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# FUNGI ISOLATED FROM THE EEZ OF INDIAN COAST

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### Abstract

Seventy eight water samples from suface to 1000 m were collected at 15 stations during FORV Sagar Sampada Cruise No. 24. From these, 208 yeast isolates were obtained belonging to the genera Candida, Rhodotorula, Sporobolomyces, Sterigmatomyces, Trichosporon, Cryptococcus, Saccharomyces and Debaryomyces. Candida was the dominant genus followed by Rhodotorula.

Seventeen yeast isolates were obtained from the benthic sediment samples and in association with euphausiids from eight stations during FORV Sagar Sampada Cruise No. 35. They belonged to the genera Candida, Rhodotorula, Trichosporon and Debaryomyces. Filamentous fungi isolated during the cruise belonged to the genera Aspergillus, Penicillium, Cladosporium, Fusarium and Alternaria. Here the yeast Rhodotorula was dominant and Aspergillus amongst filamentous fungi. C. tropicalis was found commonly associated with euphausiids.

## INTRODUCTION

Occurrence of yeasts in the sea and open ocean has been reported by several workers (Bhat and Kachwalla, 1955; Fell and van Uden, 1963; Kriss et al., 1967). Bhat and Kachwalla (1955) were the first to isolate yeasts from Indian waters. Fell (1967) reported yeasts from Indian ocean. Similarly there have been a few reports on the filamentous fungi from the sea and open ocean (Johnson and Sparrow, 1961; Roth et al., 1964; Willingham and Buck, 1965; Cvetkovic' and Ristanovic', 1980). Fell (1967) reported the occurrence of filamentous fungi while studying the distribution of yeasts in the Indian Ocean. During the last decade too, there had been sporadic reports of yeasts and filamentous fungi from sea water and sediments (Paula et al., 1983; Bruni et al., 1983; Fedorak et al., 1984). There had been reports of yeasts isolated from amphipods (Roth et al., 1962), from copepods (Seki and Fulton, 1969), from shrimps (Phaff et al., 1952) and in association with shrimp (Pagnocca et al., 1989). Till now no systematic study of the fungal flora of the EEZ of the Indian coast has been taken up.

This paper comprises of the results of the two cruises of FORV Sagar Sampada, one in the EEZ of the west coast of India in the Arabian Sea (Cruise No.24 in November, 1986) and the other in the EEZ along the east eoast of India in the Bay of Bengal in September, 1987. During these two cruises we had tried to investigate the different aspects of marine mycology. Fungal flora, both filamentous fungi (Prabhakaran and Ranu Gupta - in press) and the yeasts were isolated from the water samples collected from different depths of the water column from 15 stations during Cruise No. 24. From the benthic sediment samples too, both the fungal flora were isolated from eight stations during FORV Sagar Sampada Cruise No. 35. Yeasts associated with the zooplankton (euphausiids) were also isolated in the latter cruise.

## MATERIALS AND METHODS

The sampling stations of Cruise No. 24 is given in Fig. 1. The water samples were collected from 15 stations at different depths from 0 to 1000 m by the Rosette sampler available onboard the ship. The Rosette bottles were cleaned and rinsed with alcohol (95%) before every operation. Hydrographic parameters such as temperature, salinity, dissolved oxygen and pH of the water were recorded simultaneously. Processing of the samples was started immediately after all the collections were over at each station. 500 ml of each sample was filtered through a millipore filter membrane with  $0.45 \,\mu m$  porosity. After filtration, each membrane was placed in a petri plate on nutrient medium containing antibiotic mixture to prevent bacterial growth (van Uden and Fell, 1968). The plates were then incubated at 25±1°C for the samples collected down to 100m depth and 10±1°C for the samples collected below that depth (Jones, 1976) for two weeks when maximum colonies





Fig. 1. Map showing location of sampling stations (FORV Sagar Sampada cruise No. 24).

showed up. The colony counts were taken through a colony counter.

