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MINI-REVIEW

Application of eukaryotic and prokaryotic laccases in biosensor and biofuel cells: recent advances and electrochemical aspects

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Abstract
Laccases exhibit a wide range of applications, especially in the electrochemical field, where they are regarded as a potential biotic component. Laccase-based biosensors have immense practical applications in the food, environmental, and medical fields. The application of laccases as biocathodes in enzymatic biofuel cells has promising potential in the preparation of implantable equipment. Extensive studies have been directed towards the potential role of fungal laccases as biotic components of electrochemical equipment. In contrast, the potential of prokaryotic laccases in electrochemistry has been not fully understood. However, there has been recent and rapid progress in the discovery and characterization of new types of prokaryotic laccases. In this review, we have comprehensively discussed the application of different sources of laccases as a biocatalytic component in various fields of application. Further, we described the potential of different types of laccases in bioelectrochemical applications.

Keywords Fungal laccase · Bacterial laccase · Biosensor · Enzymatic biofuel cells · Immobilization · Direct electron transfer

Introduction

Biosensors have been widely used in the detection of pollutants and other chemicals and also as disease markers owing to their sensitivity and efficiency. The traditional monitoring techniques are often time-consuming and require specific technical knowledge (Long et al. 2013; Luong et al. 2008; Mehrotra 2016; Perumal and Hashim 2014; Su et al. 2011). It is expected that the global market for biosensors will reach 20 billion dollars by the end of 2020 (Dzyadevych et al. 2008). In particular, laccase biosensors have been used to monitor some toxic and/or harmful chemicals like phenolic compounds, pesticide residues, and hormones due to their clear catalytic mechanism, high redox potential, and special affinity for phenolic substrates (Leonowicz et al. 2001; Rodríguez-Delgado et al. 2015). Another widely used bioelectrochemical equipment are the enzymatic biofuel cells (EBFCs), which have been used in implantable devices in the healthcare industry for powering devices like pacemakers. However, the development of laccase-based EBFCs is still limited because of the low stability of the enzymes (Beneyton et al. 2011a, b; Kim et al, 2006).

Generally, laccases (EC 1.10.3.2, benzenediol: oxygen oxidoreductases) are divided into eukaryotic and prokaryotic types with different biochemical properties according to the source (Baldrian 2006; Santhanam et al. 2011). Initially, fungal laccase-based bioelectrochemical equipment were extensively developed due to their versatile, environment friendly properties, and substrate specificity (Mehrotra 2016; Perumal and Hashim 2014; Portaccio et al. 2013; Świetlikowska et al. 2013; Wang et al. 2012). Since then, more and more bacterial laccases have been discovered and characterized; the characteristics of bacterial laccases used in the electrochemical field were revealed. The results indicated that bacterial laccases exhibit better stability than fungal laccases in response to changes in environmental factors, such as temperature and pH (Singh et al. 2011). Therefore, the study of bacterial laccases for building bioelectrodes for EBFCs and biosensors

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may have great potential for use in normal and adverse condition (Beneyton et al. 2011a, b).

However, there are very few reviews about the recent applications and the features of these two different types of laccases have not been compared in the context of the electrochemical field. In this review, we have specifically focused on the recent advances and on the comparison of bioelectrodes based on eukaryotic and prokaryotic laccases, which are used as cathodes for EBFCs and as biosensors in the beverage industry, healthcare, and for monitoring environment pollution.

Laccases obtained from different sources

Laccases belong to the blue mulother phenolic compounds ti-copper-oxidase family, which have been studied for bioelectrochemical applications for decades. A majority of laccase biosensor employed the commonly used fungal enzymes from *Trametes versicolor*, *Rhusvernicifera* and *Pleurotus ostreatus* (Myasoedova et al. 2017; Liu et al. 2015). In general, most fungal laccases are extracellular enzymes, which can be inducibly expressed by adding some commonly used inducers like ferulic acid, vanilic acid, guaiacol, and other phenolic compounds (Cambria et al, 2000; Giatti Marques De Souza et al. 2004; Leonowicz et al. 2001). After the purification procedures, which include commonly used methods such as ion-exchange chromatography (Ko et al. 2001) and ammonium sulfate precipitation (Forootanfar et al. 2011), the obtained enzyme was immobilized on the electrode using several methods, such as entrapment, cross-linking, physical adsorption, and covalent bonding (Brady and Jordaan 2009; Fernandez-Fernandez et al. 2013). However, the production of fungal laccases is usually limited due to the long fermentation period, low yield, and preference for acidic conditions.

Bacterial laccases exist intracellularly as functional proteins, which makes the purification complicated (Dwivedi et al. 2011). However, the bacterial laccases have better adaptability for application in industrial processing along with additional advantages, including short production cycles and excellent storage stability (Chauhan et al. 2017). Currently, the best characterized bacterial laccase is the endospore coat protein CotA from *Bacillus subtilis*, which has been produced with excellent stability and activity towards substrates in *E. coli* (Mohammadian et al. 2010) and *Pichia pastoris* (Lu et al. 2013;Hullo et al. 2001). In addition, the bacterial laccase CueO from *E. coli* has been successfully expressed in *P. pastoris* with high yield, and the recombinant CueO was thermostable with a half-life of 60 min at 70 °C (Ma et al. 2017). Recently, laccase from *Geobacter metallireducens* was obtained through a heterologous expression system in *E. coli* BL21 with optimum temperature and pH of 75 °C and 6.0 for the oxidation of ABTS and 2,6-DMP (Berini et al. 2018).

Properties of laccases

Laccases perform several functions, including lignin degradation, cuticle tanning, and sclerotization among others (Arakane et al. 2005; Enguita et al. 2003; Gavnholt and Larsen 2002; Sanchez 2009). These peculiar properties have been exploited thoroughly and extended to several applications in many different areas, like textile or dye decolorization, food pretreatment, environment biodegradation, and detection of substrates (Baldrian 2006; Pandey et al. 2007; Sayut and Sun 2010). Particularly, laccases are considered potential candidates for use as biological electrochemical components due to some specific advantages, such as a broad spectrum of substrates (Giardina et al. 2010) and the ability to catalyze electron transfer reactions without the addition of cofactors (Munteanu et al. 1998), and without the generation of byproducts like hydrogen peroxide (Munteanu et al. 1998). This eco-friendly enzyme seems particularly suitable for online monitoring or biofuel cell application in a non-toxic environment.

