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Characterisation and bioprospecting of cold adapted yeast from water samples of Kongsfjord, Norwegian Arctic

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A total of 34 yeast isolates were characterized from 4 water samples collected from Kongsfjord at Ny Alesund region (79° N, 12° E) of Norwegian Arctic during the Indian Arctic Summer Expedition of 2009. They were studied for the effect of temperature and salt concentration on growth as well as for their ability to produce various hydrolytic enzymes at two different temperatures. Result showed that 5 out of 8 genera were common to all the stations. *Cryptococcus* was the predominant genera (>30%) followed by *Trichosporon* and *Rhodotorula*. Eighty two percentages of the yeast isolates were oxidative in nature and except *Filobasidium*, all the isolates used nitrate as a nitrogen source for growth. Yeast isolates from all the stations showed growth at 4°C and 20°C. These temperatures were chosen as most of the bacterial and yeast isolates showed psychrotrophic than true psychrophilic nature. Ninety four percentage of the yeast isolates showed growth at 2.0 M concentration of NaCl. While all the isolates were capable of producing gelatinase and lipase at 20°C, gelatinolytic (88%) and lipolytic activity (97%) were marginally less at 4°C. None of the isolates produced amylase enzyme when incubated at 4°C and 20°C. The present study highlights the wide tolerance of the psychrotrophic yeast isolates to temperature and salinity as well as their potential in biotechnology.

[Keywords: Kongsfjord, Arctic, Marine yeast, Hydrolytic enzymes]

Introduction

Polar oceans are distinct from other oceanic environments in a number of ways, but the presence of sea ice is a major habitat difference. Sea ice affects polar microbial communities by limiting light penetration into the euphotic zone and by providing a unique sea surface habitat. Sea ice serves as a support matrix for a diverse and dynamic assemblage of microbes, including phytoplankton and prokaryotes, often referred to as the sea ice microbial community. Ice forms the structural basis for many types of microbial ecosystem in the polar regions; small changes in temperature across the melting point can therefore have pronounced impacts on polar habitats and communities[1]. And the yeast diversity in the ecosystems is highly affected by a variety of abiotic and biotic factors, such as temperature, pressure, UV radiation (UVR), salinity, fauna, flora, soil run-off and anthropogenic effluents[2]. A wide range of psychrophilic and cold-tolerant microorganisms contributes essentially to the processes of nutrient turnover, biomass production and litter decomposition. Moreover, these microorganisms and their cellular constituents or products provide a large biotechnological potential.

There are approximately 100 genera and 800 described species of yeasts estimates suggest that these numbers represent 1% of the species diversity that exist in nature and the rest being non culturable[3]. At present, a total of 530 species belonging to 321 genera are considered marine[4]. Psychrophilic yeasts play an essential role in nutrient cycling and biomass production processes in cold ecosystems[5]. Interest on marine yeasts increased recently mainly due to the potential of marine yeasts as sources of enzymes with industrial applications and biologically active natural products[6,7,8,9].

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The two Arctic fjords Kongsfjorden and Krossfjorden located off the west coast of Spitsbergen have attracted the attention of scientists as a model site for studies on the impact of climate change in the Arctic[10] and are regarded as key European sites for Arctic biodiversity monitoring[11]. Each year the glacier inputs 2.6×10^5 m^3 of mineral material into fjord waters per season[12] as the melting of ice during the arctic summer releases considerable quantity of the terrogenous material into the fjords. Thus, the inflow of water and minerals causes unique temperature and salinity zones such that the surface water layer is of relatively high temperature and very low salinity compared to the lower water layer[13].

Studies on the yeasts of Arctic with respect to their abundance, distribution and taxonomy are very much limited[14]. Despite the fact that Arctic has a number of fjords comparatively little is known about the yeast diversity of the fjords. Only a few years back, the first report on the presence of yeast populations in sub glacial ice of an Arctic glacier was published[15]. Thus there is a need to carry out studies on the yeasts of Arctic with respect to their biodiversity and phylogeny. Since bioprospecting of these yeast isolates could reveal their biotechnological potential, they are also screened for cold active enzymes such as gelatinae, amylase and lipase. The present study reports the results of the investigations carried out during the Indian Arctic expedition 2009.

