# Chapter 22
# Fungi from Extreme Environments: A Potential Source of Laccases Group of Extremozymes

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## 22.1 Introduction

Laccases are a mixture of synergistic enzymes and include laccase, phenol oxidase, phenol peroxidase, lignin peroxidase, manganese peroxidase, tyrosinase, etc. They are copper-containing blue phenol oxidase and are common among various groups of organisms including bacteria, fungi and plants (Mayer 2006). Various groups of plants, animals and microbes produce phenol oxidases, both intracellular and extracellular, for a variety of purposes. Fungi, the second largest members of eukaryotic kingdom produce variety of phenol oxidases. Among all the fungi, *Ascomycota* and *Basidiomycota* particularly produce intracellular phenol oxidases and use them to synthesize protective compounds like melanin (pigmentation), in spore formation and detoxification of toxic compounds from their environment. Phenol oxidase enzymes are also responsible for fungal pathogenicity due to their plant cell wall lignin degradation potential. These enzymes hydrolyze lignocelluloses present in agro-waste, especially facilitating the degradation of lignin component which is the most complex constituent of plant cell wall. These are non-specific enzymes which can act on variety of phenolic substances. Hence, they are flexible and can be used in a range of industrial processes (Nigam 2013). These enzymes are well known in bioremediation, industrial effluent treatment containing hazardous chemicals like dyes, phenols and other xenobiotic compounds (Robinson et al. 2001a, b; Robinson and Nigam 2008; Dahiya et al. 2001). It is quite popular that the leather industry has adopted eco-friendly methods for tanning process using keratinases instead of chemicals. Similarly pulp and paper industries have adopted treatment of wood pulp by ligninolytic enzymes for lignin degradation. These ligninolytic enzymes are also

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being used in the wine, fruit juice and denim industries (Dahiya et al. 1998). Recently, concerns for decontamination of hazardous chemicals and environmental contaminants have been increased, especially in developing countries. India is also taking immense efforts in this direction to treat these hazardous compounds before releasing them in the environment. There are many compounds which are causing toxic effects on health and also damaging environment due to their presence in water bodies. This adversely affects soil and water microbiota, aquatic life, plants and human health. In humans, it is reported to cause cardiac toxicity, liver and kidney damage, neurotoxicity, reproductive and developmental toxicity and reduced blood pressure. Such compounds have long persistence in the environment and also show bioaccumulation and biomagnification in plants and animals. As discussed earlier, these are naturally present in fruits and part of plant components. Many phenolic compounds are also produced by humans through manufacturing of various day-to-day materials. At present, aquatic environments including rivers, pond, lakes, etc. are the most affected habitats in terms of phenolic contamination. These compounds enter the water bodies through natural, industrial, domestic and agricultural practices (Wallace 1996). Decomposition is not a problem for naturally produced phenols of plants and fruits origin because nature has developed the mechanism for their degradation. Wastewater from industries is really a matter of concern as it accumulates phenolic compounds in large quantity, which is difficult to manage. These compounds are also present in effluent of chemical and pharmaceutical industries, including petroleum refineries, petrochemical industries, coal gasification, resin manufacturing industries, dye synthesis units, pharmaceutical industries, pulp and paper mills, etc. In several countries, due to lack of standard norms, these compounds are gradually increasing in water bodies and creating health hazards in rivers. In addition, phenolic compounds also undergo transformation due to presence of other compounds in the aquatic body and microbial activities (Kulkarni and Kaware 2013). Due to their harmful effects, there is an urgent need to remove them from the environment (Huang et al. 2015; Nuhoglu and Yalcin 2005). Phenolic compounds are diverse in nature but in this chapter we will give the emphasis on three main types of compounds, namely phenol, cresol and alkyl phenol.

Many industries like pulp and paper are nowadays focusing on research on fungal and bacterial based bioremediation strategies due to their ability to synthesize polyphenol oxidase. It can be used for degradation of lignocelluloses and residual polyaromatic hydrocarbons for production of pulp, transformation of fuels and bioremediation of soils contaminated with toxic products (Duran et al. 2002; Claus 2003, 2004; Rabinovich et al. 2004; Masai et al. 2007) (Table 22.1). Fungi are the most potent producers of enzymes involved in lignin degradation. They require these enzymes for penetration into the plants cell wall, degradation of wood and litter biomass, etc. Pathogenic fungi and wood-rotting fungi have been studied in detail for the production of laccases (Robinson et al. 2001a, b; Robinson and Nigam 2008). These enzymes have been produced economically using several agricultural wastes as substrates on a large scale (Nigam and Pandey 2009).

**Table 22.1** Use of laccases in bioremediation process of various environmental pollutants

| Serial No. | Substrates | References |
|---|---|---|
| 1 | Xenobiotic compounds | Ullah et al. (2000), Schultz et al. (2001), and Bollag et al. (2003) |
| 2 | Synthetic dyes | Abadulla et al. (2000), Nagai et al. (2002), Claus et al. (2002), Soares et al. (2002), Peralta-Zamora et al. (2003), Wesenberg et al. (2003), and Zille et al. (2003) |
| 3 | Pesticides | Jolivalt et al. (2000), Torres et al. (2003) |
| 4 | Polycyclic aromatic hydrocarbons | Majcherczyk and Johannes (2000), Cho et al. (2002), and Pozdnyakova et al. (2004) |
| 5 | Bleaching of kraft pulp | Balakshin et al. (2001), Lund et al. (2003), and Sigoillot et al. (2004) |
| 6 | Detoxify agricultural soil | D'Annibale et al. (2000), Tsioulpas et al. (2002), and Velazquez-Cedeno et al. (2002) |

Among fungi, they are mainly produced by members of *Basidiomycota* and *Ascomycota* while *Zygomycota*, *Chytridiomycota* and *Glomeromycota* are not reported to produce them. Several members of *Ascomycota* and *Basidiomycota* produce these enzymes, *viz., Podospora anserine, Sclerotinia sclerotium, Pleurotus ostreatus, P. sapidus, Agaricus bisporus, Lentinus edodes, Schizophyllum commune, Trichoderma versicolor* and many other wood-rotting fungi (Bodke et al. 2012). There is only one report of slime moulds, *Physarum polycephalum* (Daniel et al. 1963), for this activity.

Fungi from extreme environment have also been studied to obtain suitable enzymes which can work in the harsh conditions of fermentation and in the conditions of paper-pulp delignification and waste treatment. Pulp and paper industries usually use thermophilic enzymes. Five thermophilic laccase enzyme isoforms were isolated, purified and characterized from xerophytic plants *Cereus pterogonus* and *Opuntia vulgaris* (Gali and Kotteazeth 2012, 2013; Kumar and Srikumar 2011, 2012). Different forms of laccases with extraordinary properties have been obtained from fungi like *Steccherinum ochraceum* and *Polyporus versicolor* (Chernykh et al. 2008; Nigam 2013). Several fungi, *viz., Curvularia lonarensis, Penicillium* sp., and *Trametes* sp., have been reported from various extreme environments (thermophiles, alkaliphiles, psychrophiles, marine fungi, etc.) which have been studied for the laccase enzyme production potentials (Sharma et al. 2016; Dhakar et al. 2014; Dhakar and Pandey 2013) which we discuss below in detail.

Fungal based biotechnology is still in the developmental stage since past few decades and has improved significantly. Fungi from terrestrial origin have diverse properties and are used in the production of antibiotics, extracellular enzymes, organic acids, etc. (Pointing and Hyde 2001) (Fig. 22.1a). In the past couple of decades, fungi have also been used as 'cell factories' due to the advancement in molecular and genetic tools (Punt et al. 2002). Only few studies are available on fungal laccases from extremophilic fungi as compared to terrestrial and mesophilic

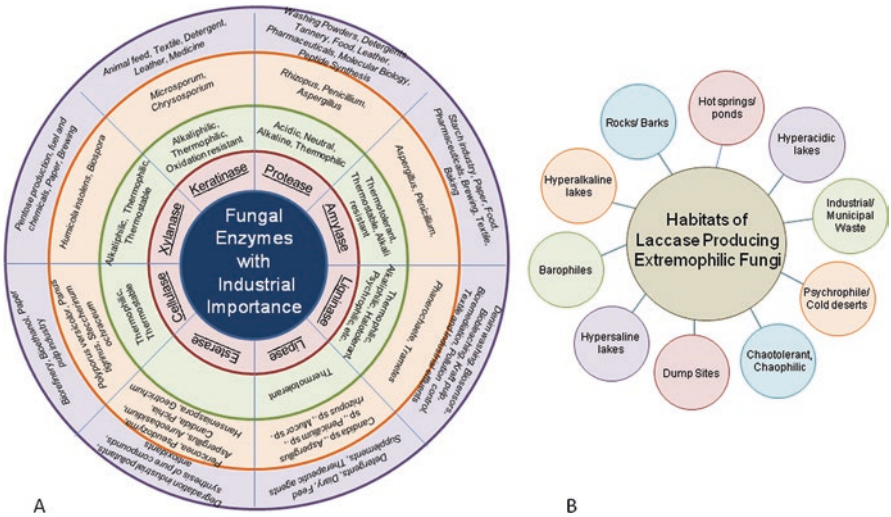


**Fig. 22.1** (**a**) Scope of industrial applications of various enzymes produced by fungi in industries, viz., textile, paper pulp, food and feed, and pharmaceutical industries. (**b**) Habitats of laccase-producing fungi from extreme environments

counterparts. What prospects do the extremophilic fungi have in such situation? Why and how these fungi have become more important target group of organisms for pharmaceutical and environmental perspectives? This chapter discussed importance of extremophilic fungi in production of laccases in association with description of fungal diversity from extremophilic environments as it helps to understand the distribution of laccases producing fungi. The emphasis is on (a) molecular biology and genetics of fungal laccases, (b) factors affecting production of laccases from extremophilic fungi and (c) recent advances on fungal laccases and potential of fungal laccases from extreme habitats.

