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Laccases from Marine Organisms and Their Applications in the Biodegradation of Toxic and Environmental Pollutants: a Review

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Abstract The discharge of industrial effluent creates environmental problems around the world and so necessitates the need for the economically expensive and sometimes technically problematic treatment of the wastewater. Laccases have enormous potential for the oxidative bioremediation of toxic xenobiotic compounds using only molecular oxygen as the sole cofactor for their reaction, and their application is regarded as environmentally friendly. Due to the low substrate specificity of laccases, they can oxidize a variety of substrates. Moreover, by using appropriate mediators, laccases can degrade a wide range of substrates, including those with structural complexity. Thus, laccases are an attractive alternative for wastewater treatment. Marine environments are rich in microorganisms that are exposed to extreme conditions, such as salinity, temperature, and pressure. Laccases from these microorganisms potentially have suitable properties that might be adaptive to bioremediation processes. This review provides the latest information on laccases from marine environments, their sources, biochemical properties, media composition for laccase production, and their applications in the bioremediation of industrial waste, especially focusing on dye decolorization.

Keywords Laccase · Bioremediation · Xenobiotic compounds · Marine environment · Dye decolorization

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Introduction

Laccases (benzenediol: oxygen oxidoreductase EC 1.10.3.2) are a type of lignin-modifying enzymes that are able to oxidize a wide range of molecules. Substrates of laccase are phenolic compounds, such as ortho- and para-diphenols, aminophenols, polyphenols, polyamines, and aromatic diamines. The efficiency of oxidation by a particular laccase is influenced by the nature and position of the substituents on the phenolic ring, where the substrate is oxidized with simultaneous radical formation that can then spontaneously rearrange to cleave the aromatic rings or promote their polymerization [1, 2].

Laccases have a lower redox potential (450–800 mV) than ligninolytic peroxidases (> 1 V) [3]. Some substrates cannot be oxidized directly by laccases, but in those cases, a mediator can often overcome the limitation. Mediators are suitable compounds that act as intermediate substrates for the laccase, whose oxidized radical forms are able to interact with the bulky or high redox-potential substrate [4]. For example, the degradation of the herbicide isoproturon by *Trametes versicolor* laccase was found to occur at a very low rate when using the laccase alone, but in the presence of the mediator 1-hydroxybenzotriazole (HBT), the isoproturon was completely degraded within 24 h [5].

The potential application of laccases encompasses various fields, including decolorization and detoxification of industrial dyes, biobleaching of pulp and paper industries, pretreatment of palm oil mill wastewater, remediation of endocrine-disrupting chemicals, ethanol production, biosensor fabrication, organic synthesis, and drug synthesis [5–11]. The effluents from these industries contain numerous inorganic chemicals, including sulfates, sulfides, carbonates, chlorides (Cl^-), chlorine bleach compounds, peroxides, and heavy metals [12]. New sources of laccases with special properties, such as high salt and temperature tolerance, and cold adaptivity, are desired for industrial applications.

Marine environments have a rich diversity of microorganisms and natural resources, with an estimated 3.67×10^{30} microorganisms [13], as well as many extreme conditions [14], including variations in light, salinity, temperature (− 35–350 °C) and pressure (up to 111 MPa). Accordingly, within each extreme marine environment, the local microbes have adapted to these various different environmental conditions, and their enzymes are, therefore, very attractive in unusual bioprocesses because of their potential habitat-related characteristics, including salt and pH tolerance, psychrotolerant, thermostability, and barophilicity. The salinity of the bulk marine environment is around ≥ 0.6 M NaCl. Extreme halophiles can grow at NaCl concentrations above 1.7 M and moderate halophiles at 0.85–1.7 M.

Biological processes that can be performed at high temperatures can have various advantages, such as decreased microbial contamination, high substrate solubility, decreased viscosity of the reaction mixture, and a faster reaction rate [15]. Whereas the advantages of cold-active biocatalysts from psychrophilic microorganisms include energy saving, due to high catalytic activity rates at low temperatures, low optimum temperature, and less resistance to thermal inaction. An example of this benefit can be seen in the bioremediation of crude oil pollution in a low temperature environment [16]. Marine microbes can degrade a diversity of organic matters and pollutants, and so they are an appealing choice for environmental and biotechnological applications [17–19].

The first part of the present review is intended to outline the current sources, general properties, and factors that influence the production of laccase from marine organisms. Their applications are then summarized in the second part.

Laccases from the Marine Environment

Distribution of Laccase in Marine Environments

Laccase and phenoloxidase (PO) play a role in the anti-bacterial host defense in invertebrates, which do not have a humoral immunity system, such as antibody production and complete immune memory, like in vertebrates. Instead, the melanin-synthesis pathway and different types of POs are major components of their immunity. The POs are a family of enzymes composed of laccases, tyrosinases (EC 1.14.18.1) and catecholases (EC 1.10.3.1), which play a role in the killing and inhibition of bacteria by initiating oxidation reactions, and are also involved in wound healing and circulating immune cells. The conversion of phenol into quinone by PO leads to the formation of melanin, which is involved in the encapsulation of pathogens and parasites [20–23]. Laccase has been reported to play a role in the anti-bacterial defense mechanism in sponges [24].

The list of marine organisms from which laccase and laccase-like activities have been found is shown in Table 1. Cerenius et al. [35] reported that the PO preparation from the freshwater crayfish, *Pacifastacus leniusculus*, showed an antibacterial activity against *Aeromonas hydrophila*, *Escherichia coli*, *Streptococcus pneumonia*, *Bacillus cereus*, *Psuedomonas aeruginosa* and *Staphylococcus aureus*. Mydlarz et al. [20] tested the PO of seven Caribbean hard coral species from four different families. They found two corals, *Montastraea annularis* and *Sidastrea siderea*, displayed a laccase-like activity, whereas four corals showed catecholase activity and four species presented strong cresolase activity. The laccase (LvLac) in the white shrimp, *Litopenaeus vannamei*, is also involved in the anti-bacterial host defense, since knock-down of LvLac increased the sensitivity of shrimps to *Vibrio parahaemolyticus* and *Micrococcus lysodeikticus* infections [34]. The effect of bacterial inhibition depends on the type of PO and substrate used. The POs from the sea cucumber, *Apostichopus japonicas*, were also found to inhibit the growth of *Vibrio splendidus*, *Staphylococcus aureus* and *Vibrio harveyi* [36]. Three laccase-type POs have also been found in *A. japonicas*, and are involved in the protection against infections from Gram-negative bacteria and double-stranded RNA viruses [33].

Laccases show a widespread occurrence in terrestrial fungi, especially in white-rot fungi [17, 18]. However, marine fungi are also an interesting source of laccase and novel bioactive metabolites that are not found in terrestrial strains of the same species, due to their adaption to the low temperature, high salinity, high pressure and oligotrophic nature of the marine environment [32, 37]. Fungi play a role in the energy flow from detritus to higher trophic levels and are involved in nutrient regeneration cycles [6]. Fungi growing in a marine environment have adapted to grow under high saline (15–34 ppt) and alkaline conditions, characteristics that potentially make their laccases of interest in the bioremediation of effluents from the pulp and paper industries, tanneries, molasses-based distilleries and textile industries, since they usually have a high salt and alkaline content [38]. However, reports focused on laccases from the marine environment and their potential applications are quite limited.

Many fungal strains have been isolated from mangrove, seagrasses, salt march grass and sponges, such as *Cerrena unicolor* [12], *Peniophora* sp. CBMAI 1063 [39], *Trichoderma harzianum/Hypocrealixii* TSK8 [9], *Nigrospora* sp. CBMAI 1328 [17], *Arthopyrenia* sp. CBMAI 1330 [17] and *Pestalotiopsis* sp. J63 [32]. Almost all of them belong to the Ascomycota and Basidiomycota, with only a few reports on white-rot fungi.

