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Review

Marine yeasts — a review

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Abstract

Yeasts are ubiquitous in their distribution and populations mainly depend on the type and concentration of organic materials. The distribution of species, as well as their numbers and metabolic characteristics were found to be governed by existing environmental conditions. Marine yeasts were first discovered from the Atlantic Ocean and following this discovery, yeasts were isolated from different sources, viz. seawater, marine deposits, seaweeds, fish, marine mammals and sea birds. Near-shore environments are usually inhabited by tens to thousands of cells per litre of water, whereas low organic surface to deep-sea oceanic regions contain 10 or fewer cells/litre. Aerobic forms are found more in clean waters and fermentative forms in polluted waters. Yeasts are more abundant in silty muds than in sandy sediments. The isolation frequency of yeasts fell as the depth of the sampling site is increased. Major genera isolated in this study were *Candida, Cryptococcus, Debaryomyces* and *Rhodotorula*. For biomass estimation ergosterol method was used. Classification and identification of yeasts were performed using different criteria, i.e. morphology, sexual reproduction and physiological/biochemical characteristics. Fatty acid profiling or molecular sequencing of the IGS and ITS regions and 28S gene rDNA ensured accurate identification. **Copyright © 2008 John Wiley & Sons, Ltd.**

Keywords: marine yeasts; distribution; oceans and seas; isolation; classification; molecular taxonomy; FAME; ergosterol

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Introduction

Yeasts are a polyphyletic group of basidiomycetous and ascomycetous fungi with a unique characteristic of unicellular growth. The term 'yeast' is derived from the old Dutch word *gist* and the German word *gischt*, which refers to fermentation. There are approximately 100 genera and 800 described species of yeasts [80] and estimates suggest that these numbers represent only about 1% of the species that exist in nature, the rest being non-culturable. [42]

Yeasts have been used by the food industry principally for the production of ethanol and carbon dioxide, which are important to the brewing, wine distilling and baking industries. Their environmental role is similar to many other fungi, acting as saprophytes by converting plant and animal organics to yeast biomass and by-products, which may have commercial importance. Some yeasts are pathogenic to plants and animals. Yeasts are rich with proteins, lipids and vitamins. Biotransformation of raw material into yeast biomass (single-cell protein) is highly significant, due to the nutritional quality of yeast and its possible utilization

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as animal or aquaculture feed. Yeasts also have immunostimulatory properties by virtue of their complex carbohydrate and nucleic acid components. They can be produced very efficiently and economically because of their shorter generation time and use of inexpensive culture media. Lipids, pullulans and enzymes from yeasts are extracellular metabolites of commercial importance.

Ecology and distribution

Yeasts are distributed in almost every part of the aquatic environment, i.e. oceans and seas, estuaries, lakes and rivers [42] (Table 1). Studies on the distribution of yeasts world-wide are presented in Figure 1. A truly marine yeast must be able to grow on or in a marine substrate. Direct examination of living marine invertebrates, however, has demonstrated the presence of parasitic and pathogenic yeasts [54,126,131] and, if such species have grown *in situ* in the animal and its native habitat, they could rightly be called indigenous marine species. So far, no physiological clues have been found to explain why marine-occurring yeasts are able to live in this special habitat. Salinity tolerance does not distinguish marine species from terrestrial species because almost all yeasts can

Table 1. Details of ecological studies on marine yeasts worldwide

Location/Sample	Generic composition	Reference
(a) Sea water		
Central Pacific	*Debaryomyces*	V Vitiaz (1957–1958)
Pacific Ocean	*Candida, Torulopsis*	144
Pacific Ocean	*Metschnikowia*	146
Loma Trough, off San Diego, California	*Cryptococcus, Rhodotorula*	146
Pacific Ocean	*Candida*	120
Pacific Ocean	*Rhodotorula, Cryptococcus*	156
Indo-Pacific and Pacific Ocean	*Leucosporidium, Rhodosporidium, Sympodiomyces*	41
Rendaiji, Shizouka Prefecture	Torulaspora, Dekkera, Candida	142
Atlantic Ocean	Torula, Mycoderma	52
Biscayne Bay	Candida, Rhodotorula	49
Southern Florida	Candida, Rhodotorula	126
Atlantic Ocean	*Kluyveromyces*	2
Gulf Stream, Bahamas	*Candida, Rhodotorula, Cryptococcus, Debaryomyces*, black yeasts	44
Gulf stream off Florida	Candida, Rhodotorula	36
Atlantic Ocean	*Cryptococcus*	116
Atlantic Ocean	*Metschnikowia*	119
Atlantic Ocean	*Sterigmatomyces*	40
Chesapeake Bay	*Rhodotorula*	27
Atlantic Ocean	*Leucosporidium, Rhodosporidium, Sympodiomyces*	41
North Sea	*Debaryomyces, Candida*	3
Southern Sao Paulo, Brazil	*Candida, Cryptococcus, Rhodotorula, Torulopsis, Trichosporon, Debaryomyces, Hansenula, Pichia, Sporobolomyces*	113
Olinda, Brazil	*Candida*	89
Off Mumbai, Arabian Sea	*Saccharomyces, Debaryomyces, Pichia, Candida, Torulopsis, Rhodotorula, Cryptococcus*	15
Indian Ocean	*Rhodotorula, Candida,Sporobolomyces*	39
Indian Ocean	*Sterigmatomyces*	40
Off Cochin, Arabian Sea	*Candida, Rhodotorula, Leucosporidium*	123
Off Mumbai, Arabian Sea	Yarrowia	108
Indian EEZ	*Candida, Filobasidium, Leucosporidium, Mastigomyces, Lodderomyces, Debaryomyces, Rhodotorula, Dekkera, Hormoascus, Cryptococcus, Schizosaccharomyces, Kluyveromyces, Williopsis, Aciculoconidia, Pichia, Torulaspora, Saccharomycopsis, Lipomyces, Geotrichum, Arxioxyma, Oosporidium, Dipodascus*	128
Antarctic Sea	*Leucosporidium, Rhodosporidium, Sympodiomyces*	49

