

Novel Enzymes Isolated from Marine-derived Fungi and its Potential Applications

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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.

Keywords: Marine-derived fungi, lignin, biotechnology, bioremediation, myco-technology, enzymes

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, 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 (Parte et al. 2017). 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 (Kohlmeyer and Kohlmeyer 1979; Hawksworth and Lucking 2017) while the latter group cannot survive on land and complete their life cycles only within the sea (Kohlmeyer and Volkmann-Kohlmeyer 2003; Li and Wang 2009; Parte et al. 2017). 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 (Osterhage 2001). 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 (Jones 2000; Jones et al. 2009; Jones et al. 2011; Jones and Pang 2012; Richards et al. 2012; Bonugli-Santos et al. 2015). 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 to their terrestrial counterparts (Méjanelle et al. 2000; Morrison-Gardiner 2002).

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 (Jones 2000; Gomes et al. 2008; Rämä et al. 2014; Bonugli-Santos et al. 2015; Raghukumar 2017). 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 (Calmels et al. 1991; Ward 2012; Benoit-Gelber et al. 2017). 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 (Mayer et al. 2013).

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

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 (Robinson 2001) the outer stratosphere (Wainwright et al. 2003) deep-sea sediments (Raghukumar et al. 2004a; Edgcomb et al. 2011), deep anoxic basins (Edgcomb et al. 2009) in hot springs with temperature of almost of 60 °C (Maheshwari et al. 2000) and below the deep sub seafloor (Liu et al. 2017). 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 barophilic 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. Eventhough 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 (Velmurugan and Lee 2012). 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 (Zhang and Kim 2010). 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 stereochemical properties. These novel characteristics of enzymes make these fungi to thrive well in these conditions where most of similar land fungal strains cannot withstand (Saleem et al. 2007; Zhang and Kim 2010) and these unique enzymes produced by these fungi have tremendous potential for biotechnological applications.

Important Enzymes Isolated From Marine-Derived Fungi

Fungi growing in extreme marine environment have the potential of producing industrially important extracellular novel enzymes (Synnes 2007; Dang et al. 2009) 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 adaptation to various marine-extreme conditions (Abe et al. 2001; Abe et al. 2006). Among enzymes from marine fungi, lignin-degrading enzymes (lignin peroxidases, manganese peroxidases, and laccases) gained much attention, as with the help of lignocellulase, fungi can degrade lignin. The application of lignocellulase to degrade lignin as a renewable source of fuel, attracted researchers the most (Kuhad et al. 1997; Raghukumar et al. 1999; D'Souza-Ticlo et al. 2009; Bonugli-Santos et al. 2010; Atalla et al. 2013). 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 (Kiiskinen et al. 2004; Mtui and Nakamura 2004). Fungi derived from marine mangrove are considered to be an important source of lignin-degrading enzymes (Raghukumar et al. 1994; Grant et al. 1996; Pointing et al. 1998; Pointing and Hyde 2001). It is also suggested that decaying activity in the marine environment is mainly performed by soft-rot fungi as compared to white-rot fungi (Pointing and Hyde 2001). Similarly, Raghukumar et al. (1994) mentioned that several mangroves-derived obligate and marine-derived fungi produce laccase, xylanase, and cellulase enzymes. Raghukumar et al. (1999) 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, an other 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 (D'Souza-Ticlo et al. 2006).

Fungi and its isolates are also used to degrade and modify pollutants in different effluents. Verma et al. (2010) explained that marine-derived ascomycetes and basidiomycetes produced laccase which can be used for the decolorization 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) to lerantendopolygalacturonase (PGase) (Abe et al. 2006) which have been widely used in food industries especially for the extraction and clarification of fruit juices. Marine-Derived fungus, *Cryptococcus* strain N6, produce a high level of superoxide dismutase which showed tolerance to high concentration of copper (Abe et al. 2001). Similarly, cold tolerant and alkaline proteases have been isolated from various deep-sea marine fungi, *Aspergillus ustus* (Damare et al. 2006), *Aureobasidium pullulans* (Chi et al. 2007), *Rhodotorula mucilaginosa* (Duarte et al. 2013), *Acremonium* sp. (Nascimento et al. 2015) this enzyme also has some other beneficial properties which make it unique from its terrestrial counterpart, for example, it has a wide range of pH of 6–10, with an optimum activity at pH 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 (Daniel et al. 2006). 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 (2012) 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* (Raghukumar et al. 2004b; D'Souza-Ticlo et al. 2009). Baker et al. (2010) isolated cold tolerant endoglucanases from

