

## Laccases – enzymes with an unlimited potential

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**Abstract:** *Laccases (EC 1.10.3.2) are among the few enzymes, the history of which dates back to the 19<sup>th</sup> century. These oxidoreductases are present in almost all known fungi, some species of higher plants and insects. Moreover, in recent years, these enzymes have also been found in some bacterial organisms. Due to their significant properties and structure of the catalytic centre, laccases have been classified as the multicopper oxidases (MCOs). These enzymes are able to catalyse the oxidation of phenolic and non-phenolic compounds, with the aid of small molecules referred to as mediators. Thanks to their diverse nature, laccases have gained attention of both scientists and entrepreneurs from all over the world. Their significance is reflected in countless scientific and industrial applications, wherein laccases have become inseparable from chemical syntheses, the food industry, textile industry, biosensor design and the environmental protection. This paper gathers the most important information and the latest scientific discoveries relating to this desirable biocatalyst.*

**Keywords:** *laccases, multicopper oxidases, reactions, properties, applications.*

### Introduction

Laccase (EC 1.10.3.2), *p*-diphenol oxidase (systematic name – benzenediol: oxygen oxidoreductase), is one of the few enzymes, the history of which dates back to the 19<sup>th</sup> century. In 1883, Yoshida [1] was the first person who described a plant laccase extracted from the resin of the tree *Rhus vernicifera*, which is widespread in Asia (Japan, China and India). Not until much later, in 1896, Bertrand and Laborde described the first fungal laccase, which commenced the domination era of enzymes from such organisms. The literature provides many examples of laccases, both extracellular and intracellular. These are glycoproteins, mainly dimeric or tetrameric, which comprise subunits, the mass of which ranges from 50 kDa to 100 kDa [2, 3].

Laccases, popularly referred to as the *blue oxidases*, are included in multicopper oxidases (MCOs) and are able to oxidize organic and inorganic compounds such as mono-, di-, poly-, amino- and methoxyphenols, and a number of aromatic amines. The final effect of the reactions catalysed by the oxidoreductases

is the reduction of molecular oxygen to two water molecules [2]. The active centre of laccases contains four copper atoms, each of which, in the native enzyme form, occurs in the oxidation state +2. As the redox potential of laccases ranges from 450 mV to 800 mV, these enzymes are able to catalyse the oxidation of simple phenol derivatives [2, 4]. The spectrum of laccase substrates can be considerably expanded using low-molecular compounds, the so-called redox mediators, which directly participate in the reaction.

Within the last two decades, laccases have joined the group of the most searched enzymes due to their wide range of applications. They can be successfully used in the following sectors: food industry, paper industry, textile industry, detoxification and biodegradation of pollutants, and synthetic organic chemistry. Moreover, they play a vital role in the process of natural delignification and show a great potential in bio-refining, too. Laccases, purified and isolated from ever new sources, are characterised by their unique properties, extending possible applications of this group of oxidoreductases [5, 6].

This paper gathers the most important scientific information on the occurrence, molecular structure, catalytic properties and applications of laccases.

## Occurrence of laccase

Laccases are widespread in the world of living organisms. The first isolated laccase was of plant origin. It was isolated from the resin of the Japanese tree *Toxicodendron vernicifluum* (formerly *Rhus vernicifera*) [3], used to obtain lacquer, hence the name of this enzyme. While continuing the research, it was revealed that *p*-diphenol oxidases are common in many species of trees as well as vegetables and fruits. The main function of these plant oxidoreductases is the synthesis of lignins and regeneration of damaged tissues, which is aided by releasing the enzyme to the apoplast – a system formed by dead plant elements, used to transport water [7]. Applicability of plant laccases is very limited due to their low oxidoreductive potential (approx. 430 mV) and considerably more difficult extraction of plant enzymes as compared with the methods used to obtain microbiological biocatalysts.

Laccases have also been found in organisms of many insects, in particular in the species of *Calliphora*, *Diptera* and *Drosophila*. It is known that they play a substantial role at initial stages of insect sclerotisation, which occurs during each stage of insect development. The first stage of the process involves the production of highly reactive tannins by the oxidation of *o*-diphenyls to corresponding electrophilic *o*-benzoquinones. This key reaction is catalysed by laccases and requires molecular oxygen acting as the hydrogen acceptor removed from the substrate [8].

The highest number of the laccases described so far were isolated from filamentous fungi, mainly those classed as *Ascomycetes* and *Deuteromycetes* as well as higher fungi in the class of *Basidiomycetes* [9]. Apart from the typical fungal environment such as the soil, municipal sewage and tree bark, some laccase-positive microorganisms were isolated from less anticipated environments,

e.g. sea water (basidiomycetes, e.g. *Corioloropsis byrsina*, *Cerrena unicolor*, and fungal plant pathogens, e.g. *Pestalotiopsis uvicola*). The first described and characterised laccase, isolated from *Ascomycetes*, was the enzyme obtained from *Monocillium indicum* [10]. This finding, and the fact that *Ascomycetes* are an easier target of genetic modifications, are the reason for which this microorganism group is more often recognised as a perfect source of laccases [11].

In recent years, the interest in bacterial laccases, the majority of which includes intracellular enzymes, has increased. The outermost polypeptide coat (S-layer) – in *Bacillus* sp. spores – contained a laccase named after its encoding gene, *cotA*, which participates in pigment production in this densely cross-linked protein structure. CotA laccases show certain structural analogies to fungal multicopper oxidases [12]. Recently, it has been possible to identify more laccases produced by Gram-negative bacteria such as *Azospirillum*, *Xanthomonas*, *Shewanella*, *Pseudomonas*, *Escherichia*, as well as those which are interesting due to their applications, laccases from extremophilic archaea (e.g. *Haloferax volcanii* and *Pyrobaculum aerophilum*) [13]. CueO laccase from *Escherichia coli* participates in the removal of excessive Cu from cells (copper efflux). It is worth mentioning that the activity of the laccase is strictly dependent on the concentration of exogenous Cu sources [14]. It is anticipated that the application potential of laccases from prokaryotes may be more comparable to those obtained from fungi because of their higher stability at a wider range of temperatures and pH values, and easier production as heterogeneous proteins [15,16].

### Classification of multicopper oxidases

The superfamily of multicopper oxidases (MCOs) is divided into four families as follows: laccases (EC 1.10.3.2), L-ascorbate oxidases (EC 1.10.3.3), bilirubin oxidases (EC 1.3.3.5) and ferroxidases (e.g. ceruloplasmins; EC 1.16.3.1) [17]. The LccED database (<https://lcced.biocatnet.de/>) provides more than 10400 MCO proteins divided into 16 superfamilies, based on sequential data (Table 1).

