Screening of Laccase-Mediator Systems by White-Rot Fungi Laccases for Biocatalytic Benzyl Alcohol Oxidation

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Research Article

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Abstract

Production of added value compounds from waste materials is of utmost importance for the development of a sustainable society especially regarding their use as catalysts in industrially relevant synthetic reactions. Herein, we show the production of laccases from four white-rot fungi, which were grown on agricultural residues, specifically *Trametes versicolor* 11269, *Pleurotus ostreatus* 1020, *Panus tigrinus* 707 and *Lentinula edodes* SC-495. The produced laccases were tested on biphasic laccase-mediator system (LMS) for the biocatalytic oxidation of the model substrate benzyl alcohol into benzaldehyde. The biphasic LMS was carried out in the presence both of tetrahydrofuran as co-solvent and of the mediator 2,2,6,6-tetramethyl-1-piperidinyloxyl (TEMPO) due to its high redox potential and its ability to perform the oxidation. Tolerance studies showed that the dialyzed solutions were able to tolerate 1% of co-solvent, while a concentration of 10% *v/v* negatively affected their activity. Performances in the biocatalytic oxidation of laccase solutions from different purification steps were compared. Similar conversion was observed for laccase in dialysis and gel filtration product versus commercial *T. versicolor* laccase. The latter oxidized almost 99% of substrate while the other laccase solutions were able to reach a conversion from 91% for the laccase solution from *P. tigrinus* 707 after dialysis, to 50% for the laccase solution from *P. ostreatus* 1020 after gel filtration.

This work highlights the potential of unpurified laccase solutions to be used as catalysts in synthetic reactions.

Statement Of Novelty

Biomass and agro-industrial waste can be a great alternative to produce chemicals and biocatalysts which can increase the sustainability of industrial processes. The agro-industrial waste has been reported both in submerged and solid-state fermentation due to the accessibility of biomass to produce biocatalysts. The activity of crude and purified laccase-containing mixtures produced from four edible mushrooms which were grown using wheat straw as raw material was benchmarked on the model reaction of the oxidation of benzyl alcohol to benzaldehyde through the laccase/TEMPO LMS with a biphasic system. Our data show that raw laccase solutions can be used as biocatalyst agents, thus speeding up and making cheaper their application from agro-industrial waste.

Introduction

Nowadays, the economic scenario is mostly based on fossil resources with a continuous increase of both their demand and their relative market price with a negative impact on the environment. The Green Transition is pushing towards the use of renewable resources to produce heat and energy, for a better employment of natural resources, such as solar, wind and hydropower energy. To produce chemicals, biomasses are used as sustainable alternatives to fossil resources by biorefining, which is one of the main drivers for the bio-based economy (Ciliberti et al. 2020; Dubois and Gomez San Juan 2016). The European Directive 2009/23/EC has defined biomass as “the biodegradable fraction of products, waste

and residues from biological origin from agriculture (including plant and animal substances), forestry and related industries including fisheries and aquaculture, as well as the biodegradable fraction of industrial and municipal waste"(EUR-Lex 2009). The use of agro-industrial waste has been reported both in submerged and solid-state fermentation (SSF) due to the accessibility of insoluble biomass (Adekunle et al. 2016). SSF has many advantages, such as higher product titer, lower wastewater output, simpler fermentation media, reduced energy requirements (Lizardi-Jiménez and Hernández-Martínez 2017).

Industrially relevant enzymes can be produced by solid-state fermentation for their deployment in organic synthesis. In particular, the selective oxidation of alcohols to the corresponding carbonyl compounds is of utmost importance in both academia and industry. Primary alcohols are directly oxidized to carboxyl acids by H_2CrO_4 or $KMnO_4$. A selective oxidation to aldehyde, instead, can be achieved using pyridinium chlorochromate (PCC) in stoichiometric amounts in aprotic solvents such as dichloromethane. Catalytic oxidation, instead, requires metal catalysts which are expensive and toxic (Hunsen 2005). A sustainable alternative is the use of Laccase/TEMPO-mediator systems, that has been used for the biocatalytic conversion of alcohols to aldehydes (Díaz-Rodríguez et al. 2014).