The track of FORV *Sagar Sampada* cruise No. 35 is given in Fig. 2, showing the sampling stations near the coast from where the benthic sediment samples were collected and also the euphausiid samples from the regular zooplankton hauls. The depth ranged from 32 to 57 m. As the deep sea grab was not available onboard, coastal stations with lesser depths were chosen for sediment collection.

A Van Veen grab was used to collect the sediment samples. The grab was thoroughly cleaned and then wiped with cotton wool soaked in alcohol (95%) before each collection. After every collection the whole lump of the sediment was immediately taken up in a pre sterilized polythene bag. The samples were taken out aseptically inside a sterile chamber from the centre of the lump to avoid any chance of external contamination. One gram of this sediment sample was shaken with 100 ml of sterilized sea water and allowed to stand for 10 minutes to let the particles to settle down. The supernatant was then filtered through 0.45 µm porosity cellulose acetate



Fig. 2. Map showing location of sampling stations (FORV Sagar Sampada cruise No. 35).

membrane filter which was placed in a petri plate with nutrient medium containing antibiotic mixture (Van Uden and Fell, 1968). Medium used for the isolation of filamentous fungi was GY-Agar (Johnson and Sparrow, 1961) with 0.01% chloramphenicol to avoid bacterial growth. Triplicate samples were taken from each collection and each membrane was placed in one plate for the yeast isolation and similar process was carried out for the isolation of filamentous fungi. For each set, blank plates were put in triplicate as controls. The plates were incubated at  $25\pm1^{\circ}$ C for a week for the yeast and for 3 to 4 days for the filamentous fungi. The colony counts were taken as before.

For each of the eight stations five samples of healthy looking euphausiids were taken from the regular hauls and washed well with sterilized sea water to remove the loosely adhering micro-organisms from them. Then each sample was asepetically planted on nutrient medium with antibiotic mixture (van Uden and Fell, 1968), in petri plates and incubated at  $25 \pm 1^{\circ}$ C for a week. The growth appearing out from the euphausiids on the medium was isolated and identified.

The identification of yeasts was carried out on the basis of detailed cultural, morphological, physiological and biochemical characteristics (Kreger van-Rij, 1984). The identification of filamentous fungi was carried out according to the standard mycological books.

## **RESULTS AND DISCUSSION**

Out of the 78 water samples collected, 71 were positive for the presence of yeasts. Table 1 gives the list of yeast species isolated. Table 2 gives the hydrographical parameters and the species of yeasts isolated from different samples at each of the 15 stations. Yeast concentration ranged from 0 to 260 colony forming units (cfu) per litre.

Yeast population was denser down to 100 m depth after which the density decreased. A total of 24 species belonging to eight genera were isolated (Table 1) of which 16 species occurred infrequently. *Candida membranaefaciens* was the dominant yeast species followed by *Rhodotorula rubra*. Bhat and Kachwalla (1955) reported *C. tropicalis* as the most common species of marine yeast from Bombay coastal waters, but for Fell (1967) *C. atmospherica* and *R. rubra* were the ubiquitous species of yeasts in the Indian Ocean.

Most of the yeast species were isolated down to 100 m depth. Exceptions were *R. graminis*, *Cryptococcus* sp. and *Candida* sp. from 500 m depth at different stations and an isolate of *Candida* sp. even from 1000 m depth.

Of all the yeasts isolated *Sterigmatomyces indicus* was the only obligate marine yeast, while the others were facultatively marine yeasts (Kohlmeyer and Kohlmeyer, 1979). Amongst the eight genera isolated *Debaryomyces* and *Saccharomyces* were the two ascosporogenous ones while all the others asporogenous.

It is obvious from Table 2 that dissolved oxygen and temperature of the collection site had direct influence on the incidence of the yeast species whereas pH and salinity had apparently no effect.