Novel Enzymes Isolated from Marine-derived Fungi and its Potential Applications

several marine-derived fungi. Similarly, a cold-tolerant xylanase was reported from marine-derived *Cladosporium* species (Del-Cid et al. 2014). This enzyme was also produced by genetically modified marine strain (a psychrotrophic fungus from the Yellow Sea; Hou et al. (2006)). Fenice et al. (1998) and (Velmurugan et al. 2011) 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 (Duarte et al. 2013). Salt tolerant fungi and their enzymes especially lignin-degrading enzymes have been extensively used in the bioremediation of different environmental pollutants (Passarini et al. 2011). 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 (Bonugli-Santos et al. 2015). 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

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, thermostability and metal-tolerance	26	--	300 U/L	Raghukumar et al. (1999)
	<i>Cerrena unicolor</i>	Glycine and fructose		70	3.0	24,000 U/L	D'Souza-Ticlo et al. (2009)
	<i>Mucor racemosus</i>	Wheat bran + glucose		--	5.0	898.15 U/L	Bonugli-Santos et al. (2010)
	<i>Trematosphaeria mangrovei</i>	Sucrose, peptone, yeast extract		25	6.0	184.84 U/mg	Atalla et al. (2013)
Lignin Peroxidase (LiP)	<i>Flavodon flavus</i>	Sugarcane bagasse	Salinity tolerance	26	--	380 U/L	Raghukumar et al. (1999)
	<i>Mucor racemosus</i>	Malt extract		--	3.0	75,376 U/L	Bonugli-Santos et al. (2010)
	<i>Cladosporium cladosporioides</i>		28	--	17,419 U/L		
	<i>Aspergillus sclerotiorum</i>					17,957 U/L	
Manganese Peroxidase (MnP)	<i>Flavodon flavus</i>	sugarcane bagasse	Salinity tolerance	26	--	420 U/L	Raghukumar et al. (1999)
	<i>Mucor racemosus CBMAI 847</i>	Wheat bran + glucose		--	4.5	44,84 U/L	Bonugli-Santos et al. (2010)
Versatile peroxidase	<i>Pleurotus eryngii</i>	Wheat straw	Mnp and Lip properties	--	--	--	Camarero et al. (1999)
Protease	<i>Aspergillus ustus</i>	Skim milk powder	Alkaline and cold tolerance	45	9.0	1639 ACU mL ⁻¹	Damare et al. (2006)
	<i>Aureobasidium pullulans</i>	Starch+ NaNO ₃		45	9.0	7.2 U/ml	Chi et al. (2007)

Novel Enzymes Isolated from Marine-derived Fungi and its Potential Applications

	<i>Rhodotorula mucilaginosa</i>	Sabouraud and skimm milk	Low temperature active	25	--	11.12 U/mL	Duarte et al. (2013)
	<i>Acremonium sp</i>	Cactus and pear	Low temperature active (10-40°C)	50	8.0	445.48 U/mL	Nascimento et al. (2015)
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. (2001); Abe et al. (2006)
Xylanase	<i>Aspergillus niger</i> , <i>Penicillium</i> , <i>Cladosporium</i>	Oat spelt xylan, sugarcane bagasse	Alkaline and cold tolerance	50	3.5	580 U/L	Raghukumar et al. (2004b); Hou et al. (2006); Del-Cid et al. (2014)
	<i>Candida davisiana</i> , <i>Cryptococcus adeliensis</i> , <i>Guehomyces pullulans</i>	Birchwood xylan and yeast nitrogen base	Active at low temperature	15	--	0.75 U/mL 0.43 U/mL 0.43 U/mL	Duarte et al. (2013)
	<i>Phoma sp.</i>	beechwood xylan and peptone	salt tolerance	45	5.0	1322.82 U/mg	Wu et al. (2018)
Lipases	<i>Penicillium Oxalicum</i> , <i>Aspergillus flavus</i> , <i>Candida intermedia</i> , <i>Candida parapsilosis</i> , <i>Lodderomyces elongisporus</i> , <i>Rhodotorula mucilaginosa</i> , <i>Aureobasidium pullulans</i> , <i>streptomycete</i>	olive oil, yeast extract, Lard, peanut oil, soybean oil	Cold tolerance	35-40	6.0-8.5	--	Kirsh (1935); Wang et al. (2007)
	<i>Leucosporidium Scottii</i> <i>Cryptococcus adeliensis</i>	Peptone and yeast extract, olive oil	Cold tolerance	15	--	0.230 U mL ⁻¹ , 0.143 U L ⁻¹	Duarte et al. (2013)
L-Glutaminase	<i>Penicillium brevicompactum</i>	Czapek Dox agar + Glucose	Thermally stable at 70 °C, and antitumor activity	50	8.5	869.1 U/ mg	Elshafei et al. (2014)
Amylase	<i>Mucor sp.</i>	Starch, Casein	Thermally stable	60	5.0	41,840 U/L	Mohapatra et al. (1998)

