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RESEARCH ARTICLE

Eur J Biol 2022; 81(1): 50-57

Yeast Diversity in the Mangrove Sediments of North Kerala, India

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Please cite this article as: Vidya P, Sebastian CD. Yeast Diversity in the Mangrove Sediments of North Kerala, India. Eur J Biol 2022; 81(1): 50-57. DOI: 10.26650/EurJBiol.2022.1027475

ABSTRACT

Objective: Mangrove sediments, due to their unique environment, are considered to be a crucial habitat in identifying yeast strains with potent industrial, biotechnological and bioremediation properties. The goal of the current study was to understand the presence, diversity and hydrolytic enzyme properties of yeasts from the mangrove sediments of North Kerala.

Materials and Methods: Sampling was done during the period 2018-2020 from mangrove sediments of 5 districts along the North Kerala coast. Isolation of yeast was done on yeast glucose peptone agar, and isolates were tested for their potential for the production of extracellular enzymes viz. amylase, cellulase, chitinase, DNase, lipase, ligninase, pectinase, protease and urease, using standard media with specific substrates. Morphological assessment, biochemical characterization and molecular identification of the isolates were performed. The phylogenetic tree of the selected yeast strains was constructed with the Maximum Likelihood method using MEGA X software.

Results: A total of 482 yeast strains belonging to 12 genera were obtained from the mangrove sediment samples, the most dominant genera being Candida (56.3%). *Kluveromyces, Debaromyces, Torulaspora, Trichosporon, pigmented yeast Rhodotorula, Cyberlindnera, Wickerhamiella, Pichia, Trichoderma, Meyerozyma* and *Kodamaea* were the other genera identified. The majority of the yeast present in the mangrove sediment samples were lipolytic (68%), followed by ureolytic (23%), ligninolytic (16%), cellulolytic (9%), DNAlytic (9%), proteolytic (8%), amylolytic (6%), pectinolytic (4%) and chitinolytic (2%) forms. All 12 genera of yeast obtained had positive forms for extracellular lipase.

Conclusion: The yeast strains obtained from mangrove sediments in the study were found to be ecologically important and have great biotechnological potential.

Keywords: Yeast, mangrove sediments, hydrolytic enzymes, phylogeny, North Kerala

INTRODUCTION

Mangroves are a unique ecosystem present in the tropical and subtropical parts of the world, and are considered the most productive coastal ecosystems. The sediments of mangroves harbor a great number of microbial communities composed mainly of bacteria, fungi and actinomycetes, which perform nutrient transformation of the ecosystem (1). The yeasts present in mangrove sediments have gained attention during recent years by virtue of their potential characteristics. Due to their ecological flexibility, they can tolerate extreme conditions like varying salinity, different environmental temperatures, oxygen concentrations and changes in acidity (2). They reproduce and grow quickly on simple substrates, which are decomposed with the help of hydrolytic enzymes they produce (3).

The hydrolytic enzymes that are produced by yeasts have a wide range of applications in industries as well as in bioremediation processes, as they take part in various transformational activities (4). The enzymes produced by yeasts from mangrove sediments are believed to have the capability to withstand extreme climatic

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Submitted: 24.11.2021 • **Revision Requested:** 25.03.2022 • **Last Revision Received:** 04.04.2022 • **Accepted:** 05.05.2022 • **Published Online:** 16.05.2022

and cultural conditions, which would help in their large-scale production (5). Moreover, during scale up cultures, they require comparatively cheap substrates, like industrial byproducts and wastes, as nutrient sources (6). Thus, mangrove sediment yeasts can be considered the best for the commercial production of industrially important enzymes due to easier production in terms of genetic and environmental manipulation, cost effectiveness, non-toxic properties and stability (7).

The diversity and identification of yeasts present in mangrove sediments primarily helps in understanding their properties which can be applied in industrial and biotechnological processes and in bioremediation. Assessment of the hydrolytic enzyme potential of the yeasts and analyzing the factors affecting it would be a great advancement in the mass production of commercially important enzymes. Though the Northern part of Kerala, India is bestowed with large mangrove areas, they are not sampled enough to provide any well-vetted information on the yeasts present in the sediments. For these reasons, this work was focused on isolation, identification and evaluation of the enzymatic capability of yeasts present in the sediments of the mangrove ecosystem along the coast of North Kerala.