In general, laccases from fungi and bacteria have different biochemical and electrochemical characteristics. Fungal laccases typically exhibit pH optima in the acidic pH range and most bacterial laccases can act and are more stable at a wider pH (6.0–8.5) range (Baldrian 2006). In addition, the thermostability of bacterial laccase is generally better than that of fungal laccases (Singh et al. 2011). On the other hand, most fungal laccases are glycosylated, with ranges between 10 and 25% (Baldrian 2006), but glycosylation of bacterial laccases has not yet been investigated. The practical utilization of prokaryotic laccases in the biosensor field is reduced due to the low-redox potential (usually below 0.55 V) with the limited range of substrates. Furthermore, the low-redox potential may lead to lower current density and power density in EBFCs (Mot and Silaghi-Dumitrescu 2012). However, bacterial laccases are of special interest since their activity and stability at wider pH range and thermal environment can make them preferable than fungal laccases in bioelectrode development for use under harsh conditions (Vaz-Dominguez et al, 2008).

The electrochemical catalytic mechanism of laccases

The catalytic mechanism of fungal or bacterial laccase towards substrates depend on the various copper centers of laccases, which contain four types of copper atoms (type I–III copper atoms) and drive electrons from a reducing substrate to molecular oxygen (Kumar et al. 2003). The first step is the reduction of the T1 copper site by accepting an electron from the oxidized substrate. Then, the internal electron from the reduced T1 is transferred to the trinuclear T2/T3 cluster with water generated by reduction of molecular oxygen (Fig. 1) (Mate and Alcalde 2015). The phenolic compounds

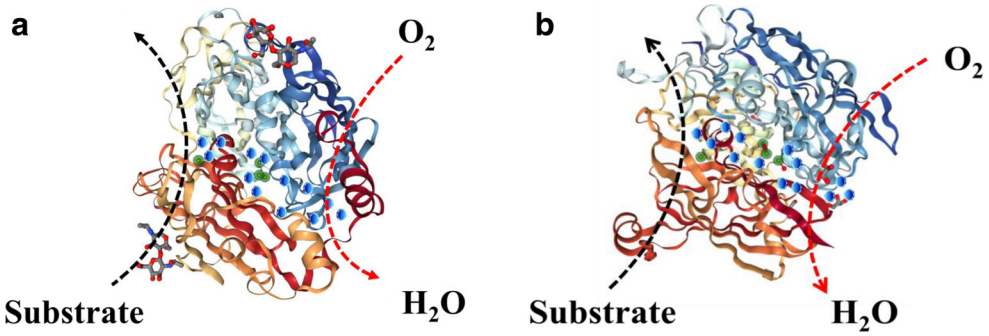

Fig. 1 The schematic of the catalytic mechanism and electron transfer of fungal laccase from *Trametes trogii* (**a**) and bacterial laccase CotA from *B. subtilis* (**b**). The substrate oxidation is held close to the mononuclear T1 Cu atoms (represented as sphere colored green), followed by shuttling electrons (blue spheres) to the trinuclear Cu center where dioxygen reduction occurs with the concomitant release of water molecules

that are important substrates can be oxidized directly by laccase and can be detected by a laccase-based biosensor. The oxidation reaction of polyphenols catalyzed by laccase is as follows:

$$PH_2 + {}^{1}/_{2}O_2 \xrightarrow{\text{lacasse}} P + H_2O$$

where, P and PH_2 are oxidized and reduced, respectively. Thus, the use of laccases is not limited to treatment processes with phenol compound desorption, but also helps in the detection systems. In addition, only water is generated, which is a great advantage compared to other catalytic process, especially for implementing the use of biosensors to keep a check on quality and safety in the beverage industry (Fig. 2).

The coordination geometry of the T1 copper site determines the redox potential of fungal and bacterial laccases, which may lead to the catalysis of different substrates (Martins et al. 2015). However, Moya R. et al. found that the redox potential is not the only factor that affects the oxidation rate; the selectivity and affinity towards the substrates of laccase also play important roles (Moya et al. 2011). For example, the CotA with low-redox potential (~ 525 mv) has faster rates of oxidation of azo dye reactive black five or aromatic amines than the fungal laccases (Martins et al. 2015).

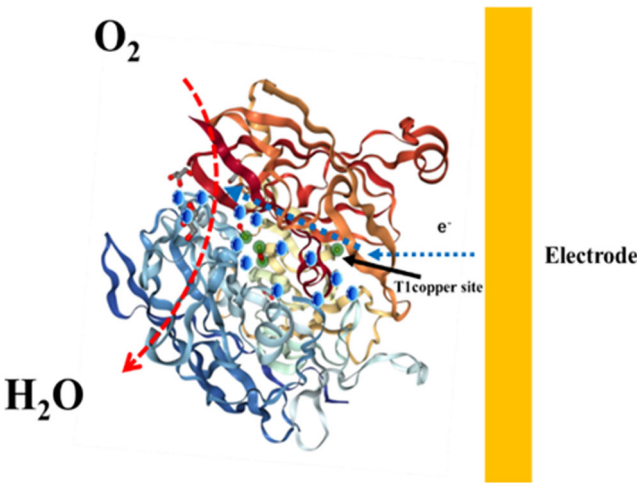

Fig. 2 A possible mechanism of the direct electron transfer (DET) for bacterial laccase CotA adsorbed on the electrode

Laccase biosensors used in human-related applications

In recent years, many laccase biosensors have been developed for the quantitative analysis of beverages, for immunoassays, hormone monitoring in clinical diagnosis, and as a phenolic compound determinant for environmental protection. These areas of application are receiving substantial attention because they are all closely related to human life. Inspired by this, different types of laccase biosensors have been developed to determine the target compounds.

