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Diversity and physiology of deep-sea yeasts: A review

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ABSTRACT

Yeasts are unicellular form of ascomycetous and basidiomycetous fungi present ubiquitously in various habitats. They play significant role of saprotrophy, mineralization and biological degradation, forming an important constituent of different ecological niches. Most of the studies have focused on the diversity of yeasts from terrestrial environments till date, while their marine and deep-sea counterparts have not received sufficient attention. Being a promising source of biotechnologically active products owing to their capability of tolerating extreme environmental conditions, the diversity of yeasts from deep-sea environment needs to be explored in detail. This review is an approach to summarize the available diversity assemblage of yeast from various deep-sea habitats reported till date based on culture-dependent as well as culture-independent methods. In addition, the potential novel yeast species reported from the above habitat, their physiology and applications are documented. Detailed diversity studies of yeasts from various deep-sea habitats in order to get deeper insight on novel strains is recommended for future studies. Efforts to get uncultivable forms in culture may be one of the future prospects for obtaining strains with enormous biotechnological potentials.

Keywords: Culture-dependent approach, culture-independent approach, deep-sea habitat, elevated hydrostatic pressure, psychrotolerant yeast.

INTRODUCTION

Deep-sea environment is characterized by the extreme conditions of temperature, hydrostatic pressure and nutrient conditions, covering around 65% area of the total earth surface (Munn, 2011). The hydrostatic pressure increases at a rate of 0.1 MPa (Mega Pascal) for every 10 m depth, ranging from 2 - 100 MPa for different deep-sea habitats. In contrast, temperature decreases with increasing depth and remains at a constant value of ~2 - 3 °C in deep-sea habitats, except for the transition zones between deep-sea hot (hydrothermal vent environments) and cold waters (cold seeps). Deep-sea environment is also characterized by low nutrient conditions and is supposed to be physically stable (Sanders, 1968). There are several reports about the existence of diverse types of microbes from the deep-sea habitats e.g., bacteria (Li *et al.,* 1999; Kato and Qureshi, 1999), archaea (DeLong, 1992; 2004; Massana *et al.,* 2000) and protists (Edgcomb *et al.,* 2002; Li *et al.,* 2011).

Fungi are ubiquitous in the terrestrial and aquatic environments. They occur in the form of unicellular yeasts, polymorphic and filamentous fungi either free living or as symbiotic forms. They are also found to occur as potential pathogenic forms of plants and animals. Fungi in marine ecosystem occupy an important position as they are involved in the decomposition and mineralization of organic matter (Kohlmeyer and Kohlmeyer, 1979; Hyde, 1989; Newell, 2001). There are approximately 80,000 species of described fungi (Kirk *et al.,* 2001) till date, representing only about 5% of the estimated 1.5 million species worldwide (Hawksworth, 1991). However, compared with archaea and bacteria, reports on fungi from deep-sea habitats are still at the inventory stage.

Deep-sea fungi (mostly filamentous forms) were first reported in shells collected from deep-sea waters of 4610 m depth (H□hnk, 1969). Other report on isolation of fungi is from water samples, collected from subtropical Atlantic Ocean, from the surface to a depth of 4,500 m using sterile van Dorn bags. or Niskin samplers (Roth *et al.,* 1964). Fungi from deep-sea were obtained only as preserved specimens by directly submerging wooden panels at 1615-5315 m depth (Kohlmeyer, 1977). Presence of fungi has also been reported from Mariana trench at a depth of 11,000 m in the Pacific Ocean (Takami *et al.,* 1997). Various filamentous fungi were isolated from calcareous sediments and were also observed to show germination of spores at elevated hydrostatic pressure (Raghukumar and Raghukumar, 1998). Fungal filaments were directly detected in calcareous fragments using the fluorescent brightener calcofluor under epifluorescence microscope (Raghukumar *et al.,* 2004). Over 200 fungal isolates were obtained in culture form from deep-sea sediments of the Central Indian Basin using different techniques like dilution plating, particle plating and pressure enrichment techniques (Damare *et al.,* 2006). Several other reports have also recovered fungal signatures from different deep-sea habitats such as methane hydrate bearing deep-sea sediments (Lai *et al.,* 2007), hydrothermal vents (Le Calvez *et al.,* 2009) and anoxic sites (Jebaraj *et al.,* 2010).

Only a few studies have focused on diversity and physiological studies of unicellular yeasts from deep-sea environments. Yeasts have been reported in several deep-sea environments (Nagahama *et al.,* 2006), including hydrocasts near hydrothermal plumes from the Mid-Atlantic Ridge near the Azores (Gadanho and Sampaio, 2005) and in Pacific sea-floor sediments (Nagahama *et al.,* 2001a). Both of these habitats were found to be dominated by unicellular forms, commonly designated as yeasts. Several of the fungi isolated from the deep ocean have remained as undescribed species. For example, Nagahama and coworkers have described a number of novel species of yeasts from deep-sea sediments (Nagahama *et al.,* 2001b; 2003a; b; 2006). Undescribed species of yeasts were also reported from Atlantic hydrothermal plume waters (Gadanho and Sampaio, 2005). Several marine yeasts such as *Debaryomyces* sp., *Rhodotorula* sp. and *Rhodosporidium* sp. were isolated in culture form over a range of temperature and hydrostatic pressure conditions

(Lorenz and Molitoris, 1997). These marine yeast species are known to be as potential mycoparasites and phytopathogens. Some cellular structures known as colacosomes are found in these parasitic species which enable them for host-parasite interactions (Bauer and Oberwinkler, 1991). A pathogenic black yeast in mussel and other animals have been reported from hydrothermal vent sites (Moreira and Lopez-Garcia, 2003; Van Dover *et al.,* 2007; Whalen and Carnegie, 2007).

Considering the significance and potential biotechnological applications, the diversity studies of yeasts from deep-sea environment needs more attention in future. In this review we have attempted to document the collective information on diversity, physiology and applications of yeasts, isolated from various deep-sea habitats till date.

