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Isolation of Marine Yeast from Muthupet Mangrove Environment

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ABSTRACT

Four sea water samples were collected from different locality of Saviyarmunnai, Vavalthottam, Manakkattai and Devararul Muthupet mangroves environs by using YM medium was used. A total of 13 isolates were isolated such as *Aureobasidium nulluns, Candida elaebora, C. sake, C. zeylanoides, Cryptococcus hansenii, C. victoriae, Dioszella crocera, Dioszella aurantiace, Leucosporidilla fragaria, L. auscorum, Rhodotorula glacialis, R. larvngis* and *Saccharomyces cerevisiae* was frequently distributed from Saviyarmunnai, Vavalthottam, Manakkattai and Devarraul villages of Muthupet environs. Physicochemical properties of water sample also analysed and correlated. The parameters such as air temperature, sea water temperature, transparency, dissolved oxygen, pH, total alkalinity, total hardness, conductivity, TDS, ammonia nitrate, nitrite, orthophosphates, iron, cross primary productivity, net primary productivity and community respiration were determined.

INTRODUCTION

Yeasts exhibit excellent survival in water and they are widely distributed in almost every part of aquatic environments. The popular of yeast in cosmopolitan and distribution and used by the food industry for production of ethanol and carbon dioxide which are important to the brewing wine distilling and baking industry yeasts are rich with proteins, lipids and vitamins. They can be produced very efficiently and economically because of the shorter generation time and use of inexpensive culture medium.

Marine yeasts are ubiquitous in marine environment. They are frequently found in the digestive tract of marine organisms and in seawater and beach sand.[(1)] It is therefore considered that the factors affection to distribution of marine yeast includes currents migration of marine organisms and contamination from terrigenous sources[(2)]. Indigenous marine yeast need to grow on marine substrate and salinity tolerance does not distinguish marine species from terrestrial species because some terrestrial species can grow in sodium chloride concentration exceeding those normally present in the sea[(3)] (Yamagata and Fujita, 1970).

Yeasts are widely distributed in the terrestrial environment, including plants and soil, as well as in wine and various foods [(4,5,6,7,8 and 9)] . However, yeasts with different metabolic attributes have been reported to occur in aquatic environments, such as oceans and seas, estuaries, lakes, and rivers [(2)] .

MATERIALS AND METHODS

Sampling: Seawater samples were collected four different sites from east coast of mangrove environs of Thiruvarur District, Tamilnadu. Sea water samples (from 10 to 15 cm) were collected at each station by walking along the shoreline. Each sample was collected in a sterilized bottle, which was rinsed twice with the sea water before use. Samples were analysed in the 5 h of acquisition at these stations. In addition to isolated yeasts, the salts and physicochemical parameters were analysed in eluding pH (by hand pH meter).

Isolation: Isolation was serially diluted Waksman and Fred, 1922 and 10^{-3} to 10^{-5} dilution samples were taken and spread over in YM agar medium. The plates were incubated for 5-7 days at 25±1^{o}C. After the incubation period, the yeast colonies were observed with the colony forming unit (CFU).

Morphological and micrographic investigation: The colonies were observed and described on NA and MYGPA medium. The isolates were grown in NA and MYPGA broth for determination of these cultural characteristics (pellicle, sedimentation or ring formation, colony of colony, elevation of colony, texture of colony and shape of colony) under microscope.

RESULTS AND DISCUSSION

The analysis of sea water samples revealed that the ranges from 2.5 to 3.6% and pH values 8.1 to 8.3 in each sets. Air temperature was 24.8, 32.3, 30.8 and 32.1^{o} C recorded with representing sites. Dissolved oxygen was 2.0, 3.3, 2.9 and 3.0 with Manakkattai, Saviyarmunnai, Vauvalthottam and Deveraraul respectively. Totally seventeen parameters were analysed and determined including salt content (Table 1). *Nadsonia commutata* has a low maximum growth temperature (22-27^{o}C) and is considered to be psychrophilic yeast[10]. Most of these mesophilic yeasts were isolated at low frequency in water samples [11] .

Isolation of yeast from four different localities were isolated and identified by using standard manual were followed, initially morphologically investigated and tabulated with some morphological characterization such as colour, surface margin, elevation, ascospore, pseudomycelium and true mycelium with respective 13 isolates were observed (Table 2). Relatively few studies have investigated marine yeasts, and this group of Mycota is still poorly understood [2]. According to Ruisi *et al*., (2007) the majority of yeasts present in Antarctic habitats are psychrotolerants, and others are psychrophiles.

