

## Novel Enzymes Isolated From Marine-Derived Fungi and Its Potential Applications

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### 1. Abstract

Marine environments provide habitats to a diverse group of microorganisms which play an important role in nutrient recycling by decomposing dead organic matters. In this regard, marine-derived fungi can be considered a great source of novel bio-active molecules of environmental and industrial importance. The morphological and taxonomical diversity of marine-derived fungi as compared to their terrestrial counterpart make it more interesting candidate to be explored and utilized in marine biotechnology. Fungi isolated from different marine habitats produce important enzymes with interesting characteristics. As marine-derived fungi have adapted well through evolution to thrive in the extreme marine conditions, they exhibited tremendous level specialization in the form of producing important secondary metabolites particularly novel enzymes which can be considered a better prospect for many future applications. This article discusses novel marine-derived enzymes, isolated from different marine fungi. From recent researches, it is cleared that marine-derived fungi have the potential to produce novel enzymes and important secondary metabolites. Lignin-degrading enzymes are one of the most important products produced by most marine-derived fungi. Future research that concentrates on culturing of rare and unique marine fungi with novel products, with an understanding of their biochemistry and physiology may pave the path for marine myco-technology.

### 3. Introduction

Marine environments (coastal, open-ocean water and sediments and the deep-sea subsurface) harbor a wide diversity of microorganisms which are involved in important biogeochemical processes. Recently, scientists showed great interest in the diversity of microorganism living in these habitats and the bioactive molecules they produced. As fungi are heterotrophic eukaryotic organisms and can be found in almost all sorts of niches such as oceans, sediments, and mangroves, coastal and terrestrial regions. According to recent predictions, there are almost 5.1 million fungal species on earth and out of which more than 1500 species belong to the marine environments [1]. Marine fungi are either freely floating entities or lodge onto sunken wood pieces, rocks, sand, plants, animals etc. On the basis of growth and sporulation characteristics, marine fungi are classified into two distinct groups, i.e. facultative marine fungi and obligate marine fungi. The first group has the

potential to grow and reproduce both in marine and terrestrial environment [2,3] while the latter group cannot survive on land and complete their life cycles only within the sea [1,4,5]. A common term Marine-derived fungi is now used for all fungi derived from marine environments including both obligate and facultative fungi as most of the fungi isolated from marine samples are not demonstrably classified as obligate or facultative marine microorganisms [6]. Marine fungi have been isolated from different substrates such as, sponges, algae, wood pieces, tunicates, sediments, mollusks, corals, plants, fish etc., and their ecology, diversity, and phylogeny have been severely discussed [7-12]. Similarly, a great number of fungal communities have also been recovered from the coastal habitats, like mangrove, sand, beach, water, river, and sediments, giving a strong proof of environmental influences such as winds, floods, and air, on fungi migration from land toward marine environments. That is why marine fungi usually demonstrate morphological characteristics similar

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to their terrestrial counterparts [13,14]. Marine-derived fungi adapted well to the extreme environment of the sea and enzymes produced by these fungi are also different from their terrestrial counterparts. Elevated pressure, salinity, low temperature, extreme pH, mineral content variation, and the partial or complete absence of light are the conditions responsible for the diversity of the enzymes produced by marine-derived fungi and homologous enzymes from terrestrial fungi [7,15,16,12,18]. As fungi found almost everywhere in nature, and have great influence on human in various aspects and have many advantages in the pharmaceutical, agricultural and food industries, for instance, *Trichoderma reesei*, *Aspergillus niger*, and *Aspergillus oryzae* have complex posttranslational processing and high protein secretion ability, therefore, widely used in industries to produce different enzymes and proteins [18-20]. A wide range of bioactive molecules have been isolated from marine environments which have the potential to perform different activities such as, antibacterial, antiviral, anti-diabetics, anti-inflammatory, and antitumor and many of these functions are due to specific enzymes produced by marine fungi [21]. This review will discuss novel enzymes isolated from marine-derived fungi and its biotechnological importance. The use of marine-derived fungi and their enzymes in different sectors will also be discussed in this review.