MATERIALS AND METHODS

Sample Collection

Mangroves of 5 districts along the North Kerala coast were selected for sediment collection. The sampling was done during the period 2018-2020 at 8 sites (Table 1). 10-20 g of sub surface sediment was collected using a hand corer, transferred aseptically into sterile polythene bags, transported in ice boxes and processed within 4 hours of collection. Five sub-samples were collected from each site, which were then homogenized to one composite sample per site.

Isolation of Yeast

Sediment samples were spread plated on Wickerham's agar medium supplemented with 200 mg/l chloramphenicol (8) in duplicates and incubated at 18±2°C for 7 days. The single colonies developed were purified by quadrant streaking. Later they were streaked onto malt extract agar slants for further studies.

Screening for Hydrolytic Enzyme Production

All the isolates were tested for the production of hydrolytic extracellular enzymes, namely protease, amylase, lipase, urease, ligninase, cellulase, DNase, pectinase and chitinase. The detection of extracellular protease, amylase, lipase and chitinase was done in nutrient agar medium containing casein (2%), starch (1%), tributyrin (1%) and colloidal chitin (5%), respectively, as substrates. The detection of DNase, cellulase, pectinase, urease and ligninase activity was tested on DNase agar, Cellulose agar, Pectin agar and Urease agar with 40% urea and Crawford's agar supplemented with 0.5% tannic acid, respectively. The plates were spot inoculated and incubated at 28±2ºC for 7 days. The plates were flooded with 1M HCl, Gram's iodine solution and 1% cetrimonium bromide (CTAB) after incubation for protease amylase and pectinase, respectively. Formation of clearance/ halo zone or brown color around the colonies was considered positive.

Identification of Yeasts

The isolates were identified to the genus level using morphological and biochemical characterization methods. Later, molecular characterization was used for species identification of selected isolates. In morphological characterization, colony characteristics on malt extract agar and microscopic appearance of methyl blue stained smear under 40x and oil immersion (100x) in compound microscope were observed. Biochemical characterization included urea hydrolysis, sugar fermentation (MOF - Microbial Oxidation Fermentation test), fatty acid hydrolysis, nitrate assimilation, starch like substance and citric acid production, Diazonium Blue B reaction and observance of growth at 37°C (9). Isolates showing similar morphological and biochemical characteristics were grouped together. Representative strains from each of these groups were selected for species identification based on their efficiency to produce extracellular enzymes determined qualitatively by screening the

Table 1. Sites of collection of mangrove sediments for the present study.

Sl. No.	Name of the collection site	Site code	Lat-Long coordinates
1	Chandragiri (Kasaragod Dt)	KGD	12°05'32'' N 75°13'39'' E
2	Edat (Kannur Dt)	EDT	12°05'32'' N 75°13'39'' E
3	Pazhayangadi (Kannur Dt)	PYD	12°02'72'' N 75°29'31'' E
4	Valapattanam (Kannur Dt)	VPT	11°93'45'' N 75°35'35'' E
5	Elathur (Kozhikode Dt)	ELR	11°19'43'' N 75°45'20'' E
6	Kadalundi (Kozhikode Dt)	KDI	11°07'43'' N 75°49'48'' E
7	Ponnani (Malappuram Dt)	PON	10°47'10'' N 75°55'30'' E
8	Chettuva (Thrissur Dt)	CTV	10°52'42'' N 76°04'79'' E

hydrolytic activity for respective substrates. The identification of these isolates was performed by sequencing of ITS region as per Harju et al. (10) with Forward ITS1 and Reverse ITS4 primers (11). The amplified fragment of approximately 580 bp, containing the ITS 1, 5.8 S and ITS 2 regions, was used for the sequence similarity search using NCBI BLAST.

Phylogenetic Analysis

Nucleotide sequences of yeasts obtained were statistically analyzed using Molecular Evolutionary Genetic Analysis Version X (MEGA X) software and a phylogenetic tree was constructed using Maximum Likelihood method. Proper outgroup (fungus - *Penicillium chrysogenum*) was selected for the construction of the phylogenetic tree and the nodal support was tested by means of 100 bootstrap pseudo replicates.