Materials and Methods

Sampling Site

The psychrotrophic yeasts were isolated from Kongsfjord at Ny Alesund region (79° N, 12° E) of Norwegian Arctic during the Indian Arctic Summer Expedition of 2009 (June - July 2009). Kongsfjord is about 25 km in length (Fig. 1) and is fed by inflows of water from Kongsbreen, the most active glacier in the Svalbard Archipelago[16]. Kongsfjorden System is an established reference site for Arctic marine studies with great potential for international, multidisciplinary collaboration due to the presence of the international research platform in Ny-Alesund. It represents a natural laboratory in the Arctic.

Sampling Method

A total of four water samples were collected from 4 different stations in sterile 1 L plastic bottles from

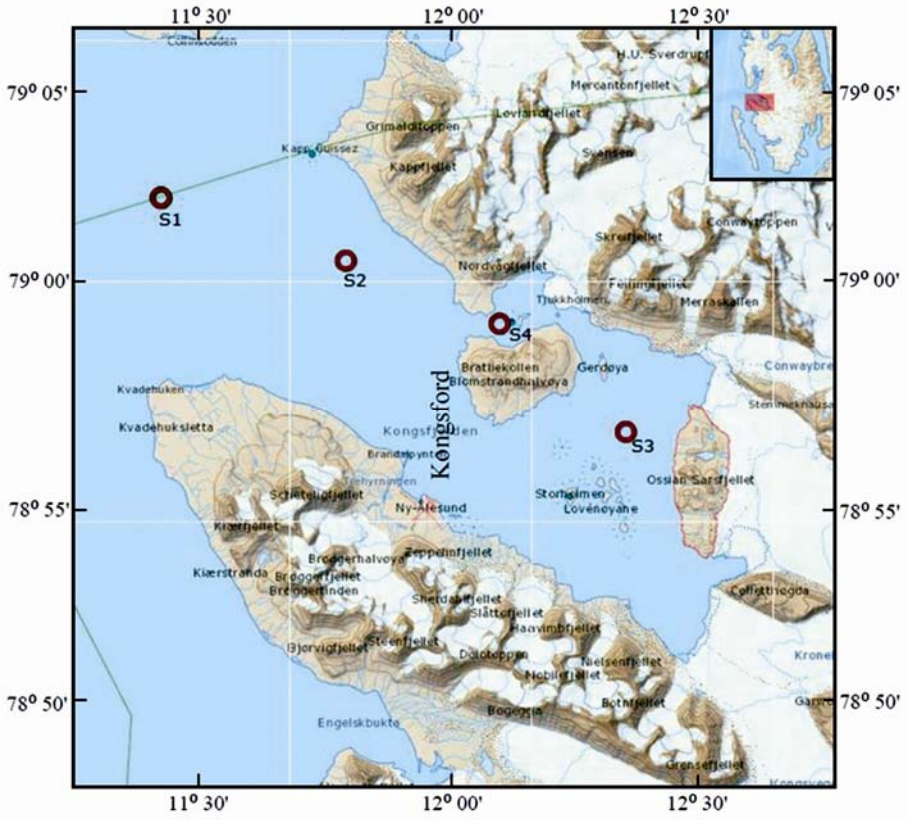

Fig. 1—Sampling site – Kongsford, Arctic Ocean

the Kongsfjord (Fig. 1). The water samples were collected by using the Research Vessel 'Teisten' with the help of Niskin sampler. The mean air temperature, water temperature and salinity were noted at the time of sampling. The samples were brought to the marine lab at Arctic research station at Ny Alesund and plated for the isolation of yeast. One set of samples were stored at 4°C and transported as cold cargo to the School of Marine Sciences, Cochin University of Science and Technology, Kerala, India, for further processing.

