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Bioprospecting Marine Fungal Enzymes-Scope and Challenges

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ABSTRACT

Marine microorganisms are promising source of enzymes with industrial applications due to their immense genetic and biochemical diversity. Availability of novel enzymes, advancements in enzymology and enzyme technology have significantly contributed to the industrial application of enzymes and the rapid expansion of the enzyme market. In this context marine fungal enzymes assume greater attention recently owing to the great demand for novel and efficient biocatalysts for industrial applications and other services. This situation has warranted exploration of marine fungal biodiversity for new enzymes. The present review focus on bioprospecting of marine fungal enzymes produced by lesser studied fungi, identification of research gaps, challenges in pursuing research in harnessing the potentials of marine fungi, and the scope for future prospects. Role of fungal enzymes in biogeochemical processes in marine environments, bioremediation, and plastic degradation is discussed indicating marine fungi as source of industrial enzymes. Scope for exploring marine fungal diversity and potentials of extremozymes, cold adaptive enzymes and halophilic fungal enzymes, besides the need for bioprocess development are discussed. Moreover, the challenges lying ahead in pursuing research on marine fungi are also discussed to draw the attention of mycologists and biotechnologists to appropriately harness the marine fungi.

Keywords: Marine fungal enzymes, Marine fungal diversity, Potential applications, Prospects

INTRODUCTION

Enzymes are biocatalysts that play key role in several industries *viz*., pharmaceuticals, food and feed, textiles, detergents, leather processing, paper and pulp processing, besides biotechnological research and development. Industrial enzymes contribute to significant cost reductions, reduced energy consumptions, in addition to improved substrate activity, product selectivity and lower physiological and environmental toxicity. Hence, there is a great demand for novel and efficient biocatalysts with varied substrate selectivity, chiral selectivity, stability and activity at different pH and temperature, and this warrants exploration of biodiversity for new and novel enzymes. Seas and oceans cover more than 70 % of the Earth's surface and harbour a vast biodiversity including 'marine microbiome' that hold immense potential for bioprospecting the economically valuable biomolecules and products. It is estimated that the discovery and recognition of new marine-derived enzymes and new applications may push the global market for marine biotechnology to $6.4 billion by 2025 (https://www.smithers.com/resources/2015/oct/global- market-for-marine-biotechnology accessed on 18th February 2023).

Microorganisms hold the largest market-share in enzyme market owing to availability, ease in large scale production, purification, and continued supply to industries. Enzymes have been screened from fungi and bacteria over a long time, ever since their industrial application was recognized. Further, salinity, high pressure, low temperature, oligotrophic conditions, pH extremes, widely ranging minerals in seawater, and special lighting conditions contribute to differences between the enzymes, produced by marine microorganisms and homologous enzymes obtained from terrestrial microorganisms (Passarini *et al*., 2013; Rämä *et al*., 2014). Due to their immense genetic and biochemical diversity, marine microorganisms are viewed as a promising source of enzymes with novel applications (Zhang and Kim, 2012). The literature on availability of novel enzymes, essentials of enzymology, and enzyme technology has significantly contributed to the industrial application of enzymes and the rapid expansion of the enzyme market. Indeed, fungal enzymes have contributed enormously to industrial bioprocesses. Velmurugan and Lee (2012) and Bonugli-Santos *et al*. (2015) have discussed marine derived fungal enzymes and their biotechnological applications. In this context, the present review focus on bioprospecting of marine fungal enzymes produced by lesser studied fungi, identification of research gaps, challenges in pursuing research in harnessing the potential of marine fungi, and the scope for future prospects.

GLOBAL ENZYME MARKET AND ENZYME INDUSTRIES

The global enzymes market was valued at $5.8 billion in 2021 and is projected to reach $10. Billion by 2031, growing at a CAGR of 6% from 2022 to 2031 (https://www.alliedmarketresearch.com/enzymes-market accessed on 1st February 23) indicating huge potential for application of enzymes in various industries and consequent great demand in the future. It is speculated that enzymes, as biocatalysts, will soon substitute almost 40% of the industrially relevant chemical reactions that require organic solvents that are harmful to the environment by 2030 (Martinez-Martinez *et al*., 2018).

The market size of global enzymes is segmented purely based on factors that include source, reaction type, application, and type. Based on reaction type, the enzymes are classified into hydrolases, oxidoreductase, transferases, lyase and others. By type, the market is dominated by protease, carbohydrase, lipase, polymerase, nuclease, and others. With respect to applications, food and beverages, feed, pharmaceuticals, confectionery, bioenergy, textile, leather processing, paper, and pulp industries, besides waste-water treatment are the primary consumers of enzymes. The enzymes of large-scale industrial applications include protease, cellulases, lipase, amylases, glucoamylase, β-glucosidase, carboxy methyl cellulases, xylanase, chitinase, lignin degrading enzymes, tannase, pectinase, inulinase, L-glutaminase, and L-asparginase.

The demand for industrial enzymes has led to proliferation of several companies that manufacture enzymes in large scale across the globe. Some of the leading enzyme manufacturers at global enzyme market include Novozymes, DuPont Danisco, Amway, BASF enzymes LLC**,** DSM, Amano, Nutritech, AB Enzymes, Roche, and Aum Enzymes. Indian enzyme market is catered by Enzyme Bioscience (P) Ltd (India), Infinita Biotech Private Limited, Lumis Biotech, Noor Enzymes, Nature BioScience, ProteoZymes, Capri Enzymes, Ultreze Enzymes Private Limited, Advance Enzyme Technologies Ltd., Antozyme Biotech Pvt. Ltd, and YSS Industrial Enzyme among the large number of producers.