## 22.2 Laccases Producing Fungi from Extreme Habitats

Fungi living in extreme environment such as high or low temperatures (thermophiles and psychrophiles), high salinity (halophiles), acidic or alkaline pH values (acidophiles and alkaliphiles, respectively), anoxygenic conditions (anaerobic fungi), high pressures (barophiles), etc. (Poli et al. 2017; Dalmaso et al. 2015; Magan 2007) are known as extremophilic fungi (Fig. 22.1b). Many reports are available on extracellular phenol oxidase by fungi from different habitats (Crognale et al. 2012) but only a few studies on laccase-producing fungi from extreme environment like marine, hot springs and soda lakes are available.

### *22.2.1 Laccase from Alkaliphilic Fungi*

The hyperalkaline habitat has both ecological and industrial significance as in high alkaline condition very few fungi can grow. It has been reported that most of the municipal wastewater treatment plants and effluents from industries have high alkalinity, and high concentration of metal ions. Hence fungi surviving in such conditions and with laccase-producing capacity can work as excellent bioinoculant for bioaugmentation-based bioremediation. Functional metagenomic studies of Soda Lake have showed that many uncultured fungi have laccases-like Cu-oxidase encoded with potential in degradation of phenolic compounds (Vavourakis et al. 2016; Ausec et al. 2011). Crognale et al. (2012) had also reported phenol oxidase-producing halotolerant fungi from olive brine wastewater. Sharma et al. (2016) isolated 104 fungal strains from Lonar lake (Fig. 22.2), a hyperalkaline habitat, and 14 were positive for enzyme production in primary screening using 2,2′-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid (ABTS) as substrate. It included *Fusarium equiseti*, *Curvularia lonarensis*, *Cladosporium funiculosum*, *Cladosporium oxysporum*, *Cladosporium halotolerans*, *Aspergillus niger*, a probable novel *Cladorrhinum* species and an unidentified fungus. Among these *Fusarium* sp. MEF008, *Curvularia lonarensis* MEF018 (Fig. 22.2), *Cladorrhinum* sp. MEF109 and *Cladosporium* sp. MEF135

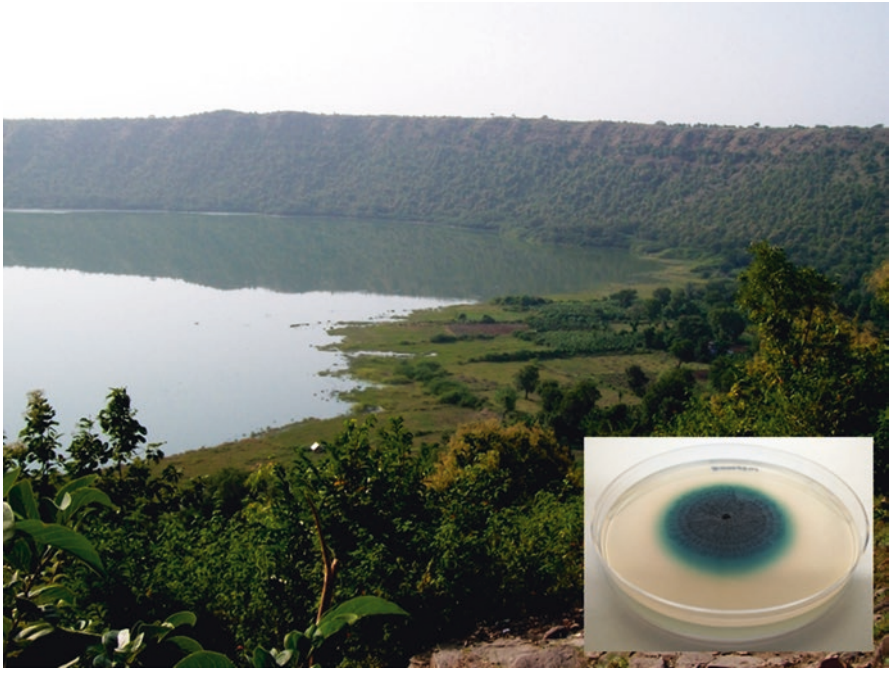

**Fig. 22.2** View of Lonar Lake, an alkaliphilic lake located at Buldhana district of Maharashtra state, India; (inset) plate showing culture (MEF018) positive for phenol oxidase production containing ABTS as substrate

**Table 22.2** List of phenol oxidase-producing fungi isolated from Lonar Lake

| Serial No. | Strain Id's | Identification | Isolated from | Phenol oxidase |
|---|---|---|---|---|
| 1 | MEF008 | *Fusarium equiseti* | Lonar Lake | +++ |
| 2 | MEF018 | *Curvularia lonarensis* | Lonar Lake | ++++ |
| 3 | MEF040 | *Cladosporium funiculosum* | Lonar Lake | + |
| 4 | MEF041 | *Cladosporium oxysporum* | Lonar Lake | + |
| 5 | MEF135 | *Cladosporium oxysporum* | Lonar Lake | +++ |
| 6 | MEF062 | Unidentified | Lonar Lake | + |
| 7 | MEF073 | *Cladosporium halotolerans* | Lonar Lake | + |
| 8 | MEF095 | *Aspergillus niger* | Lonar Lake | + |
| 9 | MEF104 | *Cladorrhinum* sp. nov. | Lonar Lake | + |
| 10 | MEF121 | *Cladorrhinum* sp. nov. | Lonar Lake | + |
| 11 | MEF127 | *Cladorrhinum* sp. nov. | Lonar Lake | + |
| 12 | MEF109 | *Cladorrhinum* sp. | Lonar Lake | +++ |
| 13 | MEF133 | *Cladorrhinum* sp. | Lonar Lake | + |
| 14 | MEF134 | *Cladorrhinum* sp. | Lonar Lake | + |

were higher phenol oxidase producer (Table 22.2). *Curvularia lonarensis* MEF018, an alkaliphilic fungus with potential to be exploited industrially, produced laccases at 40 °C, pH 12–14, and at salinity of 3%. While working on Lonar Lake, we observed that the fungi which were collected from the banks of the lake with wooden debris had showed phenol oxidase activity. The lake which is reported to be formed by meteor impact is surrounded by trees of *Acacia* sp. The wooden debris of trees falls on the lake water, leaching phenol in the lake water with time. In due course of time, fungi colonizing the wooden debris have developed the ability to produce laccase enzymes, thus playing an important role in the lake ecosystem. Such ecological adaptations of fungal strains have helped them to develop capacity to produce metabolites and enzymes which act at high temperature, pH, or salt concentrations.

### *22.2.2 Laccase from Marine Fungi*

The applications of laccases in degradation of xenobiotics by aquatic, obligate marine (and marine-derived) fungi have been observed (Martin et al. 2009; Junghanns et al. 2009; Pointing and Hyde 2000; Li et al. 2002). These marine fungi produce unique secondary metabolites and enzymes not reported from fungi residing in terrestrial habitats (Jensen and Fenical 2002). D'Souza-Ticlo et al. (2009a, b) reported that a marine isolate of *Cerrena unicolor* MTCC 5159 produces halotolerant laccase and also degrades raw textile mill effluents (Verma et al. 2010). Generally, marine fungi are able to grow on decaying lignocelluloses substrates like branches, leaves, and woods of mangroves which include mostly the members of *Ascomycota* and with few exceptions of species of *Basidiomycota* (Hyde and Jones 1988). Marine fungi play an important role in the degradation of mangrove leaves, wood pieces and other wooden

debris on the shores, thus forming detritus. These fungi play a significant role in the mineralization in the tropical marine ecosystem. However, the information related to marine laccase is still sparse and needs more work on the characterization of the type of lignin-modifying enzymes present in marine ecosystems. Raghukumar et al. (1994) isolated 17 fungi from marine habitats, out of which 12 were laccase positive which included *Gliocladium* sp., *Sordaria fimicola*, *Gongronella* sp., *Aigialus grandis*, *Halosarpheia ratnagiriensis*, *Verruculina enalia*, *Cirrenalia pygmea*, *Zalerion varium* and *Hypoxylon oceanicum.* Jaouani et al. (2014) have explored the fungal diversity of Sebkha El Melah, a Saharan salt flat located in southern Tunisia and isolated 21 moderately halotolerant fungi. It included 15 taxa belonging to 6 genera of *Ascomycota*, *viz., Cladosporium* spp., *Alternaria* spp., *Aspergillus* spp., *Penicillium* spp., *Ulocladium* sp. and *Engyodontium* sp. Three species out of 15 showed laccase activities at 10% NaCl, viz., *Cladosporium halotolerans*, *Cladosporium sphaerospermum* and *Penicillium canescens*. Laccase production at 10% salt by these strains is of biotechnological interest, especially in bioremediation of organic pollutants in high salt-contaminated environments.