**Table 1.** Classification of multicopper oxidases [17, 18]

	Superfamily	Number of domains in the structure
A	Basidiomycete laccases	
B	Ascomycete MCO	
C	insect laccases	
D	MCOs of fungal pigments	
E	fungal ferroxidase	
F	fungal and plant ascorbic oxidases	3-domain MCOs
G	plant laccases	
H	bacterial copper-resistant proteins (CopA)	
I	bacterial bilirubin oxidases	
J	bacterial laccases (CueO)	
L	bacterial MCOs	

K	SLAC homologues – type B	
M	A-type MCOs from archaea	
N	B-type bacterial MCOs	2-domain MCOs
O	C-type MCOs from archaea and bacteria	
P	Ceruloplasmins	6-domain MCOs

Bioinformatics scientists from the University of California have suggested system for classification of laccases – LacSubPred (<http://tools.bioinfo.ucr.edu/lacsubpred/>) – which takes into account the physicochemical similarity of laccases, including their properties and spectra of the substrates recognised by them [19].

## Molecular properties of laccases

### *The “family” of laccase encoding genes*

Perry et al. [20] discovered, for the first time, the presence of two genes encoding laccase isoforms in *Agaricus bisporus*. Next publications confirmed the occurrence of multi-gene systems in other laccase-positive fungi (e.g. four or five different laccase genes were found in the genomes of *Trametes villosa*, *Trametes sanguinea* and *Rhizoctonia solani*, respectively [21]). In the genome of *Coprinopsis cinerea* there were as many as 17 identified genes encoding laccases. In addition, it was proven that 16 of them could be successfully transcribed into active catalytic proteins, and as many as 15 such genes contain signal sequences responsible for the secretion of the enzyme from cells [22]. It is believed that such a high number of genes within the same species results from their participation in various physiological processes during the lifetime of the microorganism, wherein different isoforms of laccases serve for strictly specific functions [21]. Yuan et al. [23] identified three isoforms of laccases (Lac1, Lac2 and Lac3 in *Pleurotus nebrodensis*), which considerably differ in the optimum temperature of action and affinity for various substrates. Such a diversity of the laccases protects fungi from changeable living conditions. The isoforms of laccases include enzymes which are both constitutive and induced on the transcription level by the presence of various substrates. For instance, the effect of increased transcription level of *lcc1* gene in *Volvariella volvacea*, caused by the addition of specific aromatic compounds, was not observed for *lcc4* gene [24].

### *Protein sequences of laccases – key features*

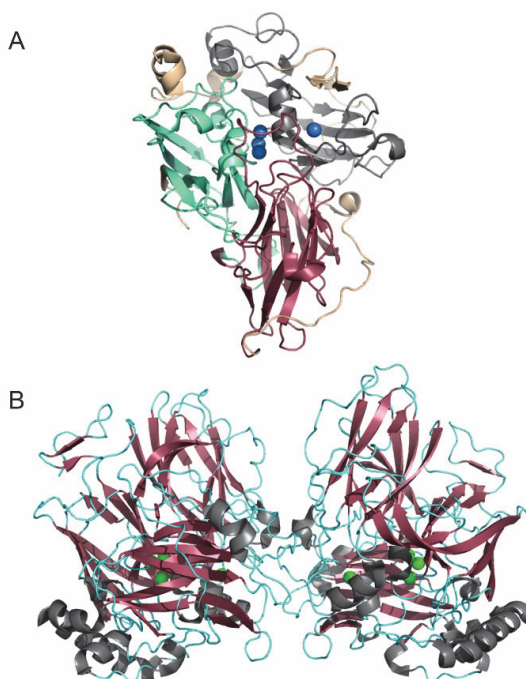
Kumar et al. [25] were the first who identify conservative sequences distinguishing laccases from other multicopper oxidases. They proved that four continuous fragments, which are conservative sequences designated as L1, L2, L3 and L4, can be distinguished in laccases, irrespective of the origin. The degree of identity for such fragments ranges from 75% to 85%. Their composition includes 12 amino acids responsible for bonding copper atoms in the catalytic centre. The regions L2 and L4 allow for classification of the protein to the superfamily of multicopper oxidases, whereas the regions L1 and L3 are characteristic for the laccase subgroup. In addition, it was possible to identify

sequences distinguishing in fungal and plant laccases from *Ascomycetes* (ascolaccases) from other laccases in the MCO superfamily. Apart from the mentioned conservative sequences, laccases of *Ascomycetes* are characterised by the presence of the so-called “SDS-gate”, which consists of two Ser residues and one Asp/Glu residue, wherein the gate is responsible for the transfer of protons during catalysis. Additionally, it was found that the laccases produced by *Ascomycetes* should have a conservative sequence of Asp-Ser-Gly (Leu/Ile/Val) at the C-terminus [25, 26].

### **The structure of laccases**

Fungal laccases are glycoproteins, which most frequently occur as isoenzymes, which, due to oligomerization of subunits, are able to form multimeric complexes. These are mainly dimeric or tetrameric forms, with the mass of individual mers ranging from 50 kDa to 110 kDa. Approximately 10% to 45% of the total subunit mass. Generally fungal and bacterial laccases have a lower percentage of saccharide components (10-25%) than plant enzymes [27]. Of course, many exceptions can be found in literature, for example laccase from *Fomes sclerodermeus* with the carbohydrates content 3% [28] and laccase produced by *Botrytis cinerea* with saccharide compound content at 91% [29]. As described in the literature, there are many *p*-diphenol oxidases occurring in the form of homodimers, for example enzymes isolated from *Rhizoctonia solani*, *Pleurotus pulmonarius* and *Phoma* sp. In case of the latter, from the phylum of *Ascomycota*, it was observed that the type of the created oligomeric form depends on pH of the environment. Trimeric structures are found only among the enzymes isolated from prokaryotes, e.g. the actinobacteria of *Streptomyces coelicolor* [30]. Tetrameric enzymes were isolated from *Agaricus bisporus*, *Armillaria mellea*, *Aspergillus nidulans* and many other fungi [21].

The first published 3D laccase structure was the enzyme containing two copper atoms in the active centre of the protein isolated from *Coprinus cinereus* [31]. In the following years, there were publications presenting spatial conformation of laccases with four copper atoms in the catalytic centre [32,33]. Currently, there are 115 records describing structures of laccases in the PDB database (as of February 2017). Most defined structures come from fungi, in particular *Trametes versicolor* (Figure 1A), *Rigidoporus lignosus*, *Lentinus tigrinus* and *Trametes trogii*, which are included in the phylum of *Basidiomycetes*. In 2002, Hakulinen et al. characterised the first three-dimensional structure of the native form of the laccase obtained from *Melanocarpus albomyces* (Figure 1B), i.e. the fungi in the phylum of *Ascomycetes* [34]. The bacterial laccases which are best-studied include those from *Bacillus subtilis*, *Bacillus halodurans* and *Escherichia coli*. For most of these enzymes, their three-dimensional structures have already been studied and characterized in detail [16, 35].