METHODS FOR CULTURING AND IDENTIFICATION OF YEASTS FROM DEEP-SEA SAMPLES

Culturing of yeasts from deep-sea sediments and waters has been reported by previous studies (Burgaud *et al.,* 2010). The first step for culturing of yeast is the collection of required deep-sea samples. Collection of deep-sea samples may be performed using routine oceanographic samplers such as Niskin or Nansen bottles or a Rossette or ZoBell sampler. These samplers do not maintain *in-situ* hydrostatic pressure. Water sampler, used to collect water sample for isolation of piezophilic bacteria which was able to retain deep-sea pressure was reported first by Jannasch *et al.* (1973).

For routine microbiological sampling of deep-sea sediments, multiple corers, long gravity corers or box corers may be used. Box corers may be used efficiently for more or less flat oceanic floors for sampling. The box corer is lowered on a ship's trawl wire till it penetrates the bottom. As the corer is pulled out of the seabed, the top and bottom of the sample box are closed. The advantage of a box corer is that it collects a sample which is generally >20 cm in length which encompasses the bulk of the vertical distribution of deep-sea organisms. Deep-sea sediments can also be collected using research submarines. A research submarine is comparatively bigger in size and can carry a pilot and one or two scientists in a pressure sphere about 2 m in diameter. Surrounding the sphere is equipment for life support, propulsion, ascent and descent, and equipments for scientific purposes such as manipulator arms, cameras and specialized payload in a carrying basket (Heirtzler and Grassle, 1976).

Another method for sampling is remotely operated vehicles (ROVs) which are self-propelled instrument packages. Some operate at the end of a cable that provides power and hosts a two-way communications link; others are untethered, carrying their own power and recording images and data. The instrument package consists of a propulsion unit, sensors (particularly television), and, in some cases, manipulator arms. These are designed either to fly or crawl, e.g. the Remote Underwater Manipulator (Thiel and Hessler, 1974) over sea bottom surface and are more suitable for seabed sampling and experimentation. After collection of samples, culturing and identification of yeasts may be done by the following methods:

Direct plate count method

In this method the water and/or sediment samples containing microbes are spread plated on selective media plates, followed by the counting of the colonies appearing after an incubation period (Damare *et al.,* 2006; Singh *et al.,* 2010). This method has limitations in terms of selection of appropriate media, pH, incubation temperature and colony-colony inhibition (Trevors, 1998), etc. Sometimes the fast growing forms can overtake slow growing ones, often resulting in biased results (Dix and Webster, 1995). A known amount of water is filtered through 0.45 µm nuclepore filters and the filters are placed directly on malt extract agar medium. Yeast colonies appearing after 7-10 days incubation are isolated in pure cultures by repeated subculturing (Raghukumar and Damare, 2008).

Community level physiological profiling

In this method the culturable yeasts can be identified on the basis of different carbon source utilization patterns. Choi and Dobbs (1999) introduced Biolog plates containing all the known carbon sources for this purpose. For analysis, samples containing yeasts are inoculated and monitored over time for their ability to utilize substrates and the speed at which these substrates are utilized. Multivariate analysis is applied to the data and relative differences between sample functional diversity can be assessed.

Fatty acid methyl ester (FAME) analysis

Fatty acids make up a relatively constant proportion of the cell biomass and signature fatty acids exist that can differentiate major taxonomic groups within a community. Therefore, a change in the fatty acid profile would represent a change in the microbial population. The fatty acid profiles can be used to study microbial community composition and population changes (Siciliano and Germida, 1998; Kelly *et al.,* 1999). This method can be used for the identification of yeasts (Gunasekaran and Hugh, 1980, Tredoux *et al.,* 1987; Marumo and Aoki, 1990).

Molecular based techniques

Among eukaryotes, 18S and internal transcribed spacer (ITS) regions of SSU rDNA are increasingly used to study fungal communities. However, the available databases are not as extensive as for prokaryotes (Prosser, 2002). In addition, the D1/D2 domain as well as other regions of Large Sub Unit (LSU) of 28S rDNA of eukaryotes can also be used for identification and diversity analysis (Pang and Mitchell, 2005; Gadanho and Sampaio, 2005; Burgaud *et al.,* 2010). Restriction fragment length polymorphism (RFLP) for the above amplified products can also be applied for the diversity analysis on the basis of DNA polymorphisms (Lai *et al.,* 2007). RFLP is the variation in DNA fragment banding patterns of electrophoresed restriction digests of DNA from different individuals of a species. The reason for the different banding patterns may be the presence of a restriction enzyme cleavage site at one place in the genome in one individual and the absence of that specific site in another individual. Therefore, on the basis of these specific banding patterns after cleavage with

a particular restriction enzyme different strains can be identified or compared.

Another method for detection of microbes in environmental samples is Fluorescent *in-situ* hybridization (FISH), which was used recently for assessment of yeasts associated with endemic animals of hydrothermal vents (Burgaud *et al.,* 2010). FISH is the method for detecting specific nucleic acids in their native environment and allows the quantification of mRNAs in yeast using fluorescently labeled single-stranded DNA probes. In this method, yeast cells are fixed and attached to coverslips and hybridized with a mixture of FISH probes, each coupled with several fluorescent dyes. FISH permits simultaneous visualization of multiple mRNA targets by using specific probes that are labeled with distinct fluorophores (Trcek *et al.,* 2012). FISH has been used as a powerful technique for *in-situ* assessment of both microbial identity and spatial distributions in complex environmental contexts using labelled oligonucleotide probes targeting specific rRNA (Yang *et al.,* 2008). However, the use of this technique to assess the target rRNA of yeast populations accurately in deep-sea environment might be a challenging task due to lower ribosomal activities at high hydrostatic pressure than at atmospheric pressure (Burgaud *et al.,* 2010). Therefore, low-level metabolic activities of yeasts living under extreme environmental abiotic factors may account for their imprecise detection and needs further standardization in future studies.

Diversity of yeasts from deep-sea habitats by culture-dependent approach

Yeasts have been isolated from different deep-sea habitats during various fungal diversity studies (Damare *et al.,* 2006; Singh *et al.,* 2010). However, a few reports focused exclusively on diversity and physiology inspection of only yeasts from such extreme environments (Nagahama *et al.,* 2001a; Burgaud *et al.,* 2010). The culture-dependent diversity studies included in the present review were based on isolation of yeasts followed by molecular identification by amplification and sequencing of 18S, ITS or D1/D2 region of 26 rRNA gene (**Table 1**). All the yeast species reported from deep-sea environment belonged to phylum *Ascomycota* and *Basidiomycota* with *Basidiomycota* being majority (**Table 1**). Among *Ascomycota*, the yeast species are affiliated predominantly to classes *Saccharomycetes* and *Dothideomycetes*. *Saccharomycetes* were isolated from oceanic habitats by culturing approaches (Fell, 1976). *Eurotiomycetes* and *Chaetothyriomycetes* are the other two classes recovered within *Ascomycota* phylum.