Novel Enzymes Isolated from Marine-derived Fungi and its Potential Applications

	<i>Aureobasidium pullulans</i>	raw potato starch		60	4.5	0.5 U/ml	Li et al. (2007)
β -glucosidase	<i>Aspergillus SA 58</i>	Starch, pectin, cellulose,	Showed good activity at low pH	35	3.0-9.0	80,000 U/L	Elyas et al. (2010)
β -D-Glucosidase	<i>Penicillium canescens</i>	Yeast extract, peptone	Thermo-stable	70	5.2	1.86 U/ml	Dubrovskaya et al. (2012)
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. (2009)
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. (2011)
Chitinase	<i>Plectosphaerella sp</i>	Colloidal chitin and malt extract	Active at low temperature	37	3.0	0.22 U/ml	Velmurugan et al. (2011)
	<i>Penicillium Janthineflum</i>			24	4.0	686 U/L	Fenice et al. (1998)
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. (2012)
Superoxide dismutase	<i>Cryptococcus</i> strain N6	Yeast extract, peptone, dextrose and CuSO ₄	High copper tolerance	24	--	110 mU/ μ g	Abe et al. (2001)
L-asparaginase	<i>Aspergillus sp.</i>	Dextrose and ammonium sulphate	Anti-tumor activity	35	7.5	185 U/ml	Sanjotha (2017)
	<i>Aspergillus terreus</i>	Dextrose and L-asparagine		35	6.0	33.86 U/mg	Farag et al. (2015)
	<i>Aspergillus sp.</i>	Glucose and asparagine		30	6.0	30.64 U/ml	Ahmed Mervat et al. (2015)
keratinase	<i>Colletotrichum capsici</i>	Dextrose and Ammonium sulfate	Alkaline in nature	--	7.5	1.858 mg/ml	Samuel et al. (2018)
	<i>Penicillium spp.</i>	Czapek's agar, rice straw, and yeast extract	Stable at pH 6.0-11.0, 26-65 °C	26	6.0	1,600 U/g	El-Gendy (2010)
Endoglucanases	<i>Various fungi isolated from marine sponge Haliclona simulans</i>	Cellobiose and yeast extract	Active at lower temperature	18-60	--	0.014-0.34 U/mg	Baker et al. (2010)
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. (2014)

Novel Enzymes Isolated from Marine-derived Fungi and its Potential Applications

L-Arginine Deiminase	<i>Aspergillus fumigatus</i>	Glucose and L-arginine	Thermo-stable and anticancer activity	45	7.0	22.0 U/ml	El-Sayed et al. (2015)
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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 (Synnes 2007; Dang et al. 2009). Enzymes produced by marine-derived fungi have many industrial applications. Some important enzymes are given below.

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 (Zhang and Kim 2010). Chi et al. (2007) 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 pH 9.5, thus performed well under high saline condition. These features make it a good candidate for detergents used under cold conditions (Raghukumar et al. 2006). Thus proteases isolated from marine-derived fungi might prove to be a valuable source of enzymes for detergent industries.

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 digestive enzymes and other clinical reagents, and as a food flavoring (Seiichi et al. 1991; Kobayashi et al. 2008; Chi et al. 2009; Zhang and Kim 2010). Kirsh (1935) 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 (Feller et al. 1990). Cold-active lipases have also been isolated from many deep-sea fungal species. For example Wang et al. (2007) 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* w18, *Rhodotorula mucilaginosa* L10-2, *Yarrowia lipolytica* N9a, and *Aureobasidium pullulans* 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.

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) (Miura et al. 2001; Abe et al. 2006). These enzymes were also tolerant at high (50 mM) concentration of CuSO₄ 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 (Daniel et al. 2006). 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.