#### 4. Fungi and the marine extreme environments

Fungi have been found to colonized substrate in many extreme habitats such as extremely cold region of polar area [22] the outer stratosphere [23] deep-sea sediments [24,25], deep anoxic basins [26] in hot springs with temperature of almost of 60°C [27] and below the deep sub seafloor [28]. One the most extreme conditions in the marine environment is the elevated hydrostatic pressure and deep-sea are considered as the home to baro-tolerant and ba-

rophilic microorganisms. Similarly, another important extreme condition is the low temperature around 2-4°C and fungi from this environment are known to produce cold-tolerant enzymes such as low-temperature active serine protease, isolated from *Aspergillus terreus* (Table 1). These enzymes are found to helpful in detergents for cold-wash, waste digestion, and degradation in cold conditions, food and industrial processing to reduce the cost of heat energy. Marine-environments provide habitat to various microorganisms including fungi; however, the origin, diversity, distribution, and important bioactive materials of marine-derived fungi have not been fully discovered. Even though the first enzyme isolated from marine-derived fungi was reported back in the 1980s but not studied extensively after that and in 1999 the detailed study on this topic started more frequently [29]. Marine environments are considered as one of the extreme environment due to various factors such as low-temperature, salinity, low or no oxygen, high pressure, salinity, and special lighting conditions are responsible for the significant differences between the enzymes generated by marine microorganisms and homologous enzymes from terrestrial microorganisms [30]. Marine organism develops a defense mechanism through evolution in order to survive in this harsh conditions and therefore, the enzymes produced by marine organisms especially fungi show unique physiological properties such as hyper-thermo-stability, baro-tolerance, salt and pH tolerance, better stability in extreme cold conditions, and unique chemical and stereo chemical properties. These novel characteristics of enzymes make these fungi to thrive well in these conditions where most of similar land fungal strains cannot withstand [30,31] and these unique enzymes produced by these fungi have tremendous potential for biotechnological applications.

**Table 1:** Important enzymes isolated from marine-derived fungi its source optimum temperature and pH, and maximum production

Enzyme	Fungal source	Substrate/Medium	Characteristics of Enzymes				Reference
			Unique property	Optimal Temperature(°C)	Optimal pH	Maximum Production	
Laccase	<i>Flavodon flavus</i>	Sugarcane bagasse	Salinity, thermo-stability and metal-tolerance	26	--	300 U/L	Raghukumar et al. [37]
	<i>Cerrena unicolor</i>	Glycine and fructose		70	3	24,000 U/L	D'Souza-Ticlo et al. [38]
	<i>Mucor racemosus</i>	Wheat bran + glucose		--	5	898.15 U/L	Bonugli-Santos et al. [39]
	<i>Trematosphaeria mangrovei</i>	Sucrose, peptone, yeast extract		25	6	184.84 U/mg	Atalla et al. [40]

