The Large Scale Production of Cerrena unicolor Laccase on Waste Agricultural Based Media

Mazur, Iwona
Department of Biochemistry, Maria Curie-Skłodowska University

Rola, Beata
Department of Biochemistry, Maria Curie-Skłodowska University

Stolarczyk, Krzysztof
Faculty of Chemistry, University of Warsaw

Nazaruk, Ewa
Faculty of Chemistry, University of Warsaw

他

https://doi.org/10.5109/1526341

出版情報：九州大学大学院農学研究院紀要. 60 (2), pp.297-302, 2015-09-18. Faculty of Agriculture, Kyushu University
バージョン：
権利関係：

J. Fac. Agr., Kyushu Univ., **60** (2), 297–302 (2015)

The Large Scale Production of Cerrena unicolor Laccase on Waste Agricultural Based Media

Iwona MAZUR[1], Beata ROLA[1], Krzysztof STOLARCZYK[2], Ewa NAZARUK[2], Renata BILEWICZ[2], Jerzy ROGALSKI[1]* and Shoji OHGA*

Laboratory of Forest Resources Management, Division of Forest Environmental Science,
Department of Agro–environmental Sciences, Faculty of Agriculture,
Kyushu University, Sasaguri, Fukuoka 811–2415, Japan
(*Received April 14, 2015 and accepted May 19, 2015*)

The effect of different agriculture waste polysaccharides as an only carbon source in culture medium of wood–degrading basidiomycete *Cerrena unicolor* C–139 were investigated. The maximal growth and laccase synthesis in shaken flask was observed in mineral salts broth containing potato starch as the carbon source and asparagine as the nitrogen source (11,000 nkat/L). When an optimized medium was stimulated by addition of 10 μM Cu^{2+} to the culture medium on days 3–5 the maximal activity 24,000 nkt/L was obtained. Next the influence of the stabilization of the medium pH after 48–h incubation on laccase activity in fermentor cultures had to be estimated. The obtained data show that use of an automatic pH control set at pH 5.0 increased laccase productivity significantly (by 12 times) as compared to that obtained in the fermentor culture with a non–stabilized pH–value. Under the new conditions, the highest enzyme activity of 290,000 nkat/L was reached after 13–day incubation.

Key words: *Cerrena unicolor*, laccase, culture factors, wood degrading, fermentor

INTRODUCTION

The lignin is one of the most abundant polymer in the nature. The best characterized and most extensively studied lignin degrading organisms are the white rot fungi (Leonowicz *et al*., 2001). The ligninolytic enzymes of white rot fungi known so far can be divided into three groups. The first one attack directly the wood constituents; this group includes enzymes acting on carbohydrate components (cellulose, hemicellulose) and lignin. The second of three above mentioned groups includes enzymes cooperates with the first one but they neither attack wood nor contribute to it. The third, very important group so called feedback type enzymes play a key role in combining the metabolic chains during biodeterioration of the wood polymer (Leonowicz *et al*., 1999).

Laccases (EC 1.10.3.2, *p*–diphenol: oxygen oxidoreductase) are multi copper–containing oxidases, catalyzing the oxidation of *ortho*– and *para*– diphenols, polyphenols, arylamines, aminophenols and some inorganic ions, while simultaneously reducing molecular dioxygen to water (Solomon *et al*., 1996; Shleev *et al*., 2005; Viswanath *et al*., 2008).