MARINE FUNGAL DIVERSITY

Marine environments include neritic (coastal), oceanic (open-ocean water), benthic (sediments in continental shelf, tidal and intertidal zones), pelagic (hydrostatic water column) deep-sea floor and their subsurface), estuaries, backwaters, mangroves, and coral reefs. Further, Polar Regions Arctic and Antarctic which are extremely cold, temperate, and tropical waters are also characteristic marine environments. These aquatic saline environments harbor neustons, sestons, phytoplankton, zooplankton, nektons, marine algae, marine plants, marine animals, and a wide range of microorganisms which constitute the core marine biodiversity. Among them, microorganisms particularly Archaebacteria, heterotrophic eubacteria, and fungi play a key role in important biogeochemical processes involving cycling of elements and nutrients which determine the primary productivity and fertility of seas and oceans. These natural processes of cycling of elements are very much mediated and facilitated by the exoenzymes produced by marine microorganisms.

After the discovery of antibiotics, bioactive molecules and enzymes from marine bacteria and some marine fungi, the scientific community and pharmaceutical and food industries are recently very keen to harness marine biodiversity towards deriving potential biomolecules with bioactivity against human pathogens and novel enzymes for varied applications. Hence, there is an intensive drive by international research community to apply the latest biotechnologies and bioinformatic tools, molecular genetics, genetic engineering, genomics, proteomics, metagenomics, transcriptomics *etc*., to recognize potential genes in both cultivable and non-cultivable microorganisms that hold immense promise to return economically valuable products of commerce and services.

Among different microorganisms, bacteria and actinomycetes isolated from marine environments are known as potential producers of bioactive molecules and enzymes over the past 50 years. Similarly, fungi are known as morphologically, phylogenetically, and functionally diverse components of the marine ecosystems particularly the water column. In fact, literature available on the occurrence and distribution of fungi inhabiting marine environments, including mangroves are rather very limited (Kalaiselvam, 2015). Compared to other groups, the marine fungi remain under-studied although they are found in almost all sorts of marine ecosystems including oceans, sediments, mangroves, coastal environments, and terrestrial environments including plants, air, animals, and human beings. Nevertheless, marine fungi remain one of the most under-studied microbial groups, with 95% of the ocean remaining mycologically unexplored (Walker *et al*., 2017; Amend *et al*., 2019). Consequently, the abundance, diversity, ecological roles, and interactions of marine fungi with other plankton remain mostly speculative and our current understanding of marine fungi, particularly planktonic fungi remain limited.

Marine fungi exist as freely floating entities or associate with sunken wood pieces, rocks, sand, plants, animals *etc.* Often, they are referred to as two distinct groups, *i.e*., facultative marine fungi and obligate marine fungi, based on their mode of growth and sporulation characteristics. The facultative marine fungi have the potential to grow and reproduce both in marine and terrestrial environments while the obligate fungi require specific marine conditions (Kohlmeyer and Volkmann-Kohlmeyer, 2003; Li and Wang, 2009). The term 'marine-derived fungi' is also often used since most of the fungi isolated from marine samples are not demonstrably classified as obligate or facultative marine microorganisms (Osterhage,

2001) and Pang *et al*. (2016) defined that 'A marine fungus is any fungus that is recovered repeatedly from marine habitats and: 1) is able to grow and/or sporulate (on a substrate) in marine environments; 2) forms symbiotic relationships with other marine organisms; or 3) is shown to adapt and evolve at the genetic level or be metabolically active in marine environments'. Parte *et al*. (2017), indicated that there are almost 5.1 million fungal species on earth and out of which, more than 1500 species belong to the marine environments.

Occurrence of marine fungi in different substrates such as sponges, algae, wood, tunicates, sediments, molluscs, corals, plants, fish besides water and sediments, and the ecology and phylogeny of this group are summarized by Jones *et al.* (2009; 2011), Jones and Pang (2012), and Richards *et al.* (2012). The global marine mycobiome is generally predominated by Ascomycota, Basidiomycota, and Chytridiomycota. Particularly, the coastal and open-ocean fungal communities show the dominance of ascomycetous classes, such as Sordariomycetes, Eurotiomycetes, Dothideomycetes, Saccharomycetes, and Pezizomycetes confirming previous culture-based studies, which have reported the prevalence of members of classes Dothideomycetes and Sordariomycetes in mangroves and coastal waters (Jones and Pang, 2012).

A high molecular diversity of fungi, with the predominance similar to Dikarya, including several unidentified and potentially novel species, isolated from the open-ocean water column, identical to that of the coastal water column has been reported for the first time in waters of the open-ocean transect from the Hawaiian coast to Australia (Wang *et al*., 2014).