### *22.2.3 Laccase from Thermophilic Fungi*

Now we know that life can exist in extreme environments and molecular studies related to their survival mechanisms in extreme condition shed new insight about their survival strategies in extreme habitats. The stabilization of processes due to thermal stress is because of multiple reasons and involves DNA, RNA, proteins, ribosomes and enzymes (Poli et al. 2017). Thermophilic fungi received immense attention due to their ability to produce enzymes suitable for industrial purposes. Species belonging to genus *Corynascus* (*Myceliopthora*) have been of interest to mycologist as it produces thermostable enzymes. For example, *Corynascus thermophilus* (basionym: *Thielavia thermophila*) produced thermostable laccases with high activity and ability to express in various hosts (Berka et al. 1997; Bulter et al. 2003; Babot et al. 2011). Laccases produced by *C. thermophilus* ATCC 42464 are completely characterized, patented and genome sequenced (Bhat and Maheshwari 1987; Roy et al. 1990; Sadhukhan et al. 1992; Badhan et al. 2007; Beeson et al. 2011). However, there is no other report of any thermophilic fungi which is so extensively studied for laccase production. It shows the scarcity of thermophilic laccase-producing strains available so far.

### *22.2.4 Laccase from Deep-Sea Sponge Fungi*

Studies on fungal diversity of marine sponges have been reported (Baker et al. 2009; Wang et al. 2008; Richards et al. 2012; He et al. 2014). Members of *Eurotiales*, *Capnoidales*, *Pleosporales* and *Hypocreales* have been identified and found to be

associated with various sponges (Suryanarayanan 2012). Aspergillus and Penicillium genera are ubiquitous with marine sponges whereas other genera which are associated with sponges but not that frequent include Alternaria, Acremonium, Beauveria, Cladosporium, Curvularia, Eurotium, Fusarium, Gymnascella, Paecilomyces, Petriella, Pichia, Spicellum and Trichoderma (Suryanarayanan 2012). Many marine fungi isolated from sponges have been screened for their lignocelluloytic activities (Bucher et al. 2004; Bianchi 2011; Richards et al. 2012). Fungi with lignocellulolytic activity from sea and other marine habitats like mangrove forests and sponges have been reported (Baker et al. 2009; Bonugli-Santos et al. 2010a, b). Batista-García et al. (2017) isolated fungi from sponges, *Stelletta normani* (*Demospongiae*, *Astrophorida*, *Ancorinidae*), from a depth of 751 m from Irish waters in the North Atlantic Ocean. Three halotolerant strains were isolated and identified which displayed laccase production along with other enzymes (CMCase and xylanase), viz., *Cadophora* sp. TS2, *Emericellopsis* sp. TS11 and *Pseudogymnoascus* sp. TS 12. These strains also showed psychrotolerance with optimal growth at 20 °C. Such strains are of immense importance both ecologically and industrially as they play significant role in maintenance of ecosystem and in development of industrial biotechnology.

### *22.2.5 Laccase from Lichen*

Lichen is a symbiotic association between a fungus and cyanobacterium. These are the microbes which preliminarily colonize on rocky substrates which is also considered an extreme environment due to lack of nutritional substrate. They are involved in the weathering of rocks and conversion and accumulation of organic matter forming the primitive soil (Nash 1996; Chen et al. 2000). Zavarzina and Zavarzin (2006) while studying the formation of primitive soil under vegetation observed that many lichens have the ability to produce and release phenol oxidases in environment.

Little information is available on the laccase isoforms from lichens. Extracellular laccase activity is considered to be due to combination of multi-copper oxidases, phenol peroxides and tyrosinases (Laufer et al. 2006a). Studies on lichen *Peltigera malacea* (a member of order *Peltigerales*) showed that the active form is a tetramer with a high molecular mass of 340 kD (Laufer et al. 2009). Work on lichens *Solorina crocea* and *Peltigera apthosa* shows that both contained a dimeric laccase (ca. 170 kD) and a monomeric form (ca.85 kD) (Lisov et al. 2007). Recently, Laufer et al. (2006b) and Zavarzina and Zavarzin (2006) reported that many lichens within the suborder Peltigerineae show high rates of extracellular laccase activity. In other groups of fungi laccases have been reported from a mass range of 60–70 kD, but laccases from lichen are heavier in the range of 200–350 kD (Baldrian 2006; Laufer et al. 2009). It has been found that almost all members of *Peltigerineae* family of lichens show some degree of laccase activity. Beckett et al. (2012, 2013) also reported strong peroxidase activity in various genera of *Peltigerales* order like

*Lobaria*, *Pseudocyphellaria* and *Sticta* and non-Peltigeralean lichens. They also showed that high laccase activity was present in the cell walls of thalli. However, their role in biology of lichens still needs more work as most of them grow on oligotrophic conditions, viz., rocks, bark, etc.

### *22.2.6 Laccase from Psychrophilic Fungi*

Several fungi are capable of producing extremozymes at varying temperature, pH and salt range. It is known that they play an important role in biodegradation in low-temperature habitats. Dhakar and Pandey (2013) and Dhakar et al. (2014) studied the production of laccases by thermotolerant *Trametes hirsuta* (MTCC 11397) and *Penicillium pinophilum* (MCC 1049) isolated from a glacial site in Indian Himalayan Region (IHR). Such features make the strains efficient for the degradation in extreme conditions. However, as per literature survey very few studies have been done on psychrophilic fungal laccases.

### *22.2.7 Laccase from Fungi Inhabiting Dumping Sites*

Ndahebwa Muhonja et al. (2018) studied the molecular and biochemical aspect of characterization of low-density polyethene (LDPE)-degrading fungi from Dandora dumpsite, Nairobi. They isolated ten fungal isolates and screened for their ability to produce extracellular laccase. *Aspergillus fumigatus* B2, 2 exhibited the highest presence of laccase which is reported to play a role in degradation of polyethene. In another study, Sumathi et al. (2016) studied the degradation of polyvinyl chloride (PVC) by laccase using a fungus *Cochliobolus* sp., isolated from plastic dumped soils near plastic industry in Renigunta near to Tirupati, Chittoor district of Andhra Pradesh, India. Plastic waste has become one of the worst man-made problems accounting for 20–30% of municipal solid waste in landfill sites. These are extreme man-made habitats wherein there is large concentration of metal toxicity, gases, etc. These studies demonstrate that fungi isolated from such habitats have potential application for bioremediation as there was significant difference in the Fourier-transform infrared spectroscopy (FTIR), Gas chromatography–mass spectrometry (GC-MS), Scanning Electron Microscope (SEM) results of control and *Cochliobolus* species-treated low-molecular-weight PVC. There is a need to conduct more studies in such extreme environments to isolate potential strains with desirable properties of bioremediations like PVC degradation and develop environment-friendly technology.

There is an immense hidden potential present in diverse group of fungi found in extreme environments (Tiquia and Mormile 2010). They are still not exploited for laccase to their complete potential due to difficulty in the culturing of such fungi in laboratory. In recent times, metagenomics has been regarded as a powerful omics tool,

by which we can conduct diversity analysis of any microbe including fungi by direct DNA extraction and sequencing from different matrix (Barone et al. 2014; Handelsman 2004). Studies on functional aspect mainly focused on enzyme encoding gene(s) and discovery of novel biocatalysts, secondary metabolites and bioactive compounds (Wong 2010). Metagenomic approach can be used to study the gene encoding novel laccase enzymes for industrial production. At present, such studies are mainly focussed on extreme habitats from which it seems too difficult to cultivate the fungal population. It has been extremely effective in the discovery of novel extremophilic enzymes discovered from marine habitats, cold-adapted enzymes, thermophilic homologs, etc. Metagenomic tools are more commonly used with bacteria as compared with fungi. Fang et al. (2012) discovered a novel laccase with alkaline activity from bacterium. Miyazaki (2005) detected copper-inducible laccase activity in *Thermus thermophilus* HB27. It became possible by searching the genome databases of aerobic thermophilic bacteria for laccases and an open reading frame (OPR) annotated TTC1370 in *T. thermophilus* HB27 (Henne et al. 2004). Suryanarayanan et al. (2012) and Kunamneni et al. (2008) have also reported laccases from fungal endophytes of plants, which may not be an extreme habitat but surely a unique one. However most of the fungal diversity of extreme environments is still unexplored because of the imitating of the extreme conditions. Moreover, it is estimated that very less fungi of extreme environments are known; hence, a lot of fungal diversity still remains to be explored and exploited for laccase production. Use of metagenomics in fungi will provide an opportunity of culture-independent study of fungal diversity of extreme environments and its biotechnological application enzymes such as laccase (Fig. 22.3a).