Isolation of yeast from four different areas with percentage of frequency also calculated. Totally 13 yeast colonies were isolated and confirmed with this characters such as *Aureobasidium nulluns, Candida elaebora, C. sake, C. zeylanoides, Cryptococcus hansenii, C. victoriae, Dioszella crocera, D. aurantiace, Leucosporidilla fragaria, L. auscorum, Rhodotorula glacialis, R. larvngis* and *Saccharomyces cerievisiae* (Table 2). Some of the species *Candida elaebora, C. sake, Cryptococcus victoriae,* and *Saccharomyces cerevisiae* were hundred percent distributed. The maximum number of yeast at Manakkattai (9) was recorded followed by Saviyarmunai and Deverarul station represented (Table 3). Several decades ago, marine-occurring yeasts were isolated from estuarine and coastal sediments in western Taiwan[12] (Cheng and Lin 1977). The genera of yeasts classified included *Saccharomyces*, *Torulopsis*, *Debaryomyces*, *Endomycopsis*, *Pichia*, *Kloeckera*, and

Rhodotorula. The genera *Saccharomyces* and *Pichia* have been found in both western and northeastern coastal waters of Taiwan[13] .

Table 1 Physicochemical properties of Water samples of Muthupet environs

Sr. No	Parameters	1	2	3	4
1	Air temperature (˚C)	24.8	32.1	30.8	29.4
2	Water temperature (˚C)	26.6	31.4	27.4	27.3
3	Transparency (cm)	23.2	24.8	24.6	22.4
4	Dissolved oxygen (mg/l)	2.0	3.3	2.9	2.7
5	pH	7.3	8.4	8.2	7.9
6	Salinity	2.5	3.0	3.2	3.6
7	Total alkalinity (mg/l as $CaCo_3$)	110	194	151	132
8	Total hardness (mg/l as $CaCo_3$)	106	159	125	119
9	Conductivity (mS)	13.5	16.8	10.4	12.9
10	Total dissolved solids (ppt)	12.3	15.9	15.1	14.5
11	Ammonia –N (mg/l)	0.3	0.8	0.6	0.7
12	Nitrite- N (mg/l)	0.02	0.1	0.1	0.08
13	Nitrate- N (mg/l)	0.09	0.4	0.2	0.1
14	Orthophosphates (mg/l)	0.4	0.7	0.6	0.5
15	Iron (mg/l)	0.1	0.5	0.3	0.6
16	Gross Primary productivity (gc/m3/hr)	0.2	0.7	0.3	0.5
17	Net primary productivity (gc/m3/hr)	0.1	0.2	0.09	0.07
18	Community respiration (gc/m3/hr)	0.02	0.16	0.01	0.04

1) Manakkattai, 2) Saviyarmanai, 3) Vauvalthottam 4) Deveraraul

Table 2 Morphological and Microscopically characteristics of isolates of yeasts

Characteristics	1	2	3	4	5	6	7	8	9	10	11	12	13
Colour	Wh	Red	Wh	Blu	W/cr	W	W	Pi	Ye	Ye	W	Re	W
Surface	Ro	Sm	Ro	Sm	Wri	Sm	Ro	Ro	Wr	Sm	Sm	Sm	Wr
Margin	En	En	Undu	En	Undu	En	Undu	En	Undu	En	En	En	Undu
Elevation	Sl co	Co	Co	Co	Co	Co	Co	Slco	Co	Slco	Co	Co	Co
Ascospore	A	A	A	A	A	A	A	A	A	A	A	A	P
Pseudomycelium	A	P	P	P	P	A	A	A	A	P	P	P	A
True mycelium	A	A	A	A	A	A	A	A	A	A	P	P	A

Wh=White; Wh/cr= White/cream; Rou.=Rough; Sm.=Smooth; Wrin.=Wrinkled; En.=Entire; Undu= Undulating; Sl. Co= Slightly convex; Co.=Convex; R.=Round ;O.=Oval; A=Absent; P=Present

Table 3 Isolation of yeast from Muthupet sea water samples

S.No	NJJUM code	Name of the yeast	A	B	C	D	% of Frequency
1	NJJUM 1	*Aureobasidium nulluns*	+	-	-	+	50
2	NJJUM 2	*Candida elaebora*	+	+	+	+	100
3	NJJUM 3	*C. sake*	+	+	+	+	100
4	NJJUM 4	*C.zeylanoides*	-	-	+	+	50
5	NJJUM 5	*Cryptococcus hansenii*	+	+	-	+	75
6	NJJUM 6	*C. victoriae*	+	+	+	+	100
7	NJJUM 7	*Dioszella crocera*	+	+	-	-	50
8	NJJUM 8	*D. aurantiace*	-	+	-	-	25
9	NJJUM 9	*Leucosporidilla fragaria*	-	-	+	+	50
10	NJJUM 10	*L. muscorum*	+	+	-	-	50
11	NJJUM 11	*Rhodotorula glacialis*	+	+	-	-	50
12	NJJUM 12	*R. larvngis*	+	-	+	+	75
13	NJJUM 13	*Saccharomyces cerevisiae*	+	+	+	+	100

A- Saviyarmunnai, B- Vavalthottam, C- Manakkattai, D- Deverarul, (+) Positive, (-) negative

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