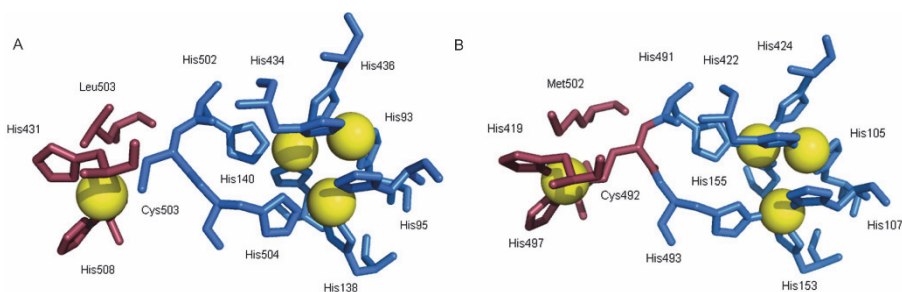


**Figure 1.** (A) Monomeric laccase from *T. versicolor* (1GYC) with marked domains: green – domain 1, grey – domain 3, pink – domain 2 (Cu atoms marked in dark blue). (B) Dimeric laccase from *M. albomyces* (2Q9O) with copper atoms (green)

Each laccase comprises  $\beta$ -sheets arranged in a characteristic Greek-key pattern (Figure 1A and 1B). The pattern occurs in each independently collapsed multicopper domain (domains: MCO 1, 2, 3) and is characteristic for both small copper proteins, including in particular arsenate reductase (EC 1.20.4.1) and plastocyanin (EC 1.10.99.1), as well as superfamilies of large blue multicopper proteins, including in particular plant ascorbic acid oxidase (EC 1.10.3.3) and mammalian ceruloplasmin [33,36]. Among the proteins in the multicopper oxidase superfamily, one can distinguish two-domain proteins (2dMCO), three-domain proteins (3dMCO) and six-domain proteins (6dMCO) (Table 1). These complex structures are formed mainly through the duplication of domains, which are classified as IV and V [37]. Laccases are mainly three-domain proteins in which the type 1 (T1) copper atom is located within the domain 3, whereas the triatomic system created by one Cu T2 atom and two T3 atoms – at the boundary between the first and third domain (seen from the N-terminus) (Figure 1A). Further, the ligand-bonding site is located between the first and second domain, whereas the oxygen-binding site – in the T3 centre. There are also two-domain proteins, which form trimeric structures, e.g. small laccase *Streptomyces coelicolor*, SLAC. Depending on the spatial arrangement of the Cu T1 atom, the

two-domain MCOs are divided into three hypothetical types. For the suggested A arrangement (UniProt Q9HQF4), the Cu T1 atom is present in both domains; however, it has been impossible so far to obtain a crystallographic structure to confirm the presence of such a system. As far as the B arrangement is concerned, Cu T1 is located within the second domain, whereas T3 – in the interdomain space (PDB 4GXF). In contrast to other variants, in the C system, the Cu T1 atom is located within the first domain (PDB 4E9V) [38].

The Cu T1 atom has a maximum of absorption at the wavelength of approx. 600 nm. The Cu T1 ion is bonded by two histidines and one cysteine residue. In addition, one of the three hydrophobic residues (Met, Leu or Phe) stabilises the position of this atom [33, 37]. The T3 atom with a distinct signal at the wavelength of  $\lambda = 330$  nm is coordinated by three histidine residues and one water molecule. The Cu T2 atom of much smaller light absorption is coordinated by two histidine molecules and one water molecule. An example structure of T1 and T2/T3 centres is presented in Figure 2 [39]. Moreover, the so-called yellow laccases were studied [40]. They provide no spectrum with the absorption at the wavelength of approx. 600 nm, which is characteristic for multicopper oxidases. At first, it was believed the enzymes are deprived of the T1 copper atom. However, signals from electron paramagnetic resonance spectroscopy clearly indicated the presence of four copper atoms in the active centre of such enzymes. Now, it is suggested that the yellow colour is caused by the bonding of aromatic lignin degradation products that are formed by the action of blue forms of such proteins [41, 42].



**Figure 2.** T1 and T2/T3 centers of the laccases with coordinating amino acids from (A) *M. albomyces* (code PDB 2Q9O) [34] and (B) *B. subtilis* (code PDB 1W6L) [35]

### Reactions catalysed by laccases

At first, it was believed that laccases were only capable of the catalysis of oxidation of simple phenolic derivatives [4]. However, it turned out that the spectrum of substrates for such enzymes could be considerably expanded by mediators interacting in the transport of electrons between the substrate and the catalytic centre of the enzyme, i.e.  $\text{Cu}^{\text{II}+}$  T1 ion [43]. The reactions catalysed by *p*-diphenol oxidases can be divided into three groups as follows:

- a) oxidation of simple phenolic derivatives without any mediator,
- b) substrate oxidation requiring an additional compound – the so-called mediator,
- c) coupling reactions.

The first stage of each laccase catalytic cycle is to split off the electron from the substrate and transfer it to the T1 copper atom [44]. Therefore, the redox potential value ( $E^{\circ}$ ) of the Cu T1 atom relative to the hydrogen electrode determines the energy necessary for bonding and collecting the electron from the reduced substrate.

Klonowska et al. [45] found that *Trametes* sp. C30 produces laccases with a high redox potential (LAC1), with a simultaneous synthesis of two other forms of the enzyme with a low potential (LAC2 and LAC3). Results of further research indicated that active centres of many fungal laccases show high structural homology, but they are distinguished by their  $E^{\circ}_{T1}$  values, which suggests that the potential depends on slight differences in the surroundings of the Cu T1 atom. As the redox potential of the studied laccases can range from +375 mV to +800 mV, the laccases are divided into three groups as follows [46]:

- I. Laccases with a low redox potential:  $E^{\circ} < 460$  mV – isolated from plants, insects and bacteria. The Cu T1 atom is in a planar arrangement, coordinately bonded in a trigonal system with nitrogen atoms from three His residues and Met sulphur
- II. Laccases with a medium redox potential: approx. 470 mV to 700 mV – isolated from *Ascomycota*, but also some bacteria and *Basidiomycetes*; typical ones contain Leu (instead of Met) in the site coordinating the Cu T1 atom
- III. Laccases with a high redox potential:  $E^{\circ}$  can range from 700 mV to 800 mV. This group includes enzymes isolated mainly from *Basidiomycetes* classified as white-rot fungi. The Cu T1 atom is usually in a planar arrangement, which creates a flat trigonal system by coordinate bonding with nitrogen atoms of the three His rings. Instead of Met, there is usually Phe providing no coordinate bonding with Cu T1