Isolation and characterisation of yeast

For the isolation of yeasts, 1 mL of the water samples were spread plated on malt-yeast-glucose-peptone agar supplemented with 200 mg/L chloramphenicol. The plates were incubated at 15°C for 14 days. The colonies developed were isolated, purified by quadrant streaking and transferred to malt extract agar slants. Morphological, physiological and biochemical properties of the isolates were determined and identified up to genera as described by Barnett *et al*.[17]. The geographical location of the sampling sites and physico-chemical properties of water samples is given in Table 1.

Determination of effect of temperature and salt concentration on growth of yeast isolates

Two sets of malt extract plates were streaked aseptically with the yeast isolates for determining the temperature on growth. One set of plate was incubated at 4°C for 10-14 days and the other set at 20°C for 5-10 days. After the incubation, plates were examined for growth of isolates. To determine the effect of salt concentrations on growth of yeast, sterile malt extract agar plates with different salt concentration were prepared by using NaCl and were streaked aseptically with the yeast isolates. The plates were then incubated at 20°C for 5-10 days and the growth in different salinity range was recorded.

Production of hydrolytic enzymes

For checking the production of gelatinase enzyme, yeast isolates were aseptically spot inoculated on sterile malt extract agar plates with 1% gelatin and incubated at 4°C and 20°C for 10-14 days and 5-10 days respectively. After incubation, the plates were flooded with mercuric chloride solution. Clear zone around the colonies indicated utilization of gelatin with the help of gelatinase enzyme. Similarly yeast cultures were aseptically spot inoculated on sterile nutrient agar plates with 1% tributyrin for checking lipase production. The plates were incubated at 4°C and 20°C for 10-14 days and 5-10 days respectively. A change in opacity of the medium around the colony indicated lipase activity. Amylase production was checked by spot inoculating the yeast cultures on sterile nutrient agar plates with 1% starch and the plates were incubated at 4°C and 20°C for 10-14 days and 5-10 days respectively. After the incubation the plates were flooded with Gram's Iodine solution. Clear zone around the colony indicates that the culture is positive for amylase production.

Results

A total of 34 yeast isolates were obtained from 3 of the 4 sampling sites. They were characterized to generic level and were subjected for appropriate studies to check the effect of temperature and salt concentration on growth as well as for their ability to produce various hydrolytic enzymes at two different temperatures.

Biochemical characterization of the yeast isolates

Thirty four yeast isolates were biochemically characterized and were classified up to generic level depending on the microscopy and biochemical tests (Table 2). The result showed that 5 out of 8 genera

Table 1—Geographical location of sampling sites and physiochemical properties of water samples from Kongsfjord

Sampling stations	Location	Water temp. (°C)	Water salinity (ppt)	No. of yeast colonies
Station I	79°02'36" N 11°19'14" E	6.13	33.69	13
Station II	79°01'22" N 11°43'06" E	6.51	33.56	8
Station III	78°57'29" N 12°19'27" E	4.14	33.13	13
Station IV	79°00'01" N 12°02'50" E	3.61	33.11	0

Table 2—Relative incidence of various genera of yeast in water samples from different stations at Kongsfjord

Genera	% of incidence at stations		
	Station I	Station II	Station III
Bullera	-	-	7.69
Candida	15.38	12.5	7.69
Cryptococcus	38.46	37.5	30.77
Debaryomyces	7.69	-	-
Filobasidium	7.69	12.5	15.38
Pichia	7.69	12.5	7.69
Rhodotorula	-	-	15.38
Trichosporon	23.08	25.0	15.38

were common to all the stations. *Debaryomyces* isolated from Station I and *Bullera* and *Rhodotorula* from Station III only. *Cryptococcus* was the predominant genera (>30%) from all the stations. *Trichosporon* was found to be the second dominant genera from staion I and II and one of the second dominant genera from Station III. Eighty two percentages of the yeast isolates were oxidative in nature and none of the isolate produced starch like substance. Except *Filobasidium* from all the stations, all the isolates used nitrate as a nitrogen source for growth.