Beverages quality testing

Measurement of tannins in wine

Nowadays, quantitative analysis in the beverage industry is becoming very important in China to ensure security and adequate quality. Almost all kinds of wine contain tannins, and a variety of phenolic compounds like cinnamic acid derivatives, catechins, and anthocyanidins, which are usually measured by the total polyphenol index. According to earlier reports, the content of polyphenolic compounds is 2 to 5 g/L in red wines on average and 100 mg/L in white wines (Montereali et al. 2010). Polyphenols are also known for their positive effect on human health due to their antioxidant properties, which are associated with reduced risk of cancer, stroke, heart disease, and diabetes (Di Fusco et al. 2010). Hence, the polyphenolic content can be used to characterize the quality of wine.

Therefore, different types of laccase biosensors have been developed to determine the tannins. Several red wine polyphenols were tested by biosensors using laccase: Massimo Di Fusco et al. compared the difference between biosensors using the laccase from *T. versicolor* and *T. hirsuta* as biocomponents

for polyphenol determination in wine (Di Fusco et al. 2010). The results obtained by using *T. hirsute* laccase-based biosensor are close to the values obtained by the Folin–Ciocalteau method, i.e., lower than 4–11% for the white wines and of the 4–8% for the red wines, which is better than the values obtained using *T. versicolor* laccase-based biosensor. The purified laccase from *Ganoderma* sp. Rckk02 was also employed to make the guaiacol detecting biosensor with a range of 0.1 to 500 μM and low detection limit of 0.05 μM. Moreover, this electrode lost just 25% of its initial activity after 5 months of storage, which is better than many earlier laccase biosensors (Chawla et al. 2012a). A novel fungal laccase-based screen-printed electrode using molybdenum disulfide and graphene quantum dots was developed and successfully applied for the detection of polyphenols like caffeic acid, chlorogenic acid and (−) epicatechin, this kind of small portable biosensor is efficient for testing red wine samples (Vasilescu et al. 2016). Zhang et al. developed a biosensor by direct adsorption of engineered *E. coli* cells, while immobilizing a bacterial laccase on the surface of electrode, this biosensor shows a linear range of 0.5–300.0 μM for catechol. It proved that the bacterial laccase-based biosensors can be employed for phenol detection in red wine, with high accuracy and recovery (Zhang et al. 2018a, b).

Polyphenol detection in tea infusions and juice

It is well known that unstable molecules, such as free radical in tea drinks, are beneficial for human health. The nutritional (Benzie and Szeto 1999), and antioxidant capacity of tea infusions has been studied using different methodologies (Sharpe et al. 2016). Among them, the detection and quantification of polyphenols can be used to evaluate the health-improving properties of teas. Early in 1992, a laccase-based sensor was developed for detecting catechols (Ghindilis et al. 1992). More recently, the screen-printed electrode using deposited polyvinyl alcohol photopolymer and *T. versicolor* laccase for the detection of phenols was developed, and the concentration range of hydroquinone detected was from 25 to 125 μM. At pH 4.7, the highest sensitivity of 9.44 nA μM^{-1} was obtained (Ibarra-Escutia et al. 2010). Another *T. versicolor* laccase-based electrode was reported to exhibit the electrochemical response in response to rutin and ascorbic acid, which were used to assess the antioxidant capacities of two herbal tea samples common in South Africa (Sabela et al. 2012). A disposable amperometric biosensor consisting of a carbon screen-printed electrode was introduced, and it could be considered a valuable tool for the monitoring of total polyphenolic content (Eremia et al. 2013).

On the contrary, phenols in fruit juice may become a serious human health threat, which can cause diseases in humans. For example, the phenol bisphenol A (BPA) widely exists in plastic bottles and can be ingested with the juice and become a toxic substance for babies, leading to teratogenicity. To address this, a laccase-thionine-carbon black biosensor was designed and tested for BPA detection. Meanwhile, the effect of enzyme loading, applied potential, and pH were investigated. The linear range of BPA concentration detected was 0.5 to 50 μM, the sensitivity was 5.0 ± 0.1 nA/μM, and the recovery ratio of obtained using tomato juice as real samples was between 92 and 120% (Portaccio et al. 2013). To monitor the quality of juice, another *Ganoderma* sp. laccase biosensor was constructed, and five commercial brands of fruit juices samples were used for evaluating the performance of the biosensor (Chawla et al. 2012b).

In Table 1, the main characteristics of several laccase-based biosensors for phenol detection in wine or other kinds of beverages are compared.

Monitoring pesticide residues in agricultural products

In the last decade, the application of agrochemicals has become one of the world's major industries, as they are very useful in removing pests or weeds. However, they are often toxic for other organisms and create serious environmental and public health problems due to the contamination of the water in the lakes and rivers with agricultural waste (Geetanjali et al. 2009). In fact, many enzymes, such as acetylcholine esterase (Liu and Lin 2006), tyrosinase (de Albuquerque and Ferreira 2007), and laccase, have been employed as the biotic components of biosensors for the monitoring of pesticide residues.

Pirimicarb, a kind of carbamate belonging to the endocrine disruptor compound, has been detected in very low concentrations in food. It is harmful for health, since it can affect some of the biological functions of the human body. A *T. versicolor* laccase/MWCNT-based biosensor with a linear range of 9.90×10^{-7} to 1.15×10^{-5} mol L^{-1} for the detection pirimicarb has been constructed and optimized by enzyme percentage and incubation time (Oliveira et al. 2013b). Under optimum conditions, the detection and quantification limits can increase up to 1.8×10^{-7} and 6.0×10^{-7} mol L^{-1}, respectively. The recovery of pirimicarb from spiked tomato and lettuce samples was greater than 90%. This group also introduced another laccase biosensor constructed using Prussian blue and graphene, the attained detection limits ranged between 5.2×10^{-9} mol L^{-1} for ziram and 1.0×10^{-7} mol L^{-1} for carbofuran using tomato and potato samples (Oliveira et al. 2013a).