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Table 1. Continued

Location/Sample	Generic composition	Reference
(b) *Sediment*		
Biscayne Bay, Bahamas	*Rhodotorula, Debaryomyces, Torulopsis, Cryptococcus, Candida, Trichosporon, Hansenula, Saccharomyces*	49
Marshes of Louisiana coast	*Pichia, Kluyveromyces*	4
Florida	*Rhodotorula*	84
Bahamas	*Cryptococcus*	1
Dry valleys of Antarctica	*Cryptococcus*	9,147,148
EEZ of south-west coast of India, Arabian Sea	*Candida, Rhodotorula*	121
Coastal Massachusetts	*Candida, Cryptococcus, Rhodotorula, Torulopsis, Trichosporon*	90
Mariana Trench	*Rhodotorula, Candida, Debaryomyces, Kluyveromyces, Pichia, Saccharomyces, Williopsis*	139
North-west Pacific Ocean	Rhodotorula,Sporobolomyces	100
Japan trench	*Cryptococcus*	1
Bahamas (mangrove)	*Lachancea*	46
Arabian Sea and Bay of Bengal	*Candida, Rhodotorula, Cryptococcus, Debaryomyces, Pichia, Trichosporon*	83
(c) Estuaries		
Swedish estuary	*Candida, Rhodotorula*	104
Suwannee estuary, Florida	*Candida, Rhodotorula, Cryptococcus, Hansenula*	84
Estuary, west coast of Taiwan	*Saccharomyces, Torulopsis, Debaryomyces, Endomycopsis, Pichia, Kloeckera, Rhodotorula*	24
Estuary, Rio de Janeiro, Brazil	*Candida, Rhodotorula, Debaryomyces, Hanseniaspora, Torulopsis, Trichosporon*	63
(d) Weeds and algae		
Algae	*Candida, Torulopsis, Trichosporon, Endomycopsis*	135
Algae and corals, Torres Strait region	*Metschnikowia, Candida, Pichia, Kluyveromyces, Torulopsis*	144
Giant kelp, Southern California	*Metschnikowia*	146
Plankton, Pacific Ocean	*Rhodosporidium*	49
Sea weeds, inshore waters	*Candida*	132
Spartina alterniflora plants, Louisiana salt marsh	*Pichia, Kluyveromyces*	92
Marsh plants, England	*Sporobolomyces*	122
Submerged seedlings of *Rhizophora mangle*	*Rhodotorula, Debaryomyces*	101
(e) Invertebrates		
Mexico shrimp (*Penaeus setiferus*)	*Trichosporon, Rhodotorula, Candida, Pichia*	117
Shrimp eggs and sponges, North Atlantic Ocean	*Debaryomyces, Torulopsis, Trichosporon*	133
Amphipod (*Podocerus brasiliensis*)	*Rhodotorula*	126
Brine shrimp (Artemia salina), Atlantic Ocean	*Metschnikowia*	44
Marine copepods (*Calanus plumchrus*)	Metschnikowia	131
Copepods (*Eurytemora velox*), Southern France	*Metschnikowia*	54
Brine shrimp (*Artemia salina*)	*Metschnikowia*	82
Fiddler crab (*Uca pugilator*)	*Rhodotorula, Torulopsis*	25
(f) Fish, birds and mammals		
Fish	Metschnikowia	45
Rainbow trout	*Debaryomyces, Saccharomyces, Rhodotorula*	7
Birds (excreta), Pacific and Atlantic Oceans	*Candida, Torulopsis*	146
Porpoise (intestine)	*Rhodosporidium*	48
Dolphin and porpoise (stomach)	*Candida*	96

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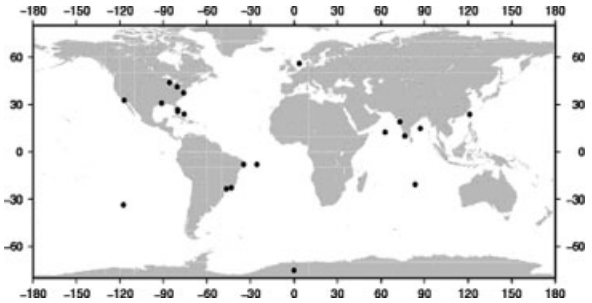

Figure 1. Worldwide study area on yeast distribution

grow in sodium chloride concentrations exceeding those normally present in the sea. Certain distinctive metabolic attributes of yeasts are associated with environmental distribution. Yeasts found in aquatic environments are generally asporogenous and oxidative or weakly fermentative. [119]

Marine yeasts are reported to be truly versatile agents of biodegradation. [36,71] They participate in a range of ecologically significant processes in the sea, especially in estuarine and near-shore environments. Among such activities, decomposition of plant substrates, nutrient-recycling, biodegradation of oil/recalcitrant compounds and parasitism of marine animals are important. Biomass data and repeated observations of microhabitat colonization by various marine-occurring yeasts support ancillary laboratory evidence for the contribution of this segment of the marine mycota to productivity and transformation activities in the sea. [93]

Oceans and seas

The discovery of marine yeasts goes back to 1894, when Fisher separated red and white yeasts from the Atlantic Ocean and identified them as *Torula* sp. and *Mycoderma* sp., respectively. [53] Following Fisher's discovery, many other workers, such as Hunter, [65] Bhat *et al*., [15,16] Suehiro [135] and van Uden and Fell, [145] isolated marine yeasts from different sources, viz. seawater, marine deposits, seaweeds, fish, marine mammals and sea birds. Zobell and Feltham [158] observed yeasts on most of their culture plates inoculated with samples of marine materials collected from land as well as from the open ocean. Russian microbiologists have reported the quantitative distribution of yeasts in the Black and Okhotsk Seas, the Pacific Ocean and the Arctic sea. [74,78,106,127] Kohlmeyer and Kohlmeyer [72] isolated yeasts from seawater, sediment, plants, animals and other organic matter in the marine habitat. They were divided into 'obligate' and 'facultative' groups. 'Obligate marine' yeasts are those yeasts that, thus far, have never been collected from anywhere other than the marine environment, whereas 'facultative marine' yeasts are also known from terrestrial habitats. Obligate marine species may be confined to marine habitats, especially if they have been collected frequently and exclusively from the sea for several years. The majority of reports on yeasts from marine environments are based on indirect collection methods, such as incubation of seawater, sediment and diverse substrates found in the sea. With such culture techniques, cells may grow *in vitro* which would have remained dormant and inactive in marine habitats. Yeast species that have also been found in fruits, soil, domestic animals and man are most likely not native to estuaries and seas, even if they were isolated from such areas many times. It is more probable that they were washed into the sea by way of rivers or sewage or with a dust-blown seaward wind. Observations such as exceptionally high yeast densities following *Noctiluca* blooms in the North Sea [94] could indicate the presence of indigenous species, but insufficient data did not allow these authors to draw definite conclusions; in addition, the area in question was polluted by sewage disposal and regular passenger traffic. [59] Kriss and Rukina [73] also found plankton blooms in the Black sea and the Pacific Ocean to be locations of greatest density of yeast populations in the sea.