The first inventory of cultured marine fungi described 209 species of higher filamentous fungi, 177 species of marine-occurring yeasts, and less than 100 species of the lower marine fungi (Kohlmeyer and Kohlmeyer, 1979). Later, reports over the years indicated occurrence of 467 (Kis-Papo, 2005), 530 (Jones *et al*., 2009), 1112 (Jones *et al*., 2015) and 1257 (Jones *et al*., 2019) species of marine fungi. Jones *et al*., (2015) updated classification of marine fungi and recorded 1,112 species, 472 genera, 129 families, and 65 orders. Major phyla include Ascomycota, Basidiomycota, Chytridiomycota (chytrids) and related phyla, Zygomycota, and Blastocladiomycota. The Halosphaeriaceae remains the largest family of marine fungi with 141 species in 59 genera, while the most specious genera are *Candida* (64 species), *Aspergillus* (47 species), and *Penicillium* (39 species) (Jones *et al*., 2015). Further update on their phylogeny led to the grouping of them into evolved branches (Ascomycota, Basidiomycota, Blastocladiomycota, and Chytridiomycota) and basal lineages (Cryptomycota, Microsporidia, and Aphelida) (Pang and Jones, 2016). Currently, about 1900 marine fungal species, distributed across seven phyla (Aphelidiomycota, Ascomycota, Basidiomycota, Blastocladiomycota, Chytridiomycota, Mucoromycota, and Microsporidia), 22 classes, 88 orders, 226 families, and 769 genera, are documented (www.marinefungi.org, accessed on 15th February 2023). The documented number (ca.1900 species) is much less than the estimated 10,000 species (Kis-Papo, 2005), which suggests that the oceans harbour a high fungal diversity, which is yet to be fully described. These discussions on marine fungal diversity testify the immense potential for possible exploration and harnessing of marine fungi for useful biomolecules and novel enzymes in future.

ECOLOGICAL FUNCTIONS IN MARINE ECOSYSTEMS INVOLVING MARINE FUNGAL ENZYMES

It is important to understand and appreciate the ecological functions of fungi in ecosystem which are catalyzed by their extracellular enzymes. Indeed, appreciation of natural processes, discussed below, that happen in marine environment would enable better understanding of the role of fungi in maintenance of ecosystem and possibility of deriving novel enzymes towards bioprospecting of marine fungal enzymes.

Biogeochemical and ecological functions of fungi

Marine microorganisms play a pivotal role in biogeochemical processes in marine environment (Sowell *et al*., 2008) in spite of the fact that their communities are very much influenced by a plethora of environmental factors that include salinity, temperature, nutrients, and dissolved oxygen. In the nutrient-rich coastal water column, there is increasing evidence for the contribution of marine fungi to biogeochemical cycling and food web dynamics on account of their saprotrophic, parasitic, hyper-parasitic, and pathogenic attributes (Sen *et al*., 2022). Fungi in the transition zones of salt marshes and mangroves are also found to play the roles of saprobes, symbionts, pathogens, and parasites, similar to their terrestrial counter parts (Jones *et al*., 2019). Two important fungal feeding strategies (*i.e.*, osmotrophy and attachment to the substrate) to the role of marine fungi as decomposers, parasites, and denitrifiers (Richards *et al*., 2012; Zhang *et al*., 2015) although studies on their ecological roles towards providing direct evidence are rather very few and thus, fungi are often neglected in the ocean ecosystem models (Wang *et al.*, 2014; Worden *et al.*, 2015). The decomposer role of fungi in aquatic ecosystems is mainly known from lotic systems, mangroves, and wetlands (Gulis *et al*., 2006; Gessner *et al*., 2007; Grossart *et al*., 2019). In fact, the frequent isolation of marine fungi from floating, sunken woody substrates, and plant detritus (Raghukumar, 2004a; Jones, 2011) indicates such a role in coastal and pelagic ecosystems. Consequently, it is speculated that fungi very much contribute to organic matter degradation in the deep sea by virtue of their dominance in the overall biomass within marine aggregates (Bochdansky *et al*., 2017). Further, these reports indicate the role of planktonic fungi in the formation and stabilization of the marine aggregates and their simultaneous degradation to dissolved organic matter (DOM). Interestingly, such a contribution highlights their possible link to the POM (particulate organic matter)-DOM cycling in the ocean. Thus, fungi play a much more important role in biological carbon pumps or ocean carbon storage than what is currently perceived. Further, applications of molecular approaches have revealed far more diverse and abundant marine fungi than those previously studied, with a growing body of evidence for their biogeochemical and ecological functions (Sen *et al*., 2022) owing to their ability to secrete a wide range of hydrolytic enzymes that catalyze bioconversions and mineralization.

Role of fungi in Bioremediation

Many fungi are known to be capable of degrading persistent pollutants including textile dyes (Haritash and Kaushik, 2009). Fungi from marine environments are adapted to high salt and pH and hence, efficiently bring about decolorization/degradation of textile effluent that has saline and alkaline conditions. Role of marine-derived fungi in significant decolorization of textile effluents and synthetic dyes (e.g., Congo red, Brilliant green, and RBBR) is demonstrated (D'Souza *et al*., 2006; Raghukumar *et al*., 2008). Marine-derived fungi could catalyze bioremediation of polluted saline environments by virtue of their tolerance to saline conditions and consequently these fungi have assumed importance as potential resources for application in the bioremediation of PAH-polluted environments, such as in ocean and marine sediments. Salt-tolerant fungi and their salt-tolerant enzymes (mainly lignin-degrading enzymes) have been used for bioremediation of environmental pollutants (Passarini *et al*., 2011).