## 22.3 Biological and Ecological Role of Laccase in Extreme Habitats

Extreme environments pose severe physicochemical conditions to the microbes accompanied by low molecular diffusion, macromolecular interactions and low metabolic rate. Hence, to survive in extreme climatic and environmental conditions, microbes including fungi need physiological adaptations. These adaptations facilitate in cellular functions and metabolic reactions. In such habitats fungi and other microbes possess proteins and enzymes which are robust in nature and help them to survive in harsh conditions. Fungi follow absorptive mode of nutrition and enzymes play an important role in breaking down the complex food material into simpler ones for absorption purpose. In addition, it also helps in invasion of plants during pathogenesis (fungal virulence factors) by plant-wounding response and pathogen defence (Beckett et al. 2005). The role of laccase has been studied in detail in white-rot fungi, where they participate in reactions that produce reactive oxygen species (ROS) such as the superoxide ($O_2^-$) and hydroxyl radicals (–OH). These molecules are involved in lignin degradation (Hammel et al. 2002; Leonowicz et al. 2001).

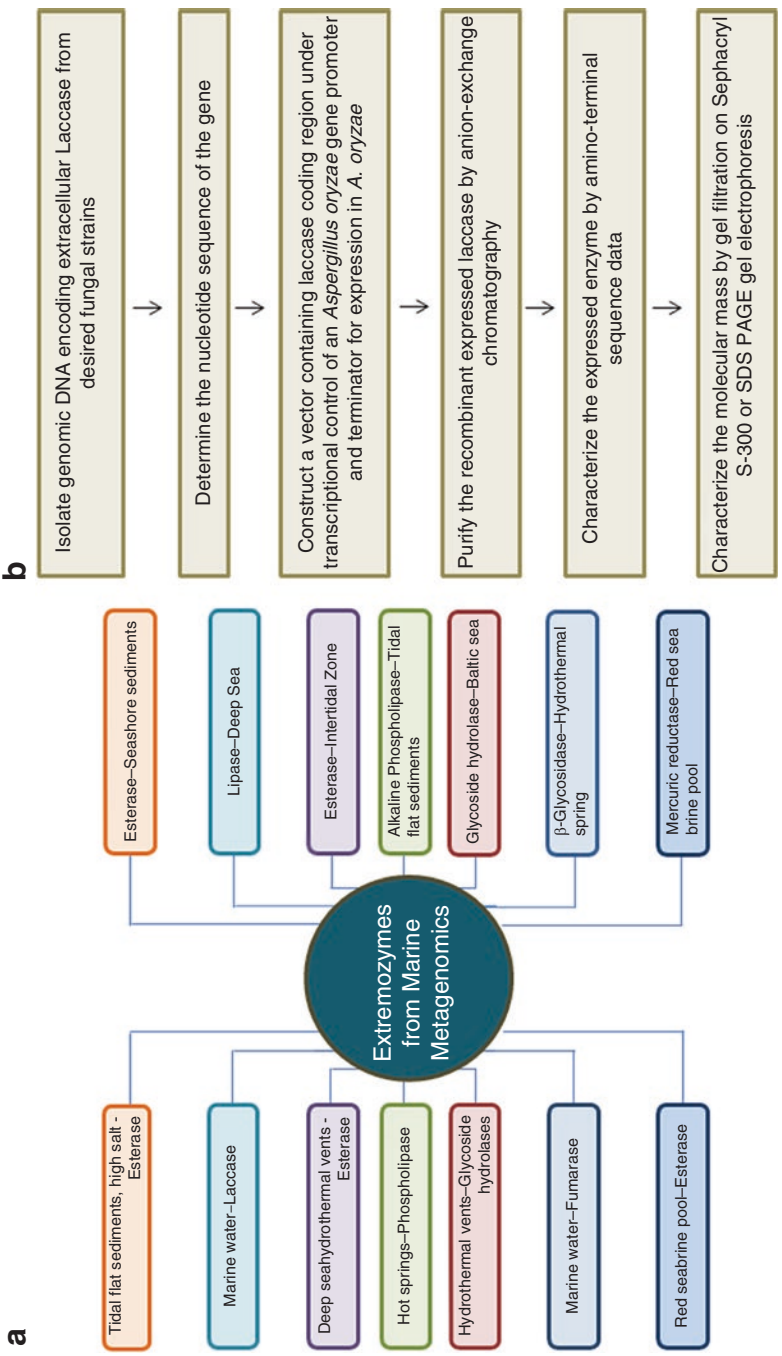


**Fig. 22.3** (**a**) Metagenomics have helped in the discovery of several extremozymes from obligate marine or associated environment; (**b**) flowchart of methodology used for characterization of gene encoding laccase enzyme

Fungal laccases are reported to be involved in development stages of fungi like morphogenesis and melanin production which is also related to the pathogen factor (Baldrian 2006). Laccases have also been reported to have other physiological roles like development of fruiting bodies, sporulation, fungal spore pigmentation and cell wall reconstitution (Alcalde 2007). In extreme environments, laccases are involved in polymerization and depolymerization of humic acids in sediments of marine habitats (Zavarzina et al. 2004). The dothideaceous black yeasts synthesize DHN-melanin. In the last step of the DHN-melanin pathway where 1,8-DHN molecules conjoin to form melanin polymer and enzymes such as phenol oxidases (tyrosinase and laccases), peroxidases are responsible for the same (Yurlova et al. 2008).

Lignin is a complex plant cell wall component consisting of phenylpropanoid units linked by C–C and C–O bonds. Since very less organisms secrete laccases extracellularly fungi with laccase production potential play an important role in lignin depolymerization. Although enzymes are substrate specific, an important aspect of laccases enzyme is that it has a broad range of substrates. This is because it consists of a group of enzymes which includes lignin peroxidase, manganese peroxidases, laccases, tyrosinases, phenol oxidase, etc. Authors observed that fungi of Lonar Lake were playing an important role by contributing in the carbon cycle by degrading the phenolic compounds released from the woods, thus playing a significant role in the alkaliphilic ecosystem. In man-made extreme environments such as plastic-dumping sites and landfills fungi are involved in polymer degradation through depolymerization and polymer is broken into smaller subunits. Some fungi growing in such habitats utilize the plastic materials as a source of carbon. *Pestalotiopsis microspora*, *Fusarium solani*, *Alternaria solani*, *Spicaria* spp., *Penicillium oxalicum*, *P. chrysogenum*, *Aspergillus fumigatus*, *A. terreus* and *A. flavus* isolates were found to grow on polyester polyurethane (PUR) as the sole carbon (Russell et al. 2011; Kale et al. 2015; Ojha et al. 2017; Ibrahim et al. 2011). The discovery of new laccases and Thurston's (1994) study have extensively reviewed the role of laccase in the biology of the fungi, but further studies on laccases using advance method will further extend our knowledge about their role in fungal biology.

## 22.4 Potential of Laccase Enzymes

The pulp consists of cellulosic fibres of wood, crops and waste paper. It is made from mechanical and chemical processes that separate cellulose fibres from rest of the wood. It includes application of harsh conditions like high temperatures (~80 °C), alkaline pH and use of chemicals. Developed countries are pushing for use of eco-friendly methods as an alternative of chemical methods, and enzymatic bio-pulping is being considered a viable option. It is eco-friendly, safer and a profitable solution for the paper and pulp industry using stable hyperthermophilic/alkaline enzymes. Sarmiento et al. (2015) have listed selected examples of in-development and commercially available hot and cold-adapted extremozymes (Table 22.3). The paper and pulp industries are using laccases for bio-bleaching and degradation

**Table 22.3** Examples of commercially available cold-active and thermostable enzymes (adopted and modified from Sarmiento et al. 2015)

| Market | Enzyme | Commercially available | Uses |
|---|---|---|---|
| *Cold-active enzymes* | | | |
| Molecular biology | Alkaline phosphatases | Antarctic phosphatase (New England Biolabs Inc.) | Dephosphorylation of 5′ end of a linearized fragment of DNA |
| | Uracil-DNA N-glycosylases (UNGs) | Uracil-DNA N-glycosylase (UNG) (ArcticZymes), Antarctic Thermolabile UDG (New England Biolabs Inc.) and | Release of free uracil from uracil-containing DNA |
| | Nucleases | Cryonase (Takara-Clontech) | Digestion of all types of DNA and RNA |
| Detergent | Lipases | Lipoclean®, Lipex®, Lipolase® Ultra, Kannase, Liquanase®, Polarzyme®, (Novozymes) | Breaking down of lipid stains |
| | Proteases | Purafect® Prima, Properase®, Excellase (Genencor) | Breaking down of protein stains |
| | Amylases | Stainzyme® Plus (Novozymes), Preferenz™ S100 (DuPont), Purafect® OxAm (Genencor) | Breakdown starch-based stains |
| | Cellulases | Rocksoft™ Antarctic, Antarctic LTC (Dyadic), UTA-88 and UTA-90 (Hunan Youtell Biochemical), Retrocell Recop and Retrocell ZircoN (EpyGen Biotech), Celluzyme®, Celluclean® (Novozymes) | Wash of cotton fabrics |
| | Mannanases | Mannaway® (Novozymes), Effectenz™ (DuPont) | Degradation of mannan or gum |
| | Pectate lyases | XPect® (Novozymes) | Pectin-stain removal activity |
| Textile | Amylases | Optisize® COOL and Optisize NEXT (Genencor/DuPont) | Desizing of woven fabrics |
| | Cellulases | Primafast® GOLD HSL IndiAge® NeutraFlex, PrimaGreen® EcoLight 1 and PrimaGreen® EcoFade LT100 (Genencor/DuPont) | Bio-finishing combined with dyeing of cellulosic fabrics |
| Food and beverages | Pectinases | Novoshape® (Novozymes), Pectinase 62L (Biocatalysts), Lallzyme® (Lallemand) | Fermentation of beer and wine, breadmaking, and fruit juice processing |