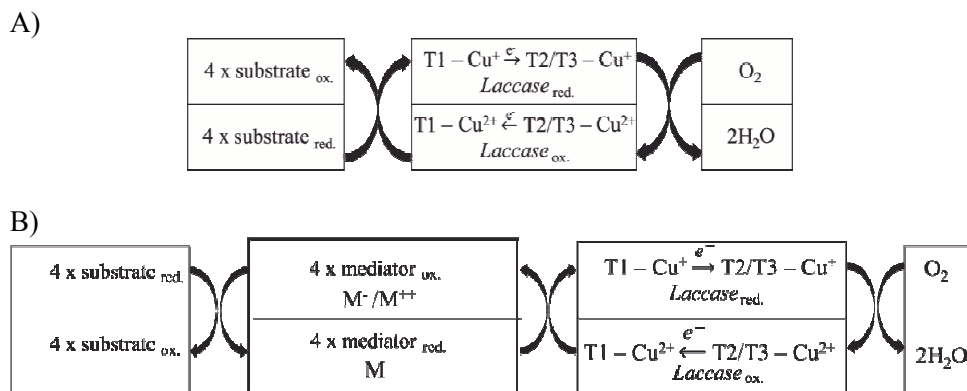
The redox potential  $E^{\circ}$  of laccases is affected not only by the presence of Leu, Phe or Met located axially relative to Cu T1 in the formed trigonal system, but also by the geometry and chemical structure of the entire active site bonding the substrate or mediator, which participates in catalysis. This phenomenon was investigated for the first time by K. Piontek et al. [47], who analysed the structure of laccase from *T. versicolor* (with a high redox potential) by comparing this enzyme with other blue oxidase representatives. They concluded that the high oxidation-reduction potential shown by this enzyme results from a significant distance of the N $\delta$ 2 His458 atom (compared to other enzymes of this type) which bonds the Cu atom coordinately into a trigonal and planar spatial system. As a result, the Cu<sup>II</sup> ion of type T1 is less stabilised, which makes it a better electron acceptor. A fragment of the  $\alpha$ -helix (455-461 residue), which has the N $\delta$ 2 His458 coordinating atom, is significantly distanced (spatially) from the Cu T1 atom.



This is caused by the presence of the conservative Glu460, which strongly interacts with the conservative Ser113. The research opened the door to modifications aimed at increasing the redox potential of laccases of different origins. Teddase et al. [48] studied the laccases from *Trametes villosa* ( $E^{\circ}$  0.79 V) and *Myceliophthora thermophila* ( $E^{\circ}$  0.46 V) with Phe and Leu in the T1 centre, respectively. They found that the catalytic efficiency of laccases may be reduced by hindered access (steric hindrance) of substrate molecules to the acceptor of the Cu(II) electron in T1 [25, 46]. Therefore, bacterial laccases, whose CuT1 centre is located just under the molecule surface, despite a low redox potential, are very efficient oxidizers, for the case of polymeric compounds as well [13].

### Oxidation mechanism of simple phenolic derivatives

Oxidation of simple phenolic derivatives without any mediator is the earliest discovered group of reactions catalysed by laccases. Relatively low substrate specificity of laccases hindered explanation of the mechanism of oxidation of various compounds including mono-, di-, and polyphenols and their derivatives containing amino, carboxyl, methoxyl and sulphone functional groups. For most laccases, the affinity for different substrates ranges from 1 mM to 10 mM (Michaelis constant,  $K_m$ ); all of them show strong affinity for molecular oxygen ( $K_m$  approx.  $10^{-8}$  mM) [49]. It is assumed that laccases catalyse consecutively four single-electron substrate oxidations, which is accompanied by molecular oxygen reduction to water in order to restore the native oxidised enzyme form (Figure 3) [39, 50].



**Figure 3.** General mechanism of oxidation catalyzed by A) laccase and by B) a coupled laccase-mediator system – compiled by the authors, based on [51]

For the phenolic substrate, at the first stage of catalysis, the proton is removed from the hydroxyl group and transformed into a phenoxyl radical. At the same time, one released electron interacts with the T1 copper atom and transforms (reduces) it from the native  $\text{Cu}^{+II}$  form into the  $\text{Cu}^{+I}$  reduced form. The produced

phenoxyl radicals can be further oxidised to quinones or take part in non-enzymatic reactions such as hydrogenation, disproportionation and polymerization [5, 50]. As a result of substrate oxidation, the obtained electrons on Cu T1 are transferred to the three-atom complex T2/T3, which leads to conversion of the enzyme's stationary form (fully oxidised) into an active form. For a complete copper reduction in the active centre of laccases, as many as 4 single-electron oxidation reactions of 4 molecules of phenolic substrates are necessary [39, 44, 52]. At first, it was believed that the only factor limiting activity of laccases was the difference in the redox potential between the T1 copper atom and the phenolic substrate, directly affecting the transport rate of the electron during the first stage of the catalytic cycle. Now, it is known that stage II (Figure 3A), i.e. transporting electrons from the Cu T1 atoms to the T2/T3 complex, also plays a significant role in the reaction mechanism and often limits the rate of the entire catalytic cycle [5].

### **Substrate oxidation with mediators**

The redox potential of laccases which is too low precludes them from direct catalysis of the oxidation reaction of complex polyphenolic structures, non-phenolic compounds and aromatic amines, i.e. compounds with a high potential  $E^{\circ}$  ( $>1.5$  V). In addition, complex molecules, in particular those in lignocellulose complexes, are not able to penetrate into the enzyme's active centre, meaning that the affinity of the enzyme for the substrates is low [53]. Kawai et al., who studied the enzyme from *T. versicolor*, proved that fungal laccases oxidize the mentioned compounds [54] using low-molecule mediators which, due to the presence (in their structure) of numerous functional groups capable of giving and accepting electrons (e.g. NO, NOH, HRNOH), mediate in their transfer from the substrate to the Cu T1 centre. During the first stage of catalysis, the mediators are oxidised by laccases, which results in a very reactive oxidised intermediate product, called the co-mediator. It can easily diffuse away from the enzyme and oxidize molecules of substrates to which the enzyme's catalytic centre is inaccessible. The systems in which reactions take place, assisted simultaneously by the laccase and specific mediators, are called laccase mediator systems (LMS) [55] (Figure 3B).

So far, more than 100 laccase mediators have been described and characterised. These mediators can be divided into *natural* and *synthetic*, based on their sources. The earliest studied natural mediator, i.e. syringaldehyde, was identified by Kawai et al. [54], who studied the spectrum of substrates accepted by the laccases from *T. versicolor*. Natural mediators can occur in two forms. The first one includes substances which are part of lignin complexes. As a result of initial oxidation of lignins, the compounds are gradually released into the environment and, during further stages, laccases use them as mediators to continue lignin biodegradation. Examples of such substances are as follows: veratric acid (3,4-dimethoxybenzoic acid), 3-hydroxyanthranilic acid, 4-hydroxybenzoic acid and 4-hydroxybenzyl alcohol [56]. The other type of natural mediators includes

substances which come as direct products of fungal metabolism. As an example, there is the white-rot fungus *Pycnoporus cinnabarinus* which, apart from the production of laccases, is also capable of biosynthesis of specific organic substances such as 2-amino-3-hydroxybenzoic acid (3-HAA), which can be used in bio-delignification as mediators to create a self-sufficient LMS system [57].

The most frequently applied synthetic mediators include, but are not limited to, diammonium salt 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), hydroxybenzotriazole (HBT), hydroxyanthranilic acid (HAA), violuric acid, hydroxyacetanilide (NHA) and hydroxyphthalimide (HPI). The ABTS application in biodegradation of lignins by *T. versicolor* was described by Bourbonnais and Paice in 1990 [53]. They proved that oxidation of veratryl alcohol to aldehyde is approx. 5-fold more efficient in the presence of that mediator.