Sea water

Yeast populations have been observed to decrease with increased distance from land [5] and certain yeast species frequently collected from seawater were obtained in the highest quantities from the vicinity of heavily polluted areas. [45] However, such facts could also indicate that the collected yeasts were merely contaminants from terrestrial sources, surviving passively in the sea. These incidents and the related arguments may very well question the statement that there are truly indigenous marine yeasts. Near-shore environments are usually inhabited by tens to thousands of cells/litre of water, whereas low organic surface to deep-sea oceanic regions contain 10 or fewer cells/litre, although local nutrient areas

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may foster concentrations of yeast cells that reach 3000–4000 cells/litre. Kriss and Novozhilova [77] reported that budding yeasts were observed by direct microscopic examination of water samples down to depths of 2000 m. This fact would be evidence for growth of yeasts in seawater; however, the collection technique with Nansen bottles used by Kriss and co-workers was questioned later, when such containers were found to be easily contaminated. [134] In a survey of marine-occurring yeasts, Kohlmeyer and Kohlmeyer [72] have compiled a list of 177 species that were isolated from water, sediment, algae, animals and other organic matter in the marine habitat. Of those, only 26 species were regarded as obligate marine forms. The most important genera of true marine yeasts are *Metchnikowia, Kluyveromyces, Rhodosporidium, Candida, Cryptococcus, Rhodotorula* and *Torulopsis.* From these studies it was found that marine yeasts do not belong to a specific genus or group, but that they are distributed among a wide variety of well-known genera, such as *Candida, Cryptococcus, Debaryomyces, Pichia, Hansenula, Rhodotorula, Saccharomyces, Trichosporon* and *Torulopsis*. The isolation frequency of yeasts falls with depth. Yeasts in the class Ascomycetes (e.g. *Candida, Debaryomyces, Kluyveromyces, Pichia* and *Saccharomyces*) are common in shallow waters, whilst yeasts belonging to the Basidiomycetes (*Cryptococcus, Rhodosporidium, Rhodotorula, Sporobolomyces*) are common in deep waters, e.g. *Rhodotorula* has been isolated from a depth of 11 000 m. [99]

During the cruise of the *RV Vitiaz* in 1957–1958, *Debaryomyces globosus* was isolated from a depth of 400 m in the central Pacific Ocean. Yamasato *et al*. [156] conducted an ecological survey of yeasts from the Pacific Ocean and yeasts were isolated from the surface to a depth of 4000 m and were found belonging to the genera *Rhodotorula, Cryptococcus, Debaryomyces* and *Candida. Cryptococcu*s and *Rhodotorula* species were predominant among yeasts isolated from deep-sea waters from Loma Trough, off San Diego, CA, USA. In samples collected off La Jolla, CA, USA, total yeast count varied in the range 0–1920 viable cells/l. [146] Fell and Castelo-Branco (146) reported observations on the distribution, ecology and taxonomy of yeasts isolated from the subtropical Atlantic near Miami, FL, USA and the warm temperature Pacific adjacent to La Jolla, CA, USA.

From the open ocean waters of the Gulf Stream near Bimini, Bahamas, genera such as *Candida, Rhodotorula, Cryptococcus, Debaryomyces* and black yeasts were isolated. The distribution of species as well as their numbers and metabolic characteristics were found to be governed by existing environmental conditions. Fell *et al*. [50] obtained a total of 179 yeast isolates from 45 sampling stations in the course of a qualitative yeast survey in Biscayne Bay, FL, USA. *Candida tropicalis* and *Rhodotorula rubra* were the predominant species. Roth *et al*. [126] and Fell [38] made a quantitative study on the distribution of yeast in the coastal areas of Southern Florida and in the Gulf Stream of Florida. Freshwater influx and heavy recreational bathing directly affected viable yeast counts in these areas. *C. tropicalis* and *R. rubra* were predominant in the inshore region. Yeasts were found to be widely distributed in the water and sediment of Chesapeake Bay and *Rhodotorula* sp. was frequently isolated from this region. [27]

Hagler and Mendonca [60] studied the yeasts from marine and estuarine waters with different levels of pollution in the state of Rio de Janeiro, Brazil. They found that yeast counts in clean seawater generally range from a few to several hundreds/litre, but in the case of enrichments such as pollution or algal blooms, the number may reach thousands/litre or more. In addition there is a shift from a prevalence of strictly aerobic yeasts in clean water to a presence of fermentative yeasts in polluted waters. Yeasts from polluted and unpolluted beaches in the southern area of Sao Paulo state, Baixada Santista, Brazil, were isolated and studied by Paula *et al*. [113] The isolates belonged to nine genera, *Candida, Cryptococcus, Rhodotorula, Torulopsis, Trichosporon, Debaryomyces, Hansenula, Pichia* and *Sporobolomyces*. The results point to the genus *Candida* as a probable pollution indicator for coastal seawater. Isolation and identification of yeasts from sand and seawater collected from two beaches of Olinda, Pernambuco state, Brazil, were performed by Loureiro *et al*.; [89] 292 strains of yeasts were obtained, belonging to four genera and 31 species, among which *Candida* was the most prevalent genus.

Ahearn and Crow [3] reported the species and densities of yeasts isolated from North Sea waters before and after the production of oil. *Debaryomyces hansenii* was the predominant species in

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both sets of samples, but after oil production, *Candida guilliermondii*, a hydrocarbonoclastic yeast, was more commonly isolated. Kriss [76] found that yeasts were observed not only in the oxygenated zone but also in the H_2S zone of the Black Sea. Further studies by Kriss revealed that the distribution of yeast in seawater is characterized by microzonation. In coastal waters, up to several thousand yeast cells/litre were found. [93,126] Yeasts are known to be normal components of the biota of the world oceans [38,79] and in heavily polluted waters there could be considerably more. The presence of some salt-tolerant yeasts in the open ocean has been reported by van Uden and Fell. [145] Fungi and yeasts which are filamentous in nature are usual inhabitants of marine environments. [63,70,72,86,105,118]

Fell [39] found living yeasts in the Indian Ocean from the surface down to a depth of 200 m. The yeasts were collected from 16 stations during the cruise of *RV Anton Brunn* in the Indian Ocean. The highest population of yeasts was found in the Somali Current and the species isolated were grouped according to their distribution. Ubiquitous species such as *Rhodotorula rubra* and *Candida atmospherica* were seen in all water masses. Widely distributed species occurred in all water masses except the Red Sea, which was represented by *Candida polymorpha* and *Rhodotorula glutinis*. Species such as *Sporobolomyces hispanicus, S. odonus* and *Rhodotorula crocea* were of restricted distribution. Bhat and Kachwalla [14] isolated yeasts from water samples collected 2–6 miles off the coast of Bombay. They obtained species such as *Saccharomyces italicus, S. chevalicri, S. rosei, Debaryomyces hansenii, Pichia guilliermondii, Candida tropicalis, Torulopsis glabrata, Torulopsis candida, Rhodotorula* sp., *Cryptococcus* sp. etc. Yeasts of the Indian Ocean waters were studied by Fell and van Uden, [45] D'Souza [31] and Godinho *et al*. [56] 33 strains of marine yeasts were isolated from the coastal and offshore waters off Cochin and *Candida* was the predominant genus obtained. [123] A marine hydrocarbon-degrading yeast was isolated from Mumbai (India) and was identified as *Yarrowia lipolytica*. [108] Yeasts were isolated from seawater samples collected from the west and east coast of India up to 200 m depth in the Exclusive Economic Zone (EEC). [128] The most predominant genera were *Candida, Filobasidium and Leucosporidium*. Most of the isolates were found to be fermentative in nature and filamentous growth was very common among the isolates.

Various kinds of ethanol producing marine yeasts from coastal waters were isolated and characterized by Urano *et al*., [142] who found that most of them belonged to the genera *Candida* and *Debaryomyces*. Zhang *et al*. [157] investigated the ecological distribution of marine microorganisms in the southern ocean to the north-west of the Antarctic Peninsula and isolated six genera of yeasts from seawater. A survey of the marine yeasts in the sub-Antarctic region near South Georgia conducted by Connell and Rodriguez [28] recovered 72 yeast isolates, of which 19% were psychrophilic (could not grow at or above 20 °C) and 43% grew more rapidly at 20 °C than at temperatures at which they were collected (< 4 °C).