Effect of temperature and NaCl concentration on growth of yeast

The effect of temperature and NaCl concentration on the growth of yeast isolates from station I, II and III is given in Table 3. Yeast isolates from all the stations showed growth at 4°C and 20°C. Effect of NaCl concentration on growth of yeast isolates

Table 3—Growth of yeast isolates from water samples of Kongsford at various temperatures and NaCl concentration

Sl. No	Isolate No.	Identity	Growth at		NaCl Concentration (M)							
			4°C	20°C	0.3	0.5	1.0	1.5	2.0	2.5	3.0	3.5
Station I												
1	52	*Candida*	+	+	+	+	+	+	+	-	-	-
2	64	*Candida*	+	+	+	+	+	+	+	+	-	-
3	54	*Cryptococcus*	+	+	+	+	+	+	+	+	+	-
4	56	*Cryptococcus*	+	+	+	+	+	+	+	+	-	-
5	63	*Cryptococcus*	+	+	+	+	+	+	+	+	-	-
6	69	*Cryptococcus*	+	+	+	+	+	+	+	+	-	-
7	70	*Cryptococcus*	+	+	+	+	+	+	+	+	-	-
8	53	*Debaryomyces*	+	+	+	+	+	+	+	+	-	-
9	61	*Filobasidium*	+	+	+	+	+	+	+	-	-	-
10	55	*Pichia*	+	+	+	+	+	+	+	+	-	-
11	57	*Trichosporon*	+	+	+	+	+	+	+	+	+	-
12	60	*Trichosporon*	+	+	+	+	+	+	+	+	-	-
13	62	*Trichosporon*	+	+	+	+	+	+	+	+	+	-
Station II												
14	47	*Candida*	+	+	+	+	+	+	+	+	-	-
15	34	*Cryptococcus*	+	+	+	+	+	+	+	+	-	-
16	35	*Cryptococcus*	+	+	+	+	+	+	+	-	-	-
17	49	*Cryptococcus*	+	+	+	+	+	+	+	+	-	-
18	33	*Filobasidium*	+	+	+	+	+	+	+	+	+	-
19	48	*Pichia*	+	+	+	+	+	+	+	+	-	-
20	50	*Trichosporon*	+	+	+	+	+	+	+	+	-	-
21	51	*Trichosporon*	+	+	+	+	+	+	+	+	-	-
Station III												
22	74	*Bullera*	+	+	+	+	+	+	+	+	-	-
23	77	*Candida*	+	+	+	+	+	+	+	+	-	-
24	75	*Cryptococcus*	+	+	+	+	+	+	+	+	-	-
25	76	*Cryptococcus*	+	+	+	+	+	+	+	+	+	-
26	79	*Cryptococcus*	+	+	+	+	+	+	-	-	-	-
27	86	*Cryptococcus*	+	+	+	+	+	+	+	+	+	-
28	72	*Filobasidium*	+	+	+	+	+	+	-	-	-	-
29	78	*Filobasidium*	+	+	+	+	+	+	+	+	-	-
30	73	*Pichia*	+	+	+	+	+	+	+	+	-	-
31	80	*Rhodotorula*	+	+	+	+	+	+	+	+	-	-
32	85	*Rhodotorula*	+	+	+	+	+	+	+	+	+	-
33	71	*Trichosporon*	+	+	+	+	+	+	+	+	+	-
34	81	*Trichosporon*	+	+	+	+	+	+	+	+	-	-

showed that except one isolate each of *Cryptococcus* and *Filobasidium* from Station III, the remaining 32 yeasts showed growth at 2.0 M concentration of NaCl. None of the isolate showed growth at 3.5 M NaCl concentration and only 8 isolates of yeasts showed growth at 3.0 M NaCl.

Effect of temperature on hydrolytic enzyme production by the yeast isolates

The ability of the yeast isolates to produce hydrolytic enzymes was checked at 4°C and 20°C and the results were tabulated in Table 4. All the yeast isolates from Station I showed gelatinase production at 4°C and 20°C. *Candida* and one of the *Trichosporon* from Station II and one of the genera of *Cryptococcus* and *Filobasidium* from Station III failed to produce gelatinase at 4°C. However all the isolates from Station II and III produced gelatinase at 20°C. Except one *Trichosporon* genera from Station III, all the yeast produced lipase at 4°C and 20°C. None of the isolates produced amylase enzyme at 4°C and 20°C.