Laccases (EC 1.10.3.2, benzenediol:oxygen oxidoreductases) are a well explored group of enzymes belonging to the multi-copper oxidase (MCO) family. Members of the MCO family contain four copper ions which are organized in two sites. The type 1 copper obtains one electron from the substrate, transferring it via a His-Cys-His motive to the trinuclear center. The trinuclear center contains one type 2 and two type 3 copper ions and this is the site where the oxygen reduces to water. In this reaction, four electrons from substrate molecules are transferred to one molecule of O_2, reducing it to two molecules of water (Mot and Silaghi-Dumitrescu 2012). Unlike other enzymes, laccases can oxidize various substrates, such as phenols (Majcherczyk 1999), aromatic and aliphatic amines and some inorganic ions, while reducing oxygen to water (Riva 2006). Plant laccases play an important role in the synthesis of lignin, while fungal laccases catalyze its degradation for wood-decay, pathogenesis, fungal morphology and detoxification (Zhao et al. 2013). These natural activities are exploited in several applications by different industrial processes. For example, in the food and textile industry, laccases are used to reduce the oxygen content, specifically in beer production to increase the product shelf-life and for their bleaching activities on denim fabrics, respectively (Mate and Alcalde 2017). Moreover, different studies showed the ability of laccases to produce polymeric structures (Braunschmid et al. 2021; Pollard and Bruns 2018).

Although laccases are widespread in nature, as they have been described from bacteria, fungi, higher plants and insects, fungal laccases represent the most significant group of the blue MCO family with regard to the number and extent of characterization. Typical fungal laccases are 60-70 kDa monomeric glycoproteins with a well characterized catalytic mechanism for the formation of radical species (Bassanini et al. 2020). Moreover, through the so-called "Laccase-mediator system" (LMS), radical species may play a role as mediators by oxidizing non-phenolic compounds. The catalytic cycle of laccases can only start if the substrate of interest has the proper redox potential. In fact, laccases typically possess a redox potential of about 0.5–0.8 mV vs. the normal hydrogen electrode (Witayakran and Ragauskas 2009). Due to steric hindrance and/or redox potential incompatibility, mediators can act

as redox intermediates, following a nature mimicking-fashion strategy. The produced free radicals from the oxidation of these compounds can act on bulky or high redox potential substrates.

Since the discovery of 2,2'-azine-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) to be used as mediator, the number of compounds that has this function has dramatically increased (Morozova et al. 2007). In order to be considered a proper mediator, the redox compound must not inhibit the enzyme and its conversion must be cyclic. One of the most common mediators in LMSs is the compound 2,2,6,6-tetramethyl-1-piperidinyloxyl (TEMPO), which has sufficiently high redox potential and it is more efficient than other mediators. The TEMPO mediator is present in the solution in the form of a relatively stable N-oxyl radical which is also able to oxidize non-phenolic structures (Fabbrini et al. 2002). Shortly, laccases oxidize TEMPO to produce the oxo-ammonium ion, which reacts with the substrate. Proton removal yields the oxidized product and the reduced (N–OH) form of TEMPO. The reduced TEMPO is converted by laccases to the oxidized form and then to the oxo-ammonium ion (Morozova et al. 2007). The LMS offers the possibility to convert primary and secondary alcohols into oxidized compounds, such as aldehydes, acids and ketones.

The application of enzymes in synthetic chemistry requires the use of non-conventional reaction systems to dissolve hydrophobic substrates in the presence of water, including the use of organic solvents (Bassanini et al. 2020). Both water-miscible solvents (Wan et al. 2010) and biphasic water-immiscible solvents (Nicotra et al. 2004; Ponzoni et al. 2007) have been applied to improve substrate solubility.

Utilization of by-products and residues from food and agricultural industries as raw materials for laccase production permits a more sustainable process, both economically and environmentally.

In the present work, we highlight the comparative activity of crude and purified laccase-containing mixtures produced from four edible mushrooms which were grown on SSF using wheat straw as substrate, on the model reaction of the oxidation of benzyl alcohol to benzaldehyde through the laccase/TEMPO LMS with a biphasic system.

Materials And Methods

Chemicals and reagents

All chemicals and reagents used in this work were of analytical grade. Potato Dextrose broth (PDB), ammonium sulfate, Tris-HCl, benzyl alcohol, 2,2,6,6-tetramethyl-1-piperidinyloxyl (TEMPO), 2,6-dimethoxyphenol (DMP) were purchased from Sigma-Aldrich (USA). Commercial laccase from *Trametes versicolor* (TV σ) was purchased from Sigma-Aldrich (USA). Organic solvents were purchased at the higher commercial quality and used without further purification.

