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Yeast isolates from the slope sediments of Arabian Sea and Bay of Bengal: Physiological characterization

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ABSTRACT

Marine yeasts are versatile agents of biodegradation. They act on varied substrates and helps in nutrient recycling. This study mainly focuses on the hydrolytic potential of the marine yeast isolates obtained from the slope sediments of Arabian Sea and Bay of Bengal. The optimum growth conditions like temperature, salinity and pH of the isolates were also determined. The isolates from Bay of Bengal showed more enzyme production than those of Arabian Sea. All the isolates were lipolytic. Oxidative forms were more in abundance than the fermentative forms. Black yeasts obtained from the study area, showed maximum hydrolytic potential than compared to their counter parts. Majority of the isolates preferred 30^{o}C, pH 6 and 15 ppt salinity for maximal growth.

Keywords: *Marine yeasts, Arabian Sea, Bay of Bengal, Enzymes, Sediments*

INTRODUCTION

Marine yeasts are considered to be an important category of marine microorganisms. Kohlmeyer and Kohlmeyer [14] isolated yeasts from seawater, sediment, plants, animals and other organic matter in the marine habitat. Marine yeasts are reported to be truly versatile agents of biodegradation [7,13]. They participate in a range of ecologically significant processes in the sea, especially in estuarine and near-shore localities. These activities include decomposition of plant substrates, nutrient-recycling phenomena and biodegradation of oil and recalcitrant compounds. Biomass data and repeated observations of microhabitat colonization by various marine yeasts support ancillary lab evidence for the contribution of this segment of the marine mycota to productivity and transformation activities in the sea [15].

Yeast enzymes were found to be useful in various industrial processes which emphasize their direct contribution to our day to day life. These enzymes are produced mostly extracellular by different metabolic reactions taking place inside the cell and participate in various transformation activities like mineralization of organic compounds. Studies by Paskevicius [18] showed that almost all the yeast strains produce lipase. The most active lipase producers belonged to the genera *Rhodotorula*, *Candida*, *Pichia* and *Geotrichum*. Lipases catalyse a wide range of reactions like hydrolysis, esterification, alcoholysis, acidolysis, aminolysis etc. [12]. Lipases are mainly involved in detergent industry and biodegradation, especially oil residues. Some lipases from the yeast strains could actively hydrolyze different oils, indicating that they may have potential applications in industry.

A protease producing strain isolated from the sediments of saltern near Qingdao, China, had the highest activity at pH 9 and 45ºC [6]. This principal enzyme, protease, has many applications in detergent, leather processing and feed industry besides waste treatment [16]. Yeast amylases have many applications in bread and baking industry, starch liquefaction and saccharification, paper industry, detergent industry, medical and clinical analysis, food and pharmaceutical industries [5,9]. Cellulases have application in stone washing, detergent additives, production of SCP, biofuels and waste treatment [23]. The enzyme inulinase produce fuel ethanol, high fructose syrup and inulo oligosaccharides [17]. The enzyme phytase is a component of commercial poultry, swine and fish diets and animal/human nutrition [10].

MATERIALS AND METHODS

Sediment samples were collected from 200, 500 and 1000 m depth regions of Arabian Sea and Bay of Bengal. For the isolation of yeasts, plating of the sediment samples were done on-board employing spread plate method in Wickerham's agar [22] supplemented with 200 mg/l chloramphenicol. The plates were incubated at 18±2°C for 14 days and the colonies developed were purified by quadrant streaking and transferred to malt extract agar slants for further studies. Isolates were stocked in malt extract agar vials overlaid with sterile liquid paraffin. Marine oxidation fermentation (MOF) medium was used for testing the ability of the yeast isolates to utilize dextrose aerobically (oxidative) or anaerobically (fermentative). The isolates were tested for the production of enzymes viz., amylase, lipase, protease, urease, aryl sulfatase, ligninase, cellulase, DNase, pectinase and chitinase.

Nutrient agar medium supplemented with casein (2%), starch (1%), tributyrin (1%) and colloidal chitin (5%), were prepared for the detection of protease, amylase, lipase and chitinase respectively. Plates were spot inoculated and incubated at room temperature (28±2ºC) for 7 to 10 days. Presence of clearance zone was noted as positive and the diameter of the zone was recorded. In the case of amylase, plates were flooded with Gram's iodine solution and the presence of clearance zone was noted. Pectin agar was used for testing the production of pectinase. The plates were spot inoculated and incubated at room temperature for 7 to 10 days. After incubation the plates were flooded with 1% cetavlon (cetyl trimethyl ammonium bromide) and the zone of clearance was noted.

Cellulose agar was used for testing cellulase production. The plates were spot inoculated and incubated at room temperature (28±2ºC) for 7 to 10 days. The zone of clearance around the colonies was noted as positive. For testing the production of aryl sulfatase, ZoBell's agar supplemented with 0.001M Tripotassium phenolphthalein disulfate (PDS) was used. The plates were spot inoculated and incubated at room temperature (28±2ºC) for 12 days. After incubation the agar plates were exposed to ammonia vapor and development of pink color around the colonies due to the release of phenolphthalein from PDS was recorded as positive. Crawford's agar was used as the basal medium for testing lignin degradation. The basal medium was supplemented with 0.5% tannic acid and the plates were spot inoculated and incubated at room temperature (28±2ºC) for 7 to 14 days. Formation of halo zone or brown color around the colonies was considered as positive. The isolates were tested for their optimum growth at different temperature, salinity and pH.