Another carbamate pesticide, formetanate hydrochloride, a kind of insecticide and acaricide widely used in fruits like grapes and mangoes, and in vegetables, such as potatoes, beans, watermelons, and tomatoes. Ribeiro et al. demonstrated a laccase biosensor which was successfully applied to detect the formetanate hydrochloride in fruit samples (sensitivity was

Table 1 Application of a laccase-based biosensor in monitoring of drinks quality

Laccase sources	Immobilization type/support	Target analyte	Analytical characteristics	Type of samples	Ref.
Paraconiothyrium variabile	Fe_3O_4/polyaniline/chitosan biocomposite matrix film	Catechol	Linear range from 0.5 to 80 μM, detection limit of 0.4 μM	Tea samples	(Sadeghi et al. 2015)
Trametes versicolor	Modified by molybdenum disulfide and graphene quantum dots	Caffeic acid	Range of 0.38–100 μM; detection limit of 0.32 μM and sensitivity of 17.92 nA μM^{-1}	Red wine	(Vasilescu et al. 2016)
Trametes versicolor	Terbium oxide nanoparticles and 8-hydroxypyrene-3-sulphonate trisodium	Gallic acid	Linear range 0.5–12 μM; detection limit is 0.14 μM	Wine	(Godoy-Navajas et al. 2015)
Trametes versicolor	Matrix-assisted pulsed laser evaporation technique	Total polyphenol content	Linear range from 1 to 60 μM limits of detection 1 μM	Tea infusion, black radish root, and artichoke leaves	(Verrastro et al. 2016)
Trametes versicolor	Sonogel-carbon electrodes with glutaraldehyde	Polyphenols	Sensitivity (A L mg^{-1}): gallic acid $4.7{\cdot}10^{-11}$, caffeic acid $1.0{\cdot}10^{-9}$ (+), catechin $1.6{\cdot}10^{-10}$ (−), epicatechin $8.2{\cdot}10^{-11}$, 4-methyl catechol $1.2{\cdot}10^{-9}$, ferulic acid $1.9{\cdot}10^{-10}$, vanillic acid $1.5{\cdot}10^{-11}$	Wines	(García-Guzmán et al. 2015)
Trametes versicolor	Platinum nanoparticles, reduced graphene oxide	Chlorogenic acid and caffeine	Chlorogenic acid: sensitivity 0.02 μA μM^{-1}, detection limit 2.67 μM; Caffeine: Sensitivity 1.38 μA μM^{-1} detection limit of 0.22 μM	Coffee samples	(Vasilescu et al. 2014)
Trametes versicolor	Water-soluble graphene derivative	Catechol	Linear range 200 nM to 15 μM, sensitivity of 93 mA M^{-1} cm^{-2}, low detection limit of 76 nM	Commercial herbal tea samples	(Boujakhrout et al. 2016)
Coriolopsis gallica	Nitrogen-doped multiwalled carbon nanotubes	Catechol, catechin	Detection limit 0.01 μmol L^{-1}	White wine	(Aguila et al. 2015)
Pycnoporus sanguineus	Organo-functionalized silica	Total phenol	Ranged from 12.97 to 99.14 μA mV^{-1} g^{-1}	Honey samples	(de Oliveira Neto et al, 2017)
Trametes versicolor	Graphene oxide and MWCNTs	Catechol	Sensitivity 0.31 A M^{-1} cm^{-2} Linear range 0.3–300 μM	Fruit juices	(Vlamidis et al. 2017)
Commercialized	AuNPs on carbon-paste screen-printed electrode	Azo-dye tartrazine	Linear range 0.2 to 14 μM, detection limit 0.04 μM	Commercial mango juice	(Mazlan et al. 2017)
Bacterial	Surface-display bacterial laccase with ice nucleation protein	Catechol	Linear range 0.5 to 300.0 μM	Red wine and tea samples	(Zhang et al. 2018a, b)
Bacterial	Immobilizing on *E. coli* surface and direct adsorption with cells	Several phenolics	Linear ranges of 5.0 to 500.0 μM, 1.0 to 5.0 μM	Red wine, pharmaceutical samples	(Zhang et al. 2017)

20.58 ± 0.49 A/μM and linearity ranged from 9.43×10^{-7} to 1.13×10^{-5} M) (Ribeiro et al. 2014).

Detection of harmful phenols for environment protection

With the growing demand for synthetic product due to long-term industrialization, the phenolic compounds have become ubiquitous pollutants, especially in wastewater from almost all chemical industries. Laccases, obtained from both eukaryotic and prokaryotic sources (Chandra and Chowdhary 2015; Ulcnik et al. 2013), have been widely used by researchers to biodegrade these toxic compounds (Morozova et al. 2007; Navarra et al. 2010; Singh et al. 2011; Wang et al. 2014b; Zhang et al. 2013). On the other hand, in the past decades, effective laccase biosensors have been developed and applied for many kinds of detection purposes, especially in online and in situ monitoring of phenolic compounds in wastewater (Table 2).

Recently, Sekretaryova et al. developed a novel screen-printed biosensor using *T. versicolor* laccase for in situ, total phenol estimation in tap water samples without additional pretreatment for samples. This kind of microscale biosensor showed great potential for directly monitor drinking water, which may be contaminated by wastewater intrusion (Sekretaryova et al. 2016); Another laccase biosensor was generated for catechol determination, especially the direct electron transfer (DET) behavior of laccase was enhanced by this modified strategy (Palanisamy et al. 2017). Upan J. and coworkers constructed an easily prepared carbon nanotube and *T. versicolor* laccase-modified screen-printed carbon electrode, a wide linear range between 1 and 50 mM and a detection limit of 0.1 μM for hydroquinone were obtained, and the recoveries were in the range of 91.2–103.8% during analysis of real samples. (Upan et al. 2016).

Application in healthcare monitoring

The concentration of disease biomarkers is one of the important indicators of the health status. Among them, the catecholamine neurotransmitters influence almost all human tissues and physiological processes. An amperometric laccase biosensor for the detection of catecholamine neurotransmitters using electrocatalytic substrate recycling was developed (Ferry and Leech 2005). An osmium complex with the poly(N-vinylimidazole) was introduced and the constructed sensor had the detection potential of − 0.2 V towards dopamine (DA), and detection limits of 11 nM for epinephrine, 8 nM for norepinephrine, and 4 nM for DA.