Li et al. (2014) isolated fungi from the coastal ecosystems of the Pearl River Delta, China. The largest diversity of fungal colonies were isolated from the surface sediment and seawater

Table 1 Sources of laccase and laccase-like proteins from marine organisms and their characteristics

Source of laccase	Phylum	MW (KDa)	pI	Reference
Prokaryote				
Oscillatoria curviceps BDU92191	Cyanobacteria	–	–	[25]
Marinomonas mediterranea MMB-1	Proteobacteria	–	–	[26]
Shewanella algae	Proteobacteria	–	–	[27]
Shewanella marisflavi	Proteobacteria	–	–	[27]
Phychrobacter sp.	Proteobacteria	–	–	[28]
Eukaryote				
Flavodon flavus 312	Basidiomycota	43, 99	At least 10 isozymes; one group of seven had pIs of 4.0, 4.2, 4.4, 4.75, 5.2, 5.6, and 6.0, while a second group of at least three isozymes had pIs of < 3.0	[29]
Cerrena unicolor NIOCC # 2a (MTCC 5159)	Basidiomycota	LacI: 82 LacII: 56 LacIId: 59 LacIII: 45	LacIId: 5.3	[12, 30]
Coriolopsis byrsina NIOCC # 15V	Basidiomycota	–	–	[30]
Diaporthe sp. NIOCC # 16V	Ascomycota	–	–	[30]
Pestalotiopsis maculans NIOCC # C3	Ascomycota	–	–	[30]
Aspergillus sclerotiorum CBMAI 849	Ascomycota	–	–	[31]
Cladosporium cladosporioides CBMAI 857	Ascomycota	–	–	[31]
Mucor racemosus CBMAI 847	Zygomycota	–	–	[31]
Marasmiellus sp. CBMAI 1062	Basidiomycota	–	–	[6]
Peniophora sp. CBMAI 1063	Basidiomycota	–	–	[6]
Tinctoporellus sp. CBMAI 1061	Basidiomycota	–	–	[6]
Nigrospora sp. CBMAI 1328	Ascomycota	–	–	[17]
Arthopyrenia sp. CBMAI 1330	Ascomycota	–	–	[17]
Pestalotiopsis sp. J63 (*Pestalotiopsis microspora*)	Ascomycota	–	–	[32]
Cladosporium sphaerospermum PKU F16	Ascomycota	–	–	[18]
Ascomycota sp. PKU F18	Ascomycota	–	–	[18]
Montastraea annularis	Cnidaria	–	–	[20]
Sidastrea siderea	Cnidaria	–	–	[20]
Apostichopus japonicas	Echinodermata	< 21	–	[33]
Litopenaeus vannamei	Arthropoda	71.7	6.11	[34]

samples at a 10-m depth, totaling 22 fungal and nine yeast isolates. Based on their ITS rRNA gene sequence analysis, 74% of the fungal isolates belonged to Ascomycota, 23% to Basidiomycota and 3% to Zygomycota. About 84% of these fungal isolates had cellulase and lipase activity, whereas about 38% exhibited laccase activity. The best laccase producers were isolates PKU F16 (*Cladosporium* sp.) and PKU F18 (*Ascomycota* sp.), with laccase activities on 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS) as the substrate of 14.6 units U mL^{-1} and 10.5 U mL^{-1}, respectively, after 6 days of cultivation. Verma et al. [30] isolated 40 fungal strains with laccase activity from detritus and decaying wood in mangrove swamps in India using agar medium containing ABTS or guaiacol as substrates. High laccase activities were found in two isolates of each of Ascomycota (*Diaporthe* sp. and

Pestalotiopsis sp.) and Basidiomycota (*Coriolopsis byrsina* and *Cerrena unicolor*). Bonugli-Santos et al. [31] reported the production of the ligninolytic enzymes, laccase, manganese peroxidase (MnP) and lignin peroxidase (LiP), by three marine-derived fungi (*Aspergillus sclerotiorum* CBMAI 849, *Cladosporium cladosporioides* CBMAI 857 and *Mucor racemosus* CBMAI 847). In addition, phylogenetic analysis showed that the laccase sequences from two marine-derived fungi, *Nigrospora* sp. CBMAI 1328 and *Arthopyrenia* sp. CBMAI 1330, were clustered with other laccases from Ascomycota from marine environments [17]. Three strains of marine-derived Basidiomycota (*Marasmiellus* sp. CBMAI 1062, *Peniophora* sp. CBMAI 1063 and *Tinctoporellus* sp. CBMAI 1061) produced multiple distinct laccase sequences with 73–90% DNA similarity to the laccases from terrestrial Basidiomycota [6].

The marine bacterium *Marinomonas mediterranea* MMB-1 has been reported to express different oxidases, such as a multi-copper oxidase with laccase, tyrosinase and L-lysine-epsilon-oxidase activities. Its complete genome sequence, with a size of 4.68 Mbp, was reported [26]. Recently, Moghadam et al. [28] isolated 13 bacteria exhibiting extracellular laccase activity from the Barents Sea, North of mainland, Norway, that all belonged to the genus *Phychrobacter*. Genome sequencing was performed for four strains, revealing genome sizes of 3.29–3.52 Mbp and a G + C content of around 42%. From the bioinformatics analysis, two different laccase-like multi-copper oxidase genes were found in each of the four strains. When two of these multi-copper oxidase genes (P11F6-LMCO and P11G5-LMCO2) were expressed in *Escherichia coli*, the recombinant proteins were shown to be active with ABTS and guaiacol as substrates.

Cyanobacteria are reported to biodegrade several compounds, such as ampicillin [40], lignin [41], pesticides [42] and azo dyes [43], and to use these compounds as nutrient sources. The marine cyanobacteria, *Oscillatoria curviceps* BDU92191, showed a single intense decoloration band for laccase activity in Acid Black 1 (Color Index Number 20470) native PAGE zymogram. Moreover, other enzymes involved in dye degrading ability, polyphenol oxidase and azoreductase, were also found in this species [25].

The classical culture-dependent approach to screen for microorganisms with a specific trait of interest is limited by the ability to culture the microorganism and so excludes microorganisms that are uncultivatable. In contrast, a metagenomics approach does not require culturing, but proceeds through high-throughput sequencing, promoting the exploration of new potential biocatalysts from selected environments with desirable characteristics, rather than by cloning organisms with the selected trait [44]. Using the metagenomics approach, two new bacterial laccases, *lac15* and *lac 21*, with a high Cl^- tolerance and alkaline stability, respectively, were found from the South China Sea metagenomics library. The amino acid sequences encoded by *lac15* and that by *lac 21* both share a low sequence identity of less than 40% with all other bacterial multi-copper oxidases and laccases. When these two genes were expressed in *E. coli*, the recombinant (r)Lac 21 showed a high potential for dye decolorization in the absence of redox mediators, while the rLac 15 was purified and crystallized [30, 45]. Recently, Fang et al. [46] reported the *carA* gene, which encodes for laccase and is one of the dominant genes in the degradation of organic pollutants.

Biochemical Properties of Laccases from Marine Organisms

As mentioned, marine biocatalysts are potentially attractive candidates for biological processes because of the habitat-related characteristics, such as thermostability, barophilicity and salt

tolerance. Recently, three proteins (SnPO1, SnPO2 and SnPO3) with laccase-like PO activities from the sea urchin *Strongylocentrotus nudus* were identified. The optimum temperature of SnPO1, SnPO2 and SnPO3 for the L-dopamine substrate was high for the first two at 75 and 70 °C, respectively, but lower for SnPO3 at 40 °C. The three proteins are thermophilic and were notably activated after incubation in boiling water for 60 min. Their optimum pH was 7.0, 9.0 and 8.0, respectively [47]. This implied that the PO system in *S. nudus* might have the capacity to adapt to the pH fluctuation in a marine environment.

