereas it was *Rhodotorula* sp. in Valanthakad. Earlier works have stated that marine yeasts are represented by a wide variety of well-known genera that include *Candida, Cryptococcus, Debaryomyces, Pichia, Hansenula, Rhodotorula, Saccharomyces, Trichosporon*, and *Torulopsis (*Kutty and Philip 2008).

The red pigmented yeasts were represented by the genera *Rhodotorula* spp. and *Rhodosporidiobolus* sp. and black yeasts by *Hortea werneckii*. One of the carotenoid pigment producing strains *Rhodotorula paludigena* VA242, isolated from Valanthakad, was found to be ideal for acting as a colour enhancing feed additive for the Ornamental Fish Koi Carp (Rekha et al., 2022).

Generic composition also varied with the sampling time (Fig.6). However, *Candida tropicalis* remained the predominant genera regardless of the sampling site and sampling time.

Red pigmented yeasts are oxidative in nature and their numbers were greater in Valanthakad, whereas *Candida* sp. are fermentative and their numbers greater in Puthuvype.

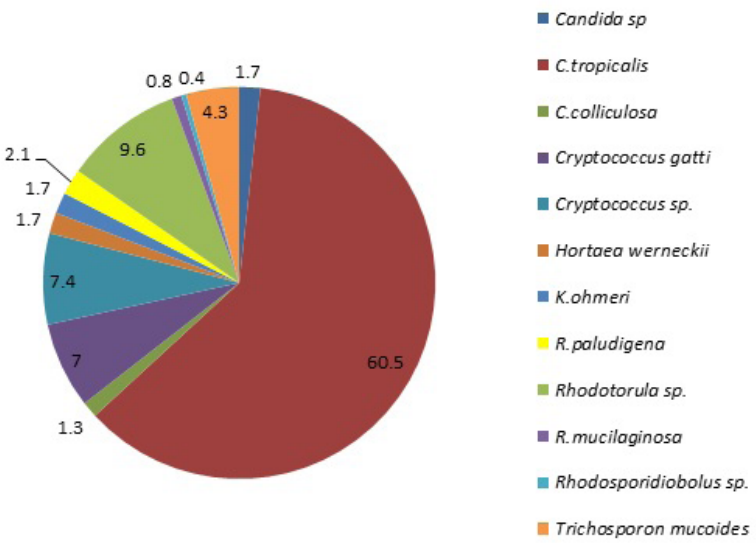

Fig. 4. Overall generic composition of yeast in the mangroves of Puthuvype and Valanthakadu