DA is related to the majority of processes in mammalian central nervous systems. It is a prognostic biomarker for clinical diagnosis of diseases, such as schizophrenia and Parkinson's disease (Ferapontova 2017). A novel biosensor was developed (Cesarino et al. 2013) using electro-co-deposition method, which is an easy and fast procedure for preparing biosensors, and the results obtained using this electrode for detecting dopamine were very similar to the data obtained by the HPLC procedure. Another laccase-based electrode was designed and synthesized using phytic acid-functionalized silica nanoparticles for dopamine detection. This biosensor had a good detection performance for DA with a wide linear range (0.99–103.10 μM) and a low detection limit (0.26 ± 0.003 μM) (Wang et al. 2014a).

The most recent advance is the identification of the bacterial laccase CotA from *Bacillus subtilis* WD23 and its expression in *P. pastoris* at a high level (891.2 U/L) (Fan et al. 2015). In addition, a mediator-free amperometric biosensor for H_2O_2 detection using this purified enzyme was designed with a linear range of 0.05 to 4.75 mM. This study investigated the ability of CotA to reduce hydrogen peroxide on the electrode, it also predicted that the bacterial laccase has great potential as a biosensor.

During the last decade, significant progress was made in the use of enzyme as aptamer electrodes, demonstrating the most promising in vivo applications for detection of disease markers like catecholamine neurotransmitters (Table 3). However, several important issues, like the electrode minimization, reusability of the physiological media, and development of the strategies for antifouling during the detection process, need to be addressed before practical in vivo applications. As the biotic components, the fungal laccases lack the ability to work under neutral conditions and at high concentration of Cl^-. Thus, the more stable bacterial laccases exhibit greater potential for use in the test chip biosensors for in vivo detection application.

Laccase-based biocathodes in EBFC

EBFCs convert biofuel into electrical energy, by utilizing the chemical energy of the biochemical pathways catalyzed by enzymes such as electro-catalysts, instead of metal catalysts, and work under mild conditions (Atanassov et al. 2007). EBFCs are considered micropower sources for implantable biomedical devices. In addition, the effectiveness of the enzymes lies in their specificity towards the substrates, including glucose and other fuels, in complex media such as physiological fluids, blood (20–40 °C within neutral pH); expensive and rare metals can be replaced with this renewable alternative biomaterial (Le Goff et al. 2015). In general, some multi-copper oxidases like laccase, ascorbate oxidase (Uzunoglu et al. 2016), and bilirubin oxidase (Santoro et al. 2016) have been developed as candidates for cathodic biocatalysis (Fig. 3). Among them, the performance of biofuel cells has been shown to be affected by using different types of laccase.

Table 2 Phenolic compound detection by laccase biosensor in environmental protection in recent years

Laccase sources	Immobilization type/support	Target analyte	Analytical characteristics	Type of samples	Ref.
Trametes versicolor	ZnO sol-gel with chitosan	Catechol	Linear range of 1.0×10^{-6} and 1.0×10^{-4} M/L; limit of 2.9×10^{-7} M/L	N.r.	(Qu et al. 2015)
Trametes versicolor	Polyaniline electrodeposited onto electrode	Catechol	Linear range 3.2×10^{-6} to 19.6×10^{-6} M, sensitivity with 706.7 mA L mol^{-1} , detection limit 2.07×10^{-6} M	N.r.	(Nazari et al. 2015)
Trametes versicolor	Palladium-copper alloyed nanocages supported	Catechol	Linear ranges from 0.005 to 1.155 mM and 1.655 to 5.155 mM for catachol detection	N.r.	(Mei et al. 2015)
Commercialized	AuNPs-crosslinked zein ultrafine fibers	Catechol	Linear range from 0.166 to 7 μM, limit of detection 0.166 μM	N.r.	(Chen et al. 2015)
Trametes versicolor	Flow injection amperometric screen-printed carbon electrode	Hydroquinone	Linear range between 1 and 50 μM; detection limit of 0.1 μM	Surface water sample	(Upan et al. 2016)
Trametes versicolor	Screen-printed carbon-fiber microelectrodes	Total phenolic compounds	Linear range from 0.2 to 10 μM, a sensitivity of 1.35 ± 0.4 A M^{-1} cm^{-2} and a dynamic range up to 43 μM	Unsupported tap water	(Sekretaryova et al. 2016)
Trametes pubescens	Situ microfluidic biosensor	ABTS	Low limit of detection 0.149 μM, sensitivity with 0.2341 nA μM^{-1}	N.r.	(Gonzalez-Rivera and Osma 2015)
Agaricus bisporus	Polythiophene-g-poly (ethylene glycol) with lateral amino groups	Catechol	Linear range 0.0025–0.05 mM and 132.45 μA mM^{-1} sensitivity retained 82% of its activity for 12 days	Artificial wastewater	(Akbulut et al. 2015)
Commercialized from Sigma-Aldrich	Anthracene-functionalized MWCNTs	Arsenite/arsenate	Limits of detection 13 mM (arsenite) and 132 μM (arsenate). Sensitivities 0.91 ± 0.07 mV/mM (arsenite) and 0.98 ± 0.02 mV/mM (arsenate)	N.r.	(Wang et al. 2016)
Trametes versicolor	Bacterial cellulose-gold nanoparticles hybrid nanofibers	Hydroquinone	Detection limit 5.71 nM, linear range 30–100 nM	Lake water	(Li et al. 2016d)
Commercialized from Sigma-Aldrich	AgNPs immobilized onto carboxymethyl cellulose-modified electrospun cellulose nanofibers	Catechol	Linear range from 4.98 μM to 3.65 mM, detection limit of 1.64 μM, current response remained 97.6% after 3 weeks stored in buffer at 4 °C	N.r.	(Fu et al. 2015a)
Trametes versicolor	Polyaniline nanorods immobilized onto cellulose nanofibers	Catechol	Linear range 0.497 μM–2.27 mM, detection limit of 0.374 μM	N.r.	(Fu et al. 2015b)
Agaricus bisporus	Copper capped magnetic core-shell nanoparticles	Catechol and phenol	Catechol and phenol in the range of 0.01–0.4 mM and 0.025–0.2 mM	Bacterial *P. putida* culture medium	(Babadostu et al. 2015)
Commercialized Sigma-Aldrich	NiCu alloy nanoparticle-loaded carbon nanofibers	Hydroquinone	Detection limit 90 nM, sensitivity 1.5 μA μM^{-1}, linear range 4×10^{-7}–2.37×10^{-6} M	Lake water	(Dawei et al. 2015)
Trametes versicolor	Porous matrix of polyaniline nanofibers	Catechol	Sensitivities for three kinds of immobilization methods: 20.3 ± 5.9, 26.6 ± 5.4 and 518 ± 11 μA mM^{-1} cm^{-2}, respectively	N.r.	(Kim et al. 2016)
Trametes versicolor	Fe_2O_3 yolk-shell particles	2,6-Dimethoxyphenol	Sensitivity 452 μA/mM/cm^2 linear ranges of detection: 2,6-dimethoxyphenol (0.025–750 μM),	Wastewater	(Patel et al. 2018)