Sediment

Relatively high yeast densities (up to 2000 viable cells/g) have been reported for marine sediments, with most of the population in the top few centimeters. [50,84] About 99 yeast strains, including 40 red yeasts were isolated from benthic animals and sediment collected from the deep sea floor in various areas in the north-western Pacific Ocean. [100]

Fell *et al*. [50] isolated yeasts from Biscayne Bay, Florida, and deep-sea sediments in the Bahamas. The most commonly isolated genera were *Rhodotorula, Debaryomyces, Torulopsis, Cryptococcus* and *Candida.* The study reported that yeasts were more abundant in silty muds than in sandy sediments. The limited deep-sea collections showed a predominance of oxidative yeasts as compared to collections made in Biscayne Bay. In the investigations of Roth *et al*., [126] sediments and surrounding waters of the grass beds showed higher cell counts and higher number of species than grasses and algae. Fell and van Uden [45] found that yeasts were confined to the upper 2 cm of the substrate at a depth of 540 m, in the Gulf Stream. In shallow Florida waters, however, where strong wave action and rapid settling of sediments prevail, yeasts were found in depths up to 9 cm. The authors concluded that availability of oxygen is the limiting factor for the growth processes of yeasts within the sediments. They occur particularly in the topmost centimeters and, according

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to Suehiro, [136] they are more frequent in the black zone than in sandy sediments. Meyers *et al*. [95] observed very high concentration of viable cells of *Spartina alterniflora* in the marshes of the Louisiana coast than in adjacent water samples. Species of *Pichia and Kluyveromyces* were predominant and occurred most commonly in the culm-sediment region of the *Spartina* plants. [4] Several hundred living yeast cells/cm^3 were found in the damp mud from the Kiel Fjord. [64]

The prevalent isolates from estuarine, littoral and deep-water marine sediments of Florida and the Bahamas have been mostly oxidative yeasts, including *Rhodotorula* and *Cryptococcus*, typical of sea water. [50,84,149] A new ascosporogenous yeast, *Lachancea meyersii* sp. nov., was isolated from mangrove regions in the Bahama Islands. [46] Yeast abundance in the sediments of 13 coastal sites of Massachusetts was quantified by MacGillivray and Shiaris. [90] The most abundant genera isolated and identified included *Candida, Cryptococcus, Rhodotorula, Torulopsis* and *Trichosporon.* Few yeasts were isolated from greater depths (11 000 m) and comparatively higher numbers from the shallower sites (1000–6500 m). The isolation frequency of yeasts fell as the depth of sampling site increased. The ratio of basidiomycetous yeasts to ascomycetous yeasts rose with increasing depth. Little diversity is observed among basidiomycetous isolates and *Rhodotorula* occupied 89% of all isolates. On the other hand, ascomycetous yeasts isolated at sites shallower than 2000 m showed a wide range of taxa, such as *Candida, Debaryomyces, Kluyveromyces, Pichia, Saccharomyces* and *Willopsis*. [139]

Hagler and Mendonca [60] suggested that polluted littoral sediments are an unfavourable environment for strictly oxidative yeasts such as *Rhodotorula* and *Cryptococcus*, which are common in less polluted sediments. Hagler *et al*. [62] studied the densities of some yeasts in intertidal sediments of a polluted subtropical estuary in Rio de Janeiro, Brazil. Highest yeast densities were found at the most polluted site, and at the upper 2 cm of sediments. *Candida krusei, Pichia membranefaciens* and similar species typically forming rugose colonies with radiating ridges were the prevalent yeasts in these sediments, and species such as *Rhodotorula rubra*, related to basidiomycetous fungi, were found in relatively low numbers. Diversity assessment of benthic yeasts was done along a longitudinal gradient in Serra Do Cipo, Brazil, to monitor organisms important in determining water contamination levels. These microbes usually feed on dissolved organic matter and multiplying rapidly under favourable conditions. [23]

Thirteen yeast strains were isolated from deep-sea sediment samples collected at a depth of 4500–6500 m in the Japan Trench. One of the strains among them, which belonged to the genus *Cryptococcus*, possessed high tolerance against Cu^{2+}. [1] Yeasts and other fungi are prevalent in marine salt marsh and mangrove ecosystems, where they play an important role in the detrital food web. [66,92]

Prabhakaran and Ranu Gupta [121] studied yeasts from sediment samples of the Indian EEZ. They found that *Candida* was the dominant group of all the species and next in abundance was *Rhodotorula.* Isolation of yeasts was done at a depth range of 200–1000 m along the continental slope sediments of Arabian sea and the Bay of Bengal and the predominant genera identified were *Candida, Rhodotorula, Cryptococcus, Debaryomyces, Pichia* and *Trichosporon.* [82]

The *Cryptococcus vishniacii* complex (yeasts of basidiomycetous affinity), isolated from the soil samples of Dr W. V. Vishniac's 1973 expedition, is peculiar to the dry valleys of Antarctica, constituting the only heterotrophic biota demonstrably indigenous to the most severe cold desert on earth, [8,147,148] where they appear to have undergone sub-specific evolution. [9]

Oil slicks

Le Petit *et al*. [85] studied oil-polluted littoral marine areas in the Mediterranean and found seven species which were able to metabolize hydrocarbon fractions. From non-polluted test sites, only one hydrocarbonoclastic species was isolated. Biodegradation was very slow and the authors concluded that yeasts probably play only a minor role in the elimination of hydrocarbons from the sea. Ahearn *et al*. [6] tested selected yeasts isolated from oil-polluted habitats for their ability to use hydrocarbons as sole source of carbon. A *Trichosporon* sp. was found to emulsify the oil. The responses of yeast populations to oil pollution were investigated by Ahearn and Meyers. [4] Plots of a *Spartina alterniflora* salt marsh in Louisiana were selected as test areas saturated with oil. Compared

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with adjacent control sites, a considerable increase in yeast densities was noticed in the oil-soaked plots, and the predominant yeasts of the marshland were replaced by hydrocarbonoclastic strains, especially *Pichia ohmeri* and *Trichosporon* sp. In the nutrient-rich sediments of the estuary, populations of yeasts continued to increase in the presence of oil. In offshore areas, however, yeast populations declined after an initial increase, perhaps due to lack of nutrients and vitamins. It was suggested that the tested organisms may have relatively low capacity to decompose crude oil at oil spillage sites. In general, yeasts isolated from oil-polluted regions exhibited much higher hydrocarbonoclastic property than the same species from non-polluted areas.