RESULTS

After isolation and purification, a total of 482 yeasts strains were obtained from the mangrove sediment samples of 8 sites in North Kerala. Based on their morphological, physiological, biochemical and molecular characterization, it was found that they belonged to 12 genera of yeast. 20 species were identified when selected isolates were sequenced and the sequences were deposited in the GenBank (Table 2). The total number and distribution of different genera of yeast obtained in the study is summarized in Figure 1. 272 of the total isolates (56.31%) belonged to genera *Candida*, in which 7 different species have been identified, among which *Candida tropicalis* was the predominant species found (84.19%). Other genera of yeast obtained from the mangrove sediments were *Kluveromyces* (12.01%), *Debaromyces* (9.52%), *Torulaspora* (9.11%), *Trichosporon* (3.73%), pigmented yeast *Rhodotorula* (3.52%), *Cyberlindnera* (2.07%), *Wickerhamiella* (1.66%), *Pichia* (1.24%), *Trichoderma* (0.62%) and one isolate each of *Meyerozyma* (0.21%) and *Kodamaea* (0.21%) (Table 3).

The majority of the yeast present in the mangrove sediment samples was lipolytic (68%), followed by ureolytic (23%), ligninolytic (16%), cellulolytic (9%), DNAlytic (9%), proteolytic (8%), amylolytic (6%), pectinolytic (4%) and chitinolytic (2%) forms

Table 2. NCBI GenBank accession details of identified yeast strains from mangrove sediments during the present study.

Sl. No.	Isolates	Organism	GenBank Accession
1.	CUMB VP –PN2-03	*Rhodotorula mucilaginosa*	MT 131387
2.	CUMB VP –PN2-10	*Candida pseudolambica*	MT 131389
3.	CUMB VP –KL1-10	*Candida tropicalis*	MT 131391
4.	CUMB VP –KL1-01	*Candida sorboxylosa*	MT 136728
5.	CUMB VP –PN2-12	*Torulaspora globosa*	MT 135124
6.	CUMB VP –ED3-04	*Torulaspora maleeae*	MT 131815
7.	CUMB VP –KG3-05	*Candida dubliniensis*	MT 132376
8.	CUMB VP –KG-4-03	*Pichia kudriavzevii*	MT 138566
9.	CUMB VP –PN4-12	*Trichoderma viride*	MT 138567
10.	CUMB VP –VL-4-03	*Trichosporon asahii*	MT 138568
11.	CUMB VP –CT-1-09	*Debaryomyces nepalensis*	MT 138571
12.	CUMB VP –PZ-4-10	*Kluyveromyces siamensis*	MT 138576
13.	CUMB VP –PZ4-07	*Cyberlindnera saturnus*	MW 617277
14.	CUMB VP –EL4-09	*Wickerhamiella infanticola*	MW 617280
15.	CUMB VP –PN4-08	*Kodamaea ohmeri*	MW 617290
16.	CUMB VP –KL5-01	*Meyerozyma carpophila*	MW 617291
17.	CUMB VP –EL5-01	*Candida membranifaciens*	MW 617296
18.	CUMB VP –VL1-05	*Candida metapsilosis*	MW 617299
19.	CUMB VP –KG4-13	*Candida orthopsilosis*	MW 617302
20.	CUMB VP – KL4-12	*Trichoderma longibrachiatum*	MW 624336

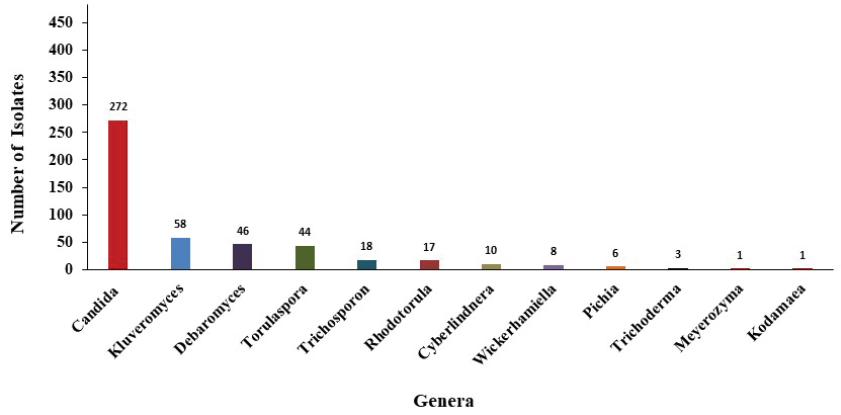

Figure 1. Distribution of different genera of yeast from the mangrove sediments.