Laccases are classified into two groups in accordance with their source: plant and fungal (Mayer and Staples, 2002). However, diphenol oxidases have also been identified in bacteria (Givuadan *et al*., 1993) and insects (Barrett, 1991). In the fungi belongs to the most effective producers of this biocatalyser were discovered three forms of laccases so called blue (Gochev and Krastanov, 2007), yellow (Leontievsky *et al*., 1999) and white ones (Palmieri *et al*., 1997). Because of their broad substrate specificity, native or immobilized laccases can be potentially used in textile dye bleaching, pulp delignification, effluent detoxification, washing of powder components, removal of phenolics from effluents, must, wine and fruit juices, transformations of steroids and antibiotics as well as in biosensors (Duran *et al*., 2002; Mayer and Staples, 2002; Minusi *et al*., 2002; Widsten and Kandelbauer, 2008; Giardina *et al*., 2010). The production of fungal laccases is associated with secondary metabolism, the main drawback of which is the limited yield of the enzyme obtained under growth–limiting conditions (Moreira *et al*., 2000). To utilize laccases more efficiently for diverse bioprocesses, large amounts of enzyme are required (Gianfreda *et al*., 1999). At present, research and application are sometimes hindered by the rather low yields of the enzyme formed by used producers as well as by the difficulties in efficiently overexpressing laccases in active form heterologously (Jönsson *et al*., 1997). The problem of increasing the yield of ligninolytic enzymes in cultures of the fungal producers is a subject of constant interest (Kantelinen *et al*., 1989), since the wild strains give extremely low concentrations of these biocatalysts when non–optimized media and culture conditions are used (Tien and Kirk, 1989).

Recently, *C. unicolor* was determined as a new fungal source of extracellular laccase, excreting the enzyme under non–induced conditions with a rate similar to the best laccase producers. Several attempts to increase its production as well a scale up process were undertaken, including optimization of the mediums composition and the physical parameters of the culture (Leonowicz *et al*., 1997; Janusz *et al*., 2007; Rogalski and Janusz, 2010; Rola *et al*., 2013). Reducing the costs of laccase production by optimising the fermentation process in finding effi-

[1] Department of Biochemistry, Maria Curie–Skłodowska University, Akademicka 19 Str., 20–031 Lublin, Poland
[2] Faculty of Chemistry, University of Warsaw, Pasteura 1 Str., 02–093 Warsaw, Poland
* Corresponding Authors (E–mail: ohga@forest.kyushu@u.ac.jp, rogal@hermes.umcs.lublin.pl)

cient and cheap carbon source in the media is the basic research for the industrial applications (Fenice *et al*., 2003; Kachlishvilli *et al*., 2014).

For these reasons the present study was conducted in order to find the best cultural conditions for the overproduction of laccase by *C. unicolor* in shaken flasks and aerated fermentor cultures, and to search for the most effective inducers of enzyme synthesis in the media containing the agricultural waste substances. The information provided by this study will add to the scarce information currently available on the production of laccase by *C. unicolor*.

MATERIALS AND METHODS

Organism

Cerrena unicolor C–139 was obtained from the culture collection of the Regensburg University and deposited in the fungal collection at the Department of Biochemistry (Maria Curie–Skłodowska University, Poland) under the strain number 139. Stock cultures of the fungus were stored at 4°C on malt agar slants. For inoculations, pieces of mycelium overgrowing agar were grown using Lindeberg and Holm (1952) medium (pH 5.5) in stationary conical flasks for 7 days at 28°C. The mycelial mats were subsequently collected, broken in a Waring blender (three times for 15s at 10,000 rpm), and homogenates were used as inoculum in shaking and aerated bioreactor cultures.