Estuaries

In littoral zones of the Crimea, Florida and California coasts, yeast population densities were found to be generally higher than adjacent open seas. [78] The apparent dominance of some yeast species in estuaries and their apparent absence in open oceans may be due to a variety of reasons. One obvious possible source of yeast in estuaries is sewage pollution and terrestrial run-off. In fact, two ecological groups encountered were yeasts such as *Rhodotorula glutinis*, which were widespread in estuaries, the open oceans and inland waters, and intestinal yeasts such as *Candida tropicalis* and *C. intermedia* from terrestrial substrates that were dominant in estuaries but rare in open seas. [29]

Taysi and van Uden [140] found that higher number of yeasts obtained from regions where there was relatively light pollution. It was found that with increase in distance from the estuaries, the number of species decreased. Ecological observations showed that estuaries had more dense yeast population than adjacent oceanic zones. Total colony counts and number of species decreased with distance from the estuaries. The species common to both estuaries and oceanic regions were the genera *Debaryomyces* and *Rhodotorula*, the species exclusively or predominantly estuarine were *Candida intermedia, C. lambica, C. silvicola* and *Torulopsis candida*. Elevated yeast densities were observed at nutrient-rich haloclines in estuaries. [104] Estuaries probably take an intermediate position, with yeast populations fluctuating between high levels in inland waters and low levels in non-estuarine regions. There are evidences that estuarine waters contain not only more yeast cells/volume but also more species than adjacent sea. [143] This may be due to the high organic load of the estuaries than the marine habitat. Numerous yeasts were identified from polluted water and sewage. [5,30,60]

Investigations on the yeast flora of the Suwannee estuary in Florida showed that *Candida* and *Rhodotorula* were the predominant genera; however, the most frequently isolated strain was *Cryptococcus laurentii*. Nine ascosporogenous species were isolated, with *Hansenula saturnus* as the predominant form. [84] The microbial flora of the estuarine and inshore environments of the west coast of Taiwan was studied by Cheng and Lin. [24] Preliminary identification of the isolates revealed that they belong to the genera *Saccharomyces, Torulopsis, Debaryomyces, Endomycopsis, Pichia, Kloeckera* and *Rhodotorula.* Hagler *et al*. [63] reported that *Candida* and *Rhodotorula* were the most frequently isolated genera from a polluted estuary; 112 yeast isolates were obtained from 31 samples of decaying vegetation in the rhizosphere of the mangrove plants, from 11 sites in the Chapora, Mandovi and Zuari estuaries of Goa, India. [32]

Weeds and algae

Several investigations deal with population of yeasts on seaweeds. Studies on zoo- and phytoplankton revealed more than 20 associated yeast species. Bunt [22] examined microbes present in the decomposing giant kelp at Macquarie island in Antarctica and found that large amounts of yeasts were present in the decomposing kelp tissue. According to Kriss, [75] the planktonosphere is richer in yeasts than other zones of the sea. Plankton catches from the black sea contained yeasts in 90% of the samples. Studies by Suehiro [135] revealed that decomposing algae constitute a suitable substrate for yeasts. The predominant species of yeasts isolated from the marine algae were *Torulopsis* sp., *Candida albicans, C. natalensis, Trichosporon cutaneum* and *Endomycopsis chodatii.* van Uden and Zobell [144] obtained yeasts from 45/62 samples collected from algal and coral growths in the Torres Strait region. Species such as *Metschnikowia reukaufii, Pichia farinose, Kluyveromyces aestuarii, Candida marina, Torulopsis torresii* and *Torulopsis maris* were obtained. Fell *et al*. [49] isolated several *Rhodosporidium* spp. from the plankton samples at various water

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depths in the Pacific Ocean. van Uden and Castelo-Branco [146] isolated yeasts from giant kelp in southern California and found *Metschnikowia zobelli* on all samples yielding yeasts except one. Suehiro *et al*. [137] estimated that more than 50% of the algal biomass (phytoplankton) was transferred into yeast biomass. He also estimated that a mixed population of yeasts may be capable of degrading and assimilating a large proportion of organic material released from decaying phytoplankton, even in the absence of bacteria.

Patel [112] found that living algae contained lower counts of yeasts compared to counts in the surrounding seawater, but when decomposition starts, yeasts in the algal material increased to higher numbers than those found in the surrounding seawater. Seshadri and Sieburth [132] reported seaweeds as a reservoir of *Candida* yeasts in inshore waters. The authors considered the possibility that the yeasts may utilize exudates of their living hosts. Meyers *et al*. [92] studied the yeast populations on living *Spartina alterniflora* plants in a Louisiana salt marsh. *Pichia spartinae* and *Kluyveromyces drosophilarum* were found on the outer surfaces of the culm, but the former species is of special interest because it occurred in great concentrations in the plants' intraculm cell liquid and viable tissue. Yeast populations of *Sporobolomyces roseus* on marsh plants in England were investigated by Pugh and Lindsay. [122] Leaves of inland plants harboured much higher cell numbers than those near shore. Newell [101] mentioned blooms of *Rhodotorula rubra* and *Debaryomyces hansenii* on submerged seedlings of *Rhizophora mangle*.

Invertebrates

Studies on invertebrates have shown that they are either devoid of yeasts or support only a small density of the population. Phaff *et al*. [117] obtained yeasts from the Mexico shrimp *Penaeus setiferus* and the yeast species isolated were *Trichosporon cutaneum, Rhodotorula glutinis, Candida parapsilosis, Pichia guilliermondii* and *Pullularia pullulans.* Siepman and Honk [133] sampled shrimp eggs, sponges and other invertebrate material collected from the North Atlantic Ocean and the species isolated were *Debaryomyces hansenii, Torulopsis candida* and *Trichosporon cutaneum.* About half the number of species found were from the internal parts of the animals and about half from surface swabs. In assimilation tests, they found strong formation of riboflavin by *Debaryomyces subglobosus (D. hansenii)*, a yeast they frequently isolated from the internal fluids of invertebrates, and the authors suggested that this yeast may serve as a vitamin source for marine animals. The whole body of the amphipod *Podocerus brasiliensis* was found to be invaded by *Rhodotorula minuta.* [126] Seki and Fulton [131] showed that the tissues of living marine copepods (*Calanus plumchrus*) were attacked by *Metschnikowia* sp. Fize *et al*. [54] reported a *Metschnikowia* sp. parasitizing living copepods (*Eurytemora velox*) in southern France.

Yeast populations from conch and spiny lobster on the Bahama Islands were studied by Voltz *et al*. [149] They isolated fewer types of yeast from the animals than from marine sand and sediment of the same habitat, and assumed that the isolates were probably ingested during feeding and did not seem to cause stress to their hosts. The commercially raised brine shrimp, *Artemia salina*, was parasitized by *Metschnikowia bicuspidate* var. *australis*, a yeast that appears to be equipped with an active predatory mechanism, attacking its host by forcible ascospore discharge. [82] Chresanowski and Cowley [25] found *Rhodotorula glutinis* and *Torulopsis ernobii* in the gut of fiddler crab, *Uca pugilator.* It was speculated that these yeasts might serve as food, but feeding experiments showed that they could not be utilized as a sole food source by the crabs. Buck *et al*., [20] investigating bivalve shellfish in Long Island Sound, noted that, in general, the liquid portion of the shellfish contained more yeasts than the internal viscera.