Strain and culture media

Four white-rot fungi were used in this work: *Lentinula edodes* SC-495 and *Panus trigrinus* 707, belonging to the culture collection of ALSIA; *Pleurotus ostreatus* 1020 and *Trametes versicolor* 11269, were

purchased from the strain collection of the DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen). These fungal cultures were cultivated in slant tubes using PDB as medium containing 25 g L^{-1} agar at 28°C for 7 days. For each strain, a liquid pre-inoculum was prepared by adding 30 mL sterile water into the slant tube to resuspend the mycelium. To disrupt the mycelium, the suspension was then homogenized at 24,000 rpm for 30 min by a T25 Ultra-Turrax (IKA, Germany). An aliquot of 25 mL was then transferred into a sterile Erlenmeyer flask with 100 mL fresh PDB medium. The strains were grown at 28°C for 7 days at 180 rpm. After further homogenization, 25 mL was transferred into a 250 mL fresh PDB medium and grown at 28°C for 5 days at 180 rpm.

Agricultural biomass

Dry Saragolla (*Triticum turgidum* subsp. *durum*) straw was collected from Metapontum Agrobios Research Center (Italy) fields. The Saragolla grain is an early durum wheat variety with an exceptionally high and stable production potential in terms of biomass and grain yield (Acquistucci et al. 2020, Danzi et al. 2021). Wheat bran was used as co-substrate for the mycelia growth and for laccase production with a composition as previously reported (Neifar et al. 2009).

Solid-state fermentation for laccase production

Grinded straw/bran mix (100 g), with a straw:bran ratio of 4:1 *w/w*, containing 70% moisture content (233 mL 0.5 mM Cu_2SO_4 solution), were inserted in an autoclavable plastic bag and sterilized by autoclave at 121°C (Neifar et al. 2011; Neifar et al. 2009). After cooling the mixture at room temperature, 30 mL liquid inoculum from a 250 mL culture, containing a concentration of 3 mg mL^{-1} from each strain, were added and properly mixed to accurately spread fungal cells. The bags were kept in the dark at 28°C for 7 days.

Extraction and purification of laccase

In order to extract the extracellular liquor containing enzymes and organic compounds from the SSF batch, 100 g of biomass was pressed through a hydraulic press (Ravaglioli Spa, Italy) with a maximum pressure of 415 bar and a maximum capacity of 15,000 kg. To remove solid particles, a volume of 150 mL extracted liquor was centrifuged at 15,000 rpm for 25 min at 4°C.

The prepared supernatant was then mixed with $(NH_4)_2SO_4$ to reach 70% *w/v* saturation. The mixture was stored at 4°C under magnetic stirring for 24 h. In order to collect precipitated proteins, the mixture was then centrifuged at 10,000 rpm for 30 min at 4°C. The protein pellet was then solubilized in 0.05 M Tris-HCl pH 7.8 and centrifuged at 4,000 rpm for 10 min at 4°C to remove impurities. In order to remove the excess of $(NH_4)_2SO_4$ from the solution, dialysis was performed into a dialysis tube of 14 kDa cut-off and dialyzed against 2 L 0.05 M Tris-HCl pH 7.8 overnight at 4°C under magnetic stirring.

To further purify the liquor of each strain, 30 mL protein solution were loaded onto an ion-exchange chromatography column packed with Sepharose Q as stationary phase connected to a Fast Protein Liquid Chromatography (FPLC) ÄKTA system (GE Healthcare, Sweden). The column was equilibrated with solution A, which contained 50 mM Tris-HCl, 15 mM NaCl pH 7. The elution was allowed using solution B,

which contained 50 mM Tris-HCl, 1 M NaCl pH 7.8 with a flow of 1 mL min^{-1} and a gradient from 0–100% solution B within 50 min. The protein elution was detected with a UV detector at 280 nm.

For each strain, fractions rich in laccase activity were combined and concentrated for the next purification step of gel filtration chromatography. A 60-cm column was packed with Toyopearl resin 50H (TosoH Bioscience, Japan) and connected to an FPLC ÄKTA system. Proteins were eluted with 50 mM Tris-HCl pH 7.8. Fractions which contained laccase activities were collected and stored at -20°C until further analysis.

Protein analysis

Protein concentration was measured by Bradford assay (Bradford 1976) using Bio-Rad Protein Assay Kit (Bio-Rad, USA). Bovine Serum Albumin (BSA) was used as protein standard. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to Laemmli (Laemmli, 1970) using a Precision Plus Protein™ All Blue (Bio-Rad, USA) as protein marker. Proteins were stained with Coomassie method.

Laccase activity assay

Enzyme solutions were spectrophotometrically assayed at 477 nm using 2,6-dimethoxyphenol (DMP) as substrate at 30°C (ε_{477} = 14600 M^{-1} cm^{-1}) (Slomczynski et al. 1995). The assay mixture consisted of 2 mM DMP in 0.1 M sodium acetate buffer pH 4.5. All experiments were performed in triplicate. One unit of enzyme activity (U) was defined as the amount of enzyme transforming 1 µmol of substrate DMP into 3,3',5,5'-tetramethoxy-*p*-diphenoquinone (cerulignone) per minute under the given experimental conditions. The laccase activity was expressed as international units (U).