RESULTS

Oxidative/fermentative nature of the yeast isolates

Arabian Sea:

Among the isolates of Arabian Sea (Cr. No. 228 & 233), only 7.4% were fermentative and 92.5% oxidative (Fig. 1a). At all the three depth zones domination of oxidative forms could be noted i.e. 97.2%, 93.2% and 88.1% at 200, 500 and 1000 m depth respectively (Fig1b). Generic wise analysis of the oxidative and fermentative forms showed that isolates belonging to the genera *Candida*, *Lipomyces, Yarrowia, Rhodotorula, Debaryomyces* and Black yeasts were cent percent oxidative in nature. More than 95% of the *Wingea* spp. was oxidative and all the *Dekkera* spp. were fermentative (Fig. 1c.).

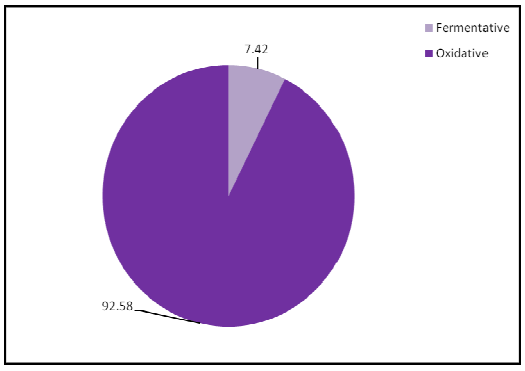

Fig. 1a **Average percentage of fermentative and oxidative among the marine yeasts from the slope sediments of Arabian Sea (Cr. No. 228 & 233)**

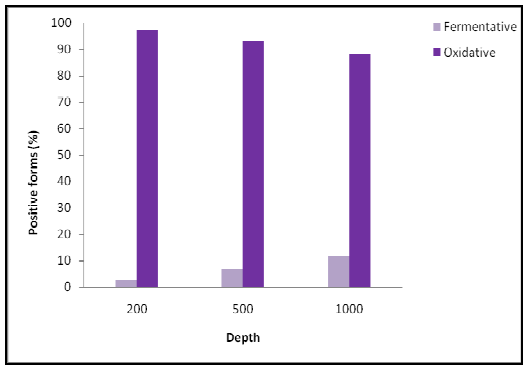

Fig. 1b Percentage of fermentative/oxidative yeasts at different depth regions in Arabian Sea (200-1000 m depth) (Cr. No. 228 & 233)

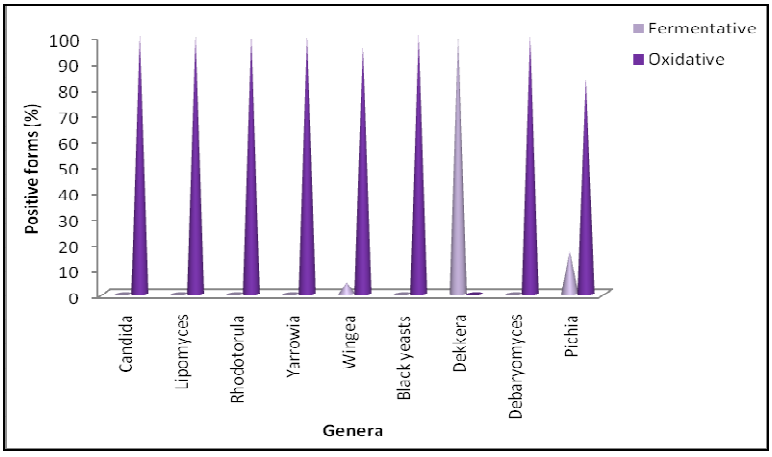

Fig 1c Percentage of fermentative and oxidative marine yeasts belonging to different genera isolated from the slope sediments of Arabian Sea (Cr. No. 228 & 233)

Bay of Bengal (Cruise 236):

Among the isolates of Bay of Bengal (Cr. No. 236) 23% were fermentative and 77% oxidative (Fig. 2a). At 200 m depth regions the fermentative (47%) and oxidative (52%) forms were in almost equal proportions. At 500 and 1000 m depth range oxidative forms were in high proportions and comprised about 87% and 76% respectively (Fig. 2b). Generic wise analysis of the oxidative and fermentative forms showed that isolates belonging to the genera *Bullera*, *Oosporidium*, *Cryptococcus*, *Pichia*, *Lipomyces*, *Yarrowia*, *Trichosporon* and Black yeasts were cent percent oxidative in nature. *Wingea* and *Dekkera* were cent percent fermentative. Isolates belonging to *Rhodotorula* (93.3%) and *Candida* (63%) were generally oxidative (Fig. 2c).

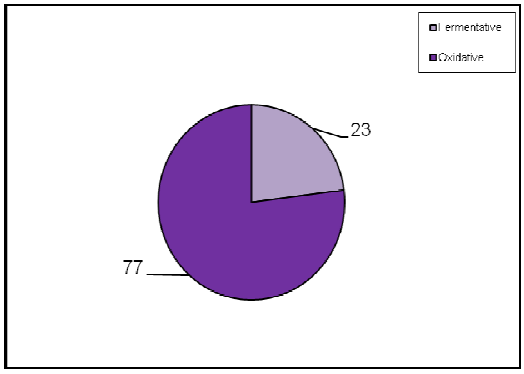

Fig. 2a **Average percentage of fermentative and oxidative forms among the marine yeasts from the slope sediments of Bay of Bengal (Cr. No. 236)**

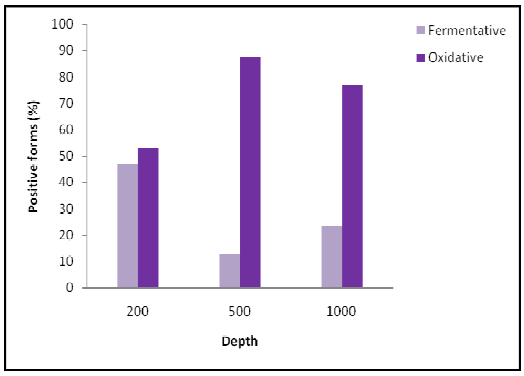

Fig. 2b **Percentage of fermentative/oxidative yeasts at different depth regions in Bay of Bengal (200-1000 m depth) (Cr. No. 236)**