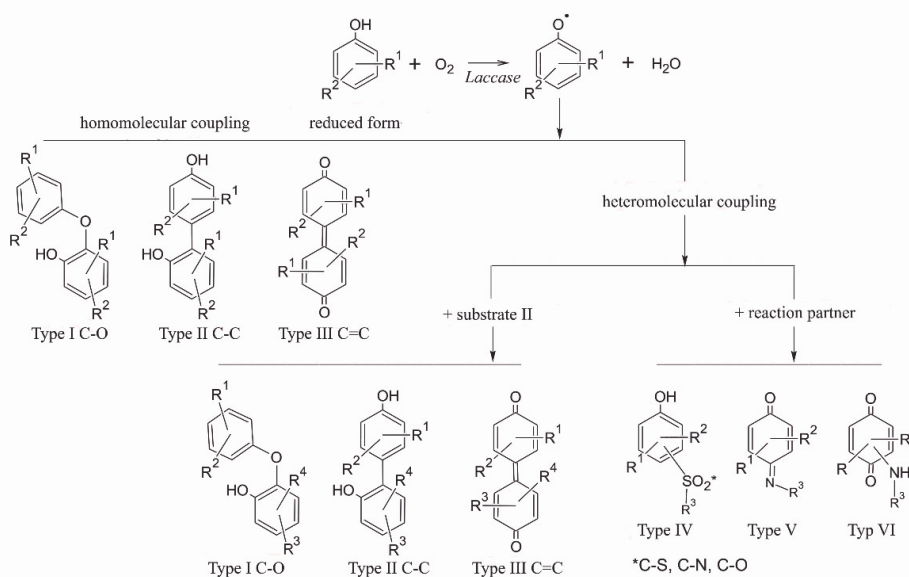
### **Coupling reactions of reactive intermediates**

The third type of reactions catalysed by laccases includes the so-called oxidative coupling, oxidative condensation, phenolic oxidative coupling and coupling reactions. The reactive radicals, produced during the first stage, can undergo spontaneous non-enzymatic reduction and/or oxidation reactions or, due to intermolecular nucleophilic attack, join each other to create complex phenolic structures. Dimeric compounds are formed, which can then be converted into oligomers, and even complex polymers [14,58].

The coupling reactions of free radicals are divided into homomolecular and heteromolecular (Figure 4). Homomolecular coupling applies to at least two molecules of the same phenolic compound, at least one of which must be in the active radical form. Heterocoupling takes place with the participation of at least two different active forms of molecules. As a result, products featuring different spatial structures and physicochemical properties are obtained. The coupling reactions in which typical substrates of laccases (containing an aromatic ring and (a) hydroxyl group(s)) are called oxidative coupling (type I), oxidative condensation (type II) and phenolic oxidative coupling (type III), all of which result in the formation of bonds such as C-O, C-C and C=C, respectively [59]. Heteromolecular coupling reactions can apply to compounds which are not typical substrates of laccases and those having groups of atoms: C-O, C-N and C-S [59]. These reactions lead to the production of phenolic derivatives (type IV), imines as quinone derivatives, wherein one of =O oxygen atoms is replaced by the =NR residue (type V) and quinone derivatives, wherein molecules are joined by the C-N bond (type VI). Table 2 presents the examples of homo- and heteromolecular coupling or condensation reactions catalysed by laccases.

Laccases catalyse coupling reactions in media with a lower water activity coefficient, e.g. coupling of 3-methyl-2-benzothiazolinone hydrazone with o-, m- and p-methoxyphenols using the laccase from *Pyricularia oryzae* was most efficient in the mixture of water and acetone (81:19) [60]. In turn, for dimerization of the semi-quinone ferulic radicals formed by the laccase from *Myceliophthora thermophila*, the water-organic environment of ethyl acetate and

phosphate buffer (volumetric ratio 4:1) was selected as the best one since it limits further polymerization of the product (adverse effect) and facilitates separation of the yellow dimer (additive to cosmetic products and food) [61].



**Figure 4.** Schematic reaction of homomolecular coupling and heteromolecular coupling using reacting substances, which are typical laccase substrates (substrate II), and changeable reacting substances, which are not typical laccase substrates (“reaction partners”): Type I – oxidative coupling; Type II – oxidative condensation; Type III – phenolic oxidative coupling; Type IV – oxidative coupling; Type V and IV – oxidative coupling and amination [59]

**Table 2.** Example homo- and heteromolecular coupling or condensation reactions catalysed by laccases. Based on [59]

Substrates	Bond Type of reaction*	Products / Applications	Source of laccases
<b>HOMOMOLECULAR COUPLING</b>			
2-hydroxydiphenyl ether and 2-hydroksybiphenyl	C-O, Type I C-C, Type II	dimers and oligomers- detoxication	<i>Pycnoporus cinnabarinus</i> [62]
chlorinated hydroxybiphenyls	C-C, Type II	dimers and oligomers- detoxication	<i>Pycnoporus cinnabarinus</i> [63]
Isoeugenol, coniferyl alcohol	C-O, Type I C-C, Type II	dimers, tetramers /synthesis of coumarine derivatives	<i>Rhus vernicifera</i> , <i>Pycnoporus</i> [49]

5,6,7,8-tetrahydro-naphtha-len-2-ol		C-C, Type II	dimers and oligomers/dimers are ingredients of metal-organic catalytic agents	<i>Myceliophthora thermophila</i> <i>Trametes pubescens</i>	[64]
17 $\beta$ -Estradiol		C-O, Type I C-C, Type II			
alkylated derivatives of salicylic acid esters		C-C, Type II	dimers – symmetric biaryls/ versatile applications	<i>Trametes</i> sp. <i>Pycnoporus cinnabarinus</i>	[65]
disubstituted and trisubstituted vanilin derivatives		C-O, Type I C-C, Type II	dimers – dilactones, dihydrobenzofurans, biphenyls/potential application in pharmaceutics	<i>Trametes versicolor</i>	[66]
HETEROMOLECULAR COUPLING					
benzoylacetone nitrile	hydroquinones	C-C Type II	benzylic nitrile products	<i>Trametes villosa</i>	[67]
3-(3, 4-dihydroxyphenyl) propionic acid	derivatives of 4-aminobenzoic acid	C-N, Type IV	dimers / synthesis in mild conditions	<i>Xylaria polymorpha</i>	[68]
mono-, di- and trichloroaniline	protocatech uic acid syringic acid vanillic acid	C-N, Type V	dimers, trimers	<i>Rhizoctonia praticola</i>	[69]
<i>p</i> -hydroquinone	single-strand aromatic amines	C-N, Type VI	dimers/ N-amino-2,5-dihydroxybenzoic acid (antibiotic)	<i>Trametes</i> sp., <i>Myceliophthora thermophila</i>	[70]
<i>p</i> -hydroquinone derivatives	<i>p</i> -aminobenzoic acid	C-N, Type VI	dimers / compounds used in pharmaceutics	<i>Pycnoporus cinnabarinus</i> <i>Myceliophthora thermophila</i>	[71]
Methylcatechol	ampicillin amoxicillin cehadroxil loracarbef	C-N, Type VI	dimers/synthesis of new antibiotics	<i>Trametes</i> sp.	[72]
<i>p</i> -hydroquinone, methylated <i>p</i> -hydroquinone	L-phenylalanine	C-N, Type VI	dimers/obtaining pharmacologically active amino acids	<i>Pycnoporus cinnabarinus</i> , <i>Myceliophthora thermophila</i>	[73]
hydroquinone or catechols	various sodium benzenesulfonates	C-S Type IV	sulfonyl benzenediols	<i>Trametes versicolor</i>	[74]