Table 3. Taxonomic list of yeast strains obtained and identified from the mangrove sediments.

Identified yeast strains	No. of isolates
Genus: *Candida*	
Candida tropicalis	229
Candida dubliensis	23
Candida matapsilosis	8
Candida pseudolambica	5
Candida sorboxylosa	4
Candida membranifaciens	2
Candida orthopsilosis	1
Genus: *Kluveromyces*	
Kluveromyces siamensis	58
Genus: *Debaromyces*	
Debaromyces napelensis	46
Genus: *Torulaspora*	
Torulaspora maleae	38
Torulaspora globosa	6
Genus: *Trichosporon*	
Trichosporon asahii	18
Genus: *Rhodotorula*	
Rhodotorula mucilaginosa	17
Genus: *Cyberlindnera*	
Cyberlindnera saturnus	10
Genus: *Wickerhamiella*	
Wickerhamiella infanticola	8
Genus: *Pichia*	
Pichia kudriavzevii	6
Genus: *Trichoderma*	
Trichoderma viride	3

(Figures 2 and 3). Generic wise hydrolytic potential for extracellular enzymes of all the isolates is summarized in Figure 4. All 12 genera of yeast obtained had positive forms for extracellular lipase. Isolates belonging to genera *Candida* and *Torulaspora* showed hydrolytic activity for all the enzymes under study, while the representatives belonging to genera *Meyerozyma* and *Kodamaea* showed only lipolytic activity. All the isolates representing genus *Trichosporon*, *Wickerhamiella*, *Trichoderma* and the pigmented red yeast *Rhodotorula* were ureolytic in nature.

Estimates of phylogenetic relatedness among species were determined using the maximum likelihood (ML) in MEGA X software. 52 sequences of yeasts were analyzed and presented as bootstrap consensus tree to illustrate the statistical strength of gene tree (Figure 5). Bootstrap support for the phylogenetic tree was determined from 1,000 replications. The evolutionary history was inferred using the Neighbor Joining method and the tree was drawn to scale, with branch lengths having the same units as those of the evolutionary distances represented and as the number of base substitutions at each site. The positions with missing data and gaps were excluded during tree construction. The fungus *Penicillium chrysogenum* was selected as an outgroup for the phylogenetic tree. The sequences were classified in 5 large clades and subclades.

DISCUSSION

In our study, around 56% of the total isolates belonged to genus *Candida*, making them the most predominant yeast present in the mangrove sediments of Northern Kerala. *C. tropicalis, C. dubliensis, C. matapsilosis, C. pseudolambica, C. sorboxylosa, C. membranifaciens* and *C. orthopsilosis* were the 7 species found under the genus *Candida*, in which *C. tropicalis* (84%) hugely outnumbered others. Reports on the yeasts present in the mangrove sediments from China and different parts of Brazil showed the domination of *Candida* spp. in the ecosystem (12,13). *Candida* is considered the most common genera of yeast present in marine environments and is isolated largely from estuarine and mangrove areas of urban regions that are polluted by human resources (14,15). Due to this reason, they

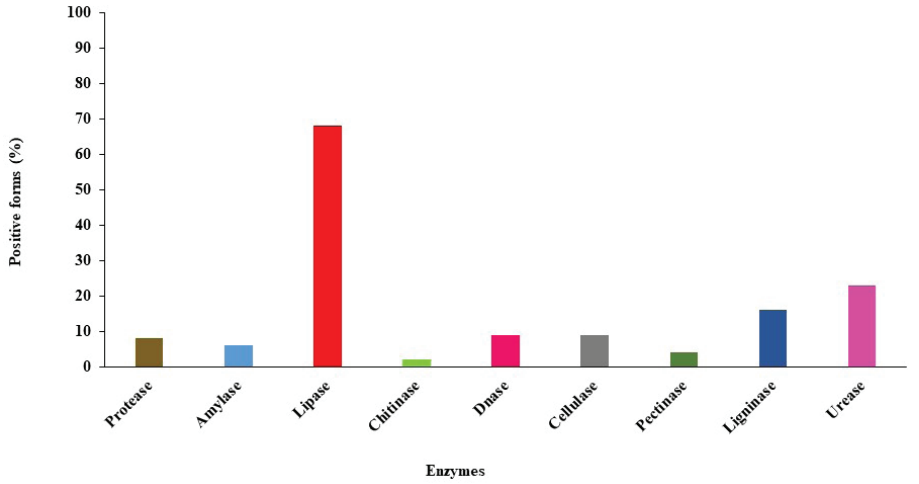

Figure 2. Percentage of yeast isolates (positive forms) showing various enzyme activities.