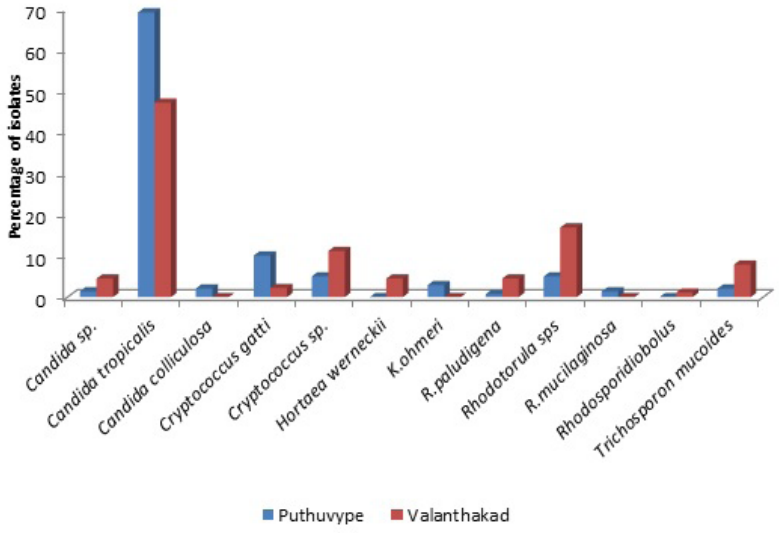

Fig. 5. Comparison of yeasts species distribution at Puthuvype and Valanthakad

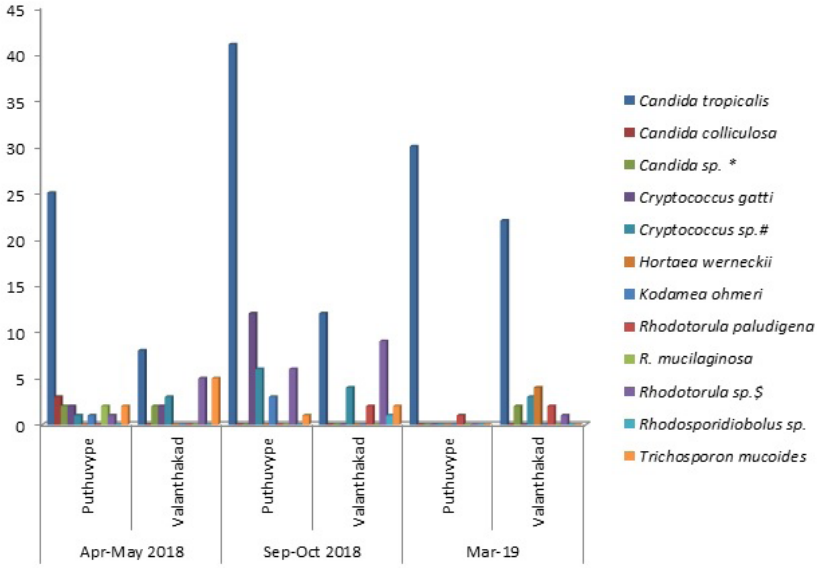

Fig. 6. Difference in the generic composition of yeasts during different sampling periods

This indicates the waters in Valanthakad were cleaner than Puthuvype as it has already been reported that aerobic forms are found more in clean waters than fermentative forms (Kutty and Philip 2008). We find that an increase in the number of *C. tropicalis* isolates and a reduction in *Rhodotorula* sp. may well indicate high anthropogenic activity in the mangrove region. This confirms the fact that Puthuvype mangroves are more polluted than Valanthakad.

3.3 Diversity

The diversity index is a quantitative measure that reflects the number of different species and how evenly the individuals are distributed among those species (Tucker et al., 2017). Between the 2 mangrove stations investigated in this study, the Shannon-wiener diversity (H'($\log_2$)) (2.04) was found to be the highest in the Valanthakad so also the values Peilou's evenness (J') and Dominance (λ) (Fig. 7a) . However the species richness was greater in Puthuvype than Valanthakad. The higher diversity noted in Valanthakad could be attributed to the pristine and undisturbed nature of the mangroves there.

Even when we analysed the diversity values during the different periods of sampling, it was found to be greater in Valanthakad than in Puthuvype (Fig. 7b, c & d). Moreover, the diversity of yeast was found to be greatest in the April-May 2018 sampling in both stations (Fig. 7b); this was the sampling that was done before the floods. After the floods, the diversity of yeasts drooped drastically at Puthuvype (Fig. 7c & d). Natural disasters like floods have been reported to have an impact on the diversity of mangrove yeasts (Nefla et al., 2022).

3.4 Hydrolytic profile

The yeast isolates obtained in this study, irrespective of the sampling time or location, exhibited lipase activity (Fig.8). Pothayi and Devasai (2020) in their study on yeasts from mangrove sediments of Northern Kerala noted that the majority of the strains were lipase positive. Isolates from Valanthakad were found to produce all the enzymes that were tested for in this study, whereas the yeasts isolated from Puthuvype exhibited only lipase, gelatinase and urease activity. This difference in hydrolytic profile could be attributed to the difference in the substrate availability in the two stations.