Several investigators have endorsed that marine fungi have the potential to degrade lignocellulosic components using their hydrolytic enzymes, such as laccase, cellulase, α-amylase, alginase, laminarinase, peroxidase, pectinase, and xylanase based on their *in vitro* studies (Kamei *et al*., 2008; Bonugli-Santos *et al*., 2010; Arfi *et al*., 2013; Wang *et al*., 2016; Mainardi *et al.*, 2018). Marine ascomycetes and basidiomycetes (*Nia vibrissa)* are reported to solubilize significant amounts of lignin from wood *in vitro* employing hydrolytic enzymes (Bucher *et al*., 2004; Jones and Choeyklin, 2008).

Plastic deterioration by marine fungi

Reports on plastic deterioration by marine fungi have indicated that polyurethanes are more susceptible to fungal attacks (Jones and Le Campion-Alsumard, 1970). *Aspergillus flavus, A. terreus, A. niger, A. fumigatus,* and *Penicillium* sp. isolated from seawater are recognized to be potential degraders of polyethylene (Alshehrei, 2017). The fungi have the potential to break down the chemical bonds of plastic polymers and degrade marine plastics using their enzymes (Zeghal *et al*., 2021). Thus, investigations have highlighted the underestimated role of planktonic fungi as degraders of marine plastic wastes. Further, marine fungi contribute to the process of microbial carbon sequestration in the ocean owing to their unique ability to produce a myriad of hydrolytic enzymes and the colonization of lignocellulosic substrates, algal biopolymers, marine snow, and plastics. Their role in long-term carbon sequestration, however, needs confirmation through further studies.

MARINE FUNGI AS SOURCE OF ENZYMES AND SCOPE FOR POTENTIAL APPLICATIONS

Based on the application, marine fungal enzymes are considered in different segments that include: (i) technical enzymes, employed in cleaning, textile, leather, biofuel, pulp, and paper industries; (ii) enzymes used in the manufacture of food and beverages and animal feed; (iii) enzymes used in environmental applications; and (v) enzymes with pharmaceutical and cosmetic applications.

Fungi as source of enzymes

Marine environment has become the focus of attention as source of enzymes for over 40 years and studies on marine fungi have been reported more frequently since 1990s. In fact, marine bacteria and fungi are routinely screened for potential industrial enzymes including chitinase, amylase, cellulases, β-glucosidase, laccase, tannase, alkaline protease, lipase, lactase, and nucleases, besides several other enzymes of economic importance (Velmurugan and Lee, 2012). Fungi isolated from different substrates in marine environments, *viz.*, invertebrates, decaying wood, seawater, sediments, and mangrove detritus, are reported to be the producers of hydrolytic and/or oxidative enzymes such as alginate lyase, amylase, cellulase, chitinase, glucosidase, inulinase, keratinase, ligninase, lipase, nuclease, phytase, protease, and xylanase (Bonugli-Santos *et al*., 2015).

The *Aspergillus* sp. and *Penicillium* sp. are recovered from marine invertebrates, seawater, deep sediments, and mangrove detritus and different species of both genera are cited as marine-derived producers of enzymes. These fungi are salt-tolerant and have been reported in the literature as invertebrate-inhabiting fungi (Da Silva *et al*., 2008; Baker *et al*., 2009; Menezes *et al*., 2010).

Alkaline xylanases and thermostable metal-tolerant laccases are produced by marine-derived strains of *Aspergillus niger* and *Cerrena unicolor* (Raghukumar *et al*., 2004b; D'Souza-Ticlo *et al*., 2009). Marine *Aspergillus oryzae* isolated from the brown alga *Dictyota dichotoma* produced an extracellular alginate lyase (EC 4.2.2) that specifically cleaves sodium alginate that results in the formation of homo polymeric blocks of polyM and polyG which may have potential use in the biomedical industry (Singh *et al*., 2011).

Extremozymes

Marine extremophiles have recently attracted a great deal of interest since they produce many economically valuable enzymes (extremozymes). In fact, several factors including competition for space and nutrients in the marine environment led to the evolution and generation of multiple enzyme systems to adapt to different environments. Thus, marine extremophiles are not only capable of surviving extreme conditions but also are ideal source of enzymes with special characteristics. Consequently, they assume great significance as biocatalysts for application in industrial processes (Rothschild and Mancinelli, 2001; Chandrasekaran and Kumar, 2010). Further, marine microbes have significantly contributed to the development of several new hydrolases such as proteases, lipases, glycoside hydrolases with novel characteristics including tolerance to extreme conditions used in industrial processes (Fulzele *et al*., 2011; Samuel *et al*., 2012).

Marine extremozymes are active at the harsh pH, temperature, and pressure conditions typical of many industrial environments and hence have potential for application in several industrial sectors such as the agricultural, chemical, food, textile, pharmaceutical, bioenergy, and cosmetic fields, besides deriving biofuel (ethanol, diesel, hydrogen) and application in bioremediation of polluted sites in consequence of accidental oil spills or release of contaminants by industrial activities (Di Donato *et al*., 2019).