## Laccases and their applications

### *Laccases in the food industry*

One of the main applications of the laccases in the food industry is the stabilization of wines and fruit juices. Their application is a better alternative to chemical and physical adsorbents (e.g. SO<sub>2</sub>) as they selectively degrade specific phenols and polyphenols, which affect flavour and scent qualities of food products [75]. Oxidised ring-type compounds undergo polymerization and aggregation, and as such they can be easily removed during clarification. Laccases are also used for cork production in the wine industry to limit the quantity of contained undesirable phenol compounds, which may react with wine [76].

Additionally, laccases are used in the bread industry for cross-linking non-amylose polysaccharides – arabinoxylans. The formation of a transverse bond network between chains of such biopolymers has a positive effect on crumb and crispness of baked products [77]. Moreover, laccases are used for cross-linking proteins to produce new food products, e.g. β-lactoglobulin (BLG) crosslinking using the laccase from *T. versicolor* reduces allergenicity, maintaining the positive effect of BLG on health at the same time [58].

During beer storage and ageing, natural complexes of polyanthocyanidins and polyphenols occur and generate protein co-precipitation, and consequently beverage turbidity. While oxidising the mentioned compounds, laccases considerably limit the effect of beer turbidity. In addition, they are used to remove oxygen at the final production stage to extend beer stability and limit the occurrence of an unpleasant odour [78].

The listed examples are just a small sample of laccase applications in the food industry. These enzymes are also used, for instance, during production of fruit juices to improve physicochemical properties of foodstuff, pectin gelling during beet sugar production, etc. [79]. Table 3 shows examples of commercially available enzymatic preparations containing laccases used in the food industry.

### *Laccases used in the wood / paper industry*

In the wood / paper industry, laccases can be used to obtain and bleach wood pulp, de-ink waste paper and treat waste water. Additionally, as recently found, they are also useful for modification of cellulose fibres.

A crucial stage of the paper-making process involves bleaching wood pulp to separate and degrade lignin complexes, which impair wood pulp properties and cause paper yellowing [80]. Conventional methods consist in using chlorine (e.g. Cl<sub>2</sub>) on the raw material or oxygen oxidising agents (ClO<sub>2</sub> and O<sub>2</sub>), which generate vast amount of harmful contaminants [81]. It appears to be a solution to use enzymatic preparations, the so-called biobleaching. As has been mentioned, in 1990, Bourbonnais and Paice performed an efficient demethylation and delignification process with wood pulp using the laccase from *T. versicolor* [53]. Currently, it is known that coupled laccase-mediator systems (LMS) are a perfect alternative to chemical bleaching agents containing chlorine [82-84], they also considerably reduce the quantity of toxic waste water.

Enzymatically catalysed, directed modification of biopolymers by means of the so-called grafting, namely the introduction of low molecular compounds, enables obtaining new materials with very specific properties. For instance, Witayakran and Ragauskas [85] examined the efficiency of amino acids introducing into a lignin-rich wood pulp using the laccase from *T. villosa* (Novo Nordisk Biochem.). Both, wood pulp treatment using the laccase and the attachment of amino acids in the presence of the enzyme, increased the number of carboxyl groups on the surface of fibres spun with the modified wood pulp. Interestingly, L-histidine grafting resulted in an increase in strength of the paper, which was manufactured using the wood pulp modified in this manner. Currently, laccase-based grafting is used to obtain unique functional materials for packaging production. Elegir et al. [86] described an innovative method of grafting non-bleached cellulose fibres with polymerization products of caffeic acid and isoeugenol, using the laccase from *Trametes pubescens*. The protective coating so obtained on the paper surface showed excellent anti-bacterial properties (LASP – laccase antibacterial surface process) [86,87]. The application of raw materials from plants in packaging production is often limited due to the hydrophilic nature and too high susceptibility of cellulose fibres to biodegradation. These properties can be changed by various enzymatic modifications. Kudanga et al. [8, 88] corrected the surface of materials for packaging production by introducing selected fluorophenol or alkylamines, using the laccase from *T. hirsuta*. Covalent bonding of such molecules using functional groups in the raw material increased hydrophobicity of the surface, which prevented too quick biodegradation of packaging. Also, an interesting example is provided by food packaging made of cardboard (or foil) coated with a film of the *T. versicolor* laccase preparation and its substrates, in particular lignosulfonates, alkaline lignin, hydrolytic lignin and lignin [89]. In the presence of lignosulfonates, the enzyme “captured” oxygen in the packaging, reducing its content even to 80% [90] and limiting food spoilage .

**Table 3.** Commercial enzymatic preparations based on laccases [91,92]

Application	Trade name	Supplier
<i>FOOD INDUSTRY</i>		
Brewing industry	Flavourstar	Novozymes A/S
Cork production	Suberase	Novozymes (Denmark)
Enhancing colours in food products	LACCASE Y120	Amano Enzyme USA Co. Ltd.
<i>PAPER INDUSTRY</i>		
Wood pulp bleaching	Lignozym®-process	Lignozym GmbH (Germany)
Wood pulp delignification	Novozym 51003	Novozymes (Denmark)

<i>TEXTILE INDUSTRY</i>		
Fabric bleaching	DeniLiteI and II	Novozymes (Denmark)
	Bleach Cut 3-S	Season Chemicals (China)
	Primagreen Base LT100	Genencor Inc. (USA)
	ZyLite	Zytex Pvt. Ltd. (India)
	Purizyme	Puridet Asia Ltd. (Hong Kong)
	APCOZYME II-S	Apollo Chemical Company, LLC (USA)
	Trilite II	Tri-Tex Co. Inc. (Canada)
	Americos laccase P	Americos Industries Inc. (India)
	Americos laccase LTC	
	Lacasa Ultratex	Proenzimas Ltda. (Colombia)
Hypozyyme	Condor Speciality Products (USA)	
Prozyme® LAC	Sunson Industry Group Co. Ltd. (China)	
PrimaGreen EcoFade LT100	Genencor International Inc. (USA)	
Fabric finishing	Cololacc BB	Colotex Biotechnology Co. Ttd. (China)
	Ecostone LC10	Ab Enzymes GmbH (Germany)
	IndiStar	
	Novoprime Base 268	Genencor Inc. (USA)
	Lava ® Zyme LITE	Novozymes (Denmark)
	IndiStar™ Color Adjust system	DyStar GmbH (Germany) Genencor International Inc. (USA)