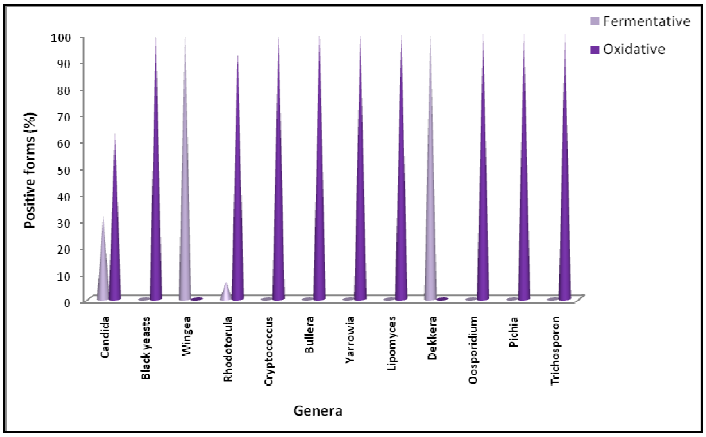

Fig 2c Percentage of fermentative and oxidative marine yeasts belonging to different genera isolated from the slope sediments of Bay of Bengal (Cr. No. 236)

Hydrolytic enzymes

All the isolates of the Arabian Sea were lipolytic, followed by ligninolytic (15.8%), ureolytic (13.3%), proteolytic (8.9%) and amylolytic (4.4%) forms (Fig. 3. None of the isolates produced aryl sulfatase, DNase, pectinase, cellulase and chitinase. Percentage of isolates producing protease, amylase and urease was more in 500 m depth zones (Fig. 4a), where as ligninase producing forms were more in 200 m depth (Fig. 4b). Isolates producing protease, amylase and ligninase were meager at 1000 m depth. None of the isolates from 1000 m depth produced urease (Fig. 4c).

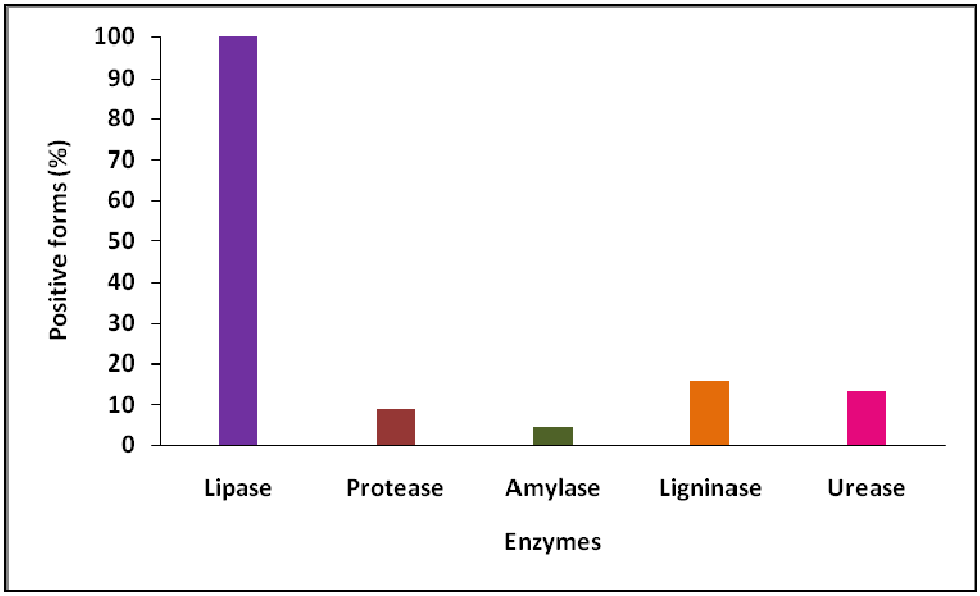

Fig. 3 Average hydrolytic enzyme production by marine yeasts from the slope sediments of Arabian Sea (Cr. No. 228 & 233)

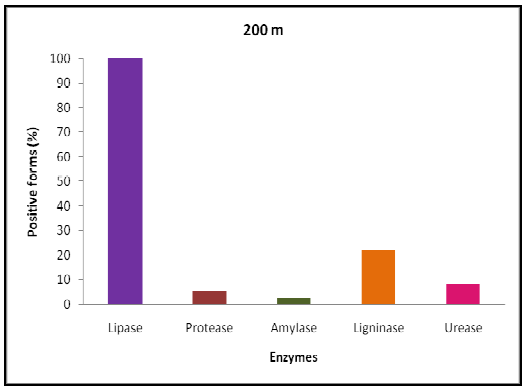

Fig. 4a (200m)

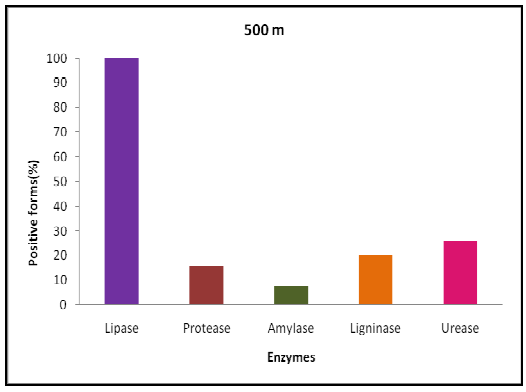

Fig. 4b (500m)