Cold-active microbial enzymes

Cold-active microbial enzymes have attracted increasing attention in recent years (Wang *et al*., 2012) since it is advantageous to use the cold-active enzymes owing to the reduced energy expenditure and processing costs associated with industrial heating steps besides the properties related to their structural characteristics (Duarte *et al*., 2013). Low-temperature active endoglucanases are obtained from several fungal strains inhabiting the marine sponge *Haliclona simulans* in Ireland (Baker *et al*., 2010), and cold-active xylanase is produced by a marine-derived *Cladosporium* sp. (Del-Cid *et al*., 2014) and by a recombinant marine fungal strain (a psychotropic fungus) from the Yellow Sea (Hou *et al*., 2006). Chitinases active at low temperatures (5 and 10^{o}C) are also reported (Velmurugan *et al*., 2011). Lipases, proteases and cellulases are reported to be produced on solid media at 15^{o}C by Antarctic marine yeast strains isolated from different marine invertebrates and sediments (Duarte *et al*., 2013).

Halophilic fungal enzymes

Many halophilic fungi show a xerotolerant tendency, growing well in low *aw* conditions beside halophilic capabilities (Gunde-Cimerman *et al*., 2000; Bonugli-Santos *et al*., 2015)**.** Most of the halophilic fungi belong to Ascomycota and Basidiomycota (Śliżewska *et al*., 2022). Halophilic species of ascomycetes namely *Aspergillus sydowii, Aspergillus versicolor, Aureobasidium pullulans, Hortaea werneckii, Penicillium chrysogenum, Phaetothecatri angularis,* and *Trimmatostromas alinum* are important (Butinar *et al*., 2005; Gunde-Cimerman *et al*., 2009**)**. Similarly, species of Basidiomycota belonging to orders Trichonosporales, Sporidiales, and Wallemiales show significant applications under high salt concentration as well in the presence of organic solvents that normally inhibit enzymatic reactions (Jin *et al.*, 2019; Ruginescu *et al.*, 2020). Further, they also maintain high stability and enzymatic activity at low water activity as low as 0.75 (De Lourdes Moreno *et al*., 2013). Halophilic enzymes demonstrate poly thermophilicity, and thus in addition to salinity conditions; they also resist various temperatures and a wide range of pH (Arifeen and Liu, 2018).

Most of the halophilic fungi secrete extracellular enzymes, which facilitate efficient and easier extraction under industrial conditions compared to halophilic bacteria. These enzymes are water-soluble and adjust to lower water activity (Primožicˇ *et al*., 2019). The enzymes produced by halophilic fungi belong mainly to the classes of hydrolases (EC 3) and oxidoreductases (EC 1) (Śliżewska *et al*., 2022).

Halophilic hydrolases are the most commonly isolated enzymes from halophilic microorganisms, and they are recognized to have potential application in biotechnological processes, that demand high stability and activity in the presence of organic solvents at high salt concentrations (Flores-Gallegos *et al*., 2018; Primožicˇ*et al*., 2019). Halophilic fungi are well known to produce amylases, lipases, cellulases, proteases, xylanases, pectinases, and others (Ruginescu *et al.*, 2020; Zhang *et al.*, 2018) that have applications as biofuel production, bioremediation, food, cosmetics, detergent, and pharmaceutical processes (Ali *et al*., 2014a; Amoozegar *et al*., 2019; González-Abradelo *et al*., 2019).

Lignin-degrading enzymes (lignin peroxidases, manganese peroxidases, and laccases) with ability to degrade lignocellulose are produced by halophilic fungi. These enzymes catalyze decolorization of dyes, treatment of colored effluents, degradation of other organic pollutants, and bioremediation owing to their ability to degrade saline and alkaline pollutants under both saline and non-saline conditions (Bonugli-Santos *et al*., 2012; Arifeen, and Liu, 2018)**.**

Halophilic fungi, unlike halophilic prokaryotes, have been observed to have the ability to grow both in salt-free conditions and in a wide range of salinity (Gunde-Cimerman and Zalar, 2014**)**. In fact, hydrolases and oxidoreductases resistant to salt presence and low water activity have potential for application in bioremediation processes and wastewater treatment (Śliżewska *et al*., 2022).

Marine *Aspergillus sclerotiorum* CBMAI 849 degrades polycyclic aromatic hydrocarbon (PAHs) during which nearly 100% deletion of pyrene and over 76% of benzo[a]pyrene occurred, indicating scope for application in bioremediation process in saline conditions (Passarini *et al*., 2011)**.** *Aspergillus gracilis* TISTR 3638 isolated from a solar saltern produce α-amylase with higher activity along with increase in salinity compared to commercial amylases and it can be used for treatment of industrial effluents contaminated with metallic ions (Ali *et al*., 2014b).

Enzyme obtained from halophilic fungi, *A. penicillioides* TISTR3639, is reported to have potential application as an additive in the laundry detergent industry (Ali *et al*., 2015). Marine fungal ligninolytic enzyme has potential for use in biomass conversion of lignin materials (Batista-García *et al*., 2017). The halophiles *A. sydowii* EXF-12860 and *A. destruens* EXF-10411 are observed to remove 100% of xenobiotics-PAHs and pharmaceutical compounds (PhC) in wastewaters under salty conditions (>1M NaCl) suggesting possible application in downstream processing of industrial effluents (González-Abradelo *et al*., 2019). Halophilic and halotolerant fungi, due to their adaptive mechanisms produce many biomolecules that are unique compounds not found in other organisms (Slizewska *et al*., (2022). Despite their many advantages and enormous potential, the mycobiota of the saline environment is still not fully explored with respect to unique biomolecules including novel enzymes.