The ascomycetous yeast communities associated with three bivalve molluscs and four crab species were studied in mangroves at Coroa Grande on Sepetiba Bay in Rio de Janeiro, Brazil. The cultures obtained were classified into 84 species, among which 44 species were novel. The ascomycetous yeast communities of the mangrove ecosystem included many new biotypes. [61]

Fish

Yeasts associated with fish were isolated from skin, gills, mouth, faeces and gut contents of the animals. Of the various species of yeasts associated with fish, *Debaryomyces hansenii* was the most dominant. This species is frequent in seawater,

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which may explain its high incidence in fish. Another important yeast species isolated from fish was *Metschnikowia zobelli*, high numbers of which were isolated from the gut contents of fish, and it has been suggested that the yeast flora of fish merely reflect their feeding habits. [45] In the Pacific, van Uden and Castelo-Branco [146] found certain fish species containing significantly higher numbers of yeast cells than the surrounding sea water, and the authors believe that these yeasts may be able to grow in the intestine of some fish. Ross and Morris [125] reported that the greatest variety and highest number of yeasts were obtained from fish skin, while gill counts gave smaller numbers. Yeasts were isolated from the intestine of farmed rainbow trout (*Salmo gairdneri*) by Andlid *et al*. [7] The dominant species were *Debaryomyces hansenii, Saccharomyces cerevisiae, Rhodotorula rubra and R. glutinis.* Red-pigmented yeasts dominated and composed about 90% of the isolates.

Birds

The gut and rectal contents of free-living gulls and terns were found to harbour yeast cells. Shore droppings of birds yielded *Torulopsis glabrata* and *Candida tropicalis* in the Pacific [146] and Atlantic [68] Oceans. These authors suggested that birds like gulls introduce yeasts through their faeces into water bodies the world over. However, yeasts occurring in gulls were not always found in seawater of the area where the birds were caught and the authors assumed that low water temperatures can prevent a build-up of detectable yeast population. Isolations from shore bird droppings on southern California beaches yielded species also occurring in the rectal contents of seagulls. [33] The occurrence of *Candida albicans* in fresh gull faeces was compared in temperate and subtropical locations. Of 239 fresh samples, 133 were obtained from south-eastern Connecticut and 106 from different sites on the south-eastern and central western coasts of Florida. Overall, 60% of all faeces contained *Candida albicans*. Of the Connecticut samples, 78% were positive, whereas only 38% of the Florida samples revealed the presence of the yeast. Only 1/24 samples of fresh brown pelican faeces contained *Candida albicans*. [19]

Mammals

van Uden and Castelo-Branco, [146] who found no yeasts in intestinal samples from eight California sea lions, reported that warm-blooded animals with a high intake of food rich in protein are, in general, unsuitable hosts for intestinal yeasts. *Rhodosporidium toruloides* was isolated from the intestine of a porpoise that died in captivity. [48] *Candida tropicalis* was found in the stomach of marine mammals such as dolphins and porpoises and would probably have been ingested with indigenous food or seawater. [96]

Isolation and cultivation of marine yeasts

Kriss (75) found that the number of yeasts estimated by direct microscopic observation were higher than those obtained by plate count. This disparity can be partly explained by the presence of non-viable and non-cultivable yeast cells. Another explanation is that numerous yeast cells may be attached to organic or inorganic particles and together will produce a single colony. Traditional methods of yeast isolation have specific limitations. The culture media and environmental growth conditions (particularly temperature) are selective, rapid-growing strains will overgrow slower-growing species and consequently rare species may not be represented. Cell numbers obtained with plate cultivation techniques do not reflect factors such as turnover rates, hyphal fragmentation, spore release or rates of consumption by various invertebrates. A variety of media and incubation conditions can be employed and designed by the researcher. The method for water sampling employs filtration through 47 mm diameter nitrocellulose filters of 0.45 μm pore size, using an autoclavable glass or plastic filter apparatus. The filter is placed face up on a nutrient agar medium. A widely used medium is Wickerham's YM medium, which contains 0.3% yeast extract, 0.3% malt extract, 0.5% peptone, 1% glucose and 2% agar prepared with sea water at a salinity equivalent to the sample site. Bacteria are inhibited by the addition of chloramphenicol (200 mg/l) to the medium prior to autoclaving. An alternative is an antibiotic mixture of penicillin G and streptomycin sulphate (each at 150–500 mg/l), added dry to autoclaved, cooled (45 °C) medium. Sediment particles can be placed

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directly on an agar medium or known quantities of sediment can be placed in a test tube with a given volume of sterile sea water, vortexed and diluted 1 : 10 in a sterile sea water series, followed by preparation of standard spread plates from each of the dilution series. Suspected yeast colonies can be picked and transferred to a microscopic slide for inspection and after confirmation can be transferred from the isolation medium to a growth medium (YM sea water agar lacking antibiotics).

A selective medium suitable for *Candida* species is the chloramphenicol malt agar and chloramphenicol cycloheximide malt agar. Some *Candida* species grow in the presence of cycloheximide, while most other species do not, so it has been used as a differential medium for *Candida* species. [26] Broad-spectrum antibiotics are both more effective in preventing bacterial growth and less harmful to yeast cells. [98,55,13,141] Various compounds have been added to media to inhibit the growth of moulds, including Rose Bengal, [67,69] dichloran [69] and propionate. [18] Oxytetracycline glucose yeast extract agar (OGYE) has been recommended for the selective isolation and enumeration of yeasts and moulds from foodstuffs. [97] It was concluded that Rose Bengal–chloramphenicol–agar is the medium of choice for samples heavily contaminated with moulds. Woods [155] used various antibiotics containing media for the enumeration of yeasts and moulds in foods and the comparative efficiency was worked out. The ability of media to suppress bacterial growth and to prevent excessive growth of fungal colonies were the two main factors considered. Malt extract agar containing oxytetracycline was recommended for samples where the main concern is enumeration of yeasts.

Yeasts are maintained on agar slopes of malt extract agar. Yeasts of certain genera (*Bensingtonia, Bullera, Cryptococcus, Leucosporidium, Rhodosporidium, Rhodotorula* and *Sporobolomyces*) generally survive longer on potato dextrose agar (Table 2). The plates are incubated at temperatures designed to maintain ambient environmental conditions. For example, polar and deep-sea samples should be incubated at ~5 °C. The temperature required for temperate and tropical samples often results in overgrowth by filamentous fungi, which can be reduced by incubation at temperatures of ~12 °C. [42] For taxonomic tests, yeasts are usually incubated at 25 °C, [21] although optimum temperatures for growth are higher for some yeasts and lower for others. [150]