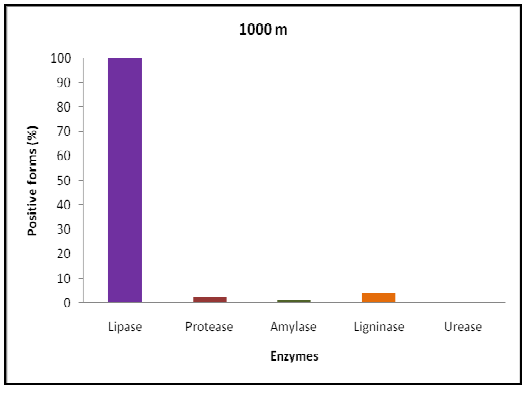

Fig. 4c (1000m)

Fig. 4a-c Hydrolytic enzyme production by marine yeast isolates at different depths along the Arabian Sea (200,500 and 1000 m depths) (Cr. No. 228 & 233)

Black yeasts were cent percent positive for lipase, protease, amylase and ligninase. They were found to be the most potent isolates in enzyme production (Fig. 5). Some of the isolates belonging to the genus *Yarrowia* were also able to produce all the enzymes. Generic wise hydrolytic potential of all the isolates are given in table 1.

Table 1 Generic wise hydrolytic potential of the isolates from the Arabian Sea

Genera/ Group	Lipase	Protease	Amylase	Ligninase	Urease
Candida	**100**	1.25	1.25	12.5	0
Lipomyces	**100**	0	0	16.2	0
Rhodotorula	**100**	0	0	37.5	37.5
Yarrowia	**100**	24.1	20.6	6.8	55.1
Wingea	**100**	16.6	0	33.3	16.6
Black yeasts	**100**	**100**	**100**	**100**	**0**
Dekkera	**100**	15.3	0	7.6	0
Debaryomyces	**100**	0	0	20	0
Pichia	**100**	0	0	60	0

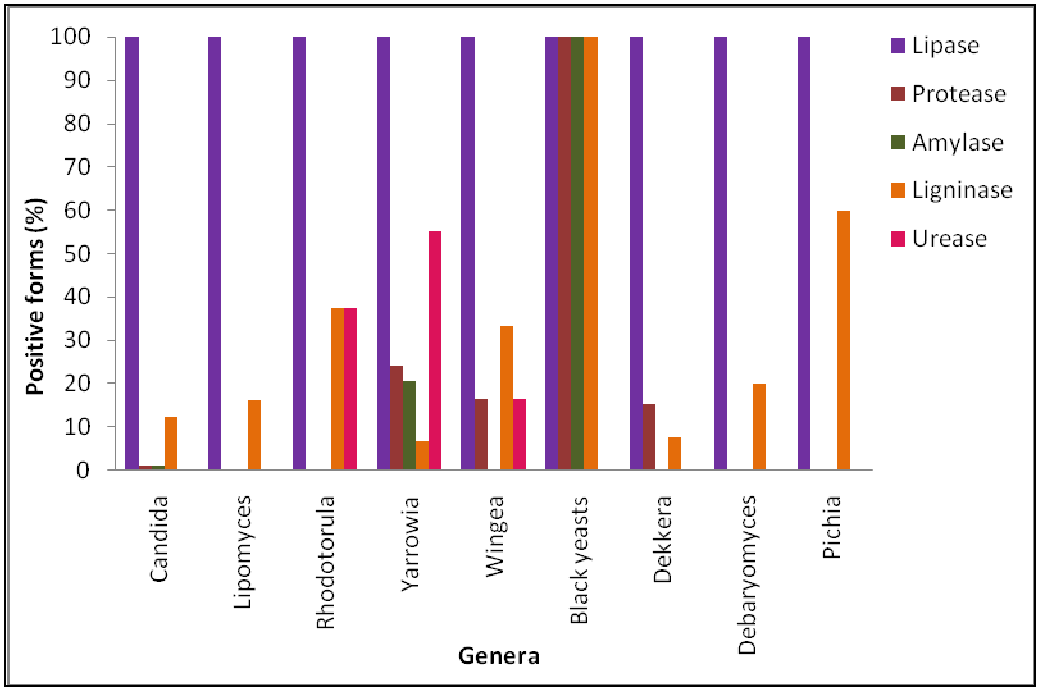

Fig. 5 Hydrolytic potential of different genera of marine yeasts isolated from the slope sediments of Arabian Sea (Cr. No. 228 & 233)

Bay of Bengal (Cruise 236):

All the isolates obtained from Bay of Bengal (Cr. No. 236) were lipolytic, followed by ligninolytic (63.7%), proteolytic (43.4%), ureolytic (36.2%), amylolytic (28.9%) and aryl sulfatase (1.45%) producing forms (Fig. 6). None of the isolates produced DNase, pectinase, cellulase and chitinase. Other than lipase production, all other enzyme production was found to be lesser in isolates from 200 m depth (Fig. 7a). The only isolate which produced aryl sulfatase was obtained from 500 m depth (Fig. 7b). Protease producing isolates were maximum at 1000 m depth (Fig. 7c).