BIOPROCESS DEVELOPMENT

High-level production in bioreactors is mainly performed using submerged-state fermentation. Whereas, Chandrasekaran and his co-workers have developed different bioprocesses including solid state fermentation (SSF), submerged fermentation, and immobilization techniques for different enzyme (chitinase, L-glutaminase, alkaline serine protease, β-glucosidase, tannase, and lipase) production by marine fungi and evaluated the potential applications of marine fungal enzymes as detailed below.

Prawn waste, a chitinous solid waste of the shellfish processing industry, was used as a substrate for chitinase production by the alkalophilic marine fungus *Beauveria bassiana* BTMF S10, isolated from marine sediment, under SSF. A maximum chitinase yield of 248.0 units/g initial dry substrate (U/gIDS) was obtained in a medium containing a 5:1 ratio (w/v) of prawn waste/sea water, at 27°C, initial pH 9.5, and after 5 days of incubation) (Suresh and Chandrasekaran, 1998). Further, studies on the impact of process parameters on chitinase production revealed that the fungus is strongly alkalophilic and produces maximum chitinase at pH 9.2. It was observed that vegetative (mycelial) inoculum is more suitable than conidial inoculum for obtaining maximal enzyme yield under SSF (Suresh and Chandrasekaran, 1999).

L-glutamine amidohydrolase (L-glutaminase, EC 3.5.1.2), a potent anti-leukemic agent and a flavour-enhancing agent used in the food industry, is produced by the marine *Beauveria bassiana* BTMF S10 under submerged fermentation (Keerthi *et al*., 1999). Later, L-glutaminase production by the same fungi was studied under SSF using polystyrene as an inert support. Maximal enzyme production (49.89 U/ml) occurred at pH 9.0, 27°C, in a seawater-based medium (Sabu *et al.*, 2000). Further the fungus was evaluated for continuous production by Ca-alginate immobilized fungi (spore inoculum) in packed bed reactor. The effect of flow rate of the medium, substrate concentration, aeration, and bed height on continuous production of L- glutaminase was studied and the results indicated scope for utilizing immobilized *B. bassiana* for continuous commercial production of L-glutaminase (Sabu *et al*., 2002).

Alkaline serine protease produced by marine fungi, *Engyodontium album* BTMFS10, under SSF was purified and partially characterized (Sreeja Chellappan *et al*., 2006). Later, alkaline protease gene (Eap) encoding subtilisin-like alkaline serine protease by the marine fungus, *Engyodontium album* BTMFS10, was isolated for the first time from a marine fungus and characterized (Jasmin *et al*., 2010). Jasmin *et al*. (2010) noted that this gene Eap consist of an open reading frame of 1,161 bp encoding a pre propeptide consisting of 387 amino acids with a calculated molecular mass of 40.923 kDa and further studies on homology comparison of the deduced amino acid sequence of Eap with other known proteins indicated that Eap encodes an extracellular protease that belongs to the subtilase family of serine protease (Family S8). The comparative homology model of the *Engyodontium album* protease (EAP) developed using the crystal structure of proteinase K revealed that EAP has broad substrate specificity similar to Proteinase K with preference for bulky hydrophobic residues at P1 and P4 (Jasmin *et al*., 2010). The enzyme was further characterized for its physicochemical properties towards evaluation of its suitability for potential industrial applications. The enzyme demonstrated considerable storage stability, and retained its activity in the presence of hydrocarbons, natural oils, surfactants, and most of the organic solvents tested. Results suggested that this marine fungal protease holds potential for use in the detergent industry and for varied applications (Chellappan *et al*., 2011).

Marine *Aspergillus sydowii* BTMFS 55 isolated from sea water produces extracellular β- glucosidase under solid state fermentation (Madhu *et al*., 2009). A sequential optimization strategy employing the two-level Plackett-Burman (PB) design was used to enhance the production of β-glucosidase under SSF with wheat bran (WB) as the growth medium. Among the 11 variables tested, moisture content, inoculum, and peptone were identified as the most significant factors for enhanced β-glucosidase production. The enzyme was purified and noted to be a monomeric protein with a molecular weight of ~95 kDa and was optimally active at pH 5.0 and 50°C (Madhu *et al*., 2009).

Marine *Aspergillus awamori* BTMFW032 isolated from seawater produces acidophilic tannase as extracellular enzyme (Beena *et al*., 2010). This enzyme catalyses synthesis of propyl gallate by direct transesterification of tannic acid using propanol as organic reaction media under low water conditions. It was noted that 699 U/ml of enzyme gives 60% solubilisation of tea cream within 1 h. Further, enzyme production medium was optimized adopting Box-Behnken design for simultaneous synthesis of tannase and gallic acid under submerged fermentation. Process variables including tannic acid, sodium chloride, ferrous sulphate, dipotassium hydrogen phosphate, incubation period, and agitation were recognized as the critical factors that influenced tannase and gallic acid production (Beena *et al*., 2011a). The fungus could produce tannase using *Garcina cambogia* leaf and seawater as substrate under slurry state fermentation (Beena *et al*., 2011b). Marine fungus, *A. awamori* BTMFW032, was also observed to produce an extracellular lipase, which reduces 92% fat and oil content in the effluent laden with oil. The enzyme was partially purified with a molecular mass of 90 kDa and optimal activity at pH 7 and 40.8°C. This lipase was noted to have potential application in bioremediation of waste water laden with oil (Basheer *et al*., 2011**).**