C. albicans, C. parapsilosis, C. atmospherica and St. indicus were found in the temperature range of 20°C to 28°C with high dissolved oxygen. Exception was at st. 834 where C. albicans was isolated from TABLE 1. Species of yeasts isolated from the EEZ of west coast of India

#### Species

Candida albicans (Robin) Berkhout

- C. atmospherica Santa Maria
- C. glabrata (Anderson) Meyer et Yarrow
- C. guilliermondii (Castellani) Langeron et Guerra
- C. membranaefaciens (Lodder et Kreger van-Rij) Wickerham et Burton
- C. parapsilosis (Ashford) Langeron et Talice
- C. polymorpha Ohara et Nonomura ex Meyer et Ahearn
- C. tropicalis (Castellani) Berkhout
- C. Voni (Desmazieres ex Lodder) Van Uden et Buckley Candida sp.

Cryptococcus laurentii (Kufferath) Skinner

Cryptococcus sp. Debaryomyces hansenii (Zopf) Lodderet Kreger-Van Rij Rhodotorula aurantiaca (Saito) Lodder R. glutinis (Fresenius) Harrison R. graminis (Di Menna) R. minuta (Saito) Harrison R. rubra (Demme) Lodder Rhodotorulas sp. Saccharomyces cerevisiae Meyen Ex hansen Saccharomyces sp. Sporobolomyces sp. Sterigmatomyces indicus Fell Trichosporon sp.

low dissolved oxygen area. Above 24°C and with high oxygen areas yeast species isolated were *C.* glabrata, *C.* polymorpha, *C.* tropicalis, *R.* aurantiaca and *R.* minuta. The versatile yeast species tolerating wide temperature and dissolved oxygen range were *C.* guilliermondii, *C.* membranaefaciens, *C.* vini, Candida sp., *Cr.* laurentii, *Cryptococcus* sp., *D.* hansenii, *R.* glutinis, *R.* garminis, *R.*rubra, *Rhodotorula* sp. *S.* cerevisiae and *Trichosporon* sp.

Table 3 gives the details of the positions of the stations for the collection of sediment samples and the filamentous fungi isolated during FORV *Sagar Sampada* Cruise No. 35. Table 4 gives the details of the yeast species isolated from the benthic sediment samples during the same cruise. Table 5 gives the details of the yeast species found associated with euphausiids from five out of eight stations. Out of the 40 samples of healthy euphausiids screened, only five samples from five different stations showed positive association with yeast.

In our investigation we find that the yeast density was restricted to the euphotic zone and very few yeast counts were obtained from 500 and 1000m depth of the water column. Fell (1967) too observed that increased density of yeast population was related to the productivity of the region in particular, corresponded with the concentration of the inverte-

Stati No	on Position ).	depth (m)	Temp. (°C)	Salinity (º/ <sub>00</sub> )	Oxygen (ml/l)	рН	Total No. of Cfu/l	Species isolated
1	2	3	4	5	6	7	8	9
001	09° 00'N	0	27.00	35.1	4.23	8.26	8	C. albicans &
021	71° 30'E	10	23.92	35.6	4.15	8.27	18	C. membranaefaciens & C. vini
		50	20.95	35.4	3.15	8.14	18	Cryptococcus sp., R. minuta & Rhodotorula sp.
		100	15.98	35.7	0.54	7.67	6	C. quilliermondii & S. cerevisiae
		500	10.05	35.5	0.99	7.57	4	Candida sp.
		1000	9.07	35.4	1.23	7.62	0	-
	11° 00'N	0	23.50	34.9	4.92	8.26	0	-
826	73° 30'E	10	23.10	35.1	4.88	8.29	18	C. quilliermondii & St. indicus
		50	22.20	35.0	0.38	8.21	28	C. membranaefaciens & Crifptococcus sp.
		100	20.50	35.2	0.26	7.79	30	C. membranaefaciens, Candida sp. Cryptococcus sp. & R. rubra
		500	13.50	34.9	0.38	7.59 <sup>.</sup>	2	R. graminis
		1000	8.50	34.8	0.61	7.58	0	- -
	11° 59' N	0	27.90	35.8	4.81	8.25	0	-
830	70° 03' E	10	22.90	36.0	4.61	8.29	158	Cr. laurentii, D. hansenii, R. glutinis & R. rubra
		50	19.50	35.4	0.45	7.85	16	Candida sp. & Trichosporon sp.
		100	16.93	35.8	0.29	7.72	2	Candida sp.
		500	13.75	34.9	0.38	7.61	6	Candida sp. & Cruntococcus sp.
		1000	9.74	34.8	0.62	7.59	4	Candida sp.
832	12° 00'N	0	28.00	34.9	4.91	8.27	58	C. membranaefaciens, Cr. laurentii &
	71° 58'E							D. hansenii
-		10	24.62	35.8	4.84	8.26	128	C. qlabrata, Candida sp., Cryptococcus sp. & S. cerevisiae