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

fungus 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 biotechnological 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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REFERENCES

- [1] Abe, F., H. Minegishi, T. Miura, T. Nagahama, R. Usami and K. Horikoshi (2006). Characterization of cold- and high-pressure-active polygalacturonases from a deep-sea yeast, *Cryptococcus liquefaciens* strain N6. *Bioscience, biotechnology and biochemistry* 70(1): 296-299.
- [2] Abe, F., T. Miura, T. Nagahama, A. Inoue, R. Usami and K. Horikoshi (2001). Isolation of a highly copper-tolerant yeast, *Cryptococcus* sp., from the Japan Trench and the induction of superoxide dismutase activity by Cu^{2+} . *Biotechnology Letters* 23(24): 2027-2034.
- [3] Ahmed Mervat, M., A. Nageh, T. Taher and H. Fareed (2015). Production, purification and characterization of L-asparaginase from marine endophytic *Aspergillus* sp: ALAA2000 under submerged and solid state fermentation. *Journal of Microbial & Biochemical Technology* 7(3): 165-172.
- [4] Atalla, M. M., H. K. Zeinab, R. H. Eman, A. Y. Amani and A. A. E. A. Abeer (2013). Characterization and kinetic properties of the purified *Trematosphaeria mangrovei* laccase enzyme. *Saudi Journal of Biological Sciences* 20(4): 373-381.
- [5] Baker, P. W., J. Kennedy, J. Morrissey, F. O'Gara, A. D. Dobson and J. R. Marchesi (2010). Endoglucanase activities and growth of marine-derived fungi isolated from the sponge *Haliclona simulans*. *Journal of Applied Microbiology* 108(5): 1668-1675.
- [6] Balabanova, L. A., Y. M. Gafurov, M. V. Pivkin, N. A. Terentyeva, G. N. Likhatskaya and V. A. Rasskazov (2012). An extracellular S1-type nuclease of marine fungus *Penicillium melinii*. *Marine Biotechnology* 14(1): 87-95.
- [7] Benoit-Gelber, I., T. Gruntjes, A. Vinck, J. G. van Veluw, H. A. B. Wösten, S. Boeren, J. J. M. Vervoort and R. P. de Vries (2017). Mixed colonies of *Aspergillus niger* and *Aspergillus oryzae* cooperatively degrading wheat bran. *Fungal Genetics and Biology* 102: 31-37.
- [8] Bonugli-Santos, R. C., L. R. Durrant, M. da Silva and L. D. Sette (2010). Production of laccase, manganese peroxidase and lignin peroxidase by Brazilian marine-derived fungi. *Enzyme and Microbial Technology* 46(1): 32-37.
- [9] Bonugli-Santos, R. C., M. R. dos Santos Vasconcelos, M. R. Passarini, G. A. Vieira, V. C. Lopes, P. H. Mainardi, J. A. Dos Santos, L. de Azevedo Duarte, I. V. Otero and A. M. da Silva Yoshida (2015). Marine-derived fungi: diversity of enzymes and biotechnological applications. *Frontiers in Microbiology* 6: 269.
- [10] Calmels, T. P. G., F. Martin, H. Durand and G. Tiraby (1991). Proteolytic events in the processing of secreted proteins in fungi. *Journal of Biotechnology* 17(1): 51-66.
- [11] Camarero, S., S. Sarkar, F. J. Ruiz-Dueñas, M. a. J. Martínez and Á . T. Martínez (1999). Description of a versatile peroxidase involved in the natural degradation of lignin that has both manganese peroxidase and lignin peroxidase substrate interaction sites. *Journal of Biological Chemistry* 274(15): 10324-10330.
- [12] Chi, Z., Z. Chi, T. Zhang, G. Liu, J. Li and X. Wang (2009). Production, characterization and gene cloning of the extracellular enzymes from the marine-derived yeasts and their potential applications. *Biotechnology Advances* 27(3): 236-255.
- [13] Chi, Z., C. Ma, P. Wang and H. F. Li (2007). Optimization of medium and cultivation conditions for alkaline protease production by the marine yeast *Aureobasidium pullulans*. *Bioresource Technology* 98(3): 534-538.
- [14] D'Souza-Ticlo, D., A. K. Verma, M. Mathew and C. Raghukumar (2006). Effect of nutrient nitrogen on laccase production, its isozyme