Table 4—Ability of yeast isolates from water samples of Kongsford to produce gelatinase, lipase and amylase at 4°C and 20°C

Sl. No	Isolate no.	Identity	Ability to produce gelatinase at		Ability to produce lipase at		Ability to produce amylase at	
			4°C	20°C	4°C	20°C	4°C	20°C
Station I								
1	52	*Candida*	+	+	+	+	-	-
2	64	*Candida*	+	+	+	+	-	-
3	54	*Cryptococcus*	+	+	+	+	-	-
4	56	*Cryptococcus*	+	+	+	+	-	-
5	63	*Cryptococcus*	+	+	+	+	-	-
6	69	*Cryptococcus*	+	+	+	+	-	-
7	70	*Cryptococcus*	+	+	+	+	-	-
8	53	*Debaryomyces*	+	+	+	+	-	-
9	61	*Filobasidium*	+	+	+	+	-	-
10	55	*Pichia*	+	+	+	+	-	-
11	57	*Trichosporon*	+	+	+	+	-	-
12	60	*Trichosporon*	+	+	+	+	-	-
13	62	*Trichosporon*	+	+	+	+	-	-
Station II								
14	47	*Candida*	-	+	+	+	-	-
15	34	*Cryptococcus*	+	+	+	+	-	-
16	35	*Cryptococcus*	+	+	+	+	-	-
17	49	*Cryptococcus*	+	+	+	+	-	-
18	33	*Filobasidium*	+	+	+	+	-	-
19	48	*Pichia*	+	+	+	+	-	-
20	50	*Trichosporon*	-	+	+	+	-	-
21	51	*Trichosporon*	+	+	+	+	-	-
Station III								
22	74	*Bullera*	+	+	+	+	-	-
23	77	*Candida*	+	+	+	+	-	-
24	75	*Cryptococcus*	+	+	+	+	-	-
25	76	*Cryptococcus*	+	+	+	+	-	-
26	79	*Cryptococcus*	-	+	+	+	-	-
27	86	*Cryptococcus*	+	+	+	+	-	-
28	72	*Filobasidium*	-	+	+	+	-	-
29	78	*Filobasidium*	+	+	+	+	-	-
30	73	*Pichia*	+	+	+	+	-	-
31	80	*Rhodotorula*	+	+	+	+	-	-
32	85	*Rhodotorula*	+	+	+	+	-	-
33	71	*Trichosporon*	+	+	-	+	-	-
34	81	*Trichosporon*	+	+	+	+	-	-

Discussion

Taxonomic characterization showed that the dominant yeasts in the Arctic are of basidiomycete affinity, among which *Cryptococcus* species was dominant. The findings agree with results of previous works from Arctic region[14,15]. In a biodiversity study of cold adapted yeasts from glacial melt water rivers in Argentina, the dominance of *Cryptococcus spp.* (50%) was reported[2,18]. Occurrence of *Cryptococcus, Rhodotorula, Bullera, Filobasidium,* and *Trichosporon* in the glacial ice core of Arctic has also been reported by Butinar *et al.*[15]. Similar genera were also encountered in the present investigation. Yeast diversity data from studies for Antarctic and Arctic regions reported occurrence of genera such as *Cryptococcus*, *Leucosporidiella*, *Dioszegia*, *Rhodotorula*, *Rhodosporidium*, *Mrakia*, *Sporobolomyces*, *Udeniomyces* and *Candida*[14,15,19]. Some of these genera were also reported in the water samples from Kongsfjord, in Norwegian Arctic. This may be because of the considerable quantity of melt water from glacier that is reaching the fjord. Among the isolates, oxidative forms were more in abundance than the fermentative forms. Studies by Fell[20] revealed that yeasts found in aquatic environments are generally asporogenous and oxidative or weakly fermentative. Hagler and Mendonca[21] reported that oxidative yeasts are seen in clean waters and fermentative ones in polluted waters. The melt water from glaciers is pristine and free of contaminants and the Kongsfjord is supplied with freshwater inflows from 4 major glaciers.