Culture Conditions

For the development of a laboratory scale inoculum the bioreactor–scale cultivations were performed at 28°C in a 3.5 L glass fermentor (BioFlo Ⅲ, New Brunswick Scientific, Edison, NY, USA) containing 2.0 L of the optimized Lindeberg–Holm medium (Rola *et al*., 2013). The fermentor equipped with pH, temperature and CO_2 sensors was sterilized (121°C, 40 min) and seeded with mycelial suspension (10% of total volume). The fungal culture was run for 14 days at the aeration rate of 1 L air min^{-1} with air and stirrer speed of 150 rpm. Antifoam B emulsion (Sigma–Aldrich Fine Chemicals, St. Louis, MO, USA) was used periodically to break the foam and the pH was not regulated. The laboratory scale fermentation was done in the 7 L fermentor BioFlow type 115 (New Brunswick Scientific, Edison, NY, USA) The fermentor vessel contained 4 L of the optimized Lindeberg–Holm medium and sterilized at 121°C by 45 min. For the inoculation the mycelial suspension from BioFlo Ⅲ fermentor (0.5 L) was added into the vessel *via* sterilized port. The fungal culture was run for 14 days at the aeration rate of 2 L air min^{-1}, 28°C and stirrer speed of 200 rpm. Antifoam B emulsion, used to break the foam, as well the samples of the medium was taken *via* sterilized ports. The fermentor was equipped with pH–, temperature and dissolved O_2 sensors.

The effect of different carbon sources on laccase synthesis

To study the different waste carbon sources on laccase production, *C. unicolor* was grown in 50 ml wide–mouth Erlenmeyer flasks with 15 ml optimized earlier Lindenberg Holm medium (Janusz *et al*., 2007) containing 10 g/l: Avicell cellulose (Sigma, St.Louis, USA), oat and barley brans (SANTE sp.j.; Warsov, Poland), wheat bran – starch free (Helsingin Mylly Oy, Helsinki), potato starch (POCH, Gliwice Poland), wheat flour (Lubella sp. Z o.o., Lublin, Poland), corn flour (BioPlanet sp. z o.o., Leszno, Poland), oat flour (Symbio s.a., Warsov, Poland), barley flour (Melvit s.a., Warsov, Poland), rice flour (BEZGLUTEN s.a., Koniusza, Poland) 10 kDa dextrane (Polfa, Kutno, Poland), cane molasse (PRO NATURA, Walcz, Poland), sugar beet molasse (Horizon Natuurvoerding BV, IJsselstein, Holland), or whey (Mogador s.r.o., Otrkovice, Czech Republic) instead glucose. The flasks were incubated on orbital rotary shaker Multitron (Inforce HT, Bottmingen/Basel, Switzerland) at 28°C and 160 rev/min for a period of 14 days.

The biomass determination

In order to determine the dynamics of mycelium growth, the after culture fluids were centrifuged at 10,000 x g by 10 min and washed two times by MilliQ water (Millipore, Bedford, USA). Next the mycelia were suspended in 15 ml MilliQ water, disrupted three times during 60 s in an ultrasonic disintegrator (Sonics Vibra–cell, Newtown, USA) and the homogenates were centrifuged at 10,000 x g for 10 min (Okunev *et al*., 1981). In cell–less extracts the content of protein was determined (Bradford, 1976), which was used as a rough index of mycelium growth. The results presented are the mean of mycelium growth obtained in two separate cultivations.

Enzyme Purification

The after culture liquid was centrifuged at 10,000 x g on CEPA LE Benchtop centrifuge (New Brunswick Scientific, Edison, NY, USA) with the flow rate 15 L/h. The supernatant was next concentrated about 25 times on the ultrafiltration system Prep/Scale TFF–6 (0.54 m^2) cartridges (Millipore, Bedford, USA) with PT polyethersulfone membrane (10 kDa cut off), desalted on Sephadex G–25 column, distributed into lyophilisation vials and lyophilised in Labconco FreeZone 12 (Labconco, Kansas, MO, USA).

Laccase Activity and Protein Measurement

Laccase activity in culture supernatant was measured spectrophotometrically at 525 nm in Shimadzu UV–Vis 160A spectrophotometer (Tokyo, Japan) or at BioTek ELx800 Absorbance Microplate Reader (Winooski, USA) controlled by KC–Junior (v. 1.41.8) software using syringaldazine as a substrate (Leonowicz and Grzywnowicz 1981). One nano katal (nkat) of laccase activity was defined as the amount of enzyme catalyzing the production of one nano mol of coloured product (quinone, ε^{M} = 65,000 $M^{-1}cm^{-1}$) per second at 25°C and pH 5.5, and expressed as nano katals per litre of culture (nkat/L). The protein concentration was determined using the Bio–Rad Protein Assay Reagent using bovine serum albumin (BSA) as standard (Bradford, 1974) or fluorometri-

caly on Qubit 2 with Qubit Protein Assay Kit (Ahnert *et al*., 2007).