The biochemical properties of the laccases isolated from marine organisms are shown in Table 2. Fang et al. [45] reported the optimum pH of the marine bacterial laccase Lac21 for syringaldazine, 2,6-dimethoxyphenol (DMP), L-dopamine, catechol and $K_4Fe(CN)_6$ as substrates was 7.5, 8.0, 7.5, 7.0 and 6.5, respectively. However, the enzyme could not oxidize ABTS and guaiacol. Lac21 was highly stable at pH 6.0–8.0, but unstable at a low pH value. The results were similar to those for Lac 15, isolated from the same habitat, which showed an optimum pH towards syringaldazine of 7.5 and stability at pH 5.5–9.0 [50]. In contrast, almost all fungal laccases cannot function very well under alkaline conditions, but are active at acidic and neutral pH conditions [49]. The optimum pH with ABTS, guaiacol or syringaldazine as the substrate for the Lac IId from the marine-adapted fungus *Cerrena unicolor* MTCC 5159 was found at 3, 6 and 6, respectively. This Lac IId was metal-tolerant and thermostable, being uninhibited by 1 mM Pb, Fe, Ni, Li, Co or Cd ions and with an optimum temperature at 70 °C and a half-life at 70 °C of 90 min. In contrast, the two laccases from *C. unicolor* 137 (isolated from a terrestrial habitat) were less stable at high temperatures, where Lacc I lost 100% activity after 20 min and Lacc II lost 90% activity after 60 min at 70 °C [12, 51]. Comparison of the characteristics of *Cerrena unicolor* laccase from a marine and a terrestrial habitat is shown in Table 3.

Lac21 showed an optimum temperature at 45 °C for all substrates tested, while the same optimum temperature was observed with Lac15 using syringaldazine as the substrate [50]. Atomic absorption spectroscopy indicated the active sites of Lac 21 contain four Cu^{2+} ions, consistent with the observation that Cu^{2+} was necessary for Lac21 activity. Some others ions, such as Li^+, K^+ and Cl^-, stimulated Lac21 activity, whereas Zn^{2+}, Co^{2+}, Mg^{2+}, Mn^{2+} and ethylenediamine tetraacetic acid disodium (EDTA) all reduced the activity. In slight contrast, Lac15 was stimulated by Mg^{2+} and Mn^{2+}, but inhibited by Zn^{2+} and Co^{2+}. Moreover, EDTA and sodium diethyldithiocarbamate were reported to inhibit the laccase activities of *A. japonicas,* but their activities were enhanced by Ca^{2+}, Mg^{2+} and Mn^{2+} [49]. Interestingly, Lac21 showed alkali stability and its activity was enhanced about 140% by the addition of 80 mM NaCl or Cl^- to < 700 mM [45, 50], indicating that Lac15 and Lac21 might be suitable for kraft pulp biobleaching and wastewater decolorization, since chlorine is used at a high concentration in those industries. However, an excess amount of Cl^- ions can inhibit laccase activity, perhaps because Cl^- is a competitive inhibitor of electron donors and hinders the entrance of substrate or by inhibiting the electron transfer at the T1 site [52].

Effect of the Medium Composition on the Production of Laccase

There is very little information on ligninolytic enzymes produced by marine-derived fungi. Several fungi have been reported to produce enzymes and novel secondary metabolites or compounds that are different from their terrestrial counterparts [53]. The most critical factors to improve or stimulate laccase production are the salinity and source and concentration of nitrogen, carbon, inducer and metal ion [17, 54, 55].

Table 2 Biochemical properties of laccase and laccase-like phenoloxidase activities from marine organisms

Organism	Protein	Optimal temperature (°C)	Optimal pH	Substrate	Special characteristic	Reference
Strongylocentrotus nudus	SnPO1	75	7	L-DOPA	Thermophilic	[47]
	SnPO2	70	9	L-DOPA	Thermophilic	
	SnPO3	40	8	L-DOPA	Thermophilic	
Lac21 from marine metagenome library expressed in *E. coli*	Lac21	45	7.5	Syringaldazine	Alkaline stability and chloride enhancing activity	[45]
			8	2,6-DMP		
			7.5	L-dopamine		
			7	Catechol		
			6.5	$K_4Fe(CN)_6$		
Lac15 from marine metagenome library expressed in *E. coli*	Lac15	45	7.5	Syringaldazine	Chloride tolerant	[45]
Cerrena unicolor MTCC 5159	LacIId	70	3	ABTS	Thermostable and metal tolerant	[12]
		–	6	Guaiacol		
		–	6	Syringaldazine		
Cerrena unicolor MTCC 5159	Partially purified laccase	60	3,6	ABTS	Grew and produced laccase at a high salinity (25–30 ppt)	[48]
Apostichopus japonicus	AjPO1	45	5	L-DOPA		[49]
	AjPO1	95	8	L-DOPA		
	AjPO1	85	8	L-DOPA		

L-DOPA: L-3,4-dihydroxyphenylalanine; ABTS: 2,2′-azino-bis (3-ethylthiazoline-6-sulfonate); 2,6-DMP: 2,6-dimethoxyphenol

Table 3 Comparison of the characteristics of *Cerrena unicolor* laccase from marine (strain MTCC 5159) and terrestrial (strain 137) habitats

	C. unicolor MTCC 5159 [12]	*C. unicolor* 137 [51]	
Molecular weight	> 20 isozymes Lac I: 82 Lac II:56 Lac IId: 59 Lac III: 45	Two laccase isoforms Lacc I: 64 Lacc II: 57	
pI	Lac IId: 5.3	Lacc I: 3.6, Lacc II: 3.7	
Optimal temperature (ABTS substrate)	Lac IId: 70 °C	Lacc I: 60 °C Lacc II: 60 °C	
Optimal pH	Lac IId	Lacc I	Lacc II
ABTS	3	2.5	2.5–3.0
Guaiacol	6	–	–
Syringaldazine	6	4.5	5.5
2,6 DMP	–	3.5	4.5
Stability at 70 °C	Lac IId: 50% of its activity at 90 min	Lacc I: 0% activity after 20 min Lacc II: 10% activity after 60 min	
Highest laccase levels	23,714 U L^{-1}	18,700 U L^{-1}	

Salinity

The terrestrial white-rot fungus *Cerrena unicolor* is also found in mangroves, where these isolates are adapted to grow and produce laccase in media containing sea water with a salinity level in the mangrove environment between 5 and 35 ppt [56]. The laccase produced by *C. unicolor* MTCC 5159 (NIOCC # 2a), isolated from a mangrove forest, can function in the presence of NaCl. A low MnP and LiP activity was also found, but was inhibited by seawater at all tested salinities [56]. Culturing *C. unicolor* MTCC5159 at a salinity of 34 and 25 ppt lead to the maximum production of biomass and laccase activity, respectively, and at a comparable level to that obtained by Chen et al. [32], with a maximum laccase production level of 30 g L^{-1}. In contrast, the marine bacterium *Marinomonas mediterranea* strain MMB-1 could only tolerate lower NaCl concentrations of between 0.17 and 0.86 M and grew between 15 and 30 °C [26].