Hydrolytic potentials of isolates were found to vary with the time of sampling (Fig.9). In the case of Puthuvype, the number of isolates possessing gelatinase and urease dropped after the April – May 2018 sampling i.e. after floods. A similar trend was also observed among the Valanthakad isolates for the enzymes gelatinase and urease, but the number of isolates possessing amylase and caseinase activity increased in this station after the April – May 2018 sampling. Yeasts with diverse metabolic capabilities have been reported to occur in aquatic environments such as oceans and seas, estuaries, lakes, and rivers (Kutty and Philip, 2008).

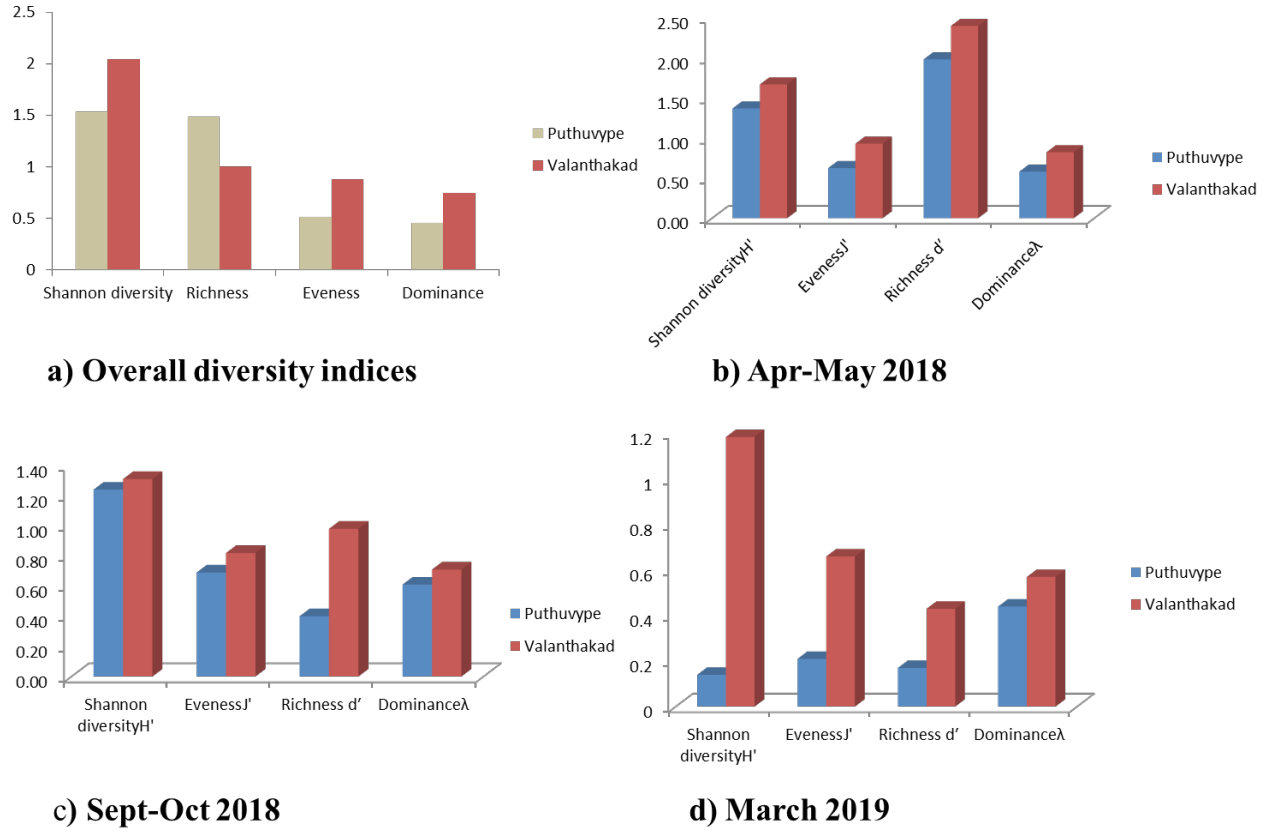

Fig. 7. Diversity Indices: a) The overall diversity indices values b) Diversity indices during April- May 2018 sampling c) Diversity indices during September-October 2018 sampling d)March 2019 sampling