(continued)

**Table 22.3** (continued)

| Market | Enzyme | Commercially available | Uses |
|---|---|---|---|
| Other | Catalase | Catalase (CAT), (Swissaustral) | Textile, cosmetic applications |
| *Thermostable enzymes* | | | |
| Food and beverages | Amylases | Avantec®, Termamyl® SC, Liquozyme®, Novamyl®, Fungamyl® (Novozymes), Fuelzyme® | Enzymatic starch hydrolysis to form syrups. Applied in processes, such as baking, brewing, preparation of digestive aids, production of cakes and fruit juices |
| | Glucoamylases | Spirizyme® (Novozymes) | Used on liquefied starch-containing substrates |
| | Glucose (xylose) isomerases | Sweetzyme® (Novozymes) | Isomerization equilibrium of glucose into fructose |
| | Proteases | Protease PLUS | Applied in brewing to hydrolyze most proteins |
| | Amyloglucosidases | GlucoStar PLUS (Dyadic) | Used in processing aids |
| | Xylanases, cellulases, pectinases, mannanases, β-xylosidases, α-l-arabinofuranosidases, amylases, protease, other | CeluStar XL, BrewZyme LP, Dyadic Beta Glucanase BP CONC, Dyadic xylanase PLUS, Xylanase 2XP CONC, AlphaStar CONC and Protease AP CONC. (Dyadic), Panzea BG, Panzea 10× BG, Panzea Dual (Novozymes), Cellulase 13P (Biocatalysts) | Hydrolysis of hemicellulose and cellulose to lower molecular weight polymers in brewing |
| | Lipases and xylanases | Lipopan® and Pentopan® (Novozymes) | Stronger dough in bakery |
| | Glucose oxidases | Gluzym® (Novozymes) | Stronger gluten in bakery |
| Pulp and paper | Xylanases | Luminase® PB-100 and PB-200 (Verenium), Xylacid® (Varuna Biocell), Xyn 10A (Megazyme) | Bio-bleaching |
| | Laccases | Laccase (Novozyme) | Bio-bleaching |
| | Lipases and esterases | Lipase B Lipozyme® CALB L, Lipase A NovoCor®AD L, Resinase™ HT and Resinase A2X (Novozymes), Optimyze® (Buckman Laboratories) | Pitch control |
| | Cellulases/hemicellulases preparations | FibreZyme® G5000, FibreZyme® LBL CONC, FibreZyme™ LDI and FibreZyme™ G4 (Dyadic) | Modify cellulose and hemicellulose components of virgin and recycled pulps |
| | Amylase | Dexamyl-HTP (Varuna Biocell) | Modified starch of coated paper |

of lignin (which renders dark colour to pulp), thus improving the colour of the product. According to Virk et al. (2012), laccases also help in removal of tacky materials containing resins from the wood. Fungal laccases have been more popular for use in bio-bleaching as compared to laccases of bacterial origin (Baldrian 2004, 2006). Fungi from extreme environment are considered as an important source of commercial enzymes such as amylase, lipases, protease and cellulase. Novozymes are such thermostable laccases produced from thermophilic fungus *Corynascus thermophilus*. AB Vista (Wiltshire, UK) has the patent for thermostable laccase enzyme effective between 30 °C and 80 °C (Paloheimo et al. 2006; Sarmiento et al. 2015). For commercial production, the laccase gene from *M. thermophila* is cloned in *Aspergillus oryzae* and the enzyme is active up to 70 °C (Xu et al. 1996; Berka et al. 1997) (Fig. 22.3b). Novozyme commercially produces laccase enzyme (EC number 1.10.32) Novozym® 51003, from fungus *Aspergillus oryzae.* It is produced in liquid form and oxidizes various phenols, anilines, benzenethiols, metal ion complexes and other compounds into quinones or other oxidized compounds, with concomitant reduction of dioxygen to water. Another product Novozymes DeniLite® is being used for enzymatic bleaching solutions altering the indigo colour through oxidation. It has made denim bleaching safer, eco-friendly and more sustainable. The rapid action of enzymes coupled with low working temperature of peroxidase enzyme helps in the production of more durable denims.

## 22.5 Current Challenges and Conclusions

Today, with increasing awareness of climate change and sustainable development, there is an inadvertent pressure on biotechnology to deliver eco-friendly solutions to processes presently employing chemical methods. Biotechnological industries are utilizing a variety of enzymes as solutions to various industrial processes. Many of these are synthesized commercially using fungal strains selected after large-scale screening. Research on extreme environments has helped in the selection of particular fungal strain with desired property. These have also been optimized for the production of high-quality laccase on a large scale for industrial applications. There is a huge requirement of laccases for the industries working in the field of waste management, whether it is biomedical, agriculture, or municipal waste and paper pulp industries (Fig. 22.4). However, there is a need to study various extreme environments with the aim of isolating fungi having potential of laccase production and capable of acting in conditions of high pH, temperature and still maintain high activity. Recent studies in molecular biology and genetics have helped in inserting and expressing the active factor or gene of the desired fungi into bacteria or yeast for rapid and easy production. This has helped in production of laccase of desired property like thermostability, tolerance to acidic or alkaline environments and metal toxicity.

As discussed above, at present most of the studies have been focussed on the laccase produced by white-rot fungi-like species of *Fomes*, *Panus* and *Phanerochaete* (Papinutti et al. 2008; Quaratino et al. 2007; Dahiya et al. 2001) which are mostly

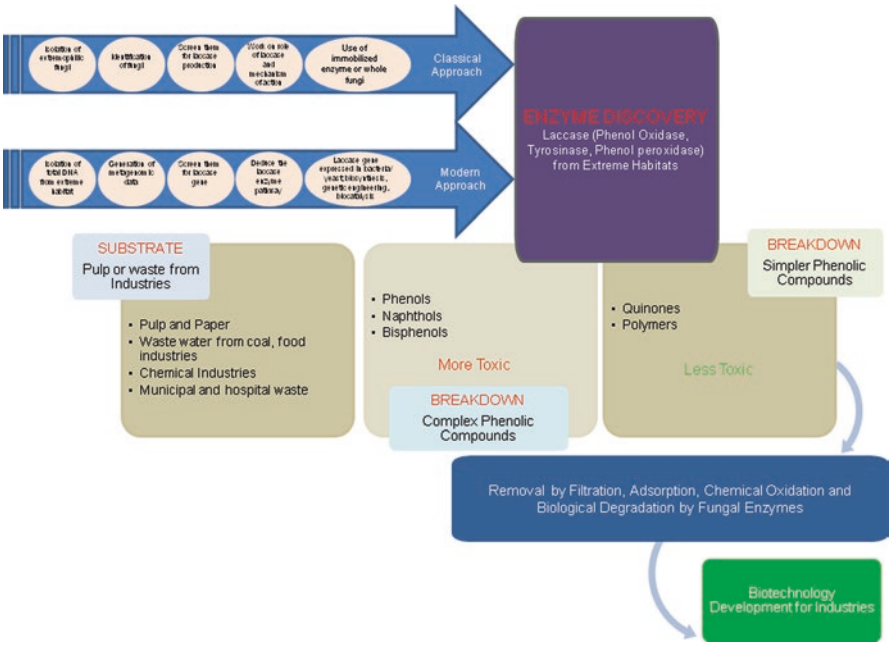


**Fig. 22.4** Extremophilic fungi have immense potentials for development of sustainable environmental technologies including biodegradation of phenolic pollutants and provide waste management solutions to various industries. This is well supported by advances in microbial techniques enabling the cloning of gene or gene clusters involved in the biosynthesis of laccases group of enzymes

mesophilic. Hence, there is a need to focus studies on fungi from extreme environments like soda lakes, hot springs, marine habitats, etc. for their isolation and screening for active laccase production. There are more studies on extremophilic bacteria as compared to extremophilic fungi. It may be due to optimized isolation strategies being used and incubation methods being practised and refined in case of bacteria but less practised in case of fungi. Recent techniques of metagenomics can also be employed to know the fungal population of such habitats and then design isolation strategies accordingly (Fig. 22.4). Many researchers have employed strategies to directly understand the functional aspect of an ecosystem using BIOLOG and API strips (Oest et al. 2018; Patel et al. 2019; Tiquia 2010, 2011). Similar strategy can be used in extreme environments that will help to know the laccase activity of the habitat, thus giving an idea of the laccase-producing abilities of the fungal strains inhabiting such habitats. Amplification of laccase gene using specific primers is another strategy for evaluating the capacity of fungal strains for laccase production. Such strategies may yield fungal strains producing laccases with unusual properties useful in industrial applications. There is a growing demand for novel and robust laccases for biotechnology in industrial applications, i.e. biofuel production, pulp and paper industries and eco-friendly municipal waste treatment. Hence, researchers should give attention and make efforts to discover the novel fungi with the capacity to produce laccases from extreme environments.