Biphasic oxidative biocatalysis

The laccase oxidation of benzyl alcohol to benzaldehyde was carried out at room temperature under magnetic stirring at 800 rpm in a final volume of 3 mL with 20 mM (0.06 mmol) benzyl alcohol, 6 mM TEMPO solution in 30 µL THF, 0.6 U laccase solution in 0.1 M citrate buffer pH 5 with addition of oxygen. The biphasic system is required due to the low solubility of TEMPO in aqueous solutions. The oxygen was produced by reacting 5% *v/v* H_2O_2 with $KMnO_4$. The addition to the reaction medium was performed at 0, 3, 6, and 24 h.

Laccase solutions from the dialysis and gel filtration steps of purification were tested on the biocatalytic model oxidation of benzyl alcohol to benzaldehyde by measuring benzaldehyde formation at 290 nm (Kawamura et al. 2016) in a 1-mL quartz cuvette against a calibration curve of the product between 0.2 and 10 mM. All experiments were performed in triplicate.

Statistical Analysis

Analysis of variance (ANOVA) of data from Benzaldehyde production was performed using Minitab ver.17 (Statistical software). Results were reported as mean of the production ± standard deviation (SD). Statistical differences ($P<0.05$) among different laccases were determined according to Tukey's test.

Results

Production and purification of laccases

Four different white-rot fungi strains were grown on the agricultural residues formed by Saragolla straw:bran with a ratio 4:1 *w/w*. The collected laccase-containing liquor was further purified through different steps performing dialysis, ion exchange chromatography and gel filtration.

The maximum laccase production was obtained for all strains at the end of 7th day of fermentation with a specific activity of liquor of 6.38 U/mg protein for *Trametes versicolor* 11269, 14.47 U/mg for *Pleurotus ostreatus* 1020, 20.75 U/mg for *Panus tigrinus* 707 and 11.65 U/mg for *Lentinula edodes* SC-495. The crude laccase of the liquor was precipitated with 70% ammonium sulfate saturation, dialyzed, purified on ion-exchange chromatography and the pooled fractions further purified by gel filtration. Laccase from *Trametes versicolor* 11269, *Pleurotus ostreatus* 1020 and *Panus tigrinus* 707 exhibited a similar specific activity of 379.68 U/mg, 377.04 U/mg and 375.78 U/mg toward DMP at the standard assay conditions with an overall fold purification of 59.48, 26.05 and 18.11 and a percentage yield of 17.39, 30.45 and 15.94, respectively. Laccase from *Lentinula edodes* SC-495 was purified with the lowest specific activity of 118.16 U/mg with a 10.14 fold purification and a percentage yield of 12.87 (Table1).

Table 1
Purification profiles of laccases from different white rot fungi

	Purification step	Volume (ml)	Total protein (mg)	Total activity (U)	Specific activity (U/mg)	Purification fold	Yield (%)
Trametes versicolor 11269	**Liquor**	150.00	283.39	1809.00	6.38	1.00	100.00
	Dialysis	30.00	136.80	1583.10	11.57	1.81	87.51
	Ion Exchange	8.00	3.78	800.00	211.81	33.18	44.22
	Gel Filtration	5.00	0.83	314.65	379.68	59.48	17.39
Pleurotus ostreatus 1020	**Liquor**	150.00	125.31	1813.50	14.47	1.00	100.00
	Dialysis	30.00	143.88	1258.50	8.75	0.60	69.40
	Ion Exchange	8.00	7.79	769.76	98.79	6.83	42.45
	Gel Filtration	10.00	1.46	552.24	37.04	26.05	30.45
Panus tigrinus 707	**Liquor**	150.00	166.95	3465.00	20.75	1.00	100.00
	Dialysis	30.00	128.71	4979.40	38.69	1.86	143.71
	Ion Exchange	8.00	6.90	1121.28	162.50	7.83	32.36
	Gel Filtration	10.00	1.47	552.40	375.78	18.11	15.94
Lentinula edodes SC-495	**Liquor**	150.00	196.99	2295.00	11.65	1.00	100.00
	Dialysis	30.00	137.22	1137.60	8.29	0.71	49.57
	Ion Exchange	8.00	9.16	367.12	40.08	3.44	16.00
	Gel Filtration	10.00	2.50	295.40	118.16	10.14	12.87

Each step showed an increase in the purity of the laccase solution, as shown also in Figure 1 for *Trametes versicolor* 11269 laccase at a molecular weight of ca. 60 kDa, as previously reported (Youshuang et al. 2011). Similar results were shown for the purification of the other laccases (data not shown). A band at around 40 kDa was present in different purification steps and in different fractions. Fractions with the same purity grade were pooled to increase the amount of laccase for biocatalytic oxidation of benzyl alcohol.