The formation of active radicals during lignin decomposition with laccases was used to “glue” wood veneer to obtain a composite necessary to manufacture boards, e.g. for furniture purposes. Beechwood chips (*Fagus sylvatica*) were treated by the laccase from *T. villosa* (Novozymes A/S) and, on the laboratory scale, it was possible to obtain fibreboards which were much stronger, compared with those obtained without the enzyme [93]. In addition, the modified chips changed chemical composition and increased hydrophobicity of the surface. This method is recommended for manufacturing medium density fibreboards and chipboards as commonly used for construction purposes. The patent [94] discloses another method of manufacturing chipboards using laccases. For that purpose, binding agents enhancing the adhesion of wood chips were obtained from kraft lignin or lignosulfonates treated with laccases. This method can successfully replace toxic binding agents (e.g. the melamine-urea-formaldehyde system or phenol-formaldehyde adhesive), still used for chipboard manufacturing [52].

Currently, there are many commercially available laccase preparations, which are successfully used in the paper/wood industry (Table 3).



### **Laccases in biorefining processes**

Due to the applied technologies, the wood, paper and food industries are target sectors for many biorefineries being erected in many countries where production is based on plant biomass. Plant lignocellulosic fractions provide a vast reservoir of new products, including biofuels (ethanol, in particular), chemicals, polymers and energy. Laccases can be used at many biorefining stages. Most importantly, they partially loosen the complex structure of lignins, facilitate the extraction of carbohydrate components – cellulose as well as hemicelluloses, and pectins which can be used to produce the 2nd generation bioethanol and various valuable chemicals. During lignin biodegradation, aldehyde/ketone and carboxyl derivatives of phenols are released which, as natural mediators, aid laccases in the degradation of non-phenolic compounds [95].

Currently, the least expensive and most frequently applied method for processing plant raw materials for ethanol fermentation is the thermochemical method. During the process, as an effect of lignin decomposition, phenol derivatives are released, what inhibits the activity of polysaccharide hydrolases and yeast growth. Laccases can be used to neutralise phenols by their polymerization [96]. Jurado M. et al. [97] proved that detoxication of hydrolysates in wheat straw using laccases induces yeast growth, considerably increases the use of glucose and the quantity of obtained ethanol.

It is anticipated that laccases will be used in the future at biorefineries for biotransformation to process lignin fractions and products of their decomposition into valuable materials, chemicals and pharmaceutical products [98].

### **Laccases in the textile industry**

It is estimated that roughly 1/3 of approximately 800,000 tonnes of the annual colour dye production is used up by the textile industry, with 10% of the quantity being released into the environment. Many of these dyes are resistant to physicochemical and microbiological degradation. Conventional removal methods (adsorption, coagulation, oxidation, filtration and electrochemical methods) are expensive and create different technical problems. It is known that laccase-positive microorganisms considerably aid the degradation of dyed substances [77, 99, 100]. There have been many studied and described examples of effective applications of LMS systems to bleaching commercially available dyes, in particular those used in the textile industry [101]. Forootanfar et al. [102] used laccases, inter alia from *A. oryzae*, *T. versicolor* and *Paraconiothyrium variabile* to degrade six commonly applied synthetic dyes. The laccase obtained from *P. variabile* de-dyed bromophenol blue in just 30 minutes, with the efficiency of 80%. After immobilisation on silica beads, the enzyme de-dyed Acid Blue 25 and Acid Orange 7 without any mediator, whereas the use of HBT additionally improves process efficiency [103]. Moreover, only a 30% decrease in the activity of this biopreparation was reported after 9 cycles of its use. Chen et al. [104] successfully applied magnetic graphene oxide (MGO) nanomaterials as a support for the immobilization of laccase. Compared with the free enzyme, MGO-laccase

exhibited better pH and thermal stabilities. The immobilized laccase was utilized in the decolorization of dye solutions and the decolorization rate of crystal violet, malachite green, and brilliant green reached 94.7%, 95.6%, and 91.4% respectively. A study carried out by Wang and Zhao [105] showed that recombinant *Bacillus subtilis* CotA laccase also can be used for efficient decolourization of dyes. Moreover this enzyme is characterized by excellent properties, which allow to reduce the costs of enzymatic processes.

The application of laccases in the textile industry is not limited to the removal of dyed compounds from waste water. Currently, laccase-based preparations are also used for decolourisation of textiles instead of chemical bleach. This method, apart from its environmental benefits, significantly improves brightening effects since no fabric yellowing and structural destruction occurs. In addition, it can be used for fabrics which are very sensitive to chemicals (e.g. stretch jeans) [91]. The first laccase-based preparation, applied in 1996 for partial decomposition of indigo used to dye jeans fabrics, was DeniLite® supplied by Novozyme. The enzyme was produced by genetically modified fungus *Aspergillus niger* [99, 102]. Currently, the offer of the biotechnological industry provides many laccase-based preparations recommended for the textile industry to bleaching fabrics (Table 3).

### **Bioremediation and biodegradation**

Microorganisms producing laccases are used (most often living cells) in bioremediation of contaminated soil and biodegradation of toxic waste [106, 107]. Saprophytic fungi and plants participate in such processes in the natural environment. They transform various phenolic compounds, other aromatic and cyclic compounds into non-toxic derivatives by oxidative transformation (e.g. chlorophenols undergo dehalogenation or are transformed into benzopyrene) and/or bind such compounds to form chemically neutral polymeric compounds (immobilisation of toxins, according to the mechanism of humus formation) [95]. It has been proven that these processes take place in the presence of enzymatic laccase preparations. Moreover, laccase-mediator systems are most often necessary to carry out these processes. Niku-Paavola and Viikari [108] demonstrated that the laccase isolated from *T. hirsuta* can be used to oxidize aliphatic and cyclic alkenes, which are a vast group of compounds polluting the environment. Oxidation reactions were catalysed only in the presence of mediators coupled with the enzyme (in particular, HBT and violuric acid), and the corresponding aldehydes and ketones were produced. Some of the studied compounds were completely oxidised in just 2 hours at 20 °C. Pesticides and herbicides are commonly used in modern agriculture and support the effective production of a variety of agricultural crops. Unfortunately, most of them produces a carcinogenic metabolite, negatively affects the environment. Zeng et al. [109] investigated that laccase from *Trametes versicolor* in the presence of HBT can be used for efficient degradation of the herbicide isoproturon. Bressler et al. [110] proved that the purified laccase from *Cariollopsis gallica*, in the

presence of ABTS, catalyses decomposition of carbazole, N-ethylcarbazole, fluorene and dibenzothiophene with an efficiency from 60% to 100%, (depending on the compound being degraded). These polycyclic hydrocarbons, produced particularly through petroleum and coal combustion, are among the main pollutants of the biosphere with an adverse effect on human health. Recent research shows that laccases can be used for efficient detoxification accompanying triclosan TCS transformation, the accumulation of which can be dangerous for the environment and human health. It has been demonstrated that laccases catalyses formation of oligomeric TCS products of less environmental concern [111].