RESULTS AND DISCUSSION

The white rot fungus *C. unicolor* belong to the one of the most effective extracellular laccase producers in not inductive conditions (Leonowicz *et al*., 2001, Janusz *et al*., 2007; Rola *et al*., 2013). This fungus can use different carbon sources as monosaccharides (glucose, fructose, galactose) as well disaccharides (maltose, fructose, cellobiose, lactose) in cultivation processes. It can suggest the possibility of them the production of β–fructofuranosidase, β–galactosidase, β–glucosidase and α–glucosidase the enzymes acting on α– and β–glicosidic bonds (Rola *et al*., 2013). The starting point of these studies was the selection of different carbon sources as the α– and β–glucans be located in different waste agriculture materials as well as molasses instead glucose in Lindenberg–Holm medium for effective production of extracellular laccase by *C. unicolor*. Titres of laccase were measured for 14 days in shaken flask cultures. The results (Table 1) indicated that the enzyme activity reached its maximum (over 11,000 nkat/l on day 10) in the medium containing potato starch. The high laccase activities according to the control condition (medium with glucose) were also observed for rye bran (10,800 nkat/l; 10 day), outs bran (7,060 nkat/l; 7 day) and cane and sugar beet molasses (6,800 nkat/l at 6 and 10 day respectively). The best carbon source (potato starch) concentration in the medium was next optimized. The concentration of carbon (added as potato starch) varied from 1 to 50 g/l while that of nitrogen (added as L–asparagine) was 1.5 g/L as in (Janusz *et al*., 2007; Rola *et al*., 2013). The results indicated that the enzyme activity reached its maximum (over 25,000 nkat/l on day 14) in cultures containing 50 g/L potato starch as a carbon source (Fig. 1). The lowest starch concentration in the medium affect lowest laccase activities but obtained much faster.

Table 1. Laccase synthesis during *C. unicolor* growth on different 1% carbon sources

Carbon source	*HLA [nkat/l]	Time of HLA [day]	Increase according to glucose [fold]
Maize flour	8560	14	1.57
Rice flour	2410	14	0.44
Rye flour	5660	14	1.03
Out flour	4280	14	0.78
Wheat flour	5400	7	0.98
Potato starch	11040	10	2.00
Oats bran	7060	7	1.28
Rye bran	10800	10	1.96
Wheat bran	1000	12	0.18
Cellulose	1290	10	0.23
Dextran	2060	11	0.36
Cane molasses	6800	6	1.23
Sugar beet molasses	6800	10	1.23
Whey	3500	8	0.64
Wheat straw	2306	11	0.42

*HLA – highest laccase activity

In the next stage of experiments optimization of the laccase production of *C. unicolor* growing in the medium with potato starch (5%) as a carbon source in 3.5 L fermentor at 200 rpm was done. For the inoculation of a 3.5 L fermentor the obtained earlier mycelium from early (5d) expotential growth phase, were used according to (Rola *et al*., 2013). The highest laccase activity was observed in the 6th day of cultivation where the pH drop down to the about 4.0 level (Fig. 2A). Parallel to the control conditions the cultivation with the sequential addition of 10 μM cupric ions doses from 3 to 5 day of growth was made (Fig. 2B). Obtained there activities were 1.5 times higher as in control fermentor and 2.2 times higher as in shaking conditions.