Saccharomycetes class consisted of *Williopsis, Candida, Debaryomyces, Kluyveromyces, Pichia* and *Clavispora* sp., isolated from different deep-sea geographic locations (**Table 1**). These ascomycetous yeasts have been found to be non-pigmented and natural inhabitant of the hydrothermal vent environments (Gadanho and Sampaio, 2005). Although being reported from other deep-sea habitats like Northwest Pacific Ocean (Nagahama *et al.,* 2001a), the utilizable organic compounds-rich environment of hydrothermal vents assist these ascomycetous yeast prosper substantially (Gadanho and Sampaio, 2005). *Candida* sp. has been reported from different deep-sea habitats as the major ascomycetous yeast. It was isolated from shrimp eggs in the North Atlantic Ocean (Siepmann and H□hnk, 1962) and from hydrothermal vent endemic fauna (Burguad *et al.,* 2010).

Class *Dothideomycetes* comprised mostly of two yeast species i.e., *Aureobasidium* and *Hortaea* (**Table 1**). Hortaea species has been reported to be potential pathogenic form of mussel from hydrothermal vent site (Van Dover *et al*., 2007). Also, this black yeast-like fungus has been characterized as halophilic or extremely halotolerant in different studies (Gunde-Cimerman *et al.,* 2000; Kogej *et al.,* 2005). *Phaeotheca* sp.has also frequently been isolated from salt environments (Gunde-Cimerman *et al.,* 2000) and characterized as halophilic. Only one yeast species i.e., *Exophiala* belonging to *Chaetothyriomycetes* class has been reported in culture both from hydrothermal vent as well as deep-sea environment of East Indian Ocean (**Table 1**). A *Capronia*-like fungus (order *Chaetothyriales*), isolated from deep-sea hydrothermal vent elicited a host immune response in mussels and was associated with tissue deterioration (Burgaud *et al.,* 2009).

Phylum *Basidiomycota* consisted of yeast species belonging to three major classes i.e., *Cystobasidiomycetes*, *Tremellomycetes* and *Microbotryomycetes* (**Table 1**). These three classes seem to be ubiquitous in various deep-sea environments and have been reported from West Pacific Ocean, Hydrothermal vents, Anoxic sites and East Indian Ocean (**Table 1**). A small proportion of yeasts have been reported from two classes *Exobasidiomycetes* and *Ustilaginomycetes* from some deep-sea habitats (**Table 1**). Generally, basidiomycetous red yeasts have been isolated more frequently from sediments from deeper regions than those collected at depths of less than 2000 m (Nagahama *et al.,* 2001a). This may be due to a difference in the amount of organic debris in the sediments or the difference in hydrostatic pressure at the sampling points.

Rhodotorula and *Rhodosporidium* have been the predominant yeast species of the class *Cystobasidiomycetes* and *Microbotryomycetes,* respectively, reported from diverse geographic locations. *Rhodotorula*, an anamorphic genus of heterobasidiomycetous yeasts (Boekhout, 1998), is characterized by the distinctive traits such as, no ballistoconidia, no fermentation ability, no starch-like compounds, and no xylose in whole-cell hydrolyzates (Fell and Statzell-Tallman, 1998). In general, *Rhodotorula* and *Rhodosporidium* are marine yeast species and are known as potential mycoparasites and phytopathogens. The reports of *Rhodotorula* from several habitats such as deep-sea vents (Gadanho and Sampaio, 2005), deep-sea sediments (Nagahama *et al.,* 2001a), coastal waters (Gadanho *et al.,* 2003, Gadanho and Sampaio, 2004) and oligotrophic lakes (Libkind *et al.,* 2003) confirms the ubiquity of this species and indicate that this strain seem to be allochthonous. *Trichosporon* sp. from the class *Tremellomycetes* is a known pathogen or parasite of marine animals, which suggests that this may be an opportunistic pathogen or parasite of deep-sea animals.

Table 1. Details regarding yeast species reported from different deep-sea habitats using culture-dependent method (Isolation of yeasts was followed by molecular identification based on amplification and sequencing of 18S, ITS or D1/D2 region of 26 rRNA gene).