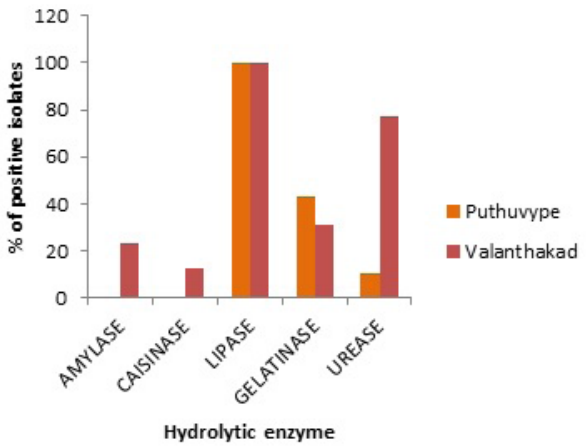

Fig. 8. Comparison of the overall hydrolytic profile of yeast isolates

4. Conclusion

It is evident from this study that the general composition of yeasts in the examined mangroves varies spatio-temporally. In comparison to Puthuvype mangroves, Valanthakad mangroves were discovered to have a higher diversity of yeasts; even the Peilou's evenness and dominance indices were found to be higher there. Valanthakad harboured more pigmented isolates. All of the findings from this study suggest that the mangroves of Valanthakad are cleaner or more pristine than those of Puthuvype. For the first time, species like *R. paludigena, Rhodosporidiobolus* sp., and *K. ohmeri* were reported from Kerala's mangroves. We found that an increase in the number of *C. tropicalis* isolates and a reduction in *Rhodotorula* sp. can serve as signs of significant anthropogenic activity in mangroves. Compared to those from Puthuvype, the strains from Valanthakad were able to hydrolyze a variety of substrates. Despite having distinct general compositions, the yeast isolates from Kochi's two mangroves were both discovered to produce a variety of hydrolytic enzymes and pigments that have the potential to be used commercially. It also highlights the necessity of adopting measures to preserve these ecosystems, which are treasure troves of biodiversity.

Acknowledgements

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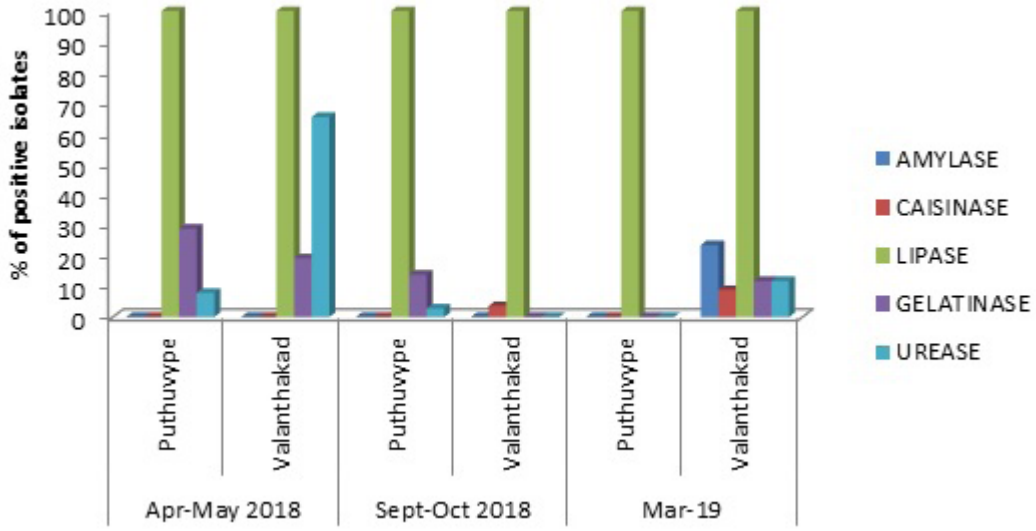

Fig. 9. Comparison of hydrolytic potentials of yeasts during different sampling periods

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