- pattern and effluent decolorization by the fungus NIOCC No. 2a, isolated from mangrove wood. *Indian Journal of Geo-Marine Sciences* 35(4): 364-372.
- [15] D'Souza-Ticlo, D., D. Sharma and C. Raghukumar (2009). A thermostable metal-tolerant laccase with bioremediation potential from a marine-derived fungus. *Marine Biotechnology* 11(6): 725-737.
- [16] Damare, S., C. Raghukumar, U.-D. Muraleedharan and S. Raghukumar (2006). Deep-sea fungi as a source of alkaline and cold-tolerant proteases. *Enzyme and Microbial Technology* 39(2006): 172-181.
- [17] Dang, H., H. Zhu, J. Wang and T. Li (2009). Extracellular hydrolytic enzyme screening of culturable heterotrophic bacteria from deep-sea sediments of the Southern Okinawa Trough. *World Journal of Microbiology and Biotechnology* 25(1): 71-79.
- [18] Daniel, I., P. Oger and R. Winter (2006). Origins of life and biochemistry under high-pressure conditions. *Chemical Society Reviews* 35(10): 858-875.
- [19] Del-Cid, A., P. Ubilla, M.-C. Ravanal, E. Medina, I. Vaca, G. Levicán, J. Eyzaguirre and R. Chávez (2014). Cold-active xylanase produced by fungi associated with Antarctic marine sponges. *Applied Biochemistry and Biotechnology* 172(1): 524-532.
- [20] Duarte, A. W., I. Dayo-Owoyemi, F. S. Nobre, F. C. Pagnocca, L. C. Chaud, A. Pessoa, M. G. Felipe and L. D. Sette (2013). Taxonomic assessment and enzymes production by yeasts isolated from marine and terrestrial Antarctic samples. *Extremophiles* 17(6): 1023-1035.
- [21] Dubrovskaya, Y. V., V. V. Sova, N. N. Slinkina, S. D. Anastyuk, M. V. Pivkin and T. N. Zvyagintseva (2012). Extracellular β -D-glucosidase of the *Penicillium canescens* marine fungus. *Applied Biochemistry and Microbiology* 48(4): 401-408.
- [22] Edgcomb, V. P., D. Beaudoin, R. Gast, J. F. Biddle and A. Teske (2011). Marine subsurface eukaryotes: the fungal majority. *Environmental Microbiology* 13(1): 172-183.
- [23] Edgcomb, V., W. Orsi, C. Leslin, S. S. Epstein, J. Bunge, S. Jeon, M. M. Yakimov, A. Behnke and T. Stoeck (2009). Protistan community patterns within the brine and halocline of deep hypersaline anoxic basins in the eastern Mediterranean Sea. *Extremophiles* 13(1): 151-167.
- [24] El-Gendy, M. M. A. (2010). Keratinase production by endophytic *Penicillium* spp. morsyl under solid-state fermentation using rice straw. *Applied Biochemistry and Biotechnology* 162(3): 780-794.
- [25] El-Sayed, A. S., M. N. Hassan and H. M. Nada (2015). Purification, immobilization, and biochemical characterization of L-arginine deiminase from thermophilic *Aspergillus fumigatus* KJ434941: anticancer activity in vitro. *Biotechnology Progress* 31(2): 396-405.
- [26] El-Sayed, A. S., A. A. Shindia, A. A. Diab and A. M. Rady (2014). Purification and immobilization of L-arginase from thermotolerant *Penicillium chrysogenum* KJ185377.1; with unique kinetic properties as thermostable anticancer enzyme. *Archives of Pharmacal Research*. <https://doi.org/10.1007/s12272-014-0498-y>.
- [27] Elshafei, A. M., M. M. Hassan, N. H. Ali, M. A. E. Abouzeid, D. A. Mahmoud and D. H. Elghonemy (2014). Purification, kinetic properties and antitumor activity of L-glutaminase from *Penicillium brevicompactum* NRC 829. *British Microbiology Research Journal* 4(1): 97-115.
- [28] Elyas, K. K., A. Mathew, R. K. Sukumaran, P. P. M. Ali, K. Sapna, S. R. Kumar and K. R. R. Mol (2010). Production optimization and properties of beta glucosidases from a marine fungus *Aspergillus*-SA 58. *New Biotechnology* 27(4): 347-351.
- [29] Farag, A. M., S. W. Hassan, E. A. Beltagy and M. A. El-Shenawy (2015). Optimization of production of anti-tumor L-asparaginase by free and immobilized marine *Aspergillus terreus*. *The Egyptian Journal of Aquatic Research* 41(4): 295-302.
- [30] Feller, G., M. Thiry, J.-L. Arpigny, M. Mergeay and C. Gerday (1990). Lipases from psychrotropic Antarctic bacteria. *FEMS Microbiology Letters* 66(1-3): 239-243.
- [31] Fenice, M., J.-L. Leuba and F. Federici (1998). Chitinolytic enzyme activity of *Penicillium janthinellum* P9 in bench-top bioreactor. *Journal of Fermentation and Bioengineering* 86(6): 620-623.
- [32] Gomes, D., M. Cavalcanti, M. Fernandes, D. Lima and J. Passavante (2008). Filamentous fungi isolated from sand and water of Bairro Novo and Casa Caiada beaches, Olinda, Pernambuco, Brazil. *Brazilian Journal of Biology* 68(3): 577-582.
- [33] Grant, W., M. Atkinson, B. Burke and C. Molloy (1996). Chitinolysis by the marine ascomycete *Corollospora maritima* Werdermann: purification and properties of a chitobiosidase. *Botanica Marina* 39(1-6): 177-186.
- [34] Guo, N., F. Gong, Z. Chi, J. Sheng and J. Li (2009). Enhanced inulinase production in solid state fermentation by a mutant of the marine