Table 2 (continued)

Laccase sources	Immobilization type/support	Target analyte	Analytical characteristics	Type of samples	Ref.
			guaiacol (0.10–250 μM), pyrogallol (0.25–250 μM), and 3,4-dihydroxy-l-phenylalanine (1.0–125 μM), with limit of detection values of 0.010, 0.052, 0.093, and 0.273 μM, respectively		
Rhus vernicifera	TiO_2 loaded copper and carbon composite nanofibers	Hydroquinone	Linear range 1 to 89.8 μM, detection limit of 3.65 μM, sensitivity of 24.6 μA/mM	N.r.	(Yang et al. 2016a)
Cerrena unicolor	Polymer dithienotetraphenylsilane	Catechol	Limit of less than 0.4 μM, linear range of 5–160 μM	N.r.	(Cabaj et al. 2016)
Trametes versicolor	Laccase were conjugated with Magnetite type nanoparticles immobilized on gold surfaces	ABTS	Sensitivity 100 mA M^{-1} cm^{-2}	N.r.	(Almeida et al. 2017)
Trametes versicolor	α-Fe_2O_3 nanocrystals incorporated carbon-paste electrode	Catechol	Linear range of 8–800 μM, limit detection 4.28 μM	Real water sample	(Sarika et al. 2016)
Trametes versicolor	Poly (3,4-ethylenedioxy-thiophene), graphene oxide nano-sheets	Catechol	Two ranges: 0.036–0.35 μM and 0.35–2.5 μM, detection limit of 0.032 μM	Real water samples	(Maleki et al. 2017)
Trametes versicolor	Titania and titania/Nafion	Catechol	Detection limit 0.75 μM, linear range 0.75 to 150 μM, correlation factor 0.9966	N.r.	(Romeroarcos et al. 2016)
Trametes versicolor	Carbon xerogel-zinc oxide composites mixed with ZnO nanoparticles	Catechol	Sensitivity 31.2 μA·mM^{-1}, detection limit 2.17 μM, linear range 6.91–453 μM	Lake water	(Li et al. 2016b)
Trametes versicolor	Optically transparent sol-gel matrices	Hydroquinone, resorcinol, and catechol	Linear ranges 1.4 and 0.2 mM, detection limit 4.5 and 0.6 μM for resorcinol and catechol, respectively	Tap water samples	(Lepore and Portaccio 2016)
Trametes versicolor	Polydopamine functionalized reduced graphene oxide-palladium nanocomposite	Catechol	Linear range 0.1 to 263 μM, sensitivity 18.4 μA mM^{-1}, detection limit 0.03 μM	Real water	(Li et al. 2016c)
Commercialized	Hybrid nanocomposite of ZnO nanoparticles/chitosan	4-Chlorophenol	Linear range 1 to 50 μM, detection limit 0.7 μM	Industrial wastewater	(Mendes et al. 2017)
Trametes versicolor	Ureasil-chalcogenide glass composite with AgNPs	ABTS	Sensitivity 67,540 A M^{-1} m^{-2}	Wastewater	(Kavetskyy et al. 2017)
Rhus vernicifera	Copper and carbon nanofibers with Ag-doped TiO2 nanoparticles	Hydroquinone	Linear range 1.20 to 176.50 μM, sensitivity of 26.05 μA/mM and a low detection limit of 3.396 μM	Real water sample	(Yang et al. 2016b)
Commercialized	Multiwalled carbon nanotube	Catechol	Linear range 0.5 to 150 μM, detection limit 0.50 μM, sensitivity 2.81 μA L/μmol	N.r.	(Romero-Arcos et al. 2017)
Trametes versicolor	Flower-shaped yolk-shell SiO2 nanospheres	Catechol	Linear range 12.5 to 450 μM, detection limit 1.6 μM	N.r.	(Zheng et al. 2018)

Table 3 The monitoring and detection of analytes of health care using laccase biosensor