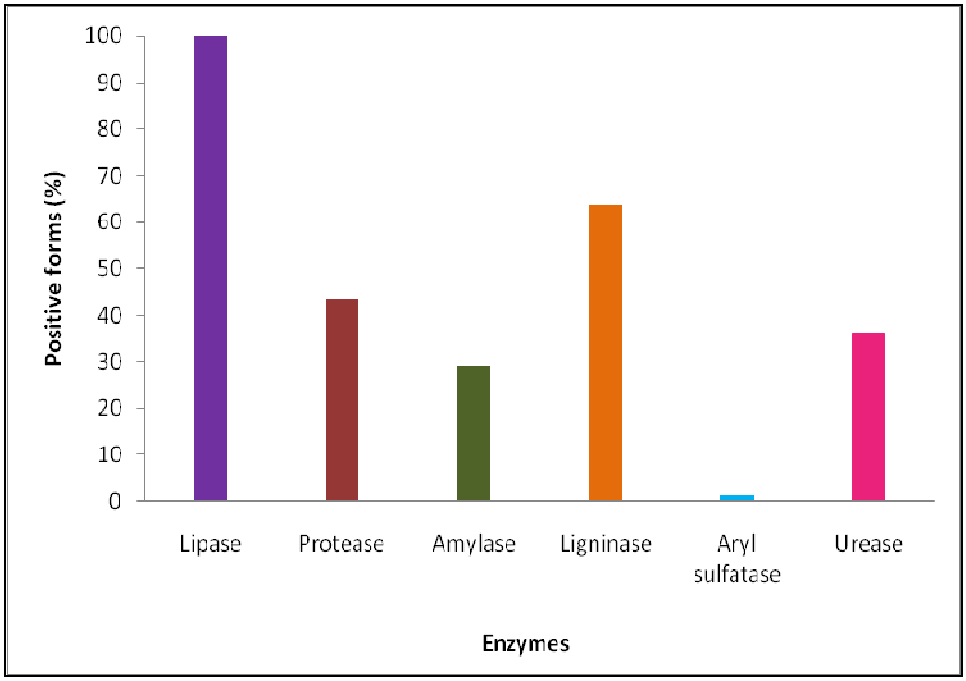

Fig. 6 Average hydrolytic enzyme production by marine yeasts from the slope sediments of Bay of Bengal (Cr. No. 236)

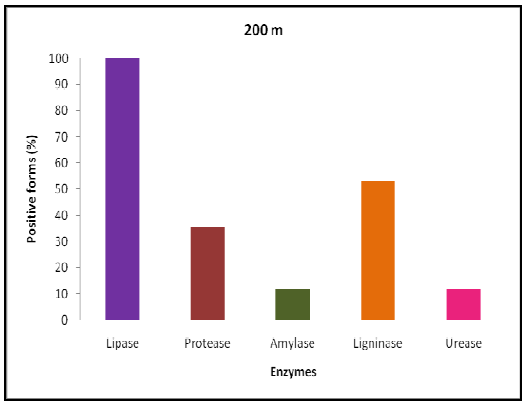

Fig. 7a (200m)

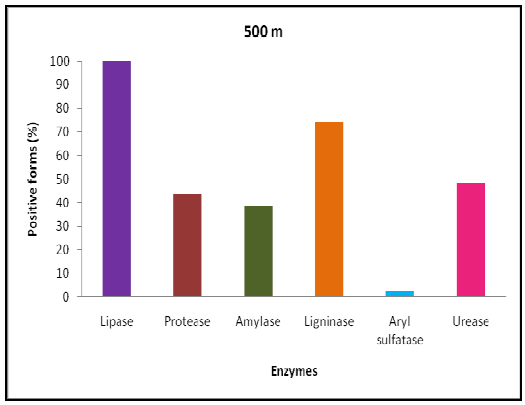

Fig. 7b (500m)

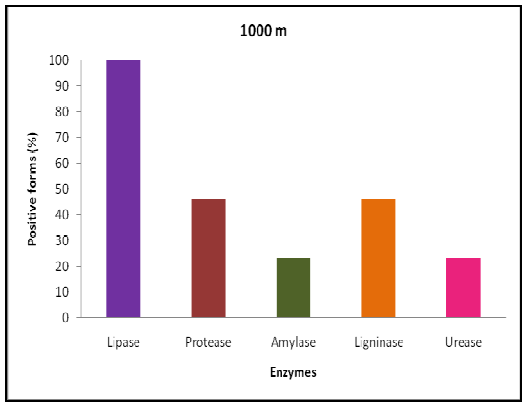

Fig. 7c (1000m)

Fig. 7 a-c Hydrolytic enzyme production by marine yeast isolates at different depths along the Bay of Bengal (200,500 and 1000 m depth) (Cr. No. 236)

Among the whole isolates only one strain produced aryl sulfatase which belonged to the genus *Cryptococcus* isolated from 500 m depth station. Black yeasts were cent percent positive for lipase, protease, amylase, ligninase

and 44.4% of them produced urease. They were found to be the most potent isolates in enzyme production (Fig. 8). Generic wise hydrolytic potential of all the isolates are given in the table 2.

Table 2: Generic/Group wise hydrolytic potential of the isolates from the Bay of Bengal (Cr. No. 236)

Genera/ Group	Lipase	Protease	Amylase	Ligninase	Aryl sulfatase
Candida	**100**	15.78	0	68.42	0
Black yeasts	**100**	**100**	**100**	**100**	**0**
Wingea	**100**	85.71	0	28.57	0
Rhodotorula	**100**	40	46.6	80	0
Cryptococcus	**100**	**50**	**50**	**50**	**25**
Bullera	**100**	20	0	60	0
Yarrowia	**100**	0	0	50	0
Lipomyces	**100**	50	0	0	0
Dekkera	**100**	0	0	100	0