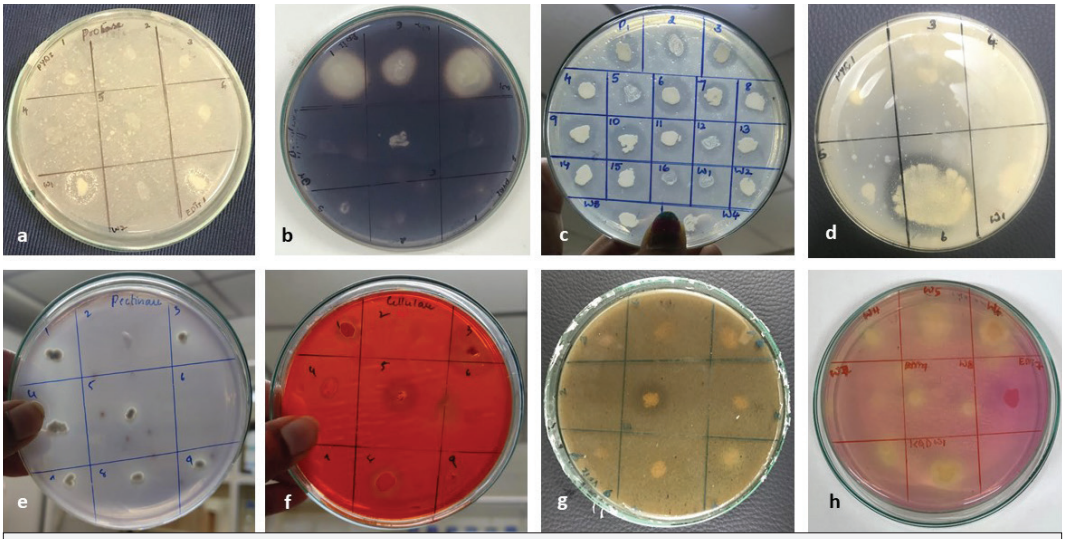

Figure 3. Plates showing various hydrolytic enzyme activities of yeast isolates. Clearance/halo zone or brown/red color around the colonies was considered positive. a) Protease b) Amylase c) Lipase d) DNase e) Pectinase f) Cellulase g) Ligninase h) Urease.

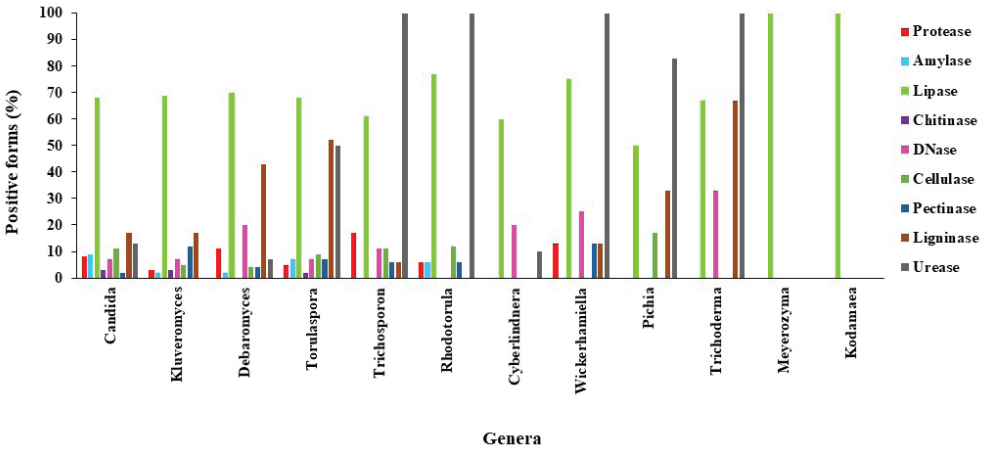

Figure 4. Generic wise distribution of hydrolytic potential of yeast isolates for various extracellular enzymes.