Lignin	<i>Flavodon flavus</i>	Sugarcane bagasse	Salinity tolerance	26	--	380 U/L	Raghukumar et al. [37]
Peroxidase	<i>Mucor racemosus</i>	Malt extract		--	3	75,376 U/L	Bonugli-Santos et al. [39]
(LiP)	<i>Cladosporium cladosporioides</i>		28	--	17,419 U/L		
	<i>Aspergillus sclerotiorum</i>		17,957 U/L				
Manganese	<i>Flavodon flavus</i>	sugarcane bagasse	Salinity tolerance	26	--	420 U/L	Raghukumar et al. [37]
Peroxidase (MnP)				--	4.5	44,84 U/L	Bonugli-Santos et al. [39]
	<i>Mucor racemosus CBMAI 847</i>	Wheat bran + glucose					
Versatile peroxidase	<i>Pleurotus eryngii</i>	Wheat straw	Mnp and Lip properties	--	--	--	Camarero et al. [70]
Protease	<i>Aspergillus ustus</i>	Skim milk powder	Alkaline and cold tolerance	45	9	1639 ACU mL <sup>-1</sup>	Damare et al. [49]
	<i>Aureobasidium pullulans</i>	Starch+ NaNO <sub>3</sub>		45	9	7.2 U/ml	Chi et al. [61]
	<i>Rhodotorula mucilaginosa</i>	Sabouraud and skimm milk	Low temperature active	25	--	11.12 U/mL	Duarte et al. [51]
	<i>Acremonium sp</i>	Cactus and pear	Low temperature active (10-40°C)	50	8	445.48 U/mL	Nascimento et al. [52]
Polygalacturonase (PGase)	<i>Cryptococcus liquefaciens</i>	Peptone, yeast extract, malt extract and glucose	Copper, cold (0-10 °C) and high pressure (100 MPa) tolerance	50	--	1.8 U/ml	Miura et al. [34]; Abe et al. [35]

Xylanase	<i>Aspergillus niger</i>	Oat spelt xylan, sugar-cane bagasse	Alkaline and cold tolerance	50	3.5	580 U/L	Raghukumar et al. [54]; Hou et al. [57]; Del-Cid et al. [56]
	<i>Penicillium, Cladosporium</i>						
	<i>Candida davisiana, Cryptococcus adeliensis, Guehomyces pullulans</i>	Birchwood xylan and yeast nitrogen base	Active at low temperature	15	--	0.75 U/mL 0.43 U/mL	Duarte et al. [51]
	<i>Phoma sp.</i>	Beech wood xylan and peptone	salt tolerance	45	5	1322.82 U/mg	
Lipases	<i>Penicillium Oxalicum, Aspergillus flavus, Candida intermedia, Candida parapsilosis, Lodderomyces elongisporus, Rhodotorula mucilaginosa, Aureobasidium pullulans, streptomycete</i>	olive oil, yeast extract, Lard, peanut oil, soybean oil	Cold tolerance	35-40	6.0-8.5	--	Kirsh [66]; Wang et al. [50]
	<i>Leucosporidium Scottii</i>	Peptone and yeast extract, olive oil	Cold tolerance	15	--	0.230 U.mL-1, 0.143 U L-1	Duarte et al. [51]
	<i>Cryptococcus adeliensis</i>						

L-Glutaminase	<i>Penicillium brevicompactum</i>	Czapek Dox agar + Glucose	Thermo-stable at 70 °C, and antitumor activity	50	8.5	869.1 U/ mg	Elshafei et al. [75]
Amylase	<i>Mucor</i> sp.	Starch, Casein	Thermo-stable	60	5	41,840 U/L	Mohapatra et al. [81]
	<i>Aureobasidium pullulans</i>	raw potato starch		60	4.5	0.5 U/ml	Li et al. [79]
β-glucosidase	<i>Aspergillus</i> SA 58	Starch, pectin, cellulose,	Showed good activity at low pH	35	3.0-9.0	80,000 U/L	Elyas et al. [76]
β-D-Glucosidase	<i>Penicillium canescens</i>	Yeast extract, peptone	Thermo-stable	70	5.2	1.86 U/ml	Dubrovskaya et al. [71]
Inulinase	<i>Pichia guilliermondii</i>	Wheat bran and rice bran	High production on solid state fermentation	30	6.5	455 U/g	Guo et al. [78]
Alginate lyase	<i>Aspergillus oryzae</i>	Yeast extract, peptone	Consisted of two polypeptides with 45 and 50 kDa	35	6.5	67.24 U/mg	Singh et al. [84]
Chitinase	<i>Plectosphaerella</i> sp	Colloidal chitin and malt extract	Active at low temperature	37	3	0.22 U/ml	Velmurugan et al. [59]
	<i>Penicillium Janthineflum</i>			24	4	686 U/L	Fenice et al. [58]
Nuclease	<i>Penicillium melinii</i>	glucose, peptone, soybean flour	Stable at 75°C and pH 2.5-8.0	37	3.7	41,250 U/mg	Balabanova et al. [69]
Superoxide dismutase	<i>Cryptococcus</i> strain N6	Yeast extract, peptone, dextrose and CuSO <sub>4</sub>	High copper tolerance	24	--	110 mU/μg	Abe et al. [80]