Yeast species	Class	Phyla	Habitat (~depth)	Reference
Williopsis, Candida, Debaryomyces, Kluyveromyces, Pichia	*Saccharomycetes*	*Ascomycota*	Sediment and invertebrates from deep-sea floors around the northwest Pacific Ocean (1000-11000 m)	Nagahama *et al*., 1999; 2001a; b; 2003a; b
Aureobasidium	*Dothideomycetes*			
Sarcinomyces	*Eurotiomycetes*			
Rhodotorula	*Cystobasidiomycetes*	*Basidiomycota*		
Cryptococcus, Trichosporon	*Tremellomycetes*			
Kondoa, Sporobolomyces, Sporidiobolus, Rhodosporidium	*Microbotryomycetes*			
Candida, Pichia	*Saccharomycetes*	*Ascomycota*	Deep-sea hydrothermal systems of the Mid-Atlantic Rift (800-2400 m)	Gadanho and Sampaio, 2005
Rhodosporidium	*Microbotryomycetes*	*Basidiomycota*		
Rhodotorula	*Cystobasidiomycetes*			
Exophiala	*Chaetothyriomycetes*	*Ascomycota*	Deep-sea samples of the hydrothermal vent ecosystem (700-3700 m)	Burgaud *et al*., 2009
Aureobasidium	*Dothideomycetes*			
Clavispora, Pichia	*Saccharomycetes*	*Ascomycota*	Active Deep Sea samples of Vailulu'u Seamount volcano, Samoa (700-1600 m)	Connell *et al*., 2009
Rhodotorula	*Cystobasidiomycetes*	*Basidiomycota*		
Cryptococcus, Dioszegia	*Tremellomycetes*			
Rhodosporidium, Sporidiobolus	*Microbotryomycetes*			
Hortaea, Aureobasidium	*Dothideomycetes*	*Ascomycota*	Animal and rock samples from deep-sea floors of hydrothermal vents (800-2600 m)	Le Calvez *et al*., 2009
Exophiala	*Chaetothyriomycetes*			
Candida, Debaryomyces, Pichia	*Saccharomycetes*	*Ascomycota*	Deep-sea samples from Mid-Atlantic Ridge, South Pacific Basins and East Pacific Rise of the hydrothermal vent ecosystem (700-2700 m)	Burgaud *et al*., 2010
Hortaea, Phaeotheca	*Dothideomycetes*			
Rhodotorula	*Cystobasidiomycetes*	*Basidiomycota*		
Rhodosporidium, Sporobolomyces, Leucosporidium	*Microbotryomycetes*			
Cryptococcus	*Tremellomycetes*			
Rhodotorula	*Cystobasidiomycetes*	*Basidiomycota*	Anoxic sites of the Arabian Sea (200 m)	Jebaraj *et al*., 2010
Graphiola	*Exobasidiomycetes*			
Sporisorium	*Ustilaginomycetes*			
Hortaea	*Dothideomycetes*	*Ascomycota*	Deep-sea sediment samples of Central Indian Ocean (4000-5500 m)	Singh *et al*., 2010; 2012a
Coniosporium	*Eurotiomycetes*			
Rhodotorula	*Cystobasidiomycetes*	*Basidiomycota*		
Sporidiobolus, Rhodosporidium	*Microbotryomycetes*			
Cryptococcus	*Tremellomycetes*			
Graphiola	*Exobasidiomycetes*			
Exophiala	*Chaetothyriomycetes*	*Ascomycota*	Deep-sea sediment samples of East Indian Ocean (4500-4800 m)	Zhang *et al*., 2014
Aureobasidium	*Dothideomycetes*			
Cryptococcus	*Tremellomycetes*	*Basidiomycota*		
Rhodotorula	*Cystobasidiomycetes*			

The reports of *Rhodotorula, Graphiola* (class *Exobasidiomycetes*) and *Sporisorium* (class *Ustilaginomycetes*) yeast species from anoxic sites suggest their probable capability of anaerobic denitrification. However, a detailed analysis of the enzymes involved in the nitrate-reducing pathways of cultured fungi and their expression profiles *in-situ* may provide a better understanding of their role in the OMZ (oxygen minimum zone) of the Arabian Sea and other anoxic aquatic systems (Jebaraj *et al.,* 2010). Several basidiomycetous yeasts have also been isolated from hydrothermal mussels, and more precisely from the byssus, which is composed of filaments with a high concentration of minerals and organic matter. These yeasts may have a role in the decomposition of organic material entrapped in mussel byssi in deep-sea vents (Burgaud *et al.,* 2010). The *Cryptococcus* species belonging to the class *Tremellomycetes* has also been reported as omnipresent in deep-sea environments and is characterized as psychrotolerant in nature (Singh *et al.,* 2010).

Diversity of yeasts from deep-sea habitats by culture-independent approach

Several recent studies have reported yeast diversity from extreme environments such as hydrothermal vents (Lopez-Garcia *et al.,* 2007), anoxic environments (Jebaraj *et al.,* 2010) and deep-sea sediments (Bass *et al.,* 2007; Nagano *et al.,* 2010; Edgcomb *et al.,* 2011) using culture-independent molecular analyses. Using fungal specific primers for construction of 18S rRNA gene libraries, Bass *et al.,* (2007) showed yeasts to be the dominant forms at several locations in deep-sea sediments of the Pacific Ocean at 1,500-4,000 m depth. Yeast species of *Lodderomyces, Malassezia, Cryptococcus, Hortaea, Pichia,* and *Candida* have been reported from methane hydrate bearing deep-sea sediments collected from water depths down to -3,000 m in South China (Lai *et al.,* 2007). Collective information on yeasts reported from different deep-sea habitats using culture-independent methods is shown in **Table 2**. Diversity of yeasts comprised of the species belonging to phyla *Ascomycota* and *Basidiomycota* (**Table 2**). Within each phylum the classes recovered were found to be more or less same as that obtained using culture-dependent method (**Table 1**). Basidiomycetous yeasts have been found to be dominating in various deep-sea environments by culture-independent methods also (Takishita *et al*., 2006; Bass *et al.,* 2007).

Within phylum *Ascomycota* the classes recovered were *Saccharomycetes*, *Dothideomycetes* and *Chaetothyriomycetes*. Unlike culture-dependent method, no yeast species belonging to *Eurotiomycetes* was detected by culture-independent method (**Table 2**). Diverse yeast species have been detected within the class *Saccharomycetes* such as *Candida, Pichia, Saccharomyces, Debaryomyces, Lodderomyces, Kodamaea, Metschnikowia, Kluyveromyces, Neurospora, Galactomyces* and *Dipodascus* (**Table 2**). The recovery of *Saccharomyces* species by culture-independent method has been reported from few deep-sea habitats (Nagahama *et al.,* 2011; Singh *et al.,* 2012a). Several studies have used this species as a model organism for high pressure stress effects at physiological, genomic and proteomic level (Guerzoni *et al.,* 1999; Abe and Horikoshi, 2000; Iwahashi *et al.,* 2001; Arao *et al.,* 2005; Fernandes, 2005; Domitrovic *et al.,* 2006; Sheehan *et al.,* 2007).

The attempts to isolate this species in culture are recommended for future studies from such extreme environments which can help in stress related studies for identification of novel genes responsible for adaptation mechanism. Detection of range of yeast sequences belonging to class *Saccharomycetes*, from methane cold seeps suggests their possible role towards utilization of methane or methanol degradation mechanisms (Lai *et al.,* 2007; Nagahama *et al.,* 2011). *Saccharomycete* yeasts are also able to cope with anoxia while producing ethanol (Prior *et al.,* 1989). Recovery of *Aureobasidium, Hortaea* and *Exophiala* species both by culture-dependent as well as culture-independent methods from various deep-sea habitats, suggests their possibility to be the native forms of such extreme environments (**Table 1 and 2**).