 TABLE 2. Species of yeasts isolated from different water samples in the EEZ of west coast of India. (FORV Sagar Sampada Cruise No. 24, 16.11.1986 - 4.12.1986)

Table	2 contd.			•				
1	2	3	4	5	6	7	- 8	9
		50	21.15	35.6	0.85	7.92	86	D. hansenii, R. graminis & R. rubra.
		100	19.05	34.9	0.23	7.73	86	C. quilliermondii, C. membrane faciens & Rhodotorula sp.
		500	14.35	34.8	0.30	7.51	6	Candida sp.
834	13º 00'N	0	28.30	35.2	4.81	8.23	136	C. membranaefaciens, Cr. laurentii, D. hansenii & St. indicus
	72º 30'E	10	26.35	35.3	4.54	8.21	160	C. tropicalis, D. hansenii, R. graminis, R. rubra, Sporobolomyces sp. & St. indicus
		20	24.95	34.9	3.46	8.20	60	Cr. laurentii, D. hansenii & R. minuta
		30	22.75	34.8	1.92	8.10	36	C. albicans &St. indicus
		40	20.70	35.1	1.07	8.10	80	Cr. laurentii, R. minuta & R. rubra
839	14º 00'N	0	27.30	35.0	5.08	8.27	81	C. membranaefaciens, R. glutinis & Rhodotorula sp.
	71º 57'E	10	26.93	34.8	4.82	8.26	38	C. polymorpha, D. hansenii, R. minuta & R. rubra
		50	20.35	35.0	0.84	7.98	40	C. membranaefaciens, R. glutinis, R. graminis & R. rubra
		100	17.36	35.2	0.46	7.73	44	C. membranaefaciens, Candida sp. & Saccharomyces sp.
		500	11.57	34.9	0.23	7.49	0	-
	15º 45' N	0	26.10	35.3	5.11	8.23	108	C. atmospherica, R. rubra & St. indicus
843	69º 14' E	10	26.52	35.6	4.92	8.22	38	R. graminis and R. minuta
		50	23.35	35.6	4.54	8.19	32	C. atmospherica, Cryptococcus sp. R. rubra & St. indicus
		100	21.23	35.4	1.46	7.69	42	C. membranaefaciens, C. vini & Candida sp.
		500	11.98	35.1	0.23	7.48	0	- -
849	17° 00' N	0	27.30	34.6	5.03	8.31	118	C. albicans, C. tropicalis & R. rubra
	72° 31' E	10	26.99	34.5	4.92	8.30	198	C. albicans, C. troicapalis, D. hansenii & R. rubra

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Table 2 Contd.