L-asparaginase	<i>Aspergillus</i> sp.	Dextrose and ammonium sulphate	Anti-tumor activity	35	7.5	185 U/ml	Sanjotha [83]
	<i>Aspergillus terreus</i>	Dextrose and L-asparagine		35	6	33.86 U/mg	Farag et al. [77]
	<i>Aspergillus</i> sp.	Glucose and asparagine		30	6	30.64 U/ml	Ahmed Mervat et al. [68]
keratinase	<i>Colletotrichum capsici</i>	Dextrose and Ammonium sulfate	Alkaline in nature	--	7.5	1.858 mg/ml	Samuel et al. [82]
	<i>Penicillium</i> spp.	Czapek's agar, rice straw, and yeast extract	Stable at pH 6.0-11.0, 26-65 °C	26	6	1,600 U/g	El-Gendy [72]
Endoglucanases	Various fungi isolated from marine sponge <i>Haliclona simulans</i>	Cellobiose and yeast extract	Active at lower temperature	18-60	--	0.014-0.34 U/mg	Baker et al. [55]
L-arginase	<i>Penicillium chrysogenum</i>	Dox's medium + L-arginine	Thermo-stable and anticancer activity	50	6.8-7.9	--	El-Sayed et al. [74]
L-Arginine Deiminase	<i>Aspergillus fumigatus</i>	Glucose and L-arginine	Thermo-stable and anticancer activity	45	7	22.0 U/ml	El-Sayed et al. [73]

## 5. Important enzymes isolated from marine-derived fungi

Fungi growing in extreme marine environment have the potential of producing industrially important extracellular novel enzymes [32,33] because of their great genetic and physiological diversity. Enzymes, such as proteases, laccases, amylases, xylanases and cellulases produced by marine fungi having many important applications in various sectors such as: i) textile, paper, leather, pulp, biofuel, medical and pharmaceuticals, food and beverage industries (ii) Environmental applications (iii) animal feed production application (iv) and for research purposes. All the important novel enzymes isolated from marine fungi and its substrate and characteristics are listed in table 1. Marine-fungal-derived enzymes showed novel characteristics as compared to those isolated from land species, due to their taxonomic diversity and ad-

aptation to various marine-extreme conditions [34,35] Among enzymes from marine fungi, lignin-degrading enzymes (lignin peroxidases, manganese peroxidases, and laccases) gained much attention, as with the help of lingo cellulase, fungi can degrade lignin. The application of lignocellulase to degrade lignin as a renewable source of fuel, attracted researchers the most [36-40]. Many marine-derived fungi produce various lignin-degrading enzymes including Lignin Peroxidase (LiP), Manganese Peroxidase (MnP) and laccase. These enzymes are not only useful to degrade lignin but also helpful in modification and degradation of other environmental pollutants such as waste derived from crude oil, textile effluents, and agricultural chemicals [41,42]. Fungi derived from marine mangrove are considered to be an important source of lignin-degrading enzymes [43-46]. It is also suggested that decaying activity in the marine environment