Tolerance of laccase systems versus tetrahydrofuran

Recently, it was reported that laccases were employed also in water-miscible organic solvents (Wu et al. 2019). Thus, laccases produced from mycelia, which were grown on agricultural residues, were compared with commercially available enzymes in the oxidation of benzyl alcohol.

The commercial TV laccase (TV σ) and the dialysate protein mixtures of the four strains were assayed for their tolerance to THF at concentrations of 1% and 10% *v/v*, after 24 h incubation time and compared with the control without THF. The TV 11269 laccase solution showed the highest tolerance to the THF 1% *v/v*, which showed a 96% activity. The enzymatic activity is almost completely lost (2.9%) at a concentration of THF of 10% *v/v*. The commercial TV laccase showed a lower tolerance (59.8%) in THF at 1% *v/v* and a slightly higher tolerance at 10% THF (17.32%), compared to TV 11269 laccase. LE SC-495, PO 1020 and PT 707 laccase solutions were more affected by the presence of the THF co-solvent compared to the TV 11269 laccase solutions (Fig. 2). In fact, they showed lower residual activity with 47%, 57% and 65% residual activity, in 1% THF and very low activity at 10% THF, respectively (Fig. 2, Table S1).

For this reason, the optimized 1% concentration THF was used as the co-solvent for the biocatalytic oxidation.

Biocatalytic oxidation of benzyl alcohol

The oxidation of benzyl alcohol to benzaldehyde was investigated as a model of an industrially relevant process. THF was used as the co-solvent with a concentration of 1% *v/v* in the presence of 0.1 M sodium citrate buffer pH 5 at room temperature, with 0.06 mmol of benzyl alcohol. Oxygen was introduced throughout the entire incubation at different times. The oxidation of benzyl alcohol to benzaldehyde through laccases was measured after 30 h. Aliquots of the dialysis and the gel filtration products were used on the reaction.

The highest performance in the conversion was observed for the commercial *T. versicolor* laccase (TV σ) with a 99% yield. Nevertheless, no significant differences were shown with the activity of laccases from all the tested strains, for both steps of purification with exception of *P. ostreatus* 1020 (Fig. 3).

PT 707 laccase showed 91% yield for the dialyzed laccase against 86% yield of the gel filtration product, followed by LE SC 495 with a 83% yield for the dialyzed laccase against 78% yield of the gel filtration product and TV 11269 that achieved a 79% yield for the dialyzed laccase against 77% yield of the gel filtration product. The lowest yield was recorded by the laccase solutions from PO 1020 which showed a 64% yield for the dialyzed laccase against 50% yield of the gel filtration product. Those data show that purification steps do not increase the activity of enzyme solutions in the biocatalytic transformation of benzyl alcohol, with a reduced production time and costs for the use of these industrially relevant biocatalysts.

Discussion

Laccases are extracellular enzymes that are expressed in the presence of specific substrates. It was previously shown that the addition of bran in solid-state fermentation can stimulate the expression of this class of enzymes due to the presence of hydroxycinnamic acids, such as ferulic acid and *p*-coumaric acid. These acids are bound to cell wall polymers, such as pectin and xyloglucans, through ester bonds and released after feruloyl esterase action (Dinis et al. 2009). Moreover, due to the presence of copper ions in the active site of the enzyme, the addition of Cu_2SO_4 has been widely used to improve their expression (Gnanamani et al. 2006).

Different purification steps were carried out and tested for differences in laccase activity. A typical trend of increase of specific activity and purification fold was observed in all fungal laccases.

Dialyzed laccase solutions and commercial *T. versicolor* laccase were tested under different concentrations of THF to identify their specific tolerance level. Interestingly, it was found that similar tolerance was shown for the commercial *T. versicolor* laccase and the dialyzed laccase solutions. Only the TV 11269 laccase solution showed a higher residual activity at 1% *v/v* THF compared to the other tested enzymes. On the other hand, at higher concentration of co-solvent (10% *v/v*), every sample showed a drop of activity. Interestingly, dialyzed and purified laccase solutions showed similar yields of the oxidized product benzaldehyde, which showed that purification steps do not increase the activity of enzyme solutions with a significant shortage of time and costs.