Laccase sources	Immobilization type/support	Target analyte	Analytical characteristics	Type of samples	Ref.
Agaricus bisporus	Complex of β-cyclodextrin and benzaldehyde generated onto graphene oxide	Dopamine	Range of 0.1–70 μM, with a low detection limit of 0.03 μM	Human urine samples	(Hua et al. 2015)
Aspergillus oryzae	AuNPs with in poly (allylamine hydrochloride)	Dopamine	Linear range from 0.49 to 23.0 μmol L^{-1}, with a limit of detection of 0.26 μmol L^{-1}	Pharmaceutical samples	(Silva and Vieira 2016)
Pleurotus ostreatus	Textile wearable electrochemical transistor in a non-invasive way	Tyrosine	Limit of detection in the order of 10^{-8} M	Aqueous solutions	(Battista et al. 2017)
Trametes versicolor	Palladium nanoparticle-bacterial cellulose hybrid nanofibers	Dopamine	Sensitivity 38.4 μA·mM^{-1}, detection limit 1.26 μM, and linear range 5–167 μM	Human urine	(Li et al. 2016a)
Trametes versicolor	Reduced graphene oxide/Rh nanoparticles	17β-estradiol	Sensitivity of 25.7 A μM^{-1} cm^{-1}	Human urine samples	(Povedano et al. 2017)
Coriolopsis gallica	Based on laccase inhibition during the oxidation of ABTS	Paracetamol	Limit detection and limit of quantification were noticed to be 0.55 μM and 8.3 μM	Real water samples	(Méndez-Albores et al. 2015)
AB Enzymes GmbH (Germany)	Boolean logic-gate principle to develop a digital biosensor	Adrenaline	Limit detection 1 nM	N.r.	(Molinnus et al. 2016)
Aspergillus oryzae	AuNPs stabilized in β-cyclodextrin	Rutin	Linear range 0.30 to 2.97 μmol L^{-1}, limit detection 0.17 μmol L^{-1}	Pharmaceutical samples	(Brugnerotto et al. 2016)
Commercialized	Nanostructured silica-phytic acid materials with diverse morphologies	Dopamine	Linear range 40–600 μM	N.r.	(Zhao et al. 2014)
Trametes versicolor	Graphene oxide or reduced graphed oxide	Dopamine	Detection limit of 91.0 nmol L^{-1}, linear ranges 0.1 to 0.4 mg mL^{-1}	Synthetic urine and plasmatic serum samples	(Kohori et al. 2017)
Bacillus sp. HR03	Surface plasmon resonance carboxymethyl dextran surface	Bromocriptine	Linear range 0.001 to 1000 ng/ml, detection limit 0.001 ng/ml	Simulated blood samples	(Jabbari et al. 2017)
Bacterial	Immobilizing on *E. coli* surface and direct adsorption with live cells	Several phenolics	Linear ranges of 5.0 to 500.0 μM, 1.0 to 5.0 μM	Red wine, pharmaceutical and wastewater samples	(Zhang et al. 2017)

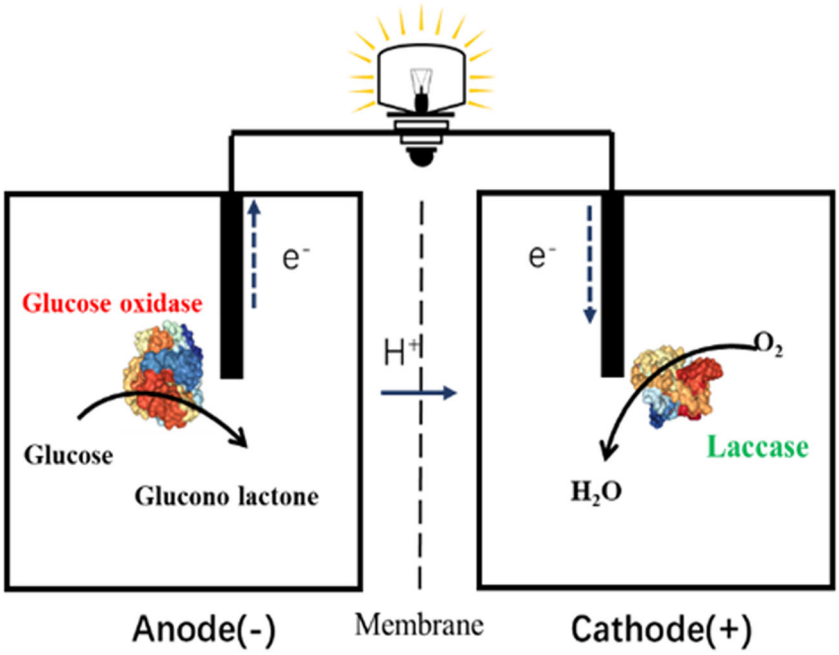

Fig. 3 Schematic representation of a biofuel cell involving glucose oxidase and laccase enzymes

When the EBFC was constructed using the fungal laccase or bacterial laccase as the cathode, the performance factors, like lifetime, stability, and power density, reveal significant differences (Table 4).

The fungal laccase as the cathode biocatalyst

Fungal laccases are mainly considered cathode biocatalyst that possess the capability to reduce oxygen at high potentials (680–850 mV) (Baldrian 2006; Mate and Alcalde 2015), and can be secreted by a variety of fungi. In recent decades, the substrate catalytic mechanism and the electron transfer process have been explained clearly (Baldrian 2006; Giardina et al. 2010; Gupta et al. 2010).

A classical miniature biofuel cell made of glucose oxidase immobilized on anode and laccase from *Coriolus hirsutus* immobilized for the oxygen reduction to water on the cathode was developed (Chen et al. 2001), the power density of the cell was 64 μW/cm^2 at 23 °C and 137 μW/cm^2 at 37 °C, and its power output was 280 nW at 23 °C and 600 nW at 37 °C. The power density of this biofuel cell was the highest when compared to other laccase-based BFC available at that time. The first implanted biofuel cell developed is continuously operating in a snail and producing electrical power over a long period of time using glucose as fuel and laccase as the biocathode without any cofactors and mediators (Halamkova et al. 2012). There was no generation of H_2O_2, since only glucose dehydrogenase was employed along with NAD^+ for the oxidation of glucose. This kind of EBFC produces continuous power supply with 530 mV open-circuit voltage and 42.5 μA short-circuit current in vivo using the laccase biocathode with metabolic glucose as the biofuel for "recharging" the living battery.