These investigations made by Chandrasekaran and team demonstrated the suitability of marine derived fungi for possible production of industrially important enzymes chitinase, L-glutaminase, alkaline serine protease, tannase, and lipase under solid state fermentation as is reported with terrestrial fungal enzyme production. It may be also noted that these marine derived fungi prefer salt water/ saline conditions and high alkaline pH for maximal enzyme production on statistical optimization of the bioprocesses. In fact, elaborate investigations are required for large scale production of these marine enzymes and there is a need to design and develop suitable bioreactors and appropriate enzyme production media for appropriate harnessing of the marine fungal enzymes.

Pectinase that catalyzes the degradation of pectic substances is considered as one of the most important enzymes in industry and it contributes about 25% in the global sales of food enzymes. This enzyme was observed to be produced by halophilic fungi, *Aspergillus terreus,* isolated from solar saltern of Marakkanam, Pondicherry, India (Rani *et al*., 2013). The authors reported that *Aspergillus terreus* enhances pectinase production under submerged fermentation using banana peel waste as substrate at 72 h of incubation at 30°C and at pH of 6.0 (Rani *et al*., 2013).

The large-scale production (e.g., in bioreactors) of glucoamylase, superoxide dismutase, lignin peroxidase, chitinase, protease, and glutaminase by marine strains is reported in the literature (Sarkar *et al*., 2010). These enzymes are produced in bioreactors largely through submerged-state fermentation. Trincone (2011) has described an overview of the bioprocess strategies adopted for the cultivation of marine-derived organisms for enzyme production, including protease, chitinase, agarase, and peroxidase.

The marine fungal derived enzymes show temperature and pH optima in the range of 35 to 70ºC, and 3.0 to 11.0, respectively. Certain other marine-derived fungal strains produce enzymes with alkaline and cold-activity characteristics, and salinity is considered an important condition in screening and production processes (Bonugli-Santos *et al*., 2015).

CHALLENGES IN BIOPROSPECTING MARINE FUNGI

Indeed apparently, it may look very easy to isolate marine fungi and bioprospect marine fungi for deriving novel enzymes with biotechnological and industrial applications. Whereas, a cursory glance of the history of bioprospecting marine fungal enzyme over the past 100 years, ever since marine mycology research was initiated, it will be clear that the progress achieved with respect to marine fungal enzymes is rather very limited compared to the quantum of literature accumulated on terrestrial fungi and their by-products including industrial enzymes. A critical assessment of the practical difficulties experienced by researchers in isolating marine fungi and maintaining themin laboratory for further studies are rather discouraging and hence, not much progress was made. This is a ground reality in marine mycological research. Nevertheless, few dedicated researchers across the globe are pursuing their goals with respect to marine fungi and bioprospecting of the same. Nevertheless, it is important to address certain issues that pose serious challenges for marine fungal research and bioprospecting of these novel fungi from marine habitats. The probable challenges in bioprospecting marine fungi for deriving novel enzymes and possible remedies are presented in **Table 1**. It is anticipated that the same may enable researchers to appreciate the challenges and design appropriate remedial measures towards efficient harnessing of marine fungi for deriving promising enzymes of biotechnological applications.

Table 1: The probable challenges in bioprospecting marine fungi for deriving novel enzymes and possible remedies.

S. No.	Challenges	Possible Remedies
1	Ideal isolation and screening methods, for both culture dependent and non- cultivation methods, employed for isolation of potential fungi producing desirable enzymes.	Development of sea water-based cultivation media for promoting slow growing fungi and identification of nutrient requirements of marine fungi that helps in rapid growth of culture dependent fungi. Metagenomic based screening programs need to be developed for recognizing potential enzyme coding genes
2	Biochemical, physical, genetic and biological characteristics of marine fungi and their enzymes are hardly available in literature. Once appropriate knowledge is available on culture preferences and the impact of environmental factors on growth and proliferation in culture conditions ideal cultivation strategies with most preferable cultivation media would be available for isolation of marine fungi from diverse marine environments including extreme marine environments	Optimization of ideal cultivation and maintenance media are mandatory requirementsfor characterizing the fungal strains immediately after isolation which would enable perfect understanding of the biology of marine fungi. Impact of environmental factors on growth and physiology of marine fungi needs to be studied before harnessing them for enzyme production
3	Molecular techniques like metagenomics and proteomics could be helpful to characterize novel enzymes and to study their functions.	Exploration of data bases on enzymes and genomics employing bioinformatics tools will facilitate design and execution of metagenomic and proteomic approaches to recognize potential enzymes of importance and understand their functions
4	Designing of appropriate strategies for evolving culture independent methods involving metagenomics and functional genomics, as well as environmental genomics for isolation of novel genes encoding novel enzymes with enormous potential for applications.	Exploration of data bases on enzymes and genomics employing bioinformatics tools will facilitate design and execution of metagenomicand functional genomics approaches forisolating novel genes encoding valuable enzymes of importance
5	Molecular crystallography, elucidation of enzyme structure, enzyme modulation, and molecular characterization	Enzymes need to be produced using ideal enzyme production media and purified for characterization studies employing conventional enzymology approach. Such approach will enrich our scientific knowledge on marine fungi and their physiology in secreting novel enzymes.
6	Fungal diversity from environments such as deep sea, cold Polar Regions, benthic environments, may prove to be the source of novel enzymes with unique properties and novel applications.	Special isolation and cultivation strategies have to be designed based on literature available on cold adapted fungi and stability of novel enzymes under extreme conditions. Knowledge on psychrophilic bacteria could be applied for designing and developing suitable cultivation media