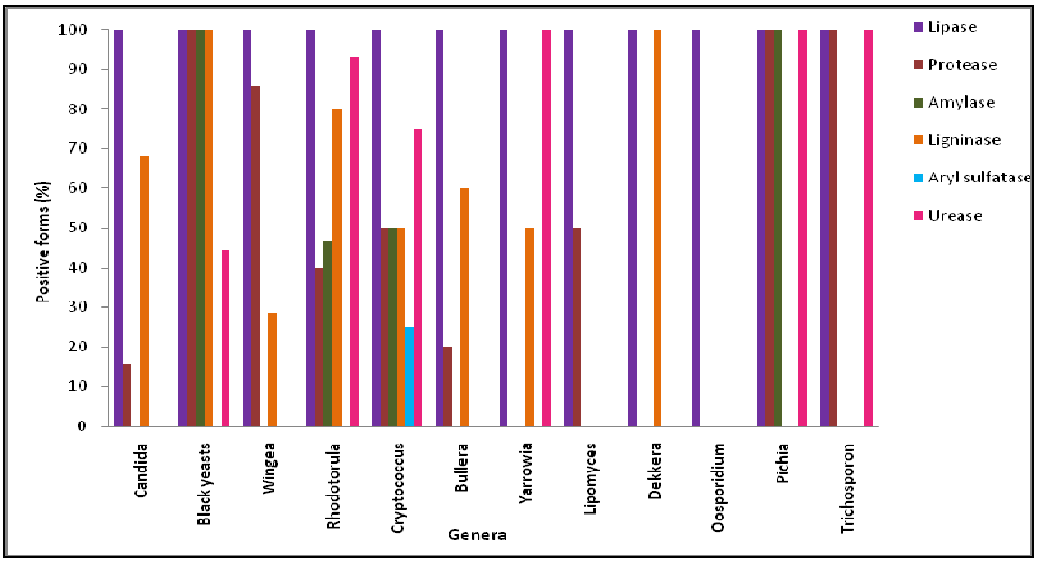

Fig. 8 Hydrolytic potential of different genera of marine yeasts isolated from the slope sediments of Bay of Bengal (Cr. No. 236)

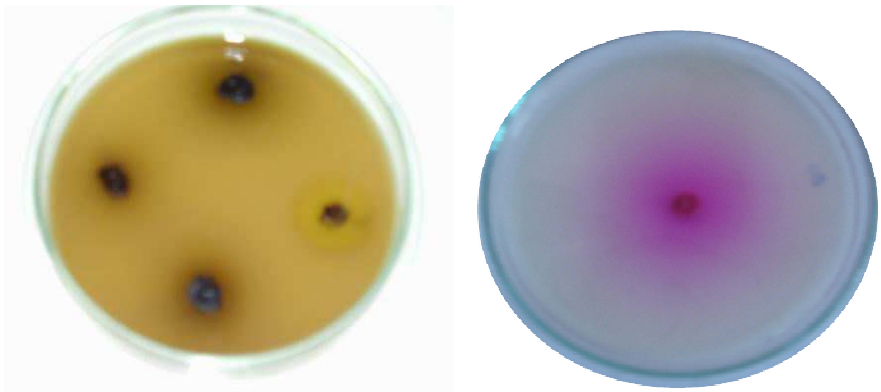

Fig. 9a **Fig. 9b**

Fig. 9a and b Hydrolytic potential of the yeast isolates a. Ligninase b. Aryl sulfatase

Growth at different temperature, salinity and pH

Temperature:

Most of the isolates preferred 30°C (69%) for maximum growth followed by 20°C (18.18%) and 40°C (12.72%) (Fig.10). The isolates did not show growth at 10 and 50°C. Percentage of isolates having maximum growth at different temperatures in three depths is given in table 3.

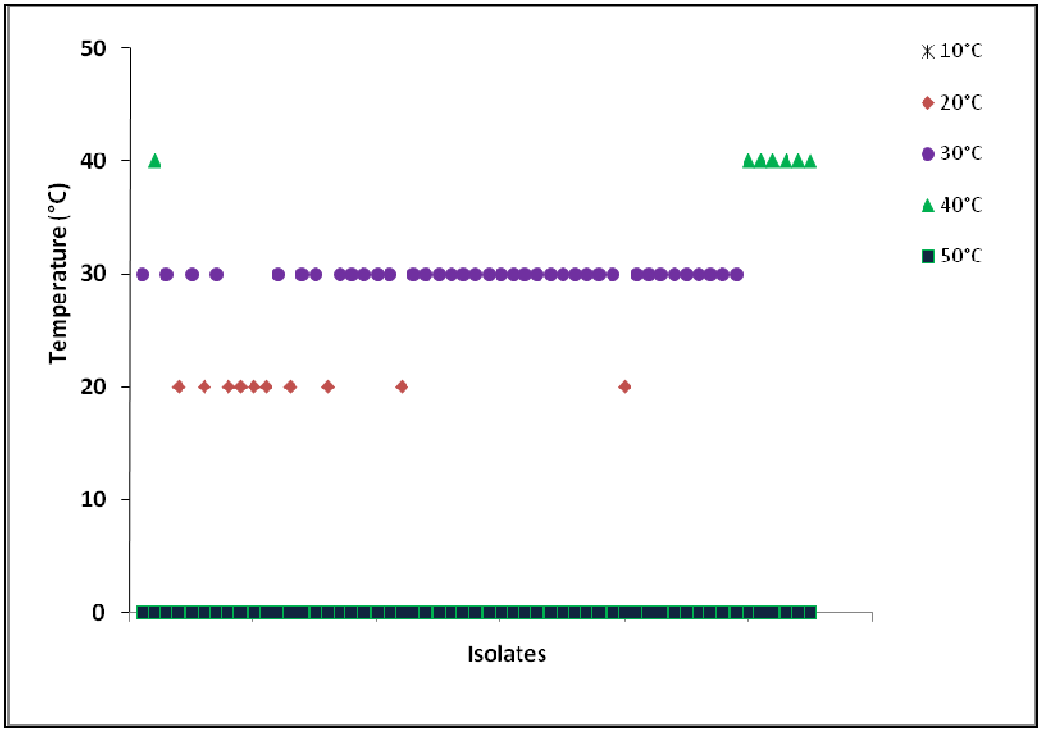

Fig. 10 Optimum temperature for the growth of various marine yeast isolates

Table 3: Percentage of isolates showing maximum growth at different temperature in three depths

Depth (m)	10°C	20°C	30°C	40°C	50°C
200	0	14.3	57.14	28.6	0
500	0	26	70.37	13.7	0
1000	0	0	100	0	0
Total (%)	0	18.18	69.09	12.72	0

Salinity:

Considerable growth could be noticed for all the isolates from 0 to 45 ppt. However 15 to 25 ppt was found to be the most preferred range (Fig. 11 and table 4).