Phylum *Basidiomycota* consisted of yeast species belonging to the classes, *Cystobasidiomycetes, Tremellomycetes, Microbotryomycetes, Exobasidiomycetes, Agaricomycetes* and *Agaricostilbomycetes* (**Table 2**). Most abundant basidiomycete yeasts species were *Rhodotorula, Rhodosporidium, Trichosporon,* etc., found as pathogenic forms of marine animals in various deep-sea habitats (**Table 2**). The sequences affiliating with *Malassezia* from the class *Exobasidiomycetes*, have been reported from a range of deep-sea habitats by culture-independent method, suggesting this species as ubiquitous and playing versatile roles in such extreme environments (**Table 2**). Members of this genus are well-known as the causative agents of skin diseases in mammals, including marine mammals, such as seals or sea lions (Guillot *et al.,* 1998; Nakagaki *et al.,* 2000; Pollock *et al.,* 2000). However, *Malassezia* sp. has not been cultured yet from deep-sea habitats (**Table 1**) and future studies targeting its recovery in culture are recommended for better understanding of host-pathogen interactions.

The yeast *Sterigmatomyces* sp. belonging to *Agaricostilbomycetes* class has been isolated previously from the marine areas at Biscayne Bay, Florida, USA (Fell, 1966). However, the occurrence of this species in deep-sea environment is scarce and has been reported in a few studies only by culture-independent method (Singh *et al*., 2012a; Zhang *et al.*, 2014). *Cryptococcus*-related phylotypes from the class *Tremellomycetes* are psychrotolerant and have been found in majority of deep sub-seafloor samples from North Pond, Hydrate Ridge, Peru Margin and Eastern Equatorial Pacific (Orsi *et al.*, 2013). Recent studies have clearly demonstrated that *Cryptococcus* was the dominant genus sequenced from sediments collected at deep methane cold seeps (Takishita *et al*., 2006; 2007).

Although a considerable diversity of yeasts has been detected by culture-independent methods, the use of different primers has been shown to impact evaluation of diversity from different habitats. Using eukaryotic specific primers, several studies have reported only a small fraction of total rDNA sequences affiliating with fungi in

Table 2. Details regarding yeast species reported from different deep-sea habitats using culture-independent method (Identification of yeasts was done by molecular amplification and sequencing of 18S or ITS region of SSU rRNA gene by eukaryotic/fungal specific primers directly from environmental samples).

Yeast species	Class	Phyla	Habitat (~depth)	Reference
Cryptococcus	*Tremellomycetes*	*Basidiomycota*	Kuroshima Knoll methane seep (640 m)	Takishita *et al*., 2006
Candida, Pichia, Saccharomyces, Debaryomyces	*Saccharomycetes*	*Ascomycota*	Deep-sea water and sediment samples from 11 different sites (250-4000 m)	Bass *et al*., 2007
Aureobasidium	*Dothideomycetes*			
Cryptococcus, Filobasidium, Trichosporon	*Tremellomycetes*	*Basidiomycota*		
Sporidiobolus, Rhodosporidium	*Microbotryomycetes*			
Rhodotorula	*Cystobasidiomycetes*			
Malassezia	*Exobasidiomycetes*			
Candida, Pichia, Lodderomyces	*Saccharomycetes*	*Ascomycota*	Methane hydrate-bearing deep-sea marine sediments in South China Sea (350-3000 m)	Lai *et al*., 2007
Hortaea	*Dothideomycetes*			
Cryptococcus	*Tremellomycetes*	*Basidiomycota*		
Malassezia	*Exobasidiomycetes*			
Aureobasidium	*Dothideomycetes*	*Ascomycota*	Lost City hydrothermal field (750-900 m)	Lopez-Garcia *et al*., 2007
Malassezia	*Exobasidiomycetes*	*Basidiomycota*		
Cryptococcus	*Tremellomycetes*	*Basidiomycota*	Oxygen-depleted sediment from a deep-sea methane cold seep of Sagami Bay, Japan (1100 m)	Takishita *et al*., 2007
Hortaea, Aureobasidium	*Dothideomycetes*	*Ascomycota*	Animal and rock samples from deep-sea floors of hydrothermal vents (800-2600 m)	Le Calvez *et al*., 2009
Exophiala	*Chaetothyriomycetes*			
Cryptococcus, Filobasidium	*Tremellomycetes*	*Basidiomycota*		
Auricularia	*Agaricomycetes*			
Kodamaea	*Saccharomycetes*	*Ascomycota*	Anoxic sites of the Arabian Sea (200 m)	Jebaraj *et al*., 2010
Rhodotorula	*Cystobasidiomycetes*	*Basidiomycota*		
Malassezia	*Exobasidiomycetes*			
Candida, Metschnikowia	*Saccharomycetes*	*Ascomycota*	Deep-sea sediment samples from five different sites off Japanese islands (1200-10,000 m)	Nagano *et al*., 2010
Cryptococcus, Trichosporon	*Tremellomycetes*	*Basidiomycota*		
Rhodotorula	*Cystobasidiomycetes*			
Cryptococcus, Trichosporon	*Tremellomycetes*	*Basidiomycota*	Deep-sea subsurface of the Peru Margin and the Peru Trench (250-420 m)	Edgcomb *et al*., 2011
Malassezia	*Exobasidiomycetes*			
Saccharomyces, Candida, Kluyveromyces, Metschnikowia	*Saccharomycetes*	*Ascomycota*	Deep-sea methane cold-seep sediments (850-1200 m)	Nagahama *et al*., 2011
Cryptococcus	*Tremellomycetes*	*Basidiomycota*		
Rhodotorula	*Cystobasidiomycetes*			
Rhodosporidium	*Microbotryomycetes*			
Malassezia	*Exobasidiomycetes*			
Candida, Pichia, Debaryomyces,	*Saccharomycetes*	*Ascomycota*	Deep-sea sediment samples of Central Indian Ocean (4000-5500 m)	Singh *et al*., 2011
Hortaea	*Dothideomycetes*			
Trichosporon	*Tremellomycetes*	*Basidiomycota*		
Malassezia	*Exobasidiomycetes*			
Rhodosporidium	*Microbotryomycetes*			
Hortaea	*Dothideomycetes*	*Ascomycota*	Deep-sea sediment samples of Central Indian Ocean (4000-5500 m)	Singh *et al*., 2012a
Saccharomyces, Debaryomyces, Neurospora	*Saccharomycetes*			
Sterigmatomyces	*Agaricostilbomycetes*	*Basidiomycota*		
Trichosporon	*Tremellomycetes*			
Rhodotorula	*Cystobasidiomycetes*			
Malassezia	*Exobasidiomycetes*			
Candida	*Saccharomycetes*	*Ascomycota*	Deep-sea sediment samples of Central Indian Ocean (4000-5500 m)	Singh *et al*., 2012b
Trichosporon	*Tremellomycetes*	*Basidiomycota*		
Rhodotorula	*Cystobasidiomycetes*			
Malassezia	*Exobasidiomycetes*			
Candida	*Saccharomycetes*	*Ascomycota*	Deep-sea sediment samples from five locations of the Pacific Ocean (5000-6900 m)	Xu *et al*., 2014
Exophiala	*Chaetothyriomycetes*			
Aureobasidium	*Dothideomycetes*			
Cryptococcus, Trichosporon	*Tremellomycetes*	*Basidiomycota*		
Malassezia	*Exobasidiomycetes*			
Auricularia	*Agaricomycetes*			
Candida, Galactomyces, Dipodascus	*Saccharomycetes*	*Ascomycota*	Deep-sea sediment samples of East Indian Ocean (4500-4800 m)	Zhang *et al*., 2014
Hortaea	*Dothideomycetes*			
Cryptococcus, Trichosporon, Guehomyces	*Tremellomycetes*	*Basidiomycota*		
Sporobolomyces	*Microbotryomycetes*			
Rhodotorula	*Cystobasidiomycetes*			
Sterigmatomyces	*Agaricostilbomycetes*			