Estimation of yeast biomass

Ergosterol is the primary sterol in the cell membrane of filamentous fungi and is either absent or a minor component in higher plants. It is also present in the yeast cell wall and mitochondria. Ergosterol is a constituent of membranes in mycelia, spores and vegetative cells. Ergosterol content has been widely used as an estimate of fungal biomass in various environments, e.g. in soil and aquatic systems, because a strong correlation has been found between ergosterol content and fungal dry mass. The concentration of ergosterol does not always correlate with absolute fungal biomass and it is influenced by both internal and external factors. The amount of ergosterol in fungi depends on the age of the culture, the developmental stage (growth phase, hyphal formation or sporulation) and growth conditions (growth medium, pH, temperature). [111] Ergosterol concentrations vary among different fungal species, among isolates of the same species and even within a strain, depending on the physiological state. [58] Because of these problems, the application of this method to environmental samples is limited. It is assumed that ergosterol is labile and undergoes a rapid degradation and cell death; a lot of environmental microbiologists use this molecule as an indicator not for total

Table 2. Media used for the isolation and cultivation of yeasts

Isolation	Cultivation	Sporulation
Malt–yeast–glucose–peptone agar [155]	Wort agar [109]	V-8 agar [152]
Malt extract agar [87]	Malt extract agar [87]	Gorodkowa agar [87]
Davis's yeast salt agar [34]	Corn meal agar [87]	Malt extract agar [87]
Oxytetracycline glucose yeast extract agar [98]	Davis's yeast salt agar [34]	Potato glucose agar [87]
	Osmophilic agar [129]	Davis's yeast salt agar [34]
	Malt yeast agar [35]	

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but exclusively for living fungal biomass. [102] The ergosterol assay is generally considered to be the most promising method for the detection of fungi because of: (a) the specificity of ergosterol to fungi; (b) the fact that it indicates live fungal mass (ergosterol becomes oxidized upon cell death); and (c) the relative constancy of the conversion factors compared to other alternative methods. [110]

Classification of yeasts

Yeasts were classified on the basis of their morphology and biochemical characteristics. The workers of the Dutch school were responsible for much of their pioneering work on the classification of yeast species known before 1950. These workers classified all the yeasts available to them on the basis of cellular morphology, spore shape and number and nature of conjugation processes, and at species level based on the ability to ferment and assimilate six sugars, to use ethanol and nitrate and to hydrolyse arbutin. The distinction between some species was rather fine as judged by these criteria.

Wickerham and Burton [154] and Wickerham, [153] at about the same time, introduced a number of refinements to the Dutch system, especially the use of a much larger number of carbon compounds. These included additional hexoses, di-, tri- and tetrasaccharides, two polysaccharides and a number of pentoses, polyhydric alcohols and organic acids. They also introduced tests for vitamin requirements. The general practice is to use approximately 30 compounds and to test for fermentation of at least 11 of these, including insulin. [12] The ability to use nitrite as well as nitrate at depressed temperatures and on media of high sugar or salt content is also used. The type and number of additional reactions tested vary with the interests and preferences of the individual investigator. Difficulties, both major and minor, accompany the use of these methods. One is the question of the stability of the biochemical criteria, e.g. *Candida* and *Torulopsis* were separated for differentiation into species solely on the ability of the former to produce pseudohyphae, until it was observed that the same species might produce two or more forms simultaneously or at different stages of growth. It has now become evident that different strains of the same species may differ in their ability to produce pseudomycelium and the value of this criterion in distinguishing the two genera approaches vanishing point; another problem is the instability of physiological characters. Scheda and Yarrow [130] observed enough variability in the fermentation and carbon-assimilation patterns of a number of *Saccharomyces* spp. to cause difficulties in the assignment of these yeast strains to different species. Another difficulty lies in the relationship of the biochemical tests to the metabolism of the organisms. It was not originally sufficiently appreciated that the various carbon compounds are not necessarily assimilated independently but may be metabolized by common pathways. Thus, yeasts that can use a particular compound can use a structurally related one by the same metabolic pathway; but Barnett [11] noted that there was a small percentage of yeasts that were exceptions to this rule. In general the conclusions were valid, that the effective number of criteria for the number of substrates reduced distinguishing yeast species metabolized by such linked mechanisms. The metabolism of most or all of the compounds used involves a few distinct central pathways and depends on the ability of the cells to convert the substrates into intermediary metabolites of one of these pathways.

Currently, the main characteristics used to classify yeasts are morphology, physiological and biochemical characteristics, [12] fatty acid profile and rDNA sequence.

Microscopical appearance

Taxonomists examine yeast cells microscopically and consider their size and shape, how they reproduce vegetatively (by multipolar, bipolar or unipolar filaments) and the form, structure and mode of formation of ascospores and teliospores.

Sexual reproduction

Some yeasts reproduce sexually by ascospores, others by teliospores and yet others by basidiospores. For ascosporogenous yeasts, taxonomic importance is given to whether asci are formed from: (a) vegetative cells; (b) two conjugating cells; or (c) a mother cell that has conjugated with its bud. For yeasts with asci borne on filaments, the arrangement of asci, whether in chains or bunches, may be used to distinguish between genera. The number of ascospores in each ascus, their shape and whether the ascospore walls are smooth or rough are factors that are used in classification.

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Physiological features

Physiological factors used for classifying yeasts are chiefly the ability to: (a) ferment sugars anaerobically; (b) grow aerobically with various compounds, such as a sole source of carbon or nitrogen; (c) grow without an exogenous supply of vitamins; (d) grow in the presence of NaCl or glucose; (e) grow at 37 °C; (f) grow in the presence of cycloheximide; (g) split fat; (h) produce starch-like substances; (i) hydrolyze urea; and (j) form citric acid.

Biochemical characteristics

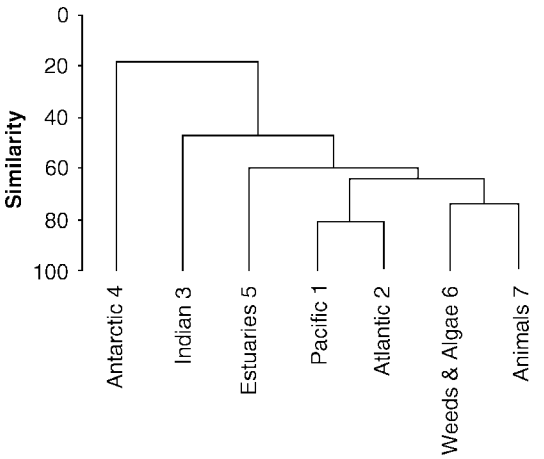
Studies of certain biochemical characters may influence taxonomic decisions, e.g. the chemical structure of cell walls, [115] particularly the cell wall mannans [10,57] and the kind of ubiquinone (coenzyme Q) present in different yeasts.