The yeast isolates encountered in the present study were psychrotrophs rather than true psychrophiles. All the isolates showed good growth at 4°C and 20°C as well as in wide range of salinity. Butinar *et al.*[15] also noted the capacity of the yeast isolated from glacier to grow at 5°C and 25°C and also at sodium concentration ranging from 5 to 340 mg/Kg. Lipolytic and gelatinolytic activity were widespread among the yeast isolates encountered in the present study. De Garcia *et al.*[18] also reported high proteolytic and lipolytic activities of yeast at 4°C than at 20°C. The study showed that the yeasts are physchrotrophic in nature and can tolerate wide range of salt concentration as reported in yeast isolates from Arctic glacier[14]. Roth *et al.*[22] stated that almost all the yeasts were able to grow in a wide range of NaCl concentration. In the present study also notably, most of the isolates were able to grow in a salinity range of 0.3-3 M. This shows that the yeast isolates are highly versatile in their salt tolerance. The fjords in the arctic, including the Kongsfjord, are subjected to freezing during winter. This results in the formation of supersaline waters covered with pack ice to thick ice sheets overlying the water column. Yeast in this region might have evolved to tolerate such high saline conditions during winter.

It is reported that cold active enzymes that function efficiently at low temperatures (0 to 25°C) have significant value in medical and pharmaceutical, food, bioscience, domestic, industrial, environmental and fine chemical synthesis areas of biotechnology[23,24]. In the present study, all the strains tested produced gelatinase and lipase enzymes at 20°C. At 4°C some strains lacked the ability to produce these enzymes. It is reported that cold-adapted enzyme producers are very valuable in regard to their potential for biotechnological application[25]. Running processes at low temperature reduces the risk of contamination by mesophiles and also saves energy. Cold active lipases are much sought after in detergent industry as cold wash can save lot of energy. Psychrophilic enzymes are both innovative and invaluable as they have high specificity at low and moderate temperatures and are extremely useful in various applications.

Previous studies on yeast extracellular enzymatic activity showed proteolytic and lipolytic activities at 4°C, 5°C and 20°C as well[2,18]. In this study yeast strains showed proteolytic and lipolytic activities at 4°C and 20°C. Almost all the yeast isolates were lipolytic which indicate the presence of lipid matter and the cycling processes of lipid moieties in the sampling region. Studies by Paskevicius[26] showed that almost all the yeast strains produce lipase at natural condition. The fact that a significant proportion of yeasts are able to hydrolyse natural compounds such as lipids and protein suggests that these strains are metabolically adapted to cold environments and have a significant ecological role in organic matter decomposition and nutrient cycling. Interestingly none of the yeast isolates in the present study were able to produce amylase. The inability of the isolates to produce amylase may be due to non-availability of easily assimilable carbohydrate source in the study area or due to the recalcitrant nature of the carbohydrates that are present. Yeast lipase draw special attention, as the organisms are considered very safe and are consumed by human population since decades[27]. According to Pathan *et al.*[14] the enzyme

activity of yeast strains collected from vicinity of Midre Lovenbreen glacier in the arctic region was better at 22°C when compared to those growing at 8°C. Cavicchioli *et al.*[28] also reported good biotechnological potential of cold adapted lipases. Cold active lipases could be a good alternative to mesophilic enzymes in brewing and wine industries as well[29].

The yeast isolates capable of producing gelatinase enzyme was marginally higher when compared to those isolates incubated at 4°C. According to Nedwell[30] the affinity of microorganisms for the substrates decreases consistently as temperature drops below the optimum temperature for growth. This effect may be because of stiffening of the lipids of the membrane below the temperature optimum, leading to decreased efficiency of transport proteins embedded in the membrane. At temperatures below the optimum temperature for growth, microorganisms will become increasingly unable to sequester substrates from their environment because of lowered affinity. This could also be a probable reason why some of the isolates were unable to show enzyme activity at 4°C.

The results of the present study revealed considerable diversity of cold adapted yeasts in the Kongsfjord water. The psychrotrophic and halotolerant nature coupled with their widespread ability to produce hydrolytic enzymes highlight their role in remineralisation of organic matter as well as their potential in biotechnological applications.

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