### **Laccases in the pharmaceutical / cosmetic industry**

Laccases provide a perfect alternative to conventional chemical synthesis as they make it possible to produce various compounds under mild process conditions, with high efficiency and slight interference in the natural environment [112]. With this unique property of the enzymes, they are more and more extensively used in the pharmaceutical industry to produce anaesthetics, anti-inflammatory drugs, tranquilisers and antibiotics [113]. While using fungal laccases, Mikolasch et al. [72] synthesised a whole group of  $\beta$ -lactam antibiotics. The laccases used for that purpose catalysed the amination of various organic compounds, in particular amino  $\beta$ -lactams, 2,5-dihydroxyphenylacetic acid, 2,5-dihydroxybenzoic acid, and catechol [114]. Another example refers to the heteromolecular coupling of imidazole derivatives and their further modifications with the fungus *T. versicolor* used to produce drugs or intermediates necessary for further synthesis [115]. More recent research showed that laccase can be used in the catalytic synthesis of coumestans (with estrogenic activity). Results obtained by Qwebani-Ogunleye et. [116] indicated that the yields of these drugs are similar to or better than that obtained by other enzymatic, chemical or electrochemical methods. Also, laccases themselves can be used as drugs reducing negative effects of aceruloplasminemia – a disease causing a disorder of iron homeostasis in the body [117].

The possibility of using laccases for inhibition of the activity of HIV-1 reverse transcriptase, without which the virus cannot transcribe the genetic material from RNA to DNA, is very promising. This activity has been discovered for laccases from many fungi, in particular *Tricholoma giganteum* [118], *Lepiota ventrisospora* [119], *Lentinus edodes* [120], *Pleurotus cornucopiae* [121], *Coprinus comatus* [122], *Lentinus tigrinus* [123] and *Agaricus placomyces* [124].

A new field of application for laccases is emerging in the cosmetic industry, too. These enzymes are used especially for the synthesis of flavonoids, pigments, cosmetic dyes as well as aromatic aldehydes and heterocyclic compounds, which are active ingredients in cosmetic products. Hair dyes based on oxidising properties of laccases are much less harmful than products with hydrogen peroxide used as the oxidising agent, which weakens hair and damages its structure [125]. Capability of laccases to polymerise phenolic substances has also found many applications in cosmetology. Joan et al. [126] used a laccase for

hetero- and homopolymerization of natural phenolic monomers and obtained stable dyes, i.e. ingredients of products used for hair colouring. For the production of hair dyes a thermostable laccase of *Thermobifida fusca*, acting in a strongly alkaline environment, was used [127]. Additionally, laccases can be used for skin lightening by reduction of the melanin content, e.g. in spots of diathesis or scars [128]. There are many patents describing dermatological skin lightening products in which laccases are among the biologically active agents [129]. Laccases can also be used for production of deodorants and personal care products [130, 131]. The laccases can be an ingredients of many cosmetic products such as toothpaste, mouthwash, detergents, face and hand soaps and shampoos, face and hand creams, and hair colourants [132].

### **Biosensors and enzymatic fuel cells**

Laccases are also used as biologically active parts of biosensors serving for, in particular, measuring the concentration of O<sub>2</sub> [5, 80], glucose, aromatic amines, phenolic compounds, even the entire lignin complexes [133, 134]. Biosensors based on laccases have been applied so far for determining the concentration of phenols, e.g. catechol, in tea and wine, as well as derivatives of phenols and lignins in waste water [92, 135]. A new interesting example is the structure of the so-called bioelectric “tongues” which apply nanostructural voltammetric biosensors containing a biologically active element consisting of a system of phenolic oxidases: laccase and tyrosinase. The enzymes were introduced into a biomimetic environment of a monolayer of arachidonic acid containing lutetium bis-phthalocyanine as an electron mediator. This multi-biosensor can be used for the detection of valuable polyphenolic antioxidants in fruit [136].

The application of laccases in nanobioelectric devices used to detect metabolites in the human body is limited due to their inhibition caused by a very high concentration of chloride ions (140-150 mM) and blood pH ( $\approx 7.4$ ). Therefore, Mate et al. [137] by means of directed evolution, obtained an enzyme of high catalytic activity under these conditions. This offers real opportunities to create non-invasive devices for precise determination of metabolites in real time.

Laccases are also immobilised at the biofuel cell cathode which directly generates electricity. A typical system of such a biofuel cell consists of two nanostructural electrodes of highly expanded surface. These are usually nanowires or carbon nanotubes on which enzymes catalysing redox reactions are immobilised. For instance, Barrière et al. [138] described a system in which glucose oxidase, immobilised at the anode, oxidises glucose, with simultaneous oxygen reduction catalysed by the laccase on the cathode causing a current flow through the external system (flow of electrons). Currently, there are more and more reports relating to the development of new, selective and efficient methods for the immobilization of laccases on the electrode surface [139].

## Summary

In the age of intensive development of the biotechnological industry and the society's increasing environmental awareness, many research units focus on laccases, i.e. enzymes included in the superfamily of multicopper oxidases, which are widespread in the world of living organisms.

This paper summarises reports available in the literature on basic molecular, structural and biochemical properties of laccases as well as their applications in various industrial sectors and biotechnology. The range of substrates acceptable by laccases can be successfully increased by specific compounds called mediators, which, together with enzymes, create coupled systems of high oxidation capabilities. For this reason, laccases are becoming a better alternative for conventional chemical processes in the paper, textile and food industry as well as biodegradation of contaminations. Also, their significant role in biorefineries, emerging in various regions of the world, is noticeable. In addition, more and more interesting innovative applications are suggested for these enzymes in cosmetology, the pharmaceutical industry, various chemical syntheses, and even as biologically active elements of biosensors and biofuel cells. The factor which limits the attempts to implement such biocatalysts on a larger scale is the high cost incurred for obtaining laccases and the problems associated with scale extension, which results in high prices of commercially available laccase preparations. Of course the prices are strictly dependent upon the purity of enzyme preparations, and thus their applications. For example the price of laccase from *Agaricus bisporus* ( $\geq 400$  U/g; Sigma Aldrich – 40452) is 666 EUR/g. On the other hand laccase preparations offered by Shandong DianMei International trade Co., Ltd cost 5-10 USD/kg. In order to ensure competitive processes using laccases relative to chemical processes, it is necessary to obtain large quantities of the enzymes of relatively high activity, but with low financial outlays. This is one of the reasons for which the interest in bacterial laccases has increased. It has been proven that they show only slightly lower activity compared to fungal laccases, being also characterised by high stability within a wide range of functional conditions [27]. Their production due to a high rate of bacterial growth can be less expensive. Currently, new alternatives emerged to produce such proteins in heterologous hosts since it offers an opportunity for their expression and further modification as well as quick and more economic laccase purification [140].

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