comparison with other eukaryotic lineages (Lopez-Garcia *et al.*, 2001; Stoeck *et al.*, 2003; 2006). However, Edgcomb *et al.* (2011) found fungal forms in majority from marine deep-sea subsurface by using eukaryotic specific primers. The use of multiple primer approach for studying diversity has revealed recovery of diverse fungal forms from oxygen depleted marine environments (Jebaraj *et al.*, 2010). While studying microeukaryotic diversity in an acidic iron river, Gadanho and Sampaio (2006) reported new fungal phylotypes with fungal-specific primers that were not detected when universal eukaryotic primers were used. These studies suggested that fungal diversity could have been underestimated in such extreme environments.

In addition, designing more yeast-specific primers to identify novel species obtained by culture-independent approach is required. It is not appropriate to speculate that all the yeast diversity obtained by using DNA amplification represent viable forms because low temperature conditions of deep-sea environment will favor preservation of DNA. Thus, it is possible that only a fraction of the uncultured yeasts will be viable. Culture-independent approach provides valuable indication of the presence of the organism/community, and in turn information gained could be utilized for their recovery in culturable form using targeted culturing methods. The methods to isolate the yet-to-be-cultured yeasts from deep-sea sediments such as improvement of isolation media, incubation techniques should be evolved so that rare and slow growing forms could be obtained. Getting insight on true yeast diversity in this extreme ecosystem may have enormous potential in the development of new products such as pharmaceuticals, molecular probes, enzymes, cosmetics and nutritional supplements (Synnes, 2007).

Novel yeast species reported from deep-sea environments

Various studies have reported isolation of yeast species from different deep-sea habitats and characterized them as new (**Table 3**). The characterization of these yeasts as novel species has been based on utilization of carbon sources, growth characteristics and molecular phylogenetic analysis of their 18S, ITS or D1/D2 region of rRNA gene (Nagahama *et al.,* 1999; 2001b; 2003a; b; 2006; 2008, Burgaud *et al.,* 2011). The novel yeast species of *Rhodotorula, Cryptococcus,* etc., showing divergent characteristics from their existing terrestrial counterparts suggests them as the specifically adapted strains for extreme conditions of deep-sea, rendering them distinct. Further physiological studies on such strains may elucidate the pathways involved in stress survival mechanism of deep-sea organisms. Recently, culture-independent approach has revealed a number of yeast phylotypes forming highly divergent clades from known sequences of the existing database (Bass *et al.,* 2007; Xu *et al.,* 2014). Singh *et al.* (2012b) reported recovery of uncultured *Malassezia* clone sequences from deep-sea sediments of Central Indian Ocean exhibiting 96% similarity with the existing database sequence. These reports indicate the probability of deep-sea environment being harbor of various novel yet-to-be cultured yeasts possessing tremendous potential in comparison with their terrestrial counterparts. However, significant inputs are required in order to culture these yet-uncultured yeast signatures in order to get deeper insight on their versatile role in ecological cycles of deep-sea habitats.

Physiology and applications of yeasts isolated from various deep-sea habitats

Pressures and temperatures of different magnitudes exert different effects on organisms. In general, elevated hydrostatic pressure in the range of several dozen MPa, have been found to be nonlethal, but may affect adversely the organisms which are adapted to atmospheric pressure (Abe *et al.,* 1999; Bartlett, 2002; Abe, 2004). Elevated pressure either inhibits or favors those biochemical processes which occur with an expansion or reduction in system volume, respectively (Somero, 1992). Likewise, mechanisms associated with low-temperature growth of microorganisms involve alterations in cellular membrane fluidity, uptake or synthesis of compatible solutes, structures of macromolecules such as ribosomes and

Table 3. Novel yeast species reported from various deep-sea habitats.

Novel yeast species	Habitat	Reference
Kluyveromyces nonferrnentans	Deep-sea samples of Suruga Bay and Sagami Bay, Japan	Nagahama *et al.*, 1999
Rhodotorula lamellibrachii	Sagami Bay, Japan	Nagahama *et al.*, 2001b
Rhodotorula benthica, Rhodotorula calyptogenae	Tubeworm and the giant white clam, collected from the deep-sea floor of the Pacific Ocean off Japan	Nagahama *et al.*, 2003a
Cryptococcus surugaensis	Deep-sea floor of Suruga Bay	Nagahama *et al.*, 2003b
Rhodotorula pacifica	Deep-sea floor of the north-west Pacific Ocean	Nagahama *et al.*, 2006
Dipodascus tetrasporeus	North-western Pacific Ocean	Nagahama *et al.*, 2008
Candida oceani	Deep-sea hydrothermal fields on the Mid-Atlantic Ridge	Burgaud *et al.*, 2011