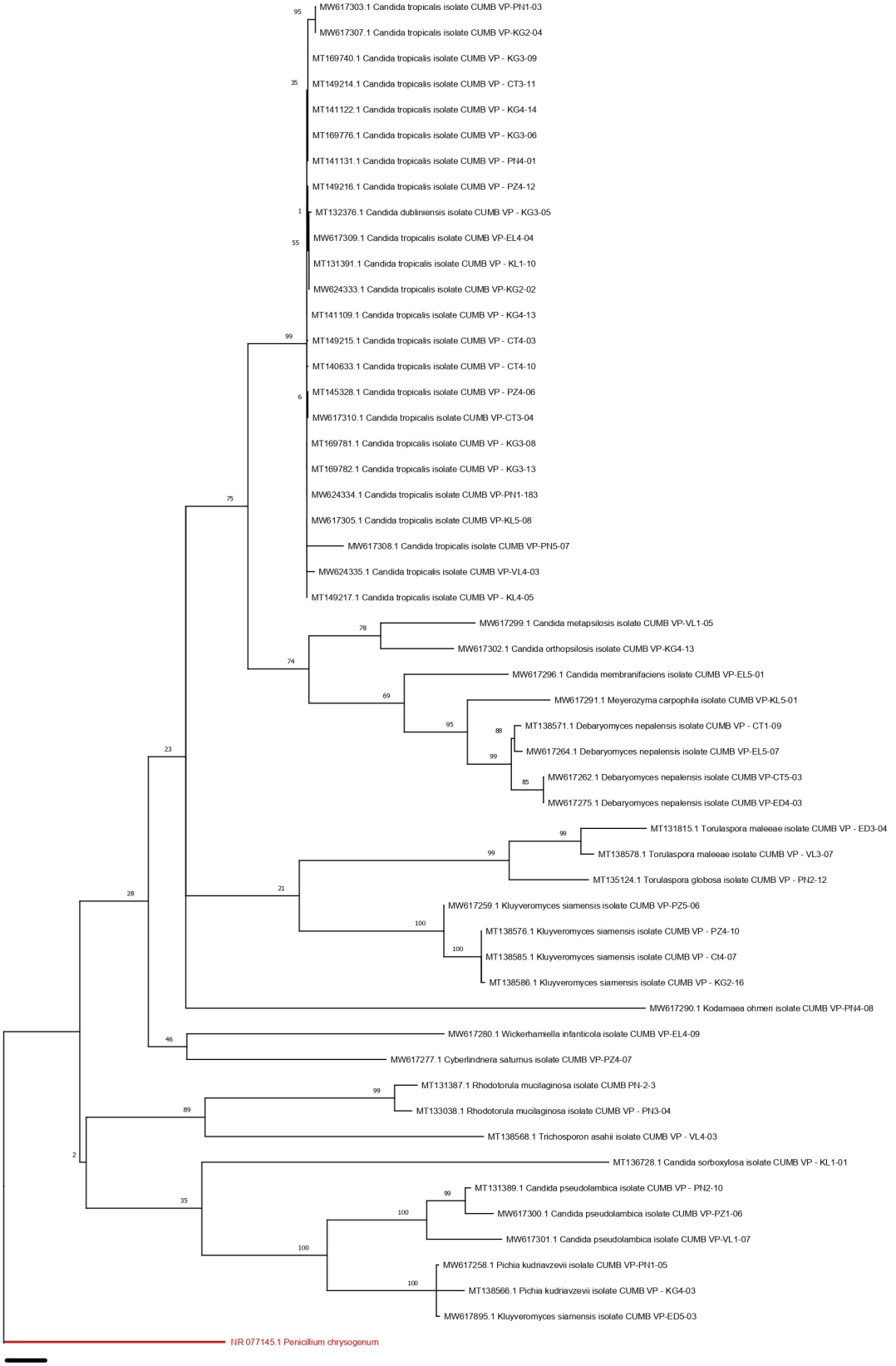

Figure 5. Phylogenetic tree representing relationship between the yeast isolates from the mangrove sediments using Neighbor Joining algorithm.