- yeast *Pichia guilliermondii* using surface response methodology and inulin hydrolysis. *Journal of Industrial Microbiology & Biotechnology* 36(4): 499-507.
- [35] Hawksworth, D. L. and R. Lucking (2017). Fungal Diversity Revisited: 2.2 to 3.8 Million Species. *Microbiology Spectrum* 5(4).
- [36] Hou, Y.-H., T.-H. Wang, H. Long and H.-Y. Zhu (2006). Novel cold-adaptive *Penicillium* strain FS010 secreting thermo-labile xylanase isolated from Yellow Sea. *Acta Biochimica et Biophysica Sinica* 38(2): 142-149.
- [37] Jones, E. G. (2000). Marine fungi: some factors influencing biodiversity. *Fungal Diversity* 4(193): 53-73.
- [38] Jones, E. G. and K.-L. Pang (2012). *Marine Fungi: and Fungal-like Organisms*. de Gruyter. pp. 528.
- [39] Jones, E., J. Sakayaroj, S. Suetrong, S. Somrithipol and K. Pang (2009). Classification of marine Ascomycota, anamorphic taxa and Basidiomycota. *Fungal Diversity* 35(1): 187.
- [40] Jones, M. D., I. Forn, C. Gadelha, M. J. Egan, D. Bass, R. Massana and T. A. Richards (2011). Discovery of novel intermediate forms redefines the fungal tree of life. *Nature* 474(7350): 200-203.
- [41] Kiiskinen, L. L., M. Rättö and K. Kruus (2004). Screening for novel laccase - producing microbes. *Journal of Applied Microbiology* 97(3): 640-646.
- [42] Kirsh, D. (1935). Lipase production by *Penicillium oxalicum* and *Aspergillus flavus*. *Botanical Gazette* 97(2): 321-333.
- [43] Kobayashi, T., O. Koide, K. Mori, S. Shimamura, T. Matsuura, T. Miura, Y. Takaki, Y. Morono, T. Nunoura and H. Imachi (2008). Phylogenetic and enzymatic diversity of deep seafloor aerobic microorganisms in organics-and methane-rich sediments off Shimokita Peninsula. *Extremophiles* 12(4): 519-527.
- [44] Kohlmeyer, J. and B. Volkmann-Kohlmeyer (2003). *Mycological research news*. *Mycol Res* 107: 385-387.
- [45] Kohlmeyer, J. and E. Kohlmeyer (1979). *Marine mycology: the higher fungi.*, Academic Press, New York; London.
- [46] Kuhad, R. C., A. Singh and K.-E. L. Eriksson (1997). Microorganisms and enzymes involved in the degradation of plant fiber cell walls. *Advances in Biochemical Engineering and Biotechnology* 57: 45-125.
- [47] Li, H., Z. Chi, X. Wang, X. Duan, L. Ma and L. Gao (2007). Purification and characterization of extracellular amylase from the marine yeast *Aureobasidium pullulans* N13d and its raw potato starch digestion. *Enzyme and Microbial Technology* 40(5): 1006-1012.
- [48] Li, Q. and G. Wang (2009). Diversity of fungal isolates from three Hawaiian marine sponges. *Microbiological Research* 164(2): 233-241.
- [49] Liu, C. H., X. Huang, T. N. Xie, N. Duan, Y. R. Xue, T. X. Zhao, M. A. Lever, K. U. Hinrichs and F. Inagaki (2017). Exploration of cultivable fungal communities in deep coal - bearing sediments from ~ 1.3 to 2.5 km below the ocean floor. *Environmental Microbiology* 19(2): 803-818.
- [50] Maheshwari, R., G. Bharadwaj and M. K. Bhat (2000). Thermophilic fungi: their physiology and enzymes. *Microbiology and Molecular Biology Reviews* 64(3): 461-488.
- [51] Mayer, A., A. D. Rodríguez, O. Tagliatalata-Scafati and N. Fusetani (2013). Marine pharmacology in 2009-2011: Marine compounds with antibacterial, antidiabetic, antifungal, anti-inflammatory, antiprotozoal, antituberculosis, and antiviral activities; affecting the immune and nervous systems, and other miscellaneous mechanisms of action. *Marine Drugs* 11(7): 2510-2573.
- [52] Méjanelle, L., J. F. Løpez, N. Gunde-Cimerman and J. O. Grimalt (2000). Sterols of melanized fungi from hypersaline environments. *Organic Geochemistry* 31(10): 1031-1040.
- [53] Miura, T., F. Abe, A. Inoue, R. Usami and K. Horikoshi (2001). Purification and characterization of novel extracellular endopolygalacturonases from a deep-sea yeast, *Cryptococcus* sp. N6, isolated from the Japan Trench. *Biotechnology Letters* 23(21): 1735-1739.
- [54] Mohapatra, B. R., U. C. Banerjee and M. Bapuji (1998). Characterization of a fungal amylase from *Mucor* sp. associated with the marine sponge *Spirastrella* sp. *Journal of Biotechnology* 60(1): 113-117.
- [55] Morrison-Gardiner, S. (2002). Dominant fungi from Australian coral reefs. *Fungal Diversity* 9: 105-121.
- [56] Mtui, G. and Y. Nakamura (2004). Lignin-degrading enzymes from mycelial cultures of basidiomycete fungi isolated in Tanzania. *Journal of Chemical Engineering of Japan* 37(1): 113-118.
- [57] Nascimento, T. C. E. d. S., A. R. d. Sena, J. E. G. Gomes, W. L. d. Santos, G. S. Agamez Montalvo, E. B. Tambourgi, E. V. d. Medeiros, L. D. Sette, A. Pessoa Junior and K. A. Moreira (2015). Extracellular serine proteases by *Acremonium* sp. L1-4B isolated from Antarctica: Overproduction using cactus pear extract with response surface methodology. *Biocatalysis and Agricultural Biotechnology* 4(4): 737-744.
- [58] Osterhage, C. (2001). Isolation, structure determination and biological activity assessment of secondary metabolites from