1	2	3	4	5	6	7	8	9
		20	26.93	34.6	4.85	8.19	134	C. membranaefaciens, C. parapsilosis, C. vini Cryptococcus sp., D. hansenii & R. minuta
		30	27.05	34.3	4.85	8.08	82	C. membranaefaciens, R. minuta & R. ruhra
		70	20.74	34.2	4.69	7.99	30	C. albicans & Candida sp.
	16° 00'N	. 0	27.00	35.3	5.30	8.22	228	C. membranaefaciens, C. tropicalis, D. hansenii & P. subra
	72° 48' E	10	27.02	35.3	4.99	8.20	260	C. membranaefaciens, C. tropicalis, D. hansenii & R. ruhra
		30	21.96	35.3	4.46	8.10	90	C. parapsilosis, Candida sp. & R. rubra
		75	19.96	35.1	0.46	8.08	28	C. quilliermondii, Cruptococcus sp. & R. glutinis
		100	19.87	34.8	0.31	7.89	38	C. vini & R. rubra
	15° 00'N	0	26.80	35.2	5.12	8.24	98	C. albicans, C. membranaefaciens, C. trovicalis & R. minuta
	73° 15' E	10	27.08	35.1	5.08	8.19	84	C. albicans, C. membranaefaciens, C. tropicalis & R. minuta
		30	26.24	35.2	5.07	8.15	56	C. tropicalis & Cruptococcus sp.
		75	20.95	35.9	0.99	8.11	34	C. quilliermondii, C. membranaefaciens & R. rubra
		100	19.85	35.9	0.77	7.91	16	C. membranaefaciens
352	14° 00N	0	27.42	35.1	4.98	8.20	140	C. albicans, R. rubra & Trichosporon sp.
	73° 34' E	10	27.35	35.1	4.92	8.16	96	C. albicans, R. rubra & Sporobolomyces sp.
		30	25.88	35.6	4.46	8.12	84	C. vini, D. hansenii & R. qlutinis
		50 ·	21.90	35.8	1.23	8.09	24	Cryptococcus sp.& S. cerevisiae
		75	22.01	35.8	1.07	8.02	26	C. membranaefaciens & Candida sp.
853	13° 00N	0	26.10	35.1	5.10	8.22	60	Cryptococcus sp., D. hansenii Rhodotorula sp. & Saccharomyces sp.

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Table	2 Contd.			-				
1	2	3	4	5	6	7	8	9
	74° 00' E	10	27.39	34.9	4.92	8.21	62	Cryptococcus sp., D. hansenii, & S. cerevisiae
		30	25.08	34.9	4.69	8.16	44	C. membranaefaciens, Cryptococcus, sp. & R. rubra
		75	24.50	35.1	2.99	8.07	50	C. membranaefaciens & Cruntococcus sp.
		90	23.79	35.1	2.92	8.02	24	Candida sp. & Cryptococcus sp.
854	12°00N	0	26.92	35.1	5.34	8.26	98	C. tropicalis, Cryptococcus sp., R. minuta & Saccharomyces sp.
	74°36'E	10	27.43	35.1	4.92	8.19	74	C. parapsilosis, C. tropicalis R. minuta & R. rubra
		30	26.20	34.8	4.61	8.15	84	C. membranaefaciens, Candida sp. & R. minuta
		50	25.34	35.2	4.54	8.06	48	Candida sp., Cryptococcus sp. & Sporobolomyces sp.
		100	22.27	35.1	2.07	7.96	0	
855	11°00N	0	27.35	34.9	5.08	8.31	56	C. albicans, C. parapsilosis, R. rubra & Trichosporan sp.
	75°09'E	10	27.83	34.8	4.85	8.27	144	C. albicans, C. guilliermondii, C. parapsilosis, C. tropicalis, & R. rubra
		30	25.82	35.0	4.23	8.14	140	C. membranaefaciens, C. vini Cryptococcus sp., R. rubra & Saccharomyces sp
		75	23.95	35.3	3.99	8.06	80	Candida sp. & R. rubra
		100	22.90	35.3	3.76	7.98	20	R. graminis
856	10°00'N	0	26.63	35.1	5.23	8.28	38	C. membranaefaciens, C.tropicalis & R. rubra
	75°40'E	10	26.92	35.0	4.69	8.23	108	C. membranaefaciens, C. tropicalis, D. hansenii,
		30	27.26	34.9	4.08	8.19	100	K. aurantiaca & R. rubra C. membranaefaciens, Crypto- coccus sp. & R. rubra
		75	26.66	34.8	3.69	8.05	70	C. membranaefaciens & Rhodotorula sp.
	-	100	25.34	35.1	3.53	7.99	32	C. membranaefaciens & Candida sp.