S. No.	Challenges	Possible Remedies
7	Genetic data on genes encoding enzymes towards possible isolation and identification of genes, molecular coning and expression of those novel genes encoding novel enzymes of economic and ecological importance.	Available methods and molecular genetic techniques experienced with terrestrial fungi could help studying marine fungal enzymes
8	Metabolomics and environmental genomics techniques and tools need to be explored for probable inference of potentials of the genetic data for prediction of applications and translation of data into technical knowhow and know why on prospects of marine fungal enzymes and their role in ecosystem	Available techniques and tools employed for Metabolomics and environmental genomics studies on terrestrial fungi and other bacteria could help studying marine fungal enzymes
9	Inadequate expertise to develop ideal bioprocess development and produce marine enzymes on large scale due to non-availability of information on behavior of marine fungi in large scale cultivation in bioreactors	Expertise has to be developed in bioreactor and bioprocesses for large scale cultivation of marine fungi using ideal enzyme production media and application of statistical modeling techniques. Strategies need to be developed for development of models for lab -scale, bench scale and pilot scale production of industrially important enzymes of marine fungi.

FUTURE PROSPECTS

Marine fungal enzyme displays novel physiological characteristics, such as high salt tolerance, thermostability, barophilicity, and cold-activity (Velmurugan and Lee, 2012) and hence, it is speculated that intensive exploratory studies will lead to discovery of new and novel enzymes with novel applications unlike their terrestrial counterparts. Considering the vast marine fungal diversity there is immense hope to derive new enzymes from the same once adequate intensive screening programs are launched (Di Donato *et al*., 2019). Moreover, with the advent of biotechnology, enzymatic engineering, and the introduction of other innovative technologies such as metabolomics, metagenomics, proteomics, and transcriptomics, it is possible to harness our rich marine microbial biodiversity towards augmenting new enzymes from marine microorganisms for effective utilization, not only as a cost-effective biocatalyst but also as an ecofriendly reagent in the coming years.

It may be worth-while to consider the following scenarios of future for assessing future prospects for bioprospecting marine fungal enzymes

(i) Hughes *et al*. (2022) opined that Omics techniques such as single-cell genomics, transcriptomics, proteomics, or metabolomics would facilitate elucidation of fungal activities and putative interactions in the intact symbiosis. These platforms will enable discovery of not only several novel industrial enzymes but also other bioactive biomolecules from non-cultivable/ culture independent microbes and fungi from unexplored horizons of marine environments.

(ii) The escalating demand for novel enzymes for applications in harsh conditions and need to have long shelf life, storage stability, and rapid catalysis indicate that high throughput microbial culturing approaches will be an option to maximize isolation and culturing of slow-growing fungi from varied marine environments.

(iii) Novel co-culturing or microcosm approaches mimicking small scale environments, such as the coral host environment or the phycosphere of algal symbionts (Raina *et al*., 2022) may also have potential for adoption.

(iv) Future scientific endeavors will totally rely on data generated through genomics, functional genomics, metabolomics, transcriptomics, and proteomics for augmentation of new and novel biomolecules of human health significance as well as understanding the functionality of such novel biomolecules for appropriate applications.

(v) There is a need to develop efficient strategies to overcome the limitations in routine isolation, screening, and cultivation of marine fungi from diverse marine habitats and those living in extreme environments. Such developments would result in efficient handling of marine fungi and appropriate harnessing of the same for desirable products and enzymes

(vi) Bonugli-Santos *et al*. (2015) indicated that there is continuous increase in demand for food and beverages enzymes due to the demand for new applications in the dairy and baking sectors, among others.

(vii) Further, marine conditions such as salinity, pressure, temperature, and light are attributed to significant differences between the enzymes produced by marine microorganisms and homologous enzymes by their terrestrial counterparts. Perhaps the adaptability of marine derived fungi to oceanic conditions can be a deciding factor in attributing unique characteristics to marine enzymes, (Bonugli-Santos *et al*., 2015) and that needs to be addressed by the scientific community through systematic investigations in future.

CONCLUSION

This review presented here unambiguously indicate the vast potential applications of fungal enzymes in expanding enzyme market that caters to the industries particularly food and beverage industry, pharmaceuticals, textile, detergent, bioremediation *etc*. Available literature on marine fungal enzymes indicated the limited availability of marine fungi as source of enzymes with novel characteristics for applications, and hence, there is a need to undertake intensive screening and harnessing of marine fungi from varied marine environments for possible bioprospecting in the future. Moreover, the serious challenges lying ahead in pursuing research on marine fungi and scope for future prospects are discussed to draw the attention of mycologists and biotechnologists to appropriately harness the novel marine fungi. It must be also mentioned that there is a need for augmentation of trained human resource for undertaking marine mycology and genomics research.

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