- marine-derived fungi. Ph.D. Thesis, Von Claudia Osterhage. pp.186.
- [59] Parte, S., V. L. Sirisha and J. S. D'Souza (2017). Chapter Four - Biotechnological Applications of Marine Enzymes From Algae, Bacteria, Fungi, and Sponges. *Advances in Food and Nutrition Research*. S.-K. Kim and F. Toldrá, Academic Press. 80: 75-106.
- [60] Passarini, M. R., M. V. Rodrigues, M. da Silva and L. D. Sette (2011). Marine-derived filamentous fungi and their potential application for polycyclic aromatic hydrocarbon bioremediation. *Marine Pollution Bulletin* 62(2): 364-370.
- [61] Pointing, S. and K. Hyde (2001). *Bio-Exploitation of Filamentous Fungi*. Fungal Diversity Press, The University of Hong Kong.
- [62] Pointing, S., L. Vrijmoed and E. Jones (1998). A qualitative assessment of lignocellulose degrading enzyme activity in marine fungi. *Botanica Marina* 41(1-6): 293-298.
- [63] Raghukumar, C., M. Shailaja, P. Parameswaran and S. Singh (2006). Removal of polycyclic aromatic hydrocarbons from aqueous media by the marine fungus NIOCC# 312: involvement of lignin-degrading enzymes and exopolysaccharides. *Indian Journal of Marine Sciences* 35(4): 373-379.
- [64] Raghukumar, C., S. Raghukumar, A. Chinnaraj, D. Chandramohan, T. D'souza and C. Reddy (1994). Laccase and other lignocellulose modifying enzymes of marine fungi isolated from the coast of India. *Botanica Marina* 37(6): 515-524.
- [65] Raghukumar, C., S. Raghukumar, G. Sheelu, S. Gupta, B. N. Nath and B. Rao (2004a). Buried in time: culturable fungi in a deep-sea sediment core from the Chagos Trench, Indian Ocean. *Deep Sea Research Part I: Oceanographic Research Papers* 51(11): 1759-1768.
- [66] Raghukumar, C., T. D'souza, R. Thorn and C. Reddy (1999). Lignin-modifying enzymes of *Flavodon flavus*, a basidiomycete isolated from a coastal marine environment. *Applied and Environmental Microbiology* 65(5): 2103-2111.
- [67] Raghukumar, C., U. Muraleedharan, V. Gaud and R. Mishra (2004b). Xylanases of marine fungi of potential use for biobleaching of paper pulp. *Journal of Industrial Microbiology and Biotechnology* 31(9): 433-441.
- [68] Raghukumar, S. (2017). *The Marine Environment and the Role of Fungi*. In: *Fungi in Coastal and Oceanic Marine Ecosystems*. Springer, Cham.
- [69] Rämä, T., J. Nordén, M. L. Davey, G. H. Mathiassen, J. W. Spatafora and H. Kausrud (2014). Fungi ahoy! Diversity on marine wooden substrata in the high North. *Fungal Ecology* 8: 46-58.
- [70] Richards, T. A., M. D. Jones, G. Leonard and D. Bass (2012). Marine fungi: their ecology and molecular diversity. *Annual Review of Marine Science* 4: 495-522.
- [71] Robinson, C. H. (2001). Cold adaptation in Arctic and Antarctic fungi. *New phytologist* 151(2): 341-353.
- [72] Saleem, M., M. S. Ali, S. Hussain, A. Jabbar, M. Ashraf and Y. S. Lee (2007). Marine natural products of fungal origin. *Natural Product Reports* 24(5): 1142-1152.