Bonugli-Santos et al. [31] investigated the enzyme activity of three fungi isolated from marine cnidarians, *Aspergillus sclerotiorum* CBMAI 849, *Cladosporium cladosporioides* CBMAI 857 and *Mucor racemosus* CBMAI 847, with different carbon sources and salinity conditions. These fungi produced laccase when they were cultured in malt extract or basal medium containing glucose and wheat bran. The result was the same for MnP, but LiP was not detected in the culture with basal medium. The highest laccase activity of *M. racemosus* CBMAI 847 (898.2 U L^{-1}) was found when they were cultured with 4.5 mg mL^{-1} wheat bran and 3.94 M NaCl, while that for *C. cladosporioides* CBMAI 857 required less wheat bran (2.4 mg mL^{-1}) and a lower salinity (2.14 M NaCl) but gave a lower optimal laccase activity (203.7 U L^{-1}). The production of MnP by *M. racemosus* CBMAI 847 was related to the salt concentration, with the highest activity at 0.86 and 2.14 M of NaCl. However, MnP was not detected in the presence of high amounts of wheat bran (4.5 mg mL^{-1}) and a low concentration of salt (0.09 M NaCl).

The mechanisms of salt-induced laccase activity are not clear yet. The reason that salt affects the laccase activity can be explained by two hypotheses. The first is that the concentration of salt could affect cell growth. Halophiles are categorized as slight halophiles, moderate halophiles or

extreme halophiles, depending on the concentration of salt required for their efficient growth. At too high a salinity condition, laccase secretion and sporulation could be delayed or inhibited. Moreover, a reduction in biomass could also occur [57]. The second is that salt might enhance the activity of laccase by involving the folding of the enzyme and perturbation of specific local sites. For example, Cl^- can bind to the sites around the T1 and T2/T3 copper centers which facilitates oxygen reduction and leads to an enhanced enzyme activity [58].

Nitrogen and Carbon Sources

The type of nitrogen source in the media affects the production of lignin-degrading enzymes and their capability for dye decolorization. In the presence of effluents, the amount of POs and laccase produced can increase several folds. The production of laccase, MnP and LiP were enhanced by 1.4, 2421 and 557 folds, respectively, in the presence of appropriate effluent and nitrogen source [59]. To study the effect of carbon and nitrogen on laccase production by the fungus NIOCC #2a isolated from decaying mangrove wood in India, different carbon and nitrogen sources were tested. The maximum laccase production was observed when fructose and glutamic acid were used as the carbon and nitrogen source, respectively, although the glutamic acid served as both a nitrogen and readily available carbon source. Using glutamic acid together with fructose probably supported the biomass build-up, giving a positive effect on the laccase production [59].

A low nitrogen medium prepared with sea water was found to be optimal for laccase production by strain *C. unicolor* MTCC 5159, with an activity of 23,714 U L^{-1} (330 U mg^{-1} protein) being obtained when using 0.5% (*w*/*v*) glycine as the nitrogen source and ABTS as the substrate for the laccase assay [12]. Furthermore, a higher laccase activity of up to 85,829 U L^{-1} was observed when cultured in the presence of 1% (*v*/v) textile mill effluent [56]. A requirement for a low nitrogen medium was also found for the production of laccase, LiP and MnP by the fungus *Flavodon flavus*, isolated from a coastal marine environment, when cultured in a low-nitrogen (2.4 mM N) medium, whereas low laccase and MnP activities were observed when cultured in a high nitrogen (24 mM N) medium [29]. However, in contrast, a high nitrogen medium was preferred by a marine strain of *Trichoderma* sp., where the optimum condition for laccase production, evaluated by response surface methodology (RSM), was 7.7 mg L^{-1} yeast extract for 12 days incubation at 29 °C and pH 5.3 [9].

The production of laccase by agro-waste has been reported for the marine-derived fungus *Pestalotiopsis* sp. J63, isolated from the oceanic sediment off the East coast of China [32]. The use of plant residues (pomelo peeling, bean pod, rice straw, corn cob, water hyacinth, wheat bran and sugarcane bagasse) by *Pestalotiopsis* sp. J63 was evaluated for laccase production, where rice straw provided the highest laccase activity (10,700 IU g^{-1} substrate) under solid state fermentation, whereas untreated sugarcane bagasse gave a maximum laccase activity (2000 IU mL^{-1}) under submerged fermentation.

Feng et al. [60] tested the effect of individual carbon sources for laccase production by *Pestalotiopsis* sp. J63. Glucose, sucrose, maltose, wheat bran, soluble starch, bean-pod powder, water hyacinth powder and rice straw were all tested, and again rice straw showed the highest laccase activity even though glucose, sucrose, maltose, wheat bran and soluble starch gave a higher biomass production. The combination of rice straw and maltose was the best carbon source for laccase production by *Pestalotiopsis* sp. J63. In addition, when different kinds of nitrogen sources were studied (ammonium sulfate, ammonium chloride, ammonium tartrate, L-glutamic acid, L-aspartic acid, urea, yeast extract, beef extract, peptone, wheat bran, water

hyacinth, powder, soybean flour and bean-pod powder), it was found that ammonium sulfate gave the highest laccase activity without any inducer supplement. Interestingly, when phenol was used as an inducer and wheat bran as the nitrogen source, a high laccase activity (5792 U L^{-1}) was observed. The result implied that the best nitrogen source depended on the inducer and that agro-residues can be used as potential nutrient sources for the production of high levels of laccase. Nitrogen and carbon sources play a role in cell growth and the transcription of laccase. However, the effects are dependent on the strain. Low nitrogen and carbon sources have been reported to reduce the transcription level of laccase. For example, when in a nitrogen-limiting condition the transcription level of *Lac7* in the white rot fungus *Cerrena* sp. strain HYB07 decreased up to 90%, which caused a reduced laccase activity, whereas the expression of *Lac4* was responsive to limiting nitrogen and the transcription of *Lac2* was highly induced under a low carbon [61]. Furthermore, different transcription patterns may be due to the combination of several parameters, such as the age of the cells and oxidative stress [62].

Inducer and Metal Ions

Copper has been reported as a strong inducer for laccase production in numerous species. Copper sulfate ($CuSO_4$), guaiacol and synthetic dyes all induced a good laccase activity from *C. unicolor* MTCC 5159, where a 100-fold increased laccase activity was obtained by the addition of 2 mM $CuSO_4$ to the medium [56]. In contrast, a lower laccase activity (18,700 U L^{-1}) was reported for *C. unicolor* 137, isolated from a terrestrial habitat [51]. Nakade et al. [63] reported the most effective inducer for *Polyporus brumalis* ibrc05015 was 0.25 mM $CuSO_4$, giving a laccase activity of 34.6 U mL^{-1}, some 20-fold higher than that obtained in the culture without $CuSO_4$. Manavalan et al. [64] also reported an improvement in the obtained laccase activity (1.5 U mL^{-1}) of *Ganoderma lucidum* when the culture was induced by 0.4 mM $CuSO_4$. Recently, Passarini et al. [17] found that liquid medium supplemented with 5 μM $CuSO_4$ lead to the highest laccase production level (25.2 U L^{-1}) by the marine fungus, *Nigrospora* sp. CBMAI 1328, whereas the highest laccase expression by the fungus *Arthopyrenia* sp. CBMAI 1330 was detected in the absence of $CuSO_4$.

The mechanism of laccase induction by Cu^{2+} involves its role in the laccase active center and its support in laccase gene transcription and post-transcription modification. Northern blot analysis showed that the transcription of two laccase genes, *poxa1b* and *poxc*, of the white rot fungus *Pleurotus ostreatus* were induced by copper. The maximum transcript levels were reported on the second day of *P. ostreatus* growth. This phenomenon could imply that the effect of copper induction occurs from the early state of fungal growth [65]. Moreover, the promoter of the *pox* genes in *P. ostreatus* contains metal responsive element consensus sequences that are similar to those found in the promoters of metallothionein genes. The expression of metallothionein genes can be induced by a variety of metal ions. The metal-regulatory protein functions as a metal receptor and transcription factor [65, 66]. Other reports showed that the transcription of the laccase gene in *Trametes velutina* 5930 can be induced by Cu^{2+} and Fe^{2+}. The putative metal-responsive elements and ACE elements in the promoter region of the lac5930-1 gene may affect the induction of lac5930-1 gene transcription by these ions [67]. Copper ions are also important for laccase production in the fungus *Cerrena* sp. strain HYB07. Without copper supplementation in the medium, the transcription level of the *Lac7* laccase gene was reduced up to 1000-fold. Apart from their effect on transcription, copper ions are also required at the active site of laccase and may decrease the proteolytic activity that is able to degrade laccase [61].