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## References

Abadulla E, Tzanov T, Costa S, Robra KH, Cavaco-Paulo A, Gubitz GM (2000) Decolorization and detoxification of textile dyes with a laccase from *Trametes hirsuta*. Appl Environ Microbiol 66:3357–3362

Alcalde M (2007) Laccases: biological functions, molecular structure and industrial applications. In: Industrial enzymes. Springer, Dordrecht, pp 461–476

Ausec L, Zakrzewski M, Goesmann A, Schlüter A, Mandic-Mulec I (2011) Bioinformatic analysis reveals high diversity of bacterial genes for laccase-like enzymes. PLoS One 6:e25724

Babot ED, Rico A, Rencoret J, Kalum L, Lund H, Romero J, José C, Martínez ÁT, Gutiérrez A (2011) Towards industrially-feasible delignification and pitch removal by treating paper pulp with *Myceliophthora thermophila* laccase and a phenolic mediator. Bioresour Technol 102(12):6717–6722

Badhan AK, Chadha BS, Kaur J, Saini HS, Bhat MK (2007) Production of multiple xylanolytic and cellulolytic enzymes by thermophilic fungus *Myceliophthora* sp. IMI 387099. Bioresour Technol 98(3):504–510

Baker PW, Kennedy J, Dobson ADW, Marchesi JR (2009) Phylogenetic diversity and antimicrobial activities of fungi associated with *Haliclona simulans* isolated from Irish coastal waters. Marine Biotechnol 11(4):540–547

Balakshin M, Capanema E, Chen CL, Gratzl J, Kirkman A, Gracz H (2001) Biobleaching of pulp with dioxygen in the laccase-mediator system–reaction mechanisms for degradation of residual lignin. J Mol Catal B 13:1–16

Baldrian P (2004) Increase of laccase activity during interspecific interactions of white-rot fungi. FEMS Microbiol Ecol 50:245–253

Baldrian P (2006) Fungal laccases—occurrence and properties. FEMS Microbiol Rev 30(2):215–242

Barone R, De Santi C, Palma Esposito F, Tedesco P, Galati F, Visone M, Di Scala A, De Pascale D (2014) Marine metagenomics, a valuable tool for enzymes and bioactive compounds discovery. Front Mar Sci 1:38

Batista-García RA, Sutton T, Jackson SA, Tovar-Herrera OE, Balcázar-López E, del Rayo Sánchez-Carbente M, Sánchez-Reyes A, Dobson AD, Folch-Mallol JL (2017) Characterization of lignocellulolytic activities from fungi isolated from the deep-sea sponge Stelletta normani. PloS one. 12(3): e0173750

Beckett RP, Minibayeva FV, Laufer Z (2005) Extracellular reactive oxygen species production by lichens. Lichenologist 37(5):397–407

Beckett RP, Minibayeva FV, Liers C (2012) Occurrence of high tyrosinase activity in the non-Peltigeralean lichen *Dermatocarpon miniatum* (L.) W. Mann. Lichenologist 44:827–832

Beckett RP, Minibayeva FV, Liers C (2013) On the occurrence of peroxidase and laccase activity in lichens. Lichenologist 45(2):277–283

Beeson WT, Iavarone AT, Hausmann CD, Cate JH, Marletta MA (2011) Extracellular aldonolactonase from *Myceliophthora thermophila*. Appl Environ Microbiol 77(2):650–656

Berka RM, Schneider P, Golightly EJ, Brown SH, Madden M, Brown KM, Halkier T, Mondorf K, Xu F (1997) Characterization of the gene encoding an extracellular laccase of *Myceliophthora thermophila* and analysis of the recombinant enzyme expressed in *Aspergillus oryzae*. Appl Environ Microbiol 63(8):3151–3157

Bhat KM, Maheshwari R (1987) *Sporotrichum* thermophile growth, cellulose degradation, and cellulase activity. Appl Environ Microbiol 53(9):2175–2182

Bianchi T (2011) The role of terrestrially derived organic carbon in the coastal ocean: a changing paradigm and the priming effect. PNAS 108(49):19,473–19,481
Bodke PM, Senthilarasu G, Raghukumar S (2012) Screening diverse fungi for laccases of varying properties. Indian J Microbiol 52(2):247–250
Bollag JM, Chu HL, Rao MA, Gianfreda L (2003) Enzymatic oxidative transformation of chlorophenol mixtures. J Environ Qual 32:63–69
Bonugli-Santos RC, Durrant LR, da Silva M, Sette LD (2010a) Production of laccase, manganese peroxidase and lignin peroxidase by Brazilian marine-derived fungi. Enzyme Microb Technol 46(1):32–37
Bonugli-santos RC, Durrant LR, Sette LD (2010b) Laccase activity and putative laccase genes in marine derived basidiomycetes. Fungal Biol 114(10):863–872
Bucher VVC, Hyde KD, Pointing SB, Reddy CA (2004) Production of wood decay enzymes, mass loss and lignin solubilization in wood by marine ascomycetes and their anamorphs. Fungal Diversity 15:1–14
Bulter T, Alcalde M, Sieber V, Meinhold P, Schlachtbauer C, Arnold FH (2003) Functional expression of a fungal laccase in *Saccharomyces cerevisiae* by directed evolution. Appl Environ Microbiol 69(2):987–995
Chen J, Blume H-P, Beyer L (2000) Weathering of rocks induced by lichen colonization—a review. Catena 39:121–146
Chernykh A, Myasoedova N, Kolomytseva M, Ferraroni M, Briganti F, Scozzafava A, Golovleva L (2008) Laccase isoforms with unusual properties from the basidiomycete *Steccherinum ochraceum* strain 1833. J Appl Microbiol 105:2065–2075
Cho SJ, Park SJ, Lim JS, Rhee YH, Shin KS (2002) Oxidation of polycyclic aromatic hydrocarbons by laccase of *Coriolus hirsutus*. Biotechnol Lett 24:1337–1340
Claus H (2003) Laccases and their occurrence in prokaryotes. Arch Microbiol 179:145–150
Claus H (2004) Laccases: structure, reactions, distribution. Micron 35:93–96
Claus H, Faber G, Konig H (2002) Redox-mediated decolorization of synthetic dyes by fungal laccases. Appl Microbiol Biotechnol 59:672–678
Crognale S, Pesciaroli L, Petruccioli M, D'Annibale A (2012) Phenol oxidase-producing halotolerant fungi from olive brine wastewater. Process Biochem 47(9):1433–1437
D'Annibale A, Stazi SR, Vinciguerra V, Sermanni GG (2000) Oxirane-immobilized *Lentinula edodes* laccase: stability and phenolics removal efficiency in olive mill wastewater. J Biotechnol 77:265–273
D'Souza-Ticlo D, Garg S, Raghukumar C (2009a) Effects and interactions of medium components on laccase from a marine-derived fungus using response surface methodology. Mar Drugs 7(4):672–688
D'Souza-Ticlo D, Sharma D, Raghukumar C (2009b) A thermostable metal-tolerant laccase with bioremediation potential from a marine-derived fungus. Marine Biotechnol 11(6):725–737
Dahiya JS, Singh D, Nigam P (1998) Characterisation of laccase produced by *Coniotherium minitans*. J Basic Microbiol 38:349–359
Dahiya J, Singh D, Nigam P (2001) Decolourisation of synthetic and spentwash-melanoidins using the white-rot fungus *Phanerochaete chrysosporium* JAG-40. Bioresour Technol 78:95–98
Dalmaso G, Ferreira D, Vermelho A (2015) Marine extremophiles: a source of hydrolases for biotechnological applications. Mar Drugs 13:1925–1965
Daniel JW, Babcock KL, Sievert AH, Rusch HP (1963) Organic requirements and synthetic media for growth of the myxomycete *Physarum polycephalum*. J Bacteriol 86(2):324–331
Dhakar K, Pandey A (2013) Laccase production from a temperature and pH tolerant fungal strain of *Trametes hirsuta* (MTCC 11397). Enzyme Res 2013:869062
Dhakar K, Jain R, Tamta S, Pandey A (2014) Prolonged laccase production by a cold and pH tolerant strain of *Penicillium pinophilum* (MCC 1049) isolated from a low temperature environment. Enzyme Res 2014:120708
Duran N, Rosa MA, Dannibale A, Gianfreda L (2002) Applications of laccases and tyrosinases (phenol oxidases) immobilized on different supports: a review. Enzyme Microb Technol 31:907–931

Fang ZM, Li TL, Chang F, Zhou P, Fang W, Hong YZ, Zhang XC, Peng H, Xiao YZ (2012) A new marine bacterial laccase with chloride-enhancing, alkaline-dependent activity and dye decolorization ability. Bioresour Technol 111:36–41