- [73] Samuel, P., M. Maheswari, J. Vijayakumar, T. Selvarathinam, K. Amirtharaj and R. Deenathayalan (2018). Bio-prospecting of marine-derived fungi with special reference to production of keratinase enzyme - A need-based optimization study. *Journal of Applied Biology & Biotechnology* 6(3): 35-41.
- [74] Sanjatha, G. and I. M., Sudheer (2017). Isolation, screening, optimization and production of Anti-tumor L-Asparaginase by fungi from karwar coastal region. *Research Journal of Recent Sciences* 6(3): 1-7.
- [75] Seiichi, A., A. Yoshida and M. Hatano (1991). Occurrence of marine bacterial lipase hydrolyzing fish oil. *Agricultural and Biological Chemistry* 55(10): 2657-2659.
- [76] Singh, R. P., V. Gupta, P. Kumari, M. Kumar, C. R. K. Reddy, K. Prasad and B. Jha (2011). Purification and partial characterization of an extracellular alginate lyase from *Aspergillus oryzae* isolated from brown seaweed. *Journal of Applied Phycology* 23(4): 755-762.
- [77] Synnes, M. (2007). Bioprospecting of organisms from the deep sea: scientific and environmental aspects. *Clean Technologies and Environmental Policy* 9(1): 53-59
- [78] Velmurugan, N. and Y. S. Lee (2012). Enzymes from marine fungi: current research and future prospects. in *Marine Fungi and Fungal-like Organisms (Marine and Freshwater Botany)*. e. E. B. G. Jones. Berlin: Walterde Gruyter, p. 441-474.
- [79] Velmurugan, N., D. Kalpana, H. Han Jung, J. Cha Hyo and Y. Soo Lee (2011). A novel low temperature chitinase from the marine fungus *Plectosphaerella* sp. strain MF-1. *Botanica Marina* 54: 75-81.
- [80] erma, A. K., C. Raghukumar, P. Verma, Y. S. Shouche and C. G. Naik (2010). Four marine-derived fungi for bioremediation of raw textile mill effluents. *Biodegradation* 21(2): 217-233.
- [81] Wainwright, M., N. C. Wickramasinghe, J. Narlikar and P. Rajaratnam (2003). Microorganisms cultured from stratospheric air samples obtained at 41 km. *FEMS Microbiology Letters* 218(1): 161-165.

Novel Enzymes Isolated from Marine-derived Fungi and its Potential Applications

- [82] Wang, L., Z. Chi, X. Wang, Z. Liu and J. Li (2007). Diversity of lipase-producing yeasts from marine environments and oil hydrolysis by their crude enzymes. *Annals of Microbiology* 57(4): 495.
- [83] Ward, O. P. (2012). Production of recombinant proteins by filamentous fungi. *Biotechnology Advances* 30(5): 1119-1139.
- [84] Wu, J., C. Qiu, Y. Ren, R. Yan, X. Ye and G. Wang (2018). Novel salt-tolerant xylanase from a mangrove-isolated fungus *Phoma* sp. MF13 and its application in Chinese steamed bread. *ACS Omega* 3(4): 3708-3716.
- [85] Zhang, C. and S.-K. Kim (2010). Research and application of marine microbial enzymes: status and prospects. *Marine Drugs* 8(6): 1920-1934.

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