are considered one of the bio indicators of pollution (16,17). The dominance of *Candida* spp. in the mangrove ecosystem, especially in the sediments, was found to have several ecological significances. It has been found that they are able to withstand the extreme conditions of the mangrove sediments, including fluctuating temperature, high salinity, low oxygen concentration and varying nutrient compositions (3). Most of the yeast belonging to genus *Candida* spp. were found to have a significant role in the process of biodegradation (18). Studies have shown that *C. tropicalis* have the ability to degrade a wide range of pollutants, including crude petroleum, oil while *C. parapsilosis* and *C. intermedia* act efficiently on all kinds of oily pollutants (19,20). Kurtzmann et al. isolated *C. pseudolambica* from the mangrove ecosystem and reported them as a cosmopolitan species in marine environments due to their active role in biodegradation (21). *Candida* spp. were also involved in converting the shell wastes of crustaceans into biomass protein and in the production of silver nano particles (22,23).

Kluveromyces siamensis, *Debaromyces napelensis*, *Torulaspora maleae* and *T. globosa, Trichosporon asahii, Rhodotorula mucilaginosa, Cyberlindnera saturnus, Wickerhamiella infanticola, Pichia kudriavzevii, Trichoderma viride, Meyerozyma carpophila* and *Kodamaea ohmeri* were the other yeast species found in the sediments. Most of the species obtained in our study are not only ecologically important but also have been found to have great biotechnological potential. *Rhodotorula, Torulaspora, Debaryomyces* and *Trichosporon* were considered bioindicators due to their role in the degradation of various pollutants and in controlling algal blooms (24). These species were also found to metabolize aromatic hydrocarbons and heavy metals present in the environment, thereby degrading them to non-toxic products (25). The pigmented yeast *Rhodotorula mucilaginosa* produce carotenoids, which makes them a potential bioindicator and biotechnological candidate. An easy method to assess the quality of sample by the colony count method was developed in which the number of pink colonies of *R. mucilaginosa* indicated the extend of pollution (16). Moreover, the carotenoids extracted from the species are widely used in food and pharmaceutical industries and also in waste water treatment (26).

The extracellular enzymes of yeast are hydrolytic in nature and they take part in a large number of transformation reactions that can be applied in industrial processes and in bioremediation. Lipase is the most common enzyme produced by marine yeast and catalyzes various chemical reactions, including hydrolysis, aminolysis, acidolysis, alcoholysis and esterification. (27,28). The majority of the isolates in our study were lipolytic, those belonging to *Candida* spp. being the highest. Hasan et al have reported that the yeast belonging to genera *Candida, Rhodotorula* and *Pichia* are active lipase producers (29). All the isolates studied belonging to genera *Trichosporon*, *Wickerhamiella*, *Trichoderma* and *Rhodotorula* were able to hydrolyze urea. The nickel containing urease enzyme has tremendous applications in medical and pharmaceutical industries (30,31). Though ligninolytic activity in yeasts is studied least by researchers, our study showed that the yeasts of genera *Debaromyces, Torulaspora* and *Trichoderma* were able to actively break down lignin substrates. The reduced production of other enzymes, including cellulase, DNase, protease, amylase, pectinase and chitinase, by the isolates under study might be due to the absence or low availability of those substrates and their recycling in the sediments of sampling sites (18). Yeast extracellular enzymes from an extreme environment like mangrove sediments should be further explored in order to make them useful in industrial processes and environmental bioremediation.

CONCLUSION

The quest for novel and improved microbial strains that can produce industrially important metabolites is a continuous process. Since, yeasts are one of the most active producers of secondary metabolites and extracellular enzymes that can be fermented using cheap substrates; they have gained massive attention during recent years. In the present study, we investigated the yeasts from mangrove sediments of North Kerala for its diversity and production of extracellular hydrolytic enzymes. The results suggest that yeasts from mangrove sediments are promising candidates for various industrial and biotechnological applications and can be effectively used in the development of strategies for the bioremediation and conservation of the ecosystem.

Informed Consent: Written consent was obtained from the participants.

Peer Review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- P.V., C.D.S.; Data Acquisition- P.V.; Data Analysis/Interpretation- P.V., C.D.S.; Drafting Manuscript- P.V.; Critical Revision of Manuscript- P.V., C.D.S.; Final Approval and Accountability- P.V., C.D.S.

Conflict of Interest: Authors declared no conflict of interest.

Financial Disclosure: Authors declared no financial support.

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