However, Cu^{2+} can be toxic due to its interaction with nucleic acids, proteins, enzymes and metabolites associated with cell functions and viability [17]. Chen et al. [32] obtained about a 13-fold increased level of laccase production by the marine-derived fungus *Pestalotioptis* sp. J63 when 0.5 mM $CuSO_4$ was used, but the laccase activity significantly decreased as the concentration of $CuSO_4$ increased above 0.5 mM. The result implied the toxicity of Cu^{2+} on fungal growth. Interestingly, the strain of *Pestalotiopsis* sp. isolated from a terrestrial forest preferred a higher $CuSO_4$ level of up to 2 mM [68].

Bonugli-Santos et al. [6] reported a small increase in the laccase activity level of three facultative marine fungi (*Tinctoporellus* sp. CBMAI 1061, *Marasmiellus* sp. CBMAI 1062 and *Peniophora* sp. CBMAI 1063) when $CuSO_4$, wheat bran and guaiacol were used, respectively. When cultured in medium prepared with artificial seawater for 21 days at 28 °C, the fungal laccases were produced at a high amount by *Marasmiellus* sp. CBMAI 1062 (971.2 U L^{-1}) and *Peniophora* sp. CBMAI 1063 (709.0 U L^{-1}).

Metal ions have also been reported to enhance or inhibit the laccase activity. The activity level of the three laccase-like phenol oxidases, SnPO1, SnPO2 and SnPO3, was greatly enhanced in the presence of Cu^{2+}, Mn^{2+} and Fe^{2+}, whereas Pb^{2+} and Cd^{2+} strongly inhibited the PO activity of SnPO1, but had no effect on SnPO2 and SnPO3, while Ca^{2+}, Mg^{2+}, Zn^{2+} and citric acid had no effect on all three proteins. These proteins might play different roles in the immune and physiological processes of *S. nudus* [47].

Aromatic compounds structurally related to lignin, such as syringic acid, tannic acid, cinnamic acid and gallic acid, could be added to fungal cultures to increase the laccase activity and laccase gene transcription. However, the induction level is highly sensitive to small differences in the chemical structures [67]. Some compounds, such as ABTS and guaiacol, were also reported to stimulate transcription of laccase genes. In the presence of ABTS, approximately twofold higher relative expression levels were found for three laccase genes in *Cerrena* sp. strain HYB07 (Lac1, Lac2 and Lac6). In addition, the transcription of Lac3 and Lac4 from this fungus was upregulated by 2.2- and 2.8-fold in the presence of guaiacol [61]. Laccase induction by these compounds may also serve as a response against toxic aromatic compounds. Laccases play a defensive role by activating the polymerization of such aromatic compounds to reduce the oxidative stress caused by the oxygen radicals that arise from the reaction of these molecules [69].

Application of Laccases from Marine Organisms in the Bioremediation of Industrial Waste

Dye Decolorization

The replacement of natural dyes by commercially synthetic dyes in the market happened rapidly from the late nineteenth century due to their low cost of production, simple dyeing process and color diversity. Nowadays, synthetic dyes are used extensively in a number of industries. Over 100,000 commercial synthetic dyes with an annual production of about 280,000 tons worldwide are available. The early synthetic dyes were classified as acidic, basic, mordant and direct. The acidic and basic dyes are comprised of anionic and cationic dyes, respectively, [70, 71]. The chromophores in anionic and non-ionic dyes are azo and anthraquinone types. Azo dyes, which are aromatic compounds with at least one -N=N- group, contribute to 60–70% of the total dyestuffs produced and are among the most widely used class of dyes in many industries [25, 72].

Furthermore, dyes with a sulfonated group substituent on the aromatic ring, which rarely occur in nature, are more difficult to biodegrade [73]. The decomposition of azo dyes may also lead to the formation of carcinogenic amines under anaerobic conditions in the environment [72]. From 2 to 50% of the applied azo dyes are lost during the dying process, generating a large volume of contaminated wastewater that needs to be treated before discharge into the environment [73].

However, it remains a difficult task to treat such wastewater due to the structural complexity, toxicity and high stability of azo dyes. Laccase substrates, such as anthraquinonic dyes, are easily degraded, whereas non-laccase substrates, like indigo and azo dyes, are more difficult to remove [30]. Physical and chemical processes, such as adsorption, precipitation, coagulation-flocculation and filtration, are commonly used for the treatment of these effluents. However, those methods suffer from the disadvantages of their high energy requirement, high operation cost, and frequent low efficiency and sometimes produce hazardous by-products [25, 74].

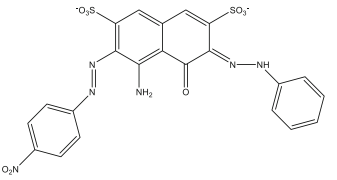
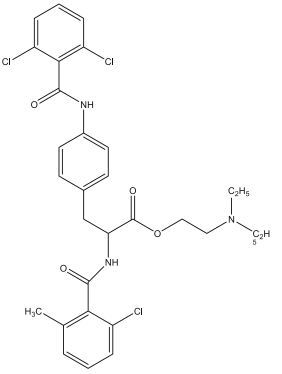
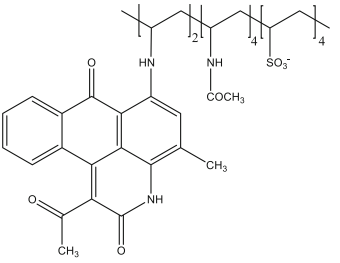
Green oxidation technologies using microbes and their enzymes to replace conventional non-biological methods are of increasing interest for biotechnological applications. Dye decolorization by bacteria strains can be due to adsorption to cell mats or to biodegradation. In the case of adsorption, the cell mats become deeply colored whereas when biodegradation takes place they retain their original color [75].

To achieve the greatest fixation of dyes to fibers, high salt concentrations (40–100 g L^{-1}) are typically used in dye baths [76], and so high salt concentrations are found in the wastewater. Although many bacterial strains have been found to be capable of decolorization of dyes, there application to wastewater treatment is limited because of the high salt concentration in the dye effluent and their sensitivity to high salinity. A concentration of more than 1% (*w*/*v*) NaCl in wastewater can cause moderate inhibition of bacterial activities due to plasmolysis or loss of activity of the cell [76]. Other industrial effluents also contain numerous inorganic chemicals, including sulfides, sulfates, chlorides and carbonates, and such effluents with a high salinity need to be diluted before treatment, so leading to an increased volume of wastewater for treatment. Consequently, screening and isolation of halotolerant microbes that produce suitable enzymes (like laccase) that are active under high saline conditions has attracted increasing interest [30, 75].