In conclusion, we have demonstrated that solid-state fermentation is a successful technique to cultivate white-rot fungi on agricultural residues in order to produce laccase-containing solutions which are tolerant to organic solvents such as THF at a concentration of 1% *v/v* and that these solutions, both crude or after thorough purification, can be used for the biocatalytic oxidation in laccase-mediator systems of the model substrate benzyl alcohol to benzaldehyde without the use of metal catalysts. Further studies will be performed to characterize the composition of the dialysate and to extend the process to the sustainable and green production of industrially relevant intermediates.

Declarations

Contributions

IM and MAMC conceived and designed this study. IM and EP conducted experiments with support of FC, CC and AB. IM, DD, EP, IP and AB analyzed data. All authors contributed to writing and editing the manuscript and approved the final version of the manuscript.

Availability of data and materials

All data generated during the current study are included into this published paper.

Ethical approval and consent to participate

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

Consent for publication

All authors declare that agree with submit the manuscript to *Waste and Biomass Valorization.*

Competing interests

The authors declare that they have no competing interests.

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References

1. Acquistucci R, Melini V, Galli V (2020) Durum wheat grain and pasta from locally-grown crops: A case-study on Saragolla (Triticum turgidum ssp. turanicum) and Senatore Cappelli (Triticum turgidum ssp. durum) wheats. Emirates Journal of Food and Agriculture 32(1):47–54. https://doi.org/ 10.9755/ejfa.2020.v32.i1.2058
2. Adekunle AE, Zhang C, Guo C, Liu C-Z (2017) Laccase Production from Trametes versicolor in Solid-State Fermentation of Steam-Exploded Pretreated Cornstalk. Waste Biomass Valor 8**:**153–159. https://doi.org/10.1007/s12649-016-9562-9
3. Bassanini I, Ferrandi EE, Riva S, Monti D (2021) Biocatalysis with Laccases: An Updated Overview. Catalysts 11(1): 26. https://doi.org/10.3390/catal11010026
4. Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry72 (1–2):248–254. https://doi.org/10.1016/0003-2697(76)90527-3
5. Braunschmid V, Binder K, Fuerst S, Subagia R, Danner C, Weber H, Schwaiger N, Nyanhongo G S Ribitsch D, Guebitz GM (2021) Comparison of a fungal and a bacterial laccase for lignosulfonate polymerization. Process Biochemistry 109:207–213. https://doi.org/10.1016/j.procbio.2021.07.001
6. Ciliberti C, Biundo A, Albergo R, Agrimi G, Braccio G, de Bari I, Pisano I (2020) Syngas derived from lignocellulosic biomass gasification as an alternative resource for innovative bioprocesses. Processes 8(12):1–38. https://doi.org/10.3390/pr8121567

7. Danzi D, Marino I, De Bari I, Mastrolitti S, Petretto G L, Pignone D, Janni M, Cellini F, Venditti T (2021) Assessment of durum wheat (Triticum durum desf.) genotypes diversity for the integrated production of bioethanol and grains. Energies 14(22)7735. https://doi.org/10.3390/en14227735
8. Díaz-Rodríguez A, Martínez-Montero L, Lavandera I, Gotor V, Gotor-Fernández V. (2014) Laccase/2,2,6,6-Tetramethylpiperidinoxyl Radical (TEMPO): An Efficient Catalytic System for Selective Oxidations of Primary Hydroxy and Amino Groups in Aqueous and Biphasic Media. Adv Synt Catal 356(10):2321–2329. https://doi.org/10.1002/adsc.201400260
9. Dinis MJ, Bezerra RMF, Nunes F, Dias AA, Guedes C v., Ferreira LMM, Cone J W, Marques G S M, Barros A R N, Rodrigues M A M (2009) Modification of wheat straw lignin by solid state fermentation with white-rot fungi. Bioresource Technology 100(20):4829–4835. https://doi.org/10.1016/j.biortech.2009.04.036
10. Dubois O, Gomez San Juan M (2016) How sustainability is addressed in official bioeconomy strategies at international, national, and regional leveles - An overview. Environment and Natural Resources Management. Working Paper (FAO) 63. https://www.fao.org/3/i5998e/i5998e.pdf
11. Fabbrini M, Galli C, Gentili P (2002) Comparing the catalytic efficiency of some mediators of laccase. Journal of Molecular Catalysis - B Enzymatic 16(5–6):231–240. https://doi.org/10.1016/S1381-1177(01)00067-4
12. Gnanamani A, Jayaprakashvel M, Arulmani M, Sadulla S. (2006) Effect of inducers and culturing processes on laccase synthesis in Phanerochaete chrysosporium NCIM 1197 and the constitutive expression of laccase isozymes. Enzyme and Microbial Technology 38(7):1017–1021. https://doi.org/10.1016/j.enzmictec.2006.01.004
13. Hunsen M (2005) Pyridinium chlorochromate catalyzed oxidation of alcohols to aldehydes and ketones with periodic acid. Tetrahedron Letters 46(10):1651–1653. https://doi.org/10.1002/chin.200526029
14. Kawamura K, Yasuda T, Hatanaka T, Hamahiga K, Matsuda N, Ueshima M, Nakai K (2016) Oxidation of aliphatic alcohols and benzyl alcohol by H2O2 under the hydrothermal conditions in the presence of solid-state catalysts using batch and flow reactors. Chemical Engineering Journal. 285:49–56. https://doi.org/10.1016/j.cej.2015.09.088
15. Laemmli UK (1970) Cleavage of Structural Proteins during the Assembly of the Head of Bacteriophage T4. Nature 227(5259):680–685. https://doi.org/10.1038/227680a0
16. Lizardi-Jiménez MA, Hernández-Martínez R (2017) Solid state fermentation (SSF): diversity of applications to valorize waste and biomass. 3 Biotech 7(1):44. https://doi.org/10.1007/s13205-017-0692-y
17. Mate DM, Alcalde M (2016) Laccase: a multi-purpose biocatalyst at the forefront of biotechnology. Microbial Biotechnology 10(6):1457–1467. https://doi.org/10.1111/1751-7915.12422
18. Morozova O v., Shumakovich GP, Shleev S v., Yaropolov YaI (2007) Laccase-mediator systems and their applications: A review. Applied Biochemistry and Microbiology 43(5):523–535. https://doi.org/10.1134/S0003683807050055