protein synthesis mechanisms (Wemekamp-Kamphuis *et al.,* 2002). The growth of mesophilic microorganisms have been shown to be inhibited at an elevated pressure of 40-50 MPa. The cessation of growth is accompanied by morphological changes such as formation of filaments in *Escherichia coli* (ZoBell, 1970) and cell chains or pseudomycelia in the marine yeast *Rhodosporidium sphaerocarpum* (Lorenz and Molitoris, 1992; Lorenz, 1993). Much higher pressures, in the range of 100 MPa may also be used for sterilization purposes (Sonoike *et al.,* 1992; Takahashi, 1992). These effects depend not only on the magnitude but also on the duration of pressure applied in combination with temperature, pH, oxygen supply and composition of culture media (Abe, 2007). Both low temperature and elevated pressure inhibit an early step of translation (Schwarz and Landau, 1972; Broeze *et al.,* 1978). The cold shock response has been suggested to be an adaptive response to facilitate the expression of genes involved in translation initiation (Jones *et al.,* 1987). Also, elevated pressure, similar to low temperature incubation, results in the continued synthesis of stringently controlled proteins, involved in transcription and translation despite the growth rate decrease and this behavior also suggests decreased translation capacity (Welch *et al.,* 1993). Finally, both low temperature and elevated pressure decrease membrane fluidity (Chong *et al.,* 1981; MacDonald, 1984), which perturbs a variety of membrane associated processes, including transmembrane ion and nutrient flux, and DNA replication.

In comparison to bacteria, only a few studies have reported the effect of elevated hydrostatic pressure and low temperature conditions on growth patterns of fungi and yeasts. Among yeasts, *Saccharomyces cerevisiae* is a facultative anaerobe and has been used in various studies for analyzing the effects of elevated hydrostatic pressure and low temperature. Being a facultative anaerobe, it has been proved as most suitable organism to cope up with the closed pressure vessel condition where the oxygen concentration is very low during incubations (Abe, 2007). The genome of this yeast encodes approximately 6000 genes out of which more than 4800 are non-essential ones. Recent large scale phenotypic screening of the *S. cerevisiae* gene-deletion library has revealed numerous unexpected genes and metabolic pathways that are involved in tolerance of environmental stresses (Martin and Drubin, 2003; Chasse and Dohlman, 2004). Analyses of genome-wide gene expression profiles of this yeast were done showing no growth at elevated pressure and low temperature conditions. However, after being recovered from the stress conditions the genes responsible for transcription of energy metabolism, cell defense and protein metabolism have been found to be highly upregulated (lwahashi *et al.,* 2003). Also, the survival of *S. cerevisiae* at elevated pressures has been shown to be enhanced by preceding heat-shock treatment (Iwahashi *et al.,* 1991). The heat shock treatments confer enhanced synthesis of heat shock proteins (Hsps) and metabolism of trehalose in this yeast (Singer and Lindquist, 1998). Among many Hsps, Hsp104 plays an essential role in acquired tolerance by unfolding denatured intracellular proteins in an ATP-dependent manner (Sanchez and Lindquist, 1990).

In addition, in *S. cerevisiae,* the rate of synthesis of several other proteins, e.g., ubiquitin, some glycolytic enzymes, and a plasma membrane protein, is strongly enhanced upon exposure of cells to stress. A plasmid carrying the *TAT2* gene, which encodes a high affinity tryptophan permease, enabled the cells of *S. cerevisiae* to grow under conditions of pressure in the range of 15 to 25 MPa. Additionally, cells expressing the Tat2 protein at high levels became endowed with the ability to grow under low-temperature conditions at 10 or 15 °C as well as at high pressure (Abe and Horikoshi, 2000). The ability of S. cerevisiae to grow under different pressures may be extended up to >50 MPa, regardless of the amino acid auxotrophy of the strain (Abe and Horikoshi, 2000). Abe and Horikoshi (1995) found that hydrostatic pressures of 40-60 MPa promoted acidification of the vacuoles in yeast cells and that expression of the tryptophan permease i.e. *TAT2* gene can be the rate limiting factor affecting the growth of tryptophan requiring yeast cells.

The growth characteristics of deep-sea fungi and yeast isolates under simulated deep-sea conditions have been assessed in several studies (Raghukumar and Raghukumar, 1998; Damare *et al.,* 2006; Damare and Raghukumar, 2008; Singh *et al.,* 2010). The marine yeasts *Rhodotorula rubra* and *Rhodosporidium sphaerocarpum* have been demonstrated to grow at 40 MPa, corresponding to the pressure experienced at depths of 4,000 m (Lorenz and Molitoris, 1997). The yeast NIOCC#PY13 (*Cryptococcus* sp.) showed comparatively higher growth at 5 °C than the other mesophilic counterparts suggesting its psychrotolerant nature and therefore better adaptation to grow at lower temperatures (Singh *et al.,* 2010). This yeast was exposed to elevated hydrostatic pressure and low temperature to explore the differentially expressed genes responsible for its survival at such extreme conditions by using the technique of suppression subtractive hybridization. The differentially expressed genes were found to be similar to the NCBI database ESTs (expressed sequence tags), coding for proteins such as arachidonic acid metabolism, amino acid transport and unsaturation of membrane fatty acids, which have been demonstrated previously to assist in the survival of microorganisms under stress conditions (Singh *et al.,* 2012c). Most of the yeasts reported from deep-sea hydrothermal vents have been reported as halophilic or halotolerant (Burgaud *et al.,* 2010). One of these halophilic yeasts was identified and characterized as *Hortaea werneckii* in the physiological studies of Burgaud *et al.* (2010). This black yeast-like fungus was characterized as halophilic or extremely halotolerant previously in different studies (Gunde-Cimerman *et al.,* 2000; Kogej *et al.,* 2005) where it has frequently been isolated from hypersaline waters of solar salterns.

An outline describing yeasts isolated from various deep-sea habitats and their applications has been represented in **Table 4**. One of the yeast strains, N6 belonging to *Cryptococcus liquefaciens*, isolated from deep-sea sediments of the Japan Trench was demonstrated to tolerate very high concentrations of Cu, which was triggered by production of an antioxidant enzyme, superoxide dismutase, as a defensive mechanism (Abe *et al.,* 2001; Teh *et al.,* 2008). Recently, another species of

Table 4. Details regarding biotechnological/industrial applications of yeasts isolated from different deep-sea environments.