Dyes can be degraded by a variety of microorganisms. The structure of dyes decolorized by laccases obtained from marine organisms and the obtained decolorization percentage are shown in Table 4. Liu et al. [27] reported two bacterial strains, *Shewanella algae* and *Shewanella marisflavi,* isolated from a marine environment that showed a higher ability to decolorize dyes than other strains from non-saline sources. The two strains decolorized Amaranth in the presence of 100 g L^{-1} NaCl or Na_2SO_4. Under these saline conditions, some organic substances, such as formate, lactate, glucose, sucrose, pyruvate, acetate and glycerine, could promote the decolorization of Amaranth by these two strains and this was enhanced at higher cell concentrations, but decreased with culture time, which might be caused by substrate limitation and salt stress accumulation. Phytotoxicity assessment data showed that the decolorization of Amaranth by *S. algae* and *S. marisflavi* produced metabolites that were less toxic than the original dye, but still toxic and so only partial detoxification of the dye was obtained. Moreover, the decolorization was inhibited by low concentrations of $NaNO_3$.

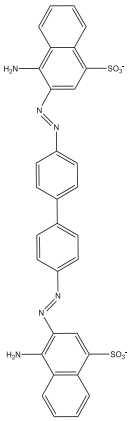
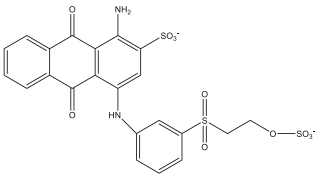
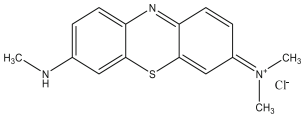
To study the effect of salinity on decolorization, three enzyme activities (azoreductase, laccase and NADH-dichlorophenol indophenol (NADH-DCIP) reductase) in the cell extracts of *S. algae* and *S. marisflavi* were also determined. The activity of the three enzymes were stimulated at a low NaCl concentration (20–30 g L^{-1}), with 83.7 and 48.5% higher laccase

Table 4 Structure of dyes (where available) degraded by laccases from marine organisms

Dye	Chemical structure	Organism	Maximum decolorization (%)	Reference
Acid Black 1		*Oscillatoria curviceps* BDU92191	84%	[25]
Poly-B-411		*Flavodon flavus* 312	> 90%	[29]
Poly-R 478		*Flavodon flavus* 312	~80%	[29]
		*Cerrena unicolor* NIOCC # 2a (MTCC 5159)	43%	[57]

activities in *S. algae* and *S. marisflavi* than in a non-saline condition. However, high performance liquid chromatography and Fourier transform infrared spectrometry analyses revealed that the azo dye degradation by these two strains was accomplished through a reductive pathway and so the laccase might not have been involved in this cellular decolorization. In

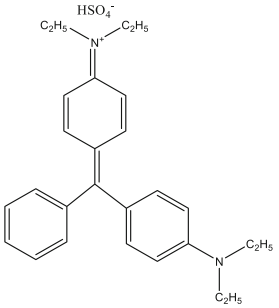
Table 4 (continued)

Congo red		*Flavodon flavus* 312	> 90%	[29]
		Cerrena unicolor NIOCC # 2a (MTCC 5159)	Decolorization in plate assay	[12]
		Cerrena unicolor NIOCC # 2a (MTCC 5159)	47%	[57]
Remazol brilliant blue R		*Flavodon flavus* 312	> 90%	[29]
		Cerrena unicolor NIOCC # 2a (MTCC 5159)	46%	[57]
Azure B		*Flavodon flavus* 312	> 90%	[29]

contrast, Wu et al. [77] observed only laccase and NADH-DCIP reductase activities in the cell free extract of *S. oneidensis* WL-7 during the decolorization of Reactive Black 5. A significant enhancement of the laccase activity (227%) was detected in the cell extract after the decolorization process, whereas the NADH-DCIP activity was not found, suggesting that the laccase was responsible for the dye decolorization.

A new marine bacterial laccase, Lac21, screened from a metagenomics library, also showed a high capacity for decolorization. Four commercial textile azo dyes (Reactive Deep Blue M-2GE, Reactive Brilliant Orange K-7R, Reactive Red KM-8B and KD-8B) could be decolorized to different degrees without a redox mediator at 20–40 °C, where 80% decolorization of Reactive Deep Blue M-2GE (50 mg L^{-1}) was achieved by Lac 21 at 15 U L^{-1} after a 24 h incubation at 20 °C [45].

Table 4 (continued)

Brilliant Green		*Flavodon flavus* 312	~70%	[29]
		Cerrena unicolor MTCC 5159 (NIOCC # 2a)	79%	[57]
Textile effluent A (TEA) (Azo dye-20)	-	*Cerrena unicolor* NIOCC # 2a (MTCC 5159)	55.6%	[30]
		Coriolopsis byrsina NIOCC # 15V	31.2%	[30]
		Diaporthe sp. NIOCC # 16V	48%	[30]
		Pestalotiopsis maculans NIOCC # C3	58%	[30]
		Cerrena unicolor NIOCC # 2a (MTCC 5159)	11%	[57]

Priya et al. [25] tested the ability of 10 cyanobacterial strains to decolorize the diazo dye, Acid Black 1. Among the strains tested, *Oscillatoria curviceps* BDU92191 showed the highest decolorization level (98%) after 8 days at 200 and 500 mg L^{-1} dye concentration, and used it as a nitrogen source. Degradation was attributed to the laccase, PPO and azoreductase activities. Recently, Bonugli-Santos et al. [39] reported Reactive Black 5 decolorization by the marine Basidiomycota fungus *Peniophora* sp. CMBAI1063. Using an integrated Plackett-Burman and central composite design with RSM analysis, the optimal rate of decolorization

Table 4 (continued)

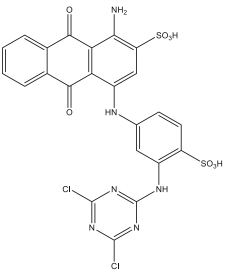
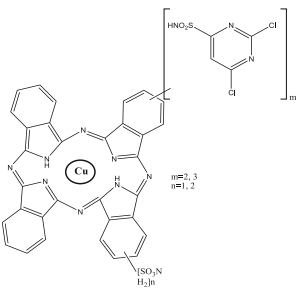
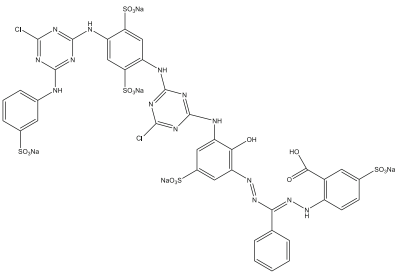
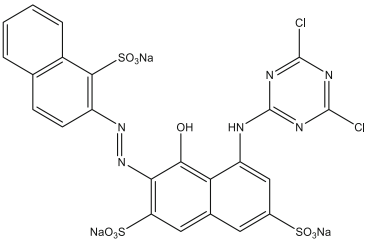
Textile effluent B (TEB) (Reactive blue 4, reactive blue 140 base, reactive blue 140, reactive blue 160 base, reactive blue 163, reactive red 11, reactive yellow 145, reactive green 19)	Reactive blue 4 	*Cerrena unicolor* NIOCC # 2a (MTCC 5159)	76.4%	[30]
		Coriolopsis byrsina NIOCC # 15V	61.5%	[30]
		Diaporthe sp. NIOCC # 16V	75.1%	[30]
	Reactive blue 140 	*Pestalotiopsis maculans* NIOCC # C3	79.3%	[30]
		Cerrena unicolor NIOCC # 2a (MTCC 5159)	22%	[57]
	Reactive blue 160 			
	Reactive red 11 			

Table 4 (continued)