is mainly performed by soft-rot fungi as compared to white-rot fungi [46]. Similarly, Raghukumar et al. [43] mentioned that several mangroves-derived obligate and marine-derived fungi produce laccase, xylanase, and cellulase enzymes. Raghukumar et al. [37] for the first time isolated marine-derived fungus *Flavodon flavus* strain 312, which has the potential to produce all the lignin-degrading enzymes (LiP, MnP, and laccase) under specific conditions. Similarly, another fungus isolated from decaying mangrove wood from marine has the ability to produce a high level of laccase when grown in phenolics and lignin substrates [47]. Fungi and its isolates are also used to degrade and modify pollutants in different effluents. Verma et al. [48] explained that marine-derived ascomycetes and basidiomycetes produced laccase which can be used for the decolonization and modification of pollutants in textile effluents. Some unique and interesting enzymes were also produced by deep-sea marine fungi. Scientists isolated yeast, *Cryptococcus liquefaciens* strain N6, from the deep marine environment which has the potential to produce cold (0-10°C) and high pressure (100 MPa) tolerant endo polyGalacturonase (PGase) [35] which have been widely used in food industries especially for the extraction and clarification of fruit juices. Marine-Derived fungus, *Cryptococcus* strains N6, produce a high level of superoxide dismutase which showed tolerance to high concentration of copper [34]. Similarly, cold tolerant and alkaline proteases have been isolated from various deep-sea marine fungi, *Aspergillus ustus* [49], *Aureobasidium pullulans* [50], *Rhodotorula mucilaginosa* [51], *Acremonium* sp. [52] this enzyme also has some other beneficial properties which make it unique from its terrestrial counterpart, for example, it has a wide range of p<sup>H</sup> of 6–10, with an optimum activity at p<sup>H</sup> 9. Its optimum temperature was 45°C and showed good activity at 2 °C, also its activity remains stable in the presence of high concentration (0.5M) of NaCl which is equal to seawater salinity. These enzymes have the potential to reduce the energy requirement for a chemical reaction by increasing the hydrostatic pressure and decreasing the temperature [53]. Enzymes like this could be very useful in the future to lower the energy cost. Apart from the ecological role marine fungi also played an essential role in organic matter degradation and decomposition. In this regard, a great diversity of hydrolytic and oxidative enzymes has been isolated from these fungi which can be used in biotechnological processes. Velmurugan and Lee [29] reported that fungi living in the marine environment can produce salt, pressure, metals, and heat-tolerant enzymes. A thermo-stable metal tolerant laccases and alkaline xylanases have been isolated from marine strains of *Cerrenaunicolor* and *Aspergillus niger* [38,54]. Baker et al. [55] isolated cold tolerant endoglucanases from several marine-derived fungi. Similarly, a cold-tolerant xylanase was reported from marine-derived *Cladosporium* species [56]. This enzyme was also produced by genetically modified marine strain (a psychrotrophic

fungus from the Yellow Sea; Hou et al. [56]). [58,59] also isolated a low-temperature active chitinase enzyme. Yeast strains isolated from different Antarctic marine samples (sediments and marine invertebrates) has the potential to produce lipases, proteases, and cellulases on solid media at 15°C [51]. Salt tolerant fungi and their enzymes especially lignin-degrading enzymes have been extensively used in the bioremediation of different environmental pollutants [60]. All important and novel enzymes isolated from marine-derived fungi are listed in table 1. Enzymes production from marine-derived fungi mainly depends on its physiology and the composition of the culture medium [12]. The source of carbon and nitrogen play the most important role in fungal enzymes production. The most common substrates used for marine-derived fungal enzymes production are: Soluble starch, peptone, pectin, yeast extract, malt extract, casein, wheat bran, sugarcane bagasse and xylan (Table 1). All the enzymes produced by marine-derived fungi isolated from different marine habitats are shown in Table 1. The optimal temperature of the enzymes ranges from 26 to 70°C while the optimal pH ranges from 3 to 9. Table 1 Important enzymes isolated from marine-derived fungi its source optimum temperature and pH, and maximum production

## 6. Application of some important novel fungal enzymes

Marine-derived fungi with novel and unique properties are considered to be a good candidate of industrially important enzymes [32,33] Enzymes produced by marine-derived fungi have many industrial applications. Some important enzymes are given below.