19. Mot AC, Silaghi-Dumitrescu R (2012) Laccases: complex architectures for one-electron oxidations. Biochemistry 77(12):1395–407. https://doi.org/10.1134/S0006297912120085
20. Neifar M, Jaouani A, Ellouze-Ghorbel R, Ellouze-Chaabouni S, Penninckx MJ (2009) Effect of culturing processes and copper addition on laccase production by the white-rot fungus Fomes fomentarius MUCL 35117. Lett Appl Microbiol 49(1):73–78. https://doi.org/10.1111/j.1472-765X.2009.02621.x
21. Neifar M, Kamoun A, Jaouani A, Ellouze-Ghorbel R, Ellouze-Chaabouni S (2011) Application of Asymetrical and Hoke Designs for Optimization of Laccase Production by the White-Rot Fungus Fomes fomentarius in Solid-State Fermentation. Enzyme Research 2011(ID 368525):12. https://doi.org/10.4061/2011/368525
22. Nicotra S, Intra A, Ottolina G, Riva S, Danieli B (2004) Laccase-mediated oxidation of the steroid hormone 17β-estradiol in organic solvents. Tetrahedron: Asymmetry 15(18):2927–2931. https://doi.org/10.1016/j.tetasy.2004.06.034
23. Pollard J, Bruns N (2018) Biocatalytic ATRP. ACS Symposium Series 1284:379–393. https://doi.org/10.1021/bk-2018-1284.ch019
24. Ponzoni C, Beneventi E, Cramarossa MR, Raimondi S, Trevisi G, Pagnoni UM, Riva S, Forti L (2007) Laccase-Catalyzed Dimerization of Hydroxystilbenes. Adv Synth Catal 349(8–9):1497–1506. https://doi.org/10.1002/adsc.200700043
25. Riva S (2006) Laccases: blue enzymes for green chemistry. Trends Biotechnol 24(5):219–226. https://doi.org/10.1016/j.tibtech.2006.03.006
26. Slomczynski D, Nakas JP, Tanenbaum SW (1995) Production and Characterization of Laccase from Botrytis cinerea 61-34. Applied and Environmental Microbiology 61(3):907-912 Slomczynski, D., Nakas, J. P., & Tanenbaum, S. W. (1995). Production and Characterization of Laccase from Botrytis cinerea 61-34. Applied and environmental microbiology, 61(3), 907–912. https://doi.org/10.1128/aem.61.3.907-912.1995
27. Wan YY, Lu R, Xiao L, Du YM, Miyakoshi T, Chen CL, Knill C J, Kennedy J F (2010) Effects of organic solvents on the activity of free and immobilised laccase from Rhus vernicifera. International Journal of Biological Macromolecules 47(4):488–495. https://doi.org/10.1016/j.ijbiomac.2010.07.003.
28. Witayakran S, Ragauskas AJ (2009) Synthetic Applications of Laccase in Green Chemistry. Adv Synth Catal 351(9):1187–1209. https://doi.org/10.1002/adsc.200800775.
29. Wu M-H, Lin M-C, Lee C-C, Yu S-M, H-J Wang A, David Ho T-H (2019) Enhancement of laccase activity by pre-incubation with organic solvents. Sci Rep 9:9754. https://doi.org/10.1038/s41598-019-45118-x
30. Youshuang Z, Haibo Z, Mingle C, Zhenzhen W, Feng H, Peiji G (2011) Production of a thermostable metal-tolerant laccase from Trametes versicolor and its application in dye decolorization. Biotechnol Bioproc E 16**,** 1027-1035. https://doi.org/10.1007/s12257-011-0129-0
31. Zhao Q, Nakashima J, Chen F, Yin Y, Fu C, Yun J, Shao H, Wang Z, Wang Z-Y, Dixon R (2013) LACCASE Is Necessary and Nonredundant with PEROXIDASE for Lignin Polymerization during