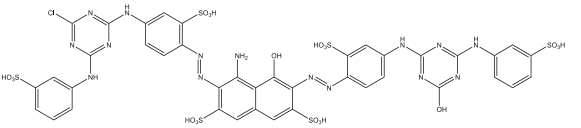
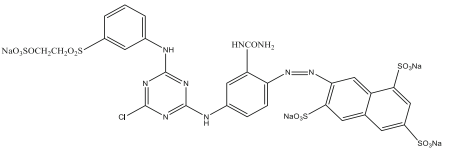
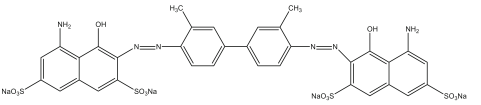
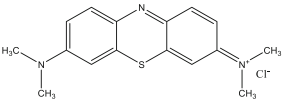
	Reactive yellow 145 			
	Reactive green 19 			
Trypan Blue		*Cerrena unicolor* NIOCC # 2a (MTCC 5159)	Decolorization in plate assay	[12]
		Cerrena unicolor NIOCC # 2a (MTCC 5159)	25%	[57]
Methylene Blue		*Cerrena unicolor* NIOCC # 2a (MTCC 5159)	Decolorization in plate assay	[12]
		Cerrena unicolor NIOCC # 2a (MTCC 5159)	5%	[57]

Table 4 (continued)

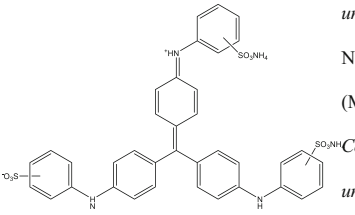
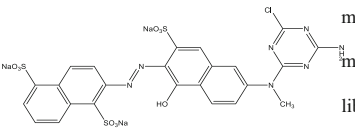
Aniline Blue		*Cerrena unicolor* NIOCC # 2a (MTCC 5159)	Decolorization in plate assay	[12]
		Cerrena unicolor NIOCC # 2a (MTCC 5159)	40%	[57]
Reactive Brilliant Orange K-7R		*Lac21* from marine metagenome library expressed in *E. coli*	~10–20%	[46]
		Lac15 from marine metagenome library expressed in *E. coli*	~8–70%	[51]
Reactive Red KM-8B	-	*Lac21* from marine metagenome library expressed in *E. coli*	~10–30%	[46]
		Lac15 from marine metagenome library expressed in *E. coli*	~15–50%	[51]

Table 4 (continued)

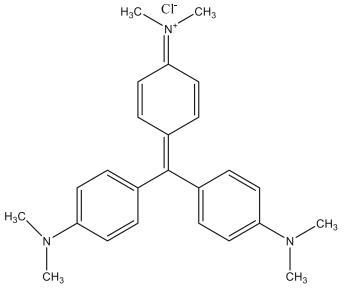
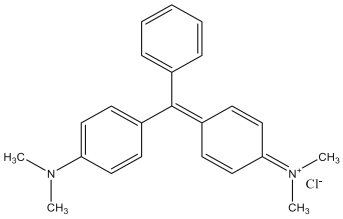
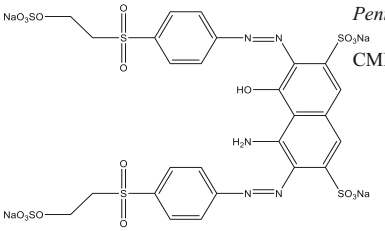
KD-8B	-	*Lac21* from marine metagenome library expressed in *E. coli*	~10–20%	[46]
		Lac15 from marine metagenome library expressed in *E. coli*	10–30	[51]
Reactive Deep Blue M-2GE	-	*Lac21* from marine metagenome library expressed in *E. coli*	80%	[46]
		Lac15 from marine metagenome library expressed in *E. coli*	~40–60%	[51]
Crystal violet		*Cerrena unicolor* NIOCC # 2a (MTCC 5159)	54%	[57]

Table 4 (continued)

Molasses spent wash	-	*Cerrena unicolor* NIOCC # 2a (MTCC 5159)	33%	[57]
Black liquor	-	*Cerrena unicolor* NIOCC # 2a (MTCC 5159)	59%	[57]
Malachite Green		*Trichoderma harzianum/Hypocrealixii* TSK8	89%	[9]
Reactive Black 5		*Peniophora* sp. CMBAI1063	94%	[40]

(up to 94%) was obtained under a saline condition. Interestingly, no mutagenic compounds were detected during the decolorization, but laccase and MnP were produced during the process.

The degradation of Reactive Black 5 occurred in two steps. Firstly, the dye concentration decreased rapidly due to the reduction of one azo bond, generating some purple colored compounds, which might have been due to the action of MnP. These intermediates still had an azo bond. Secondly, the further reduction of the azo bond intermediate to colorless compounds. This step took longer than the first one [39, 78]. These results reinforce the potential of microorganisms from marine environments, together with their enzymes, for application in saline process, such as the decolorization of textile dyes.

The fungus *Flavodon flavus* strain 312 isolated from sea grass in India can produce laccase, MnP and LiP depending on the amount of nitrogen in the medium. *Flavodon flavus* presented

an efficient degradation of five different dyes (Poly-B, Poly-R, Congo red, Remazol brilliant blue R and azure B) when the respective dye was added to a 4-day-old culture of the fungus to a final concentration of 0.02% (*v*/*v*). The best decolorization of all the tested dyes was observed in a low nitrogen medium prepared with 50% (*v*/*v*) artificial seawater [29].

Solid medium containing dyes have been used to identify the ligninolytic potential and phenolic compound degradation by fungi [74, 79, 80]. The fungus *Cerrena unicolor* MTCC 5159 (NIOCC # 2a), isolated from decaying mangrove, has been reported to produce laccase as the major lignin-degrading enzyme, and showed a high ability to decolorize Congo red, Trypan Blue, Methylene Blue and Aniline Blue in a plate assay. Moreover, its partially purified laccase could reduce lignin from sugarcane bagasse pulp by up to 36% in 24 h [12]. This fungus also showed a high decolorization capability when dyes were added to the culture medium or the cell-free culture supernatant containing laccase. The decolorization of black liquor from paper and pulp mills (at a 10% (*v*/*v*) concentration) and the molasses spent wash from distillery waste in the culture medium reached about 60 and 100%, respectively, after 6 days of culture. The culture supernatant with a laccase activity of 18 U mL^{-1} showed about 79% decolorization of brilliant green within 12 h of incubation and 79% decolorization of black liquor (at 10% (*v*/*v*) concentration) within 6 h at pH 6 and 60 °C [56].

The mechanisms of dye removal by fungi are physical adsorption and enzymatic degradation [81]. The first mechanism of dye removal in many cases is the adsorption of dye to the fungal surface [30, 82]. Saravanakumar et al. [9] observed the accumulation of dye on the surface of the marine fungus *Trichoderma harzianum/Hypocrealixii* TSK8 by scanning electron microscopy. This fungus was isolated from mangroves and achieved an 89% degradation of malachite green. Decolorization of the dye was also significant. The laccase activity in the culture solution increased over time up to a maximum on the 12th day, when addition of the dye resulted in its complete removal on the same day. The optimal condition for dye degradation, as evaluated by RSM, was 5.81 mg L^{-1} yeast extract for 10 days incubation at pH 5.8 and 30 °C. The degraded dye metabolites were less toxic than the original dye.

Raw dye-containing textile mill effluent A (TEA), containing an azo dye at pH 8.9, and textile effluent B (TEB), containing a mixture of eight reactive dyes at pH 2.5, were both decolorized by two isolates of Ascomycota (*Diaporthe* sp. and *Pestalotiopsis* sp.) and two Basidiomycota (*Coriolopsis byrsina* and *Cerrena unicolor*). These four marine fungi decolorized TEA by 30–60% and TEB by 33–80% within 6 days when used at 20–90% concentrations. Adsorption appeared to be the primary mechanism of dye removal by the two Ascomycota, whereas degradation by laccase played an important role in dye removal by the two Basidiomycota. Furthermore, the Ascomycota and Basidiomycota isolates had the ability to reduce the toxicity, chemical oxygen demand and total phenolics in the TEA and TEB [30]. Fang et al. [50] demonstrated decolorization by pure Lac15, a laccase from a marine microbial metagenome. Although Lac15 was unable to completely decolorize Reactive Brilliant Blue X-BR and K-GR, either with or without mediators, at 10 U L^{-1} and 45 °C it could completely decolorize the 50 mM reactive azo dyes, Reactive Brilliant Orange K-7R and Reactive Deep Blue M-2GE under alkalescent conditions within 1 h.