### 6.1. Proteases

Proteases are the most commonly used enzyme all over the world and have many applications in the detergents industry and lather production. It has also been used in the pharmaceutical industry as an anti-inflammatory and digestive drug [30]. Chi et al. [61] isolated yeast strain, *Aureobasidium pullulans*, from China Yellow Sea, which produced a high yield (623 U/mg) of alkaline protease. Another alkaline protease isolated from marine-derived fungus showed 45% and 25% activity at 20°C and 15°C, respectively, with the highest activity at p<sup>H</sup> 9.5, thus performed well under high saline condition. These features make it a good candidate for detergents used under cold conditions [62]. Thus proteases isolated from marine-derived fungi might prove to be a valuable source of enzymes for detergent industries.

### 6.2. Lipases

Lipases catalyze the breakdown of oil and fats and are found in a wide variety of organism. Recently this enzyme got more attention and have been isolated from various microorganisms and also produced commercially. Lipases are mainly used in the production of detergents, paper, cosmetics, in medicine as diges-



tive enzymes and other clinical reagents, and as a food flavoring [30,63-65]. Kirsh [66] isolated lipase for the first time from *Penicillium Oxalicum* and *Aspergillus flavus*. The best cold-tolerant lipase was extracted from *Moraxella*, isolated from seawater of Antarctica, can work at a temperature of 3°C with an optimum of 25°C of temperature [67]. Cold-active lipases have also been isolated from many deep-sea fungal species. For example Wang et al. [50] isolated 9 lipase producing yeast strains by screening a total of 427 marine-derived yeast strains belonging to the following groups *Pichia guilliermondii* N12c, *Candida intermedia* YA01a, *Candida parapsilosis* 3eA2, *Candida quercitrusa* JHSb, *Lodderomyces elongisporus* YF12c, *Candia rugosa* wl8, *Rhodotorula mucilaginosa* L10-2, *Yarrowia lipolytica* N9a, and *Aureobasidium pululans* HN2.3. Lipases from these fungi showed optimum activity at various low temperature and pHs. The optimum temperature was between 35 and 40 °C while the optimum pH was between 6.0 and 8.5.

### 6.3. Polygalacturonases

Polygalacturonases (PGase) are enzymes usually used in food industry for clarification of fruit juices. Two unique PGase were isolated from deep-sea (4500-6500 m) marine-derived yeast extracted from the Japan Trench, showed activity at low temperature (24°C) and high hydrostatic pressure (100 MPa) [34,35]. These enzymes were also tolerant at high (50 mM) concentration of CuSO<sub>4</sub> and showed the high activity of superoxide dismutase (superoxide radical scavenger). This pressure can shift the temperature required for a given chemical reaction towards lower temperature [53]. Thus it is possible to reduce the energy expenditure by running a chemical reaction with a mesophilic enzyme rather than thermophilic enzymes at elevated hydrostatic pressure.

## 7. Conclusions

Even though marine fungi have been extensively studied in recent researches, our understanding and scientific knowledge of marine fungi is still very limited. Marine fungi produced unique and novel enzymes which have many industrial applications. So far the major focus was on marine bacteria and regardless of the physiological adaptability to low temperature, elevated hydro pressure, and playing important roles in the ecosystem, marine fungal taxa have not been discovered that much. Marine-derived fungi have revealed much promise in terms of interesting enzymes with novel properties, unique metabolites, and secondary metabolites. Many biotechnologically important enzymes have been produced by marine-derived fungal community isolated from a variety of marine habitats. This diversity of marine fungal products is due to genetic diversity based on taxonomy and adaptability to various extreme environmental conditions. There is still a very big scope

to examine these fungi for other interesting and useful products, like extracellular polysaccharides and other secondary metabolites. Future studies should be focused on marine fungal biology to reveal interesting biochemical and physiological features useful to various new biotechnological processes, for example, finding of new techniques to study uncultured and rare marine-derived fungi and knowing about its physiology and biochemistry will definitely pave the way for future marine mycology.

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