Fatty acid profiling

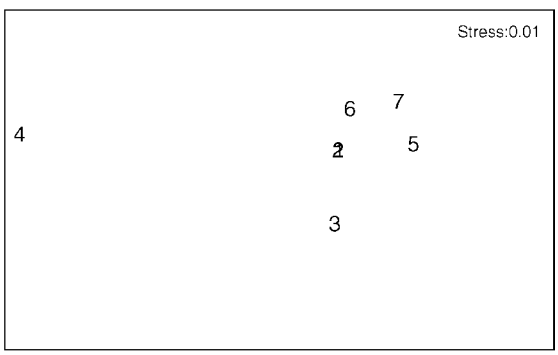
Microbial fatty acid profiles are unique from one species to another. It is known that fatty acids with 16–18 carbon atoms generally predominate in yeasts. The fatty acids occur as esters in triacylglycerol, phospholipids, glycolipids or sterols in membranes and other cytoplasmic organelles, such as the mitochondria, plasmalemma, endoplasmic reticulum, nuclei, vacuoles, spores and lipid particles. The 14 : 0 fatty acids are only seen as trace fatty acyl residues. The microbial identification system based on fatty acid methyl ester (FAME) analysis has been used in laboratories for the identification of clinical yeast strains. [114] The system analyses long-chain fatty acids containing 9–20 C atoms, identifying and quantifying the FAMEs of microorganisms. The database library searches for fatty acid composition, compares the FAME profile of the isolate with those of well-characterized strains and defines the most likely species of the isolate.

rDNA sequencing

Fell and Kurtzman [43] reported the nucleotide sequence analysis of a variable region of the large sub unit rRNA for identification of marine-occurring yeasts. The data suggest that large subunit sequences can be used for yeast identification, with the possible exception of closely related



a. Bray- Curtis similarity dendrogram



b. Multidimensional scaling plot

Figure 2. Distribution of yeasts in various oceans and flora/fauna. (a) Bray–Curtis similarity dendrogram. (b) Multidimensional scaling plot

homothallic species. The D1/D2 variable region of the large subunit rRNA was examined for nucleotide sequence signatures as a potential taxonomic tool. [37,47,52] Differentiation of strains within a species can play a significant role in ecological population analysis. Phylogenetic analysis based on molecular sequencing of the D1/D2 domain of 26S rDNA, [17,88] internal transcribed spacer (*ITS*) regions and 5.8S rRNA gene has been used to investigate the intraspecific relationships among the isolates. [46,51,100,120,124,151] Use of ITS or DNA sequences are considered to be the best tools for rapid and accurate identification of yeast isolates. ITS primers (Forward-ITS1 and Reverse- ITS4) by White *et al*., [151] which amplify a fragment of approximately 580 bp containing the ITS 1, 5.8s and ITS 2 regions are widely used for the purposes (Table 3). For

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Table 3. Primers used for the amplification and sequencing of yeast rDNA

Primer Code	Sequence	Forward/reverse	Location	Reference
ITS1	TCC GTA GGT GAA CCT GCG G	Forward	ITS1	151
NS7	GAG GCA ATA ACA GGT CTG TGA TGC	Forward	ITS1	42
ITS5	GGA ATG AAA AGT CGT AAC AAG G	Forward	ITS1	42
Hor-F	TGG ACA CCT TCA TAA CTC TTG	Forward	ITS1	103
Hor-R	TCA CAA CGC TTA GAG ACG G	Reverse	ITS1	103
LR6	CGC CAG TTC TGC TTA CC	Reverse	ITS2	42
EXO1	CTC AGA GCC GGA AAC TTG GTC	Forward	ITS2	120
EXO2	CCG CCG TCA TTG TCT TTG G	Reverse	ITS2	120
ITS3	GCA TCG ATG AAG AAC GCA GC	Forward	ITS2	151
ITS4	TCC TCC GCT TAT TGA TAT GC	Reverse	ITS2	151
NL1	GCA TAT CAA TAA GCG GAG GAA AAG	Forward	D1/D2	151,107
R635, NL4	GGT CCG TGT TTC AAG ACG G	Reverse	D1/D2	151,107
NL4A	GCG ACT TAA GAT CAT TAT GCC	Reverse	D1/D2	91
NL4A1	GCG ACT TAA GAT CAT TAT GCC AAC ATC C	Reverse	D1/D2	91
F63	GCA TAT CAA TAA GCG GAG GAA AAG	Forward	D1/D2	17
LR3	GGT CCG TGT TTC AAG ACG G	Reverse	D1/D2	17
SSU1f	CTG GTT GAT CCT GCC AGT AGT CAT	Forward	Small rDNA	81
SSU2r	ATG ATC CTT CCG CAG GTT CAC	Reverse	Small rDNA	81
SSU3f	TGG AGG GCA AGT CTG GTG CCA	Forward	Small rDNA	81
SSU4r	AAC TAA GAA CGG CCA TGC ACC A	Reverse	Small rDNA	81
LR11F	TTA CCA CAG GGA TTA CTG GC	Forward	IGS	42
LR12F	CTG AAC GCC TCT AAG TCA GAA	Forward	IGS	42
IG1F	CAG ACG ACT TGA ATG GGA ACG	Forward	IGS	42
5SF	GCA CCC TGC CCC GTC CGA TCC	Forward	IGS	42
5SR	GGA TCG GAC GGG GCA GGG TGC	Reverse	IGS	42
NS1R	GAG ACA AGC ATA TGA CTA C	Reverse	IGS	42
SR3R	GAA AGT TGA TGA GGC T	Reverse	IGS	42
SR1R	ATT ACC GCG GCT GCT	Reverse	IGS	42
26SF	ATC CTT TGC AGA ACG ACT TGA	Forward	IGS1	138
5SR	AGC TTG ACT TCG CAG ATC GG	Reverse	IGS1	138

the identification of several species, most appropriate techniques include hybridization probes with macro- and micro-arrays, which are designed to identify a large number of species.

Conclusion

Literature survey revealed that investigations on marine yeasts are comparatively few and that this group of marine mycota is still poorly understood. Study on the distribution of marine yeasts is limited in the oceanic waters of the globe and is mainly restricted to coastal waters of the Atlantic, Pacific and Indian Oceans. Polar and deep-sea studies are comparatively fewer. Genus-wise distribution showed more similarity between the yeast flora of Atlantic and Pacific waters compared to Indian waters (Figure 2a, b). Diverse yeast genera could be obtained from Indian waters, viz. *Candida, Cryptococcus, Debaryomyces, Kluyveromyces, Metchnikowia, Pichia, Hansenula, Rhodotorula, Torulopsis* [as *Torulopsis* species were without legality, Yarrow and Meyer (1978) proposed transferring them to the genus *Candida* and amended the diagnosis of *Candida* to include non-filamentous species [13]], *Trichosporon, Saccharomyces, Sporobolomyces* and black yeasts. The most frequently observed genera are *Candida, Cryptococcus, Debaryomyces* and *Rhodotorula. Candida, Debaryomyces* and *Rhodotorula* showed a cosmopolitan distribution. Studies on yeasts associated with marine animals are also limited. Although classification of yeasts can be done based on morphology and physiological/biochemical characterization, accurate identification requires either fatty acid profiling (FAME) or nucleotide sequence analysis of the D1/D2, ITS, 18S or 28S regions of rRNA. Identification using FAME is now confined to clinical yeasts and its

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application to marine yeasts is yet to be developed. Although ergosterol estimation is used in the quantification of yeast biomass, it is not a reliable method for yeast population estimation in the natural environment, due to variations in ergosterol content in different strains and at different growth stages. Moreover, the synthesis is affected by various physicochemical and nutritional conditions of the culture environment. Therefore, development of a quantitative assay is important in accounting for non-culturable forms.

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