Bioremediation of Pesticide and Xenobiotic Compounds

Pesticides and herbicides are extensively used in agriculture despite their high ecotoxicity and long persistence in nature. Although some of them, such as dichlorodiphenyltrichloroethane (DDT) and hexachlorocyclohexane (HCH), are banned for agricultural use, they are still used

and can be transported from sites of previous or current application into the ground water and environment and cause catastrophic impact to the ecosystem, human health, and the quality of soil and surface water [83]. A variety of fungi, including *Phanerochaete chrysospoium*, *P. ostreatus*, *Trametes versicolor* and *Conidiobolus 03-1-56*, have the ability to degrade pesticides and herbicides, such as γ-hexachlorocyclohexane (lindane), diuron and endosulfan [84–87]. Several recent researches presented that laccase and peroxidase are involved in the degradation of pesticides. For instance, *Trametes versicolor* was found to decrease the concentration of tribromophenol (TBP) and to contain peroxidase and laccase activities in the culture medium, which implied that these enzymes might be involved in the biodegradation process [88].

Laccases from fungi, including wild type, heterologously expressed laccase or a laccase-mediator system, have been reported as a potential tool for the biodegradation of xenobiotic compounds. For example, when the laccase from *Trametes sanguineus* was over expressed in the fungus *Trichoderma atroviride*, it efficiently removed the endocrine disruptors, benzo[α]pyrene and phenanthrene [89]. Laccase from the fungus *Trametes villosa* was also reported to remove chlorinated phenols, and so could be applied in the decontamination of wastewater polluted with chlorinated phenols [90]. Laccase-mediator systems have been found to efficiently degrade and detoxify the herbicide isoproturon. In the presence of 0.3 U mL^{-1} laccase and 1 mM of mediator HBT, isoproturon was completely degraded within 24 h [5].

The crop soil where herbicides and pesticides are frequently used sometimes has a high salinity due to irrigation or from the use of chemical fertilizers. This high salinity can make the biodegradation process become more difficult because it negatively affects the growth and activities of non-halotolerant microorganisms. Microbes that can grow in these relatively high salt conditions together with their enzymes in biological treatment could be an interesting approach for bioremediation [91, 92]. The halophilic Basidiomycota fungus *Dacryopinax elegans*, isolated from decaying wood in the Atlantic Rainforest fragments in Brazil, was found to degrade the herbicide diuron in presence of NaCl. It also produced MnP, LiP and laccase, where their production profiles were similar to that for diuron degradation [91].

Despite the fact that laccase from many terrestrial fungal and bacterial strains have been found to degrade several herbicides, pesticides and xenobiotic compounds, reports on the bioremediation of such compounds by laccase from marine organisms are quite limited. Illumina high-throughput sequencing and metagenome analysis were used to study the diversity of biodegradation genes (BDGs), dichlorodiphenyltrichloroethane degradation genes, hexachlorocyclohexane degradation genes and atrazine degradation genes in six sediment samples from Hong Kong and the South China Sea. The dominant genes for degradation of the organic pollutants were the *lip* and *mnp* genes, encoding POs, and *carA*, which encodes for laccase. The most abundant genes in the degradation of DDT, HCH and atrazine were the *hdt*, *hdg* and *atzB* genes, which encode for a hydratase, dehalogenase and ethylaminohydrolase, respectively. Most of the phyla involved in the biodegradation belonged to the Proteobacteria (49.3%) followed by the Actinobacteria (21.7%). The most abundant genera that played a role in the biodegradation in all sediments were *Plesiocystis*, *Anaerolinea*, *Jannaschia* and *Mycobacterium*. The degradation pathway of DDT, HCH and atrazine has been reported previously [46].

Polycyclic aromatic hydrocarbons (PAHs), aromatic hydrocarbons with two or more fused benzene rings, are xenobiotic pollutants that are widely distributed in various ecosystems. They are of significant concern due to their toxicity, carcinogenicity, mutagenicity and resistance towards biodegradation. The majority of PAHs come from human activity, including

the incomplete combustion of organic matter, such as fossil fuels, coal tar, wood, garbage, refuse, used lubricating oil and oil filters, waste incineration and petroleum spills and discharges [93]. In terrestrial systems, the predominant high molecular weight (HMW) PAHs are often bound to soil particulates and are difficult to degrade by bacteria, whereas some fungi have an ability to degrade HMW PAHS [94]. The bioremediation of PAHs by fungi has received increasing attention. Both intracellular and extracellular enzymes play a role in the biodegradation of PAHs, such as cytochrome P450, LiP, MnP and laccase [95]. Laccases from white-rot fungi oxidize PAHs to the corresponding PAH quinones, and then further degrade these materials to carbon dioxide [96]. *Fusarium solani*, isolated from PAH-contaminated mangrove sediments in Hong Kong, was found to biodegrade anthracene and benz[a]anthracene, while it also could use anthracene and benz[a]anthracene as a sole carbon source. After 40 days, *F. solani* could remove 40 and 60% of anthracene and benz[a]anthracene, respectively. Although laccase activity was detected during the degradation process, LiP and MnP activities were not found, implying that laccase was the important enzyme involved in the transformation of PAHs [97]. Furthermore, PAH-degrading bacterial strains have also been reported and the majority of PAH-degrading bacterial strains from marine environments, such as sediments, seawater and salt marshes, belong to the phyla Proteobacteria, Actinobacteria, Cyanobacteria, Bacteroidetes and Firmicutes [98].

In addition, PAHs are also found in sea water and sediment, where low molecular weight PAHs are predominant [99]. Biodegradation of PAH in the deep sea, which is characterized by a high pressure (10–50 MPa at 1000–5000 m) and low temperature (2–3 °C), is quite difficult. The high pressure limits the growth of bacteria, while the darkness and low temperature can cause a lower metabolic activity and reduce the PAH degradation efficiency [100, 101]. Laccases or enzymes from marine organisms that can function at such extreme conditions and have a high saline tolerance could be potential enzymes for the biodegradation of PAHs.

Conclusion

Marine ecosystems are a potential source of discovery of novel biocatalysts of industrial interest. Among such biocatalysts, laccases are very attractive due to their ability to oxidize a broad range of substrates, including phenolic and non-phenolic compounds, giving them a tremendous potential for the bioremediation and biodegradation of environmental pollutants. The capability to biodegrade pollutants varies with the source of the microorganism, type of pollutant, mediators and conditions used. Reports of microorganisms and their enzymes that showed oxidoreductase activity in saline conditions are very few, whereas industrial effluents and, especially, wastewaters from the dye industries contain a high level of saline, carbonates, chlorides and sulfates. Laccases from the marine organisms discussed here hold a high potential for bioremediation under such conditions, but certainly far more remain to be discovered. The expression of laccase is mainly effected by the salinity, amount of nitrogen, carbon, copper and metal ions. Certainly, a lot of potential marine microorganisms, and so enzymes, which could be used in bioremediation, have not been explored. To use these enzymes at an industrial scale requires the production of highly active enzymes at a large volume and acceptable cost. Screening of new laccases by classical clonal selection or metagenomics approaches and subsequent protein engineering for their hypersecretion and hyperactivation are areas of future research.

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Compliance with Ethical Standards

Conflict of Interest All authors declare that they have no conflict of interest.

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