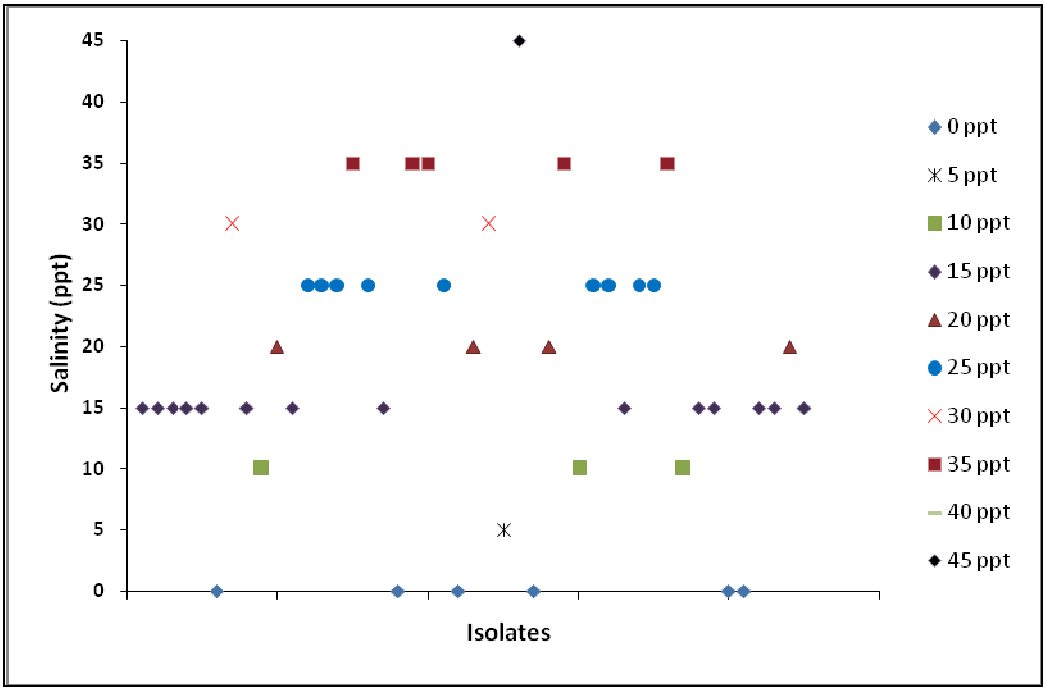

Fig. 11 Optimum salinity for the growth of marine yeast isolates

Table 4: Percentage of isolates (from various depths) showing maximum growth at different salinities

Depth (m)	0 ppt	5 ppt	10 ppt	15 ppt	20 ppt	25 ppt	30 ppt	35 ppt	40 ppt	45 ppt
200	6.25	0	6.25	18.75	12.5	31.25	0	25	0	0
500	16.6	4.16	8.3	37.5	4.16	16.6	8.3	0	0	4.16
1000	20	0	0	40	20	0	0	20	0	0
Total (%)	13.3	2.22	6.66	31.1	8.8	20	4.4	11.1	0	2.22

pH:

Most of the isolates showed maximum growth at pH 6 and 7 (Fig.12 and table 5). However, considerable growth could be recorded at a pH range 4-9.