Gali NK, Kotteazeth S (2012) Isolation, purification and characterization of thermophilic laccase from xerophyte *Cereus pterogonus*. Chem Nat Compd 48:451–456

Gali NK, Kotteazeth S (2013) Biophysical characterization of thermophilic laccase from the xerophytes: *Cereus pterogonus* and *Opuntia vulgaris*. Cellulose 20:115–125

Hammel KE, Kapich AN, Jensen KA Jr, Ryan ZC (2002) Reactive oxygen species as agents of wood decay by fungi. Enzyme Microb Technol 30(4):445–453

Handelsman J (2004) Metagenomics: application of genomics to uncultured microorganisms. Microbiol Mol Biol Rev 68:669–685

He L, Liu F, Karuppiah V, Ren Y, Li Z (2014) Comparisons of the fungal and protistan communities among different marine sponge holobionts by pyrosequencing. Microb Ecol 67:951–961

Henne A, Bruggemann H, Raasch C, Wiezer A, Hartsch T, Liesegang H, Johann A, Lienard T, Gohl O, Martinez-Arias R, Jacobi C, Starkuviene V, Schlenczeck S, Dencker S, Huber R, Klenk HP, Kramer W, Merkl R, Gottschalk G, Fritz HJ (2004) The genome sequence of the extreme thermophile *Thermus thermophilus*. Nat Biotechnol 22:547–553

Huang DL, Wang C, Xu P, Zeng GM, Lu BA, Li NJ, Huang C, Lai C, Zhao MH, Xu JJ, Luo XY (2015) A coupled photocatalytic–biological process for phenol degradation in the *Phanerochaete chrysosporium*–oxalate–Fe3O4 system. Int Biodeter Biodegr 97:115–123

Hyde KD, Jones EB (1988) Marine mangrove fungi. Marine Ecology 9(1):15–33

Ibrahim IN, Maraqa A, Hameed KM, Saadoun IM, Maswadeh HM (2011) Assessment of potential plastic-degrading fungi in Jordanian habitats. Turkish J Biol 35(5):551–557

Jaouani A, Neifar M, Prigione V, Ayari A, Sbissi I, Ben Amor S, Ben Tekaya S, Varese GC, Cherif A, Gtari M (2014) Diversity and enzymatic profiling of halotolerant micromycetes from Sebkha El Melah, a Saharan salt flat in southern Tunisia. Biomed Res Int 2014:439,197

Jensen PR, Fenical W (2002) Secondary metabolites from marine fungi. In: Hyde KD (ed) Fungi in marine environments. Fungal Diversity Press, Hong Kong, China, pp 293–315

Jolivalt C, Brenon S, Caminade E, Mougin C, Pontie M (2000) Immobilization of laccase from *Trametes versicolor* on a modified PVDF microfiltration membrane: characterization of the grafted support and application in removing a phenylurea pesticide in wastewater. J Membr Sci 180:103–113

Junghanns C, Pecyna MJ, Bohm D, Jehmlich N, Martin C, von Bergen M, Schauer F, Hofrichter M, Schlosser D (2009) Biochemical and molecular genetic characterisation of a novel laccase produced by the aquatic ascomycete *Phoma* sp. UHH 5-1-03. Appl Microbiol Biotechnol 84:1095–1105

Kale SK, Deshmukh AG, Dudhare MS, Patil VB (2015) Microbial degradation of plastic: a review. J Biochem Technol 6(2):952–961

Kulkarni SJ, Kaware JP (2013) Review on research for removal of phenol from wastewater. Int J Sci Res Publ 3(4):1–5

Kumar GN, Srikumar K (2011) Thermophilic laccase from xerophyte species *Opuntia vulgaris*. Biomed Chromatogr 25:707–711

Kumar GN, Srikumar K (2012) Characterization of xerophytic thermophilic laccase exhibiting metal ion-dependent dye decolorization potential. Appl Biochem Biotechnol 167:662–676

Kunamneni A, Camarero S, García-Burgos C, Plou FJ, Ballesteros A, Alcalde M (2008) Engineering and applications of fungal laccases for organic synthesis. Microb Cell Fact 7(1):32

Laufer Z, Beckett RP, Minibayeva FV (2006a) Co-occurrence of the multicopper oxidases tyrosinase and laccase in lichens in sub-order Peltigerineae. Ann Bot 98(5):1035–1042

Laufer Z, Beckett RP, Minibayeva FV, Lüthje S, Böttger M (2006b) Occurrence of laccases in lichenized ascomycetes of the *Peltigerineae*. Mycol Res 110(7):846–853

Laufer Z, Beckett RP, Minibayeva FV, Lüthje S, Böttger M (2009) Diversity of laccases from lichens in suborder *Peltigerineae*. Bryologist 112(2):418–426

Leonowicz A, Cho N-S, Luterek J, Wilkolazka A, Wojtas-Wasilewska M, Matuszewska A, Hofrichter M, Wesenberg D, Rogalski J (2001) Fungal laccase: properties and activity on lignin. J Basic Microbiol 41:185–227

Li X, Kondo R, Sakai K (2002) Studies on hypersaline-tolerant white-rot fungi. II. Biodegradation of sugarcane bagasse with marine fungus *Phlebia* sp. MG-60. J Wood Sci 48:159–162

Lisov AV, Zavarzina AG, Zavarzin AA, Leontievsky AA (2007) Laccases produced by lichens of the order *Peltigerales*. FEMS Microbiol Lett 275:46–52

Lund M, Eriksson M, Felby C (2003) Reactivity of a fungal laccase towards lignin in softwood kraft pulp. Holzforschung 57:21–26

Magan N (2007) Fungi in extreme environments. Mycota 4:85–103

Majcherczyk A, Johannes C (2000) Radical mediated indirect oxidation of a PEG-coupled polycyclic aromatic hydrocarbon (PAH) model compound by fungal laccase. Biochim Biophys Acta 1474:157–162

Martin C, Corvini PF, Vinken R, Junghanns C, Krauss G, Schlosser D (2009) Quantification of the influence of extracellular laccase and intracellular reactions on the isomer-specific biotransformation of the xenoestrogen technical nonylphenol by the aquatic hyphomycete *Clavariopsis aquatica*. Appl Environ Microbiol 75:4398–4409

Masai E, Katayama Y, Fukuda M (2007) Genetic and biochemical investigations on bacterial catabolic pathways for lignin–derived aromatic compounds. Biosci Biotech Bioch 71:1–15

Mayer AM (2006) Polyphenol oxidases in plants and fungi: going places? A review. Phytochemistry 67(21):2318–2331

Miyazaki K (2005) A hyperthermophilic laccase from *Thermus thermophilus* HB27. Extremophiles 9(6):415–425

Nagai M, Sato T, Watanabe H, Saito K, Kawata M, Enei H (2002) Purification and characterization of an extracellular laccase from the edible mushroom *Lentinula edodes*, and decolorization of chemically different dyes. Appl Microbiol Biotechnol 60:327–335

Nash T (ed) (1996) Lichen Biology. Cambridge Univ. Press, Cambridge

Ndahebwa Muhonja C, Magoma G, Imbuga M, Makonde HM (2018) Molecular characterization of Low-Density Polyethene (LDPE) degrading bacteria and fungi from Dandora Dumpsite, Nairobi, Kenya. Int J Microbiol 2018:4167845

Nigam PS (2013) Microbial enzymes with special characteristics for biotechnological applications. Biomolecules 3(3):597–611

Nigam P, Pandey A (2009) Biotechnology for agro-industrial residues utilisation. Springer Science Business Media B.V., pp 1–466

Nuhoglu A, Yalcin B (2005) Modelling of phenol removal in a batch reactor. Process Biochem 40(3–4):1233–1239

Oest A, Alsaffar A, Fenner M, Azzopardi D, Tiquia-Arashiro SM (2018) Patterns of change in metabolic capabilities of sediment microbial communities along river and lake ecosystems. J Inter Microbiol 2018:6234931. https://doi.org/10.1155/2018/6234931

Ojha N, Pradhan N, Singh S, Barla A, Shrivastava A, Khatua P, Rai V, Bose S (2017) Evaluation of HDPE and LDPE degradation by fungus, implemented by statistical optimization. Sci Rep 7:39,515

Paloheimo M, Puranen T, Valtakari L, Kruus K, Kallio J, Maentylae A, Fagerstrom R, Ojapalo P, Vehmaanpera J (2006) Novel laccase enzymes and their uses. US Patent No. 77321784 B2. United States Paten and Trademark Office

Papinutti L, Dimitriu P, Forchiassin F (2008) Stabilization studies of *Fomes sclerodermeus* laccases. Bioresour Technol 99(2):419–424

Patel D, Gismondi R, Alsaffar A, Tiquia-Arashiro SM (2019) Applicability of API ZYM to capture seasonal and spatial variabilities in lake and river sediments. Environ Technol. https://doi.org/10.1080/09593330.2018.1468492