Table 4 The EBFC using laccase from eukaryotic and prokaryotic microorganism

Laccase sources	Fuel	Immobilization type/support	Electrical characteristics	Type of electron transfer	Ref.
Fungal laccase					
Pycnoporus sanguineus	Methanol	Polyamidoamine dendrimers on top of a polypyrrole matrix with ABTS	Maximum power densities of 51.2 and 99.8 μW cm^{-2}, 60% of the initial activity after 60 days	MET	(Neto et al. 2016)
Trametes versicolor	Glucose/O_2	Nafion on carbon paper together with carbon nanoparticles and ABTS	The maximum voltage was 402 mV for the first cycles, the maximum power density was 160 mW/m^2	MET	(Luo et al. 2010)
Trametes versicolor	Glucose/O_2	Mediatorless cell based on compressed carbon nanotube	High power density up to 1.3 mW cm^{-2} and an open-circuit voltage of 0.95 V; remains stable for 1 month and 1 mW cm^{-2} power density under physiological conditions	DET	(Abdelkader et al. 2011)
Coriolopsis gallica	O_2	Graphite electrodes were functionalized with 4-2-aminoethyl benzoic acid hydrochloride	Current density of 2977 μA cm^{-2} and a power density of 1190 μW cm^{-2} at 0.41 V	DET	(Martinez-Ortiz et al. 2011)
Coriolopsis gallica	Ethanol methanol/O_2	Functionalized graphite electrode with4-(2-aminoethyl) benzoic acid	0.5 V at open-circuit voltage current with intensity of 250 μA cm^{-2}	DET	(Arrocha et al. 2014)
Trametes versicolor	Glucose/O_2	MWCNT	Retain 22% of its initial maximum power density after 1 year	MET	(Reuillard et al. 2015)
Bacterial laccase					
E. coli (CueO)	O_2	Highly oriented pyrolytic graphite electrode	Catalytic current density about −4 mA cm^{-2}	DET	(Miura et al. 2007)
E. coli (CueO)	O_2	Ketjen black-modified carbon paper electrodes	The current density of O_2 reduction reached values as high as 20 mA cm^{-2} in a citrate buffer (1.0 M, pH 5.0, 25 °C)	DET	(Kontani et al. 2009)
Bacillus subtilis (CotA)	O_2	Glassy carbon electrodes functionalized with aminophenyl monodiazonium salts	The reduction current −187.3 μA cm^{-2}, stable during at least 7 weeks, delivering 35% of its initial catalytic current	DET	(Beneyton et al. 2011a, b)
Streptomyces coelicolor	O_2	Using both MET and DET-type electrode schemes	Redox potential of 0.43 V; 6.25 mA/cm^2 at 0.3 V	DET/MET	(Gallaway et al. 2008)

The bacterial laccase as the cathode biocatalyst

Y. Miura and coworkers (Miura et al. 2009) investigated the direct electrochemical process of CueO from *E. coli* fixed on carbon aerogel electrodes. The redox potential of 0.28 V at pH 5.0 was derived from the type I (T1) Cu site. They also found that the mutant that forms a hydrogen bond with His443 coordinated with the T1 Cu to effectively tune the redox potential, which reduced the overpotential of catalytic O_2 reduction. Until recently, it was more promising to achieve the DET process: the bacterial laccase CueO from *E. coli* was chosen to be the cathodic biocatalyst with Ketjen black-modified carbon electrode, and the largest current density at 0 V reached values of 20 mA cm^{-2} under optimized conditions (Kontani et al. 2009). The first bioelectrochemical studies of the thermostable CotA laccase were performed by Thomas Beneyton and coworkers (Beneyton et al. 2011a, b). The T1 copper site with 460 mV redox potential was investigated by DET study of O_2 reduction. The advantages of this laccase over the fungal enzyme are that it is more stable and active across a broad range of temperatures. In this study, the three mutants were immobilized on the electrode with phenyl-NH_2 moieties. The effect of temperature on the catalytic activity was studied and the reduction current of − 187.3 μA cm^{-2} could be obtained at an optimal operation temperature of 45–50 °C. The electrode also showed long-term stability and high tolerance towards Cl^-. The favorable CotA electrochemical properties have guided the preference and focus on exploring new kinds of stable bacterial laccases that will allow the EBFC to work well in different environmental conditions, rather than to set up a specific test environment to match the fuel cell.

Further, aiming to improve the performance of laccase electrodes, enzyme engineers have considered modifying the properties of the target protein. Schwaneberg et al. focused on the direct devolution of enzymes for biofuel cell properties (Yu et al. 2011). In our previous study, the bacterial laccase CotA from *Bacillus subtilis* 168 was displayed on the surface of *E. coli* (Zhang et al. 2018a, b). There was significant improvement in thermal and alkaline stability of the surface displayed CotA, which can be considered a very important bioimmobilization strategy for EBFC. It is believed that this technology will provide new enzymes with higher catalytic activity or stability. Many researchers have focused their attention on developing a more stable bacterial laccase for use as the bioelectrochemical component on the cathode.

Nowadays, the focus of researchers is on the process by which the electrons are transferred from the electrode directly using eukaryotic or prokaryotic laccase, without the utilization of any expensive or toxic mediators (Abdelkader et al. 2011; Arrocha et al. 2014; Holzinger et al. 2012). In general, laccase provides some advantages to achieve the DET process; the catalytic parameters with the "three clusters of copper ions transfer mechanism" are clear and the only by-product is molecular water, which has no effect on the electrode or application environment. Inspired by this, laccase has been chosen as an important candidate for the biocatalysis of the cathode. Research in the field suggests that the bacterial laccase can always achieve the DET process more easily than the fungal laccase due to its structural features, i.e., the exposed T1 site that reduces the distance of electron transport.

Conclusions

Laccases from fungi or bacteria have good potential applications in the field of enzyme biosensors or enzyme biofuel cells. Among them, the fungal laccase has better catalytic activity for the substrate and seems more suitable for biosensors as the biorecognition element. These sensors can be applied to different areas and conditions like environmental protection and food monitoring. In contrast, the bacterial laccases are generally employed as the biological cathode catalytic components in enzymatic fuel cells due to their better stability and resistance, which is very important for the EBFC implant to be successful in the human body.

To enhance the practical application of laccase electrodes, there are some problems that need to be tackled, such as improving the stability of the fungal laccase or bacterial laccase and increasing the catalytic activity and redox potential. These problems can be addressed by employing advanced biocompatibility nanomaterials, optimized immobilization methods, molecular modification of protein and protein engineering, and so on. In addition, improving the DET is also critical for the development of biosensors and EBFCs. Therefore, a research strategy that reduces the distance between the laccase activity center and the electrode or improves the utilization of better conductive material is the key for future research.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human or animals performed by any of the authors.

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