Station No.	Position	depth (m)	Total No. of Cfu/g	Species isolated
1156	14° 30' N 80° 18' E	32	10	Penicillium Sp., Fusarium sp. and Aspergillus flavus
1164	15° 33' N 80° 40' E	50	8	Aspergillus fumigatus and Cladosporium sp.
1171	16° 00' N 81° 28' E Near the st.	160		
	16° 05' N 81° 30' E	40	13	Aspergillus niger, Penicillium chrysogenum, Penicillium sp. and Fusarium sp.
1172	16° 31' N 82° 25' E	34	34	Aspergillus niger, Penicillium sp., Fusarium sp., Cladospo- rium sp. C. herbarum and Alternaria sp.
1179	17° 15' N 82° 05' E	46	7	Aspergillus niger and Cladosporium sp.
1180	17° 25' N 83° 00' E	51	8	Aspergillus fumigatus and Cladosporium sp.
1185	18° 03' N 84° 04' E	57	9	Penicillium sp. and Cladosporium sp.
1186	18° 01' N 83° 54' E	40	22	Aspergillus niger, Aspergillus flavus, Penicillium sp. and Fusarium sp.

TABLE 3. Species of filamentous fungi isolated from different sediment samples in the EEZ of east coast of India (FORV Sagar Sampada Cruise No. 35)

brates. Majority of yeasts in ocean are probably saprophytes, for their growth they depend on the available organics after the death of various marine organisms (Fell, 1976). In turn they may be included in the diet of invertebrates as a supplementary source of proteins and vitamins. Our observation of the presence of denser population of yeast in the upper layers (euphotic zone) corroborates Fell's observations.

In view of the above and as is obvious from our results of the cruise more detailed study of the EEZ of India is essential to give a better understanding of the yeast population in relation to their linkage to the productivity of the region and their role in the food chain of the ecosystem. When we compare the population of yeast and filamentous fungi in the benthic sediment, we find that the filamentous fungi were more in number and variety than the yeasts, but it was reverse in water samples. As deep sea grab was not available onboard we took the sediment samples from the coastal stations. It is obvious that most of the filamentous fungi isolated were from terrigenous origin highly adapted to diverse ecological conditions.

Out of the five positive samples of euphausiid, two showed association with *C. tropicalis*, one with *R. lactosa*, another one with *Trichosporon* sp. One sample showed association with both the yeasts (*R. lactosa* and *C. tropicalis*). The relation between the yeast species and the euphausiid has to be established.

Station No.	Position	depth (m)	Total No. of Cfu/g	Species isolated
1156	14° 30' N 80° 18' E	32	18	Rhodotorula graminis
1164	15° 33' N 80° 40' E	50	9	Debaryomyces hansenii & Rhodotorula minuta
Near the st.1771	16° 05' N 81° 30' E	40	19	Rhodotorula minuta
1172	16° 31' N 82° 25' E	34	-	-
1179	17° 15' N 82° 05' E	46	12	Rhodotorula rubra
1180	17° 25' N 83° 00' E	51	6	Rhodotorula minuta & Rhodotorula graminis
1185	18° 03' N 84° 04' E	57	8	Rhodotorula minuta , Rhodotural rubra & Candida tropicalis
1186	18° 01' N 83° 54' E	40	16	Candida tropicalis

TABLE 4. Species of yeasts isolated from different sediment samples in the EEZ of east coast of India (FORV Sagar Sampada Cruise No. 35)

## TABLE 5. Species of yeasts isolated in association with euphausiids during FORV Sagar Sampada Cruise No. 35

Station No.	Position	Species isolated				
1156	14° 30' N 80° 18' E	Candida tropicalis				
1157	14° 30' N 80° 28' E	Candida tropicalis				
1165	15° 30' N 80° 46' E	Rhodotorula lactosa & Candida tropicalis				
1179	17° 15' N 82° 05' E	Trichosporon sp.				
1182 -	17° 30' N 84° 00' E	Rhodotorula lactosa				

Healthy samples were chosen, so that there is less chance of the saprophytic role of the yeasts isolated. As suggested by Pagnocca *et al*. (1989) in the case of shrimp, it could be from the intestinal flora where they are favourably lodged.

During these two cruises we had tried to explore different avenues of marine mycology in the EEZ of Indian coast and the results indicate that a detailed systematic study is needed in this less studied field of biological oceanography.

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