Source of isolation	Habitat (~depth)	Yeast species	Application	Reference
Sediment samples	Japan Trench (6500 m)	*Cryptococcus liquefaciens* strain N6	Superoxide dismutase enzyme	Abe *et al*., 2001
Sediment samples	Japan Trench (6500 m)	*Cryptococcus liquefaciens* strain N6	Polygalacturonase enzyme p36 and p40 (N6-PGases)	Abe *et al*., 2006
Mud samples	Sagami bay, Japan (1100-6500 m)	*Kondoa, Cryptococcus, Rhodotorula, Rhodosporidium, Candida, Kluyveromyces, Metschnikowia, Aureobasidium and Williopsis*	Polygalacturonase enzyme production, capable of pectin degradation	Minegishi *et al*., 2006
Sediment samples	Japan Trench (6500 m)	*Cryptococcus liquefaciens* strain N6	Cu/Zn superoxide dismutase enzyme	Teh *et al*., 2008
Deep-sea cold-seep clam	Sagami bay, Japan (1156 m)	*Pseudozyma hubeiensis*	Glycolipid biosurfactants production	Konishi *et al*., 2010
Sediment samples	Central Indian Ocean (5000 m)	*Cryptococcus* sp. (NIOCC#PY13)	Heavy metal tolerance towards metal salts of Zn, Cu, Pb and Cd	Singh *et al*., 2013

psychrotolerant yeast *Cryptococcus,* isolated from deep-sea sediments of Central Indian Ocean was demonstrated to tolerate considerable concentrations of four heavy metal salts, i.e., $ZnSO_4$, $CuSO_4$, $Pb(CH_3COO)_2$ and $CdCl_2$ (Singh *et al.,* 2013). The polymetallic nodules reported in the Central Indian Basin are rich in Ba, Co, Cu, Fe, Mg and Mn (Nath *et al.,* 1989). The recovery of yeasts from this area suggests their probable capability of tolerance to metals. Connell *et al.* (2009) have reported metal tolerant yeasts producing siderophores from deep-sea basalt rocks in Vailulu'u Seamount, Samoa. These findings indicate active role of fungi in biogeochemical cycles in the deep sea. The greater capacity of deep-sea isolates towards metal tolerance abilities may be attributed to their competance to survive under extreme conditions by expression of genes involved in combating these stress mechanisms (Abe and Minegishi, 2008; Singh *et al.,* 2013). Since the exposure to heavy metals also imposes similar stress conditions in microorganisms, deep-sea microbes for the metal tolerance studies may prove highly effective for the bioremediation of metal-contaminated sites.

Recently, deep-sea yeasts have emerged as significant sources of pectin-degrading enzymes (Abe *et al.,* 2006; Minegishi *et al.,* 2006). Pectic compounds are polysaccharides with α-1,4-glycoside linkage of polymers of galacturonic acids, having plant origin. Fungal polygalacturonases (PGases) are used mainly in food industries, especially for extraction and clarification of fruit juices. However, commercial preparations of PGases from fungi have been a challenging task due to their complex nature being a mixture of endo-, exo-PGase, pectin lyase and other nonspecific enzymes. The PGases from yeasts are comparatively simpler in profile and offer advantages over filamentous fungal PGases. Additionally, deep-sea yeasts having capability of tolerating extreme environmental conditions might be an excellent source of such industrially useful enzymes with versatile properties. For example, PGAses purified from the deep-sea yeast *Cryptococcus liquefaciens* strain N6, remain almost unchanged up to a hydrostatic pressure of 100 MPa at 24 °C (Abe *et al*., 2006), suggesting its application in the reactions accompanying high pressures. Recently, one yeast species belonging to *Pseudozyma*, isolated from deep-sea cold-seep clam at Sagami Bay, Japan has been demonstrated to produce novel form of Mannosylerythritol lipids (MELs). MELs are one of the most promising biosurfactants (BSs) known to date and are abundantly produced from vegetable oils and saccharides by yeast and fungal strains. The isolation of deep-sea yeasts producing novel varieties of such bioactive compounds may have a great impact on the developments of biotechnological industries.

CONCLUSIONS AND FUTURE DIRECTIONS

In conclusion, deep-sea habitats may harbor diverse yeast taxa that are tolerant to low temperature and elevated hydrostatic pressure which may lead to discovery of new metabolites or enzymes. However, the diversity studies of yeasts are scarce from deep-sea environments compared to filamentous fungi and their terrestrial counterparts. Lately, a few studies have attempted isolation of yeasts from sediments, water and fauna of extreme environments such as hydrothermal vents, cold seeps, anoxic sites and other geographic locations. These diversity studies have applied culture-dependent and culture-independent methods for assessment of yeast signatures. Culture-independent methods include molecular tools enabling direct detection of yeast communities from such environments and therefore provide a broad range of information regarding diversity patterns. The yeast diversity has been found to be restricted to species belonging majorly to phylum *Ascomycota* and *Basidiomycota* both by culture-dependent and culture-independent methods. Despite detection of diverse species of yeasts by culture-independent approach, most of them have not been isolated yet in culture. Isolation of yeast species in-culture, inhabiting extreme conditions of deep-sea may provide greater insight on their adaptation mechanisms. The methods to isolate the yet-to-be-cultured yeasts from deep-sea sediments such as improvement of isolation media, incubation techniques are recommended for future studies so that rare and slow growing forms could be obtained. In addition, getting insight on true yeast diversity in this extreme ecosystem may have enormous potential in the development of new biotechnologically

active products. Recently, new species of yeasts have been reported from deep-sea habitats which are highly divergent from their terrestrial counterparts. Further analysis of adaptation mechanisms in these novel forms may elucidate the ecological role played by yeasts in such extreme environments. Physiological studies of deep-sea yeasts have identified their halo-, psychro- and metal tolerant properties over wide range of salinities temperatures and metal salts. Exposure to these extreme conditions elicits expression of specific genes in deep-sea yeast strains, resulting into production of various industrially significant secondary metabolites. Detailed studies of the above genes/proteins from deep-sea yeasts are suggested for future investigations, which may shed more light on the existence and adaptation mechanisms adopted for their survival under such extreme conditions. Also, being a reservoir of yeasts producing industrially useful products, deep-sea offers a solid platform for succeeding such research outcomes.

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