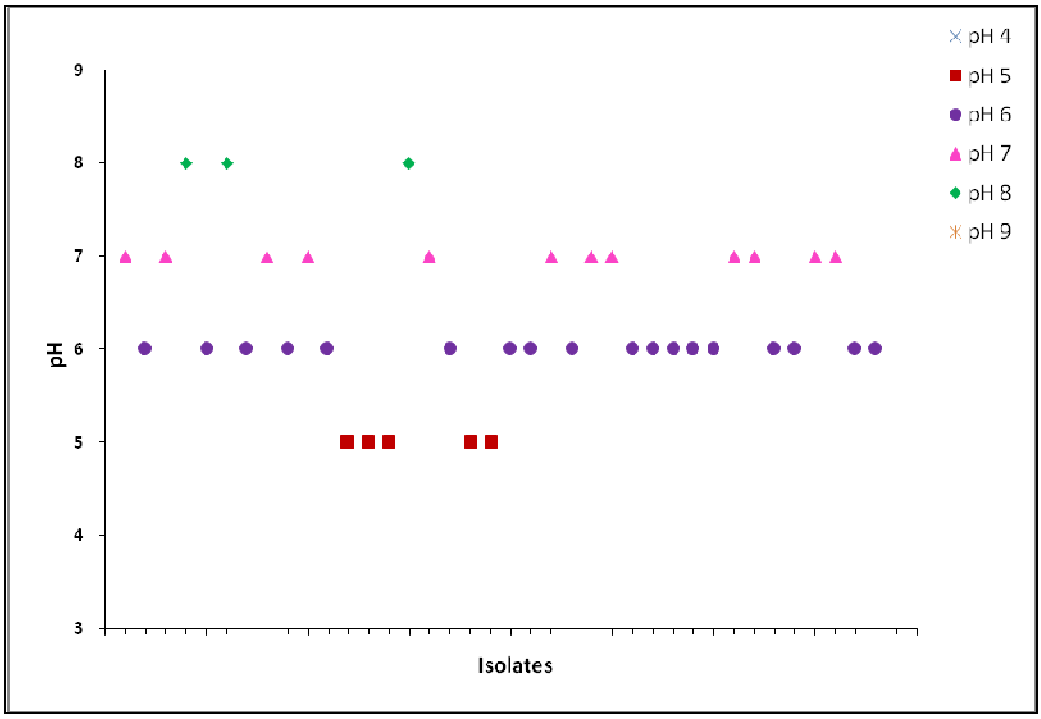

Fig. 12 Optimum pH for the growth of various marine yeast isolates

Table 5: Percentage of isolates (from various depths) showing maximum growth at different pH

Depth (m)	pH 4	pH 5	pH 6	pH 7	pH 8	pH 9
200	0	12.5	50	31.25	6.25	0
500	0	5.88	52.94	29.41	11.76	0
1000	0	40	20	40	0	0
Total (%)	0	13.15	47.36	31.57	7.89	0

In general, the isolates were able to grow considerably at a temperature range of 20-40°C. But, for almost all the isolates the maximum growth was observed at 30°C. Even though these isolates were obtained from a marine realm where the temperature ranged between 6-16°C, these organisms preferred the ambient temperature (28±2°C) for their growth. Notably, the isolates were able to grow in a wide range of salinities, with the optimum between 15- 25 ppt for most of the isolates.

DISCUSSION

Hydrolytic enzyme production was higher among isolates from Bay of Bengal where the organic matter was reported to be low. Most of the isolates preferred 30^{o}C, pH 6 and 15 ppt salinity for maximal growth. The physico-chemical data for maximal growth points to the possibility of these isolates to be of terrestrial origin which got adapted to the marine habitat. Among the isolates oxidative forms were more in abundance than the fermentative forms. Studies by Fell [8], revealed that yeasts found in aquatic environments are generally asporogenous and oxidative or weakly fermentative. Studies by Hagler and Mendonca [11] proved that oxidative yeasts are seen in clean waters and fermentative in polluted waters.

All the yeast isolates were lipolytic which indicate the presence of lipid matter and the cycling process of lipid moieties in the sampling region. Studies by Paskevicius [18] showed that almost all the yeast strains produce lipase. Lipases are the most important biocatalysts and have wide variety of industrial applications. Yeast lipases draw special attention, as these organisms are considered very safe and are consumed by human population since decades [20]. Lipases from *Yarrowia lipolytica* was found to have applications in bioremediation of environments contaminated with aliphatic and aromatic compounds, organic pollutants, 2,4,6-trinitrotoluene, and metals. Also they are industrially important in the synthesis of β-hydroxy butyrate, l-dopa, and emulsifiers [2]. The extracellular enzymes play important role in various industrial processes and also in the environment. Crude amylase from *Saccharomycopsis fibuligera* A11 was found to convert cassava starch actively into monosaccharides and oligosaccharides [3]. Yeast proteases have many applications in detergents, leather processing, feeds, chemical industry as well as waste treatment [16]. Ligninolytic enzymes from yeasts are not commonly studied. Studies by Villas Boas [21], showed that the yeast strain *Candida utilis* has lignocellulose degrading ability. Urease is a nickel containing enzyme that catalyses the hydrolysis of urea. Urease has many industrial applications like diagnostic kits for determination of urea in blood serum, in alcoholic beverages as a urea reducing agent and in biosensors of haemodialysis systems for determining blood urea [1]. The enzyme production potential showed that the isolates are truly versatile agents of biodegradation. Different enzymes from terrestrial microbes have been proved to have potential applications in various industries [4], yeasts from marine environments are also proved to be a good source of enzymes with unique properties. As marine ecosystem is the largest in the world, this needs to be explored for novel bioactive compounds.

Roth et al. [19] stated that almost all the yeasts were able to grow at wide range of NaCl concentrations. Salinity tolerance does not distinguish marine species from terrestrial species because almost all yeasts can grow in sodium chloride concentrations exceeding those normally present in the sea. All the isolates were able to grow at a pH range of 4-9, but the optima for most of the isolates were 6 and 7. Yeasts generally prefer a slightly acidic pH, which was evidenced in the case of these marine isolates also. In general, the isolates were able to grow considerably at a temperature range of 20-40°C. But, for almost all the isolates the maximum growth was observed at 30°C. Even though these isolates were obtained from a marine realm where the temperature ranged between 6- 16°C, these organisms preferred the ambient room temperature (28±2°C) for their growth. Notably, the isolates were able to grow in a wide range of salinities, with the optimum between 15- 25 ppt for most of the isolates.

CONCLUSION

Marine yeast isolates from the slope sediments were found to be predominantly oxidative. Hydrolytic enzyme production was higher among the isolates from Bay of Bengal where the organic matter is reported to be low. Most of the isolates preferred 30^{o}C, pH 6 and 15 ppt salinity for maximal growth. The physico-chemical data for maximal growth points to the possibility of these isolates to be of terrestrial origin and adaption to the marine habitat. The present study highlights the importance of black yeasts as a potent source of extracellular enzymes. Black yeasts were found to be highly versatile agents of biodegradation since cent percent of them produced protease and lipase. Further studies on this group of yeasts especially with regard to enzyme production and biogeochemical cycling of elements would be highly rewarding. Phylogenetic analysis of these groups would also be highly important to derive evolutionary relationship with other groups of yeasts.

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