Peralta-Zamora P, Pereira CM, Tiburtius ERL, Moraes SG, Rosa MA, Minussi RC, Durán N (2003) Decolorization of reactive dyes by immobilized laccase. Appl Catal B 42:131–144

Pointing SB, Hyde KD (2000) Lignocellulose-degrading marine fungi. Biofouling 15:221–229

Pointing SB, Hyde KD (2001) Bio-exploitation of filamentous fungi. Fungal Diversity Res Ser 6:1–467

Poli A, Finore I, Romano I, Gioiello A, Lama L, Nicolaus B (2017) Microbial diversity in extreme marine habitats and their biomolecules. Microorganisms 5(2):25

Pozdnyakova NN, Rodakiewicz-Nowak J, Turkovskaya OV (2004) Catalytic properties of yellow laccase from *Pleurotus ostreatus* D1. J Mol Catal B 30:19–24

Punt PJ, Biezen NV, Conesa A, Albers A, Mangnus J, Hondel C (2002) Filamentous fungi as cell factories for heterologous protein production. Trends Biotechnol 20:200–206

Quaratino D, Federici F, Petruccioli M, Fenice M, D'Annibale A (2007) Production, purification and partial characterisation of a novel laccase from the white-rot fungus *Panus tigrinus* CBS 577.79. Antonie van Leeuwenhoek 91(1):57–69

Rabinovich ML, Bolobova AV, Vasilchenko LG (2004) Fungal decomposition of natural aromatic structures and xenobiotics: a review. Appl BiochemMicrobiol 40:1–17

Raghukumar C, Raghukumar S, Chinnaraj A, Chandramohan D, D'souza TM, Reddy CA (1994) Laccase and other lignocellulose modifying enzymes of marine fungi isolated from the coast of India. Botanica Marina 37(6):515–524

Richards TA, Jones MDM, Leonard G, Bass D (2012) Marine fungi: their ecology and molecular diversity. Ann Rev Mar Sci 4:495–522

Robinson T, Nigam P (2008) Remediation of textile dye-waste water using a white rot fungus *Bjerkandera adusta* through solid state fermentation (SSF). Appl Biochem Biotechnol 151:618–628

Robinson T, Chandran B, Nigam P (2001a) Studies on the production of enzymes by white-rot fungi for the decolourisation of textile dyes. Enzyme Microb Technol 29:575–579

Robinson T, Chandran B, Nigam P (2001b) Studies on the decolourisation of an artificial effluent through lignolytic enzyme production by white-rot fungi in N-rich and N-limited media. Appl Microbiol Biotechnol 57:810–813

Roy SK, Dey SK, Raha SK, Chakrabarty SL (1990) Purification and properties of an extracellular endoglucanase from *Myceliophthora thermophila* D-14 (ATCC 48104). Microbiology 136(10):1967–1971

Russell JR, Huang J, Anand P, Kucera K, Sandoval AG, Dantzler KW, Hickman DS, Jee J, Kimovec FM, Koppstein D, Marks DH, Mittermiller PA, Nunez SJ, Santiago M, Townes MA, Vishnevetsky M, Williams NE, Nunez Vargas MP, Boulanger LA, Slack CB, Strobell SA (2011) Biodegradation of polyester polyurethane by endophytic fungi. Appl Environ Microbiol 77(17):6076–6084

Sadhukhan RA, Roy SK, Raha SK, Manna SU, Chakrabarty S (1992) Induction and regulation of alpha-amylase synthesis in a cellulolytic thermophilic fungus *Myceliophthora thermophila* D14 (ATCC 48104). Indian J Exp Biol 30(6):482–486

Sarmiento F, Peralta R, Blamey JM (2015) Cold and hot extremozymes: industrial relevance and current trends. Front Bioeng Biotechnol 3:148

Schultz A, Jonas U, Hammer E, Schauer F (2001) Dehalogenation of chlorinated hydroxybiphenyls by fungal laccase. Appl Environ Microbiol 67:4377–4381

Sharma R, Prakash O, Sonawane MS, Nimonkar Y, Golellu PB, Sharma R (2016) Diversity and distribution of phenol oxidase producing fungi from soda lake and description of *Curvularia lonarensis* sp. nov. Front Microbiol 7:1847

Sigoillot C, Record E, Belle V, Robert JL, Levasseur A, Punt PJ, van den Hondel CAMJ, Fournel A, Sigoillot JC, Asther M (2004) Natural and recombinant fungal laccases for paper pulp bleaching. Appl Microbiol Biotechnol 64:346–352

Soares GMB, Amorim MTP, Hrdina R, Costa-Ferreira M (2002) Studies on the biotransformation of novel disazo dyes by laccase. Process Biochem 37:581–587

Sumathi T, Viswanath B, Sri Lakshmi A, SaiGopal DV (2016) Production of laccase by *Cochliobolus* sp. isolated from plastic dumped soils and their ability to degrade low molecular weight PVC. Biochem Res Int 2016:9519527

Suryanarayanan TS (2012) The diversity and importance of fungi associated with marine sponges. Bot Mar 55(6):553–564

Suryanarayanan TS, Thirunavukkarasu N, Govindarajulu MB, Gopalan V (2012) Fungal endophytes: an untapped source of biocatalysts. Fungal Diversity 54(1):19–30

Thurston CF (1994) The structure and function of fungal laccases. Microbiology 140(1):19–26

Tiquia SM (2010) Metabolic diversity of the heterotrophic microorganisms and potential link to pollution of the Rouge River. Environ Pollut 158:1435–1443

Tiquia SM (2011) Extracellular hydrolytic enzyme activities of the heterotrophic microbial communities of the Rouge River: an approach to evaluate ecosystem response to urbanization. Microb Ecol 62(3):679–689

Tiquia SM, Mormile M (2010) Extremophiles—A source of innovation for industrial and environmental applications. Environ Technol 31(8-9):823

Torres E, Bustos-Jaimes I, Le Borgne S (2003) Potential use of oxidative enzymes for the detoxification of organic pollutants. Appl Catal B 46:1–15

Tsioulpas A, Dimou D, Iconomou D, Aggelis G (2002) Phenolic removal in olive oil mill wastewater by strains of Pleurotus spp. in respect to their phenol oxidase (laccase) activity. Bioresour Technol 84:251–257

Ullah MA, Bedford CT, Evans CS (2000) Reactions of pentachlorophenol with laccase from *Coriolus versicolor*. Appl Microbiol Biotechnol 53:230–234

Vavourakis CD, Ghai R, Rodriguez-Valera F, Sorokin DY, Tringe SG, Hugenholtz P et al (2016) Metagenomic insights into the uncultured diversity and physiology of microbes in four hypersaline Soda Lake brines. Front Microbiol 7:211

Velazquez-Cedeno MA, Mata G, Savoie JM (2002) Waster educing cultivation of *Pleurotus ostreatus* and *Pleurotus pulmonarius* on coffee pulp: changes in the production of some lignocellulolytic enzymes. World J Microbiol Biotechnol 18:201–207

Verma AK, Raghukumar C, Verma P, Shouche YS, Naik CG (2010) Four marine-derived fungi for bioremediation of raw textile mill effluents. Biodegradation 21(2):217–233

Virk AP, Sharma P, Capalash N (2012) Use of laccase in pulp and paper industry. Biotechnol Prog 28(1):21–32

Wallace J (1996) Phenol. In: Kroschwitz JI, Howe-Grant M (ed) Kirk-Othmer encyclopedia of chemical technology, 4th edn. Wiley, New York, pp 592–602

Wang G, Li Q, Zhu P (2008) Phylogenetic diversity of culturable fungi associated with the Hawaiian sponges *Suberites zeteki* and *Gelliodes fibrosa*. Antonie Van Leeuwenhoek 93(1–2):163–174

Wesenberg D, Kyriakides I, Agathos SN (2003) White-rot fungi and their enzymes for the treatment of industrial dye effluents. Biotechnol Adv 22:161–187

Wong D (2010) Applications of metagenomics for industrial bioproducts. In: Marco D (ed) Metagenomics: theory, methods and applications, 1st edn. Horizon Scientific Press, Norwich, UK, pp 141–158

Xu F, Shin W, Brown SH, Wahleithner JA, Sundaram UM, Solomon EI (1996) A study of a series of recombinant fungal laccases and bilirubin oxidase that exhibit significant differences in redox potential, substrate specificity, and stability. Biochim Biophys Acta 1292(2): 303–311

Yurlova NA, De Hoog GS, Fedorova LG (2008) The influence of ortho-and para-diphenoloxidase substrates on pigment formation in black yeast-like fungi. Stud Mycol 61:39–49

Zavarzina AG, Zavarzin AA (2006) Laccase and tyrosinase activities in lichens. Microbiology 75(5):546–556

Zavarzina AG, Leontievsky AA, Golovleva LA, Trofimov SYA (2004) Transformation of soil humic acids by blue laccase of *Panus tigrinus* 8/18: an *in vitro* study. Soil Biol Biochem 36:359–369

Zille A, Tzanov T, Gubitz GM, Cavaco-Paulo A (2003) Immobilized laccase for decolorization of Reactive Black 5 dyeing effluent. Biotechnol Lett 25:1473–1477