Vascular Development in Arabidopsis. The Plant Cell 25(10):3976–3987. https://doi.org/10.1105/tpc.113.117770.

Figures

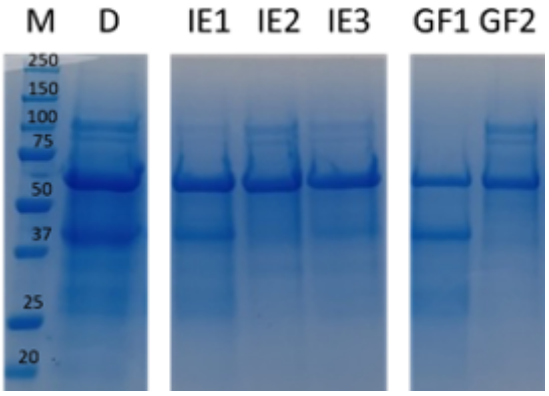

Figure 1

SDS-PAGE analysis (4–12%) of different purification fractions of TV 11269 laccase. Lane M, Precision Plus Protein™ All Blue (Bio-Rad, USA); lane D, Dialysis fraction; lane IE1, IE2, and IE3, Ion-exchange fractions; Lane GF1 and GF2, Gel-Filtration fractions.

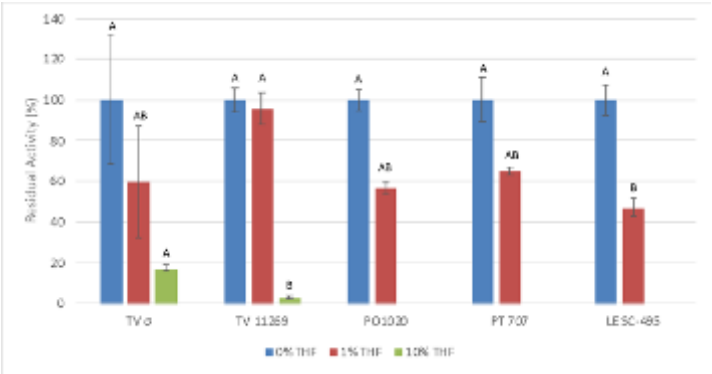

Figure 2

Tolerance to THF of the commercial laccase (TV σ) and the dialysate protein mixtures of TV 11269, PO 1020, PT 707, and LE SC-495. THF concentrations of 1% *v/v* and 10% *v/v* were tested and related to the laccase activity in aqueous solution. Data are mean values of three different measurements, different letters indicate differences according to Tukey Test at $p<0.05$.

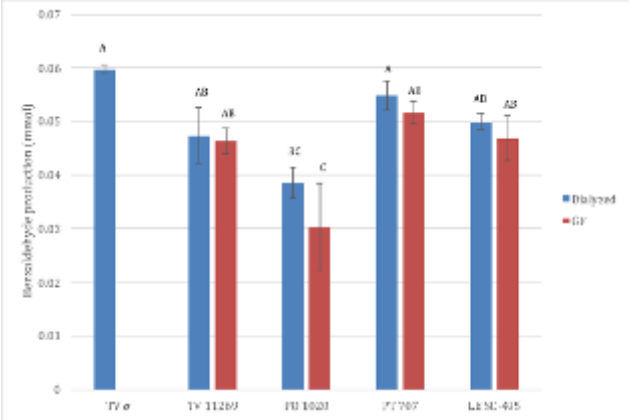

Figure 3

Conversion of benzyl alcohol to benzaldehyde by laccase solutions produced from four different white-rot fungi compared to the commercial *T. versicolor* laccase (TV σ), after 30 h from inoculation. Data are mean values of three different measurements, different letters indicate differences according to Tukey test at $p<0.05$.