Cupric ions had been reported as the strong stimulants of laccase activity by Giardina *et al*. (1999) and Galhaup *et al*. (2002), in whose experiments up to 50 times higher levels of the enzyme were obtained in induced, compared to non–induced, cultures. The optimal copper dose for the enzyme production by *C. unicolor* C–139 in shaken flask as well fermentor cultures was found to be 10 μM (Janusz *et al*., 2007; Rola *et al*., 2013). The optimal Cu^{2+} dose was significantly lower than that (2.0 mM, added after 4 days of incubation) reported by Galhaup and Haltrich (2001) for submerged cultures of *T. pubescens*, but was still within the range of 2 to 600 μM used in typical cultivation media for the production of laccase both in wild–type and recombinant strains of different basidiomycete fungi (Palmieri *et al*., 2000; Chen *et al*., 2003). It had also been reported (Palmieri *et al*., 2000) that the induction of laccase in *P. ostreatus* occurred when the fungus was cultivated in a nutrient–rich medium supplemented with 150 μM $CuSO_4$ at the time of inoculation.

It is significant that in all shaken flasks and aerated fermentor cultures carried out without pH–regulation (self–control of medium pH by the fungus took place) in

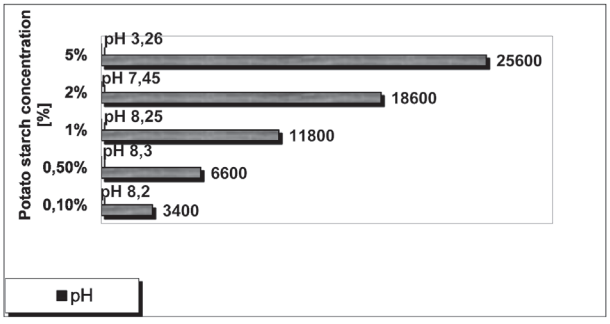

Fig. 1. The relationship between extracellular laccase synthesis and potato starch concentration in the *C. unicolor* cultivation media.
*HLA – highest laccase activity

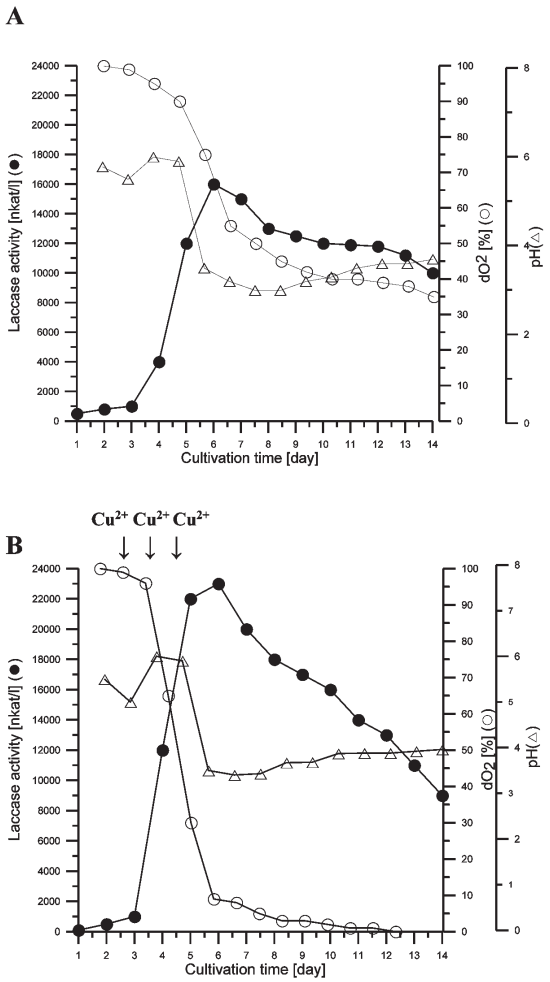

Fig. 2. The course of a typical *C. unicolor* laboratory–scale fermentation in the medium without Cu^{2+} complementation (A) and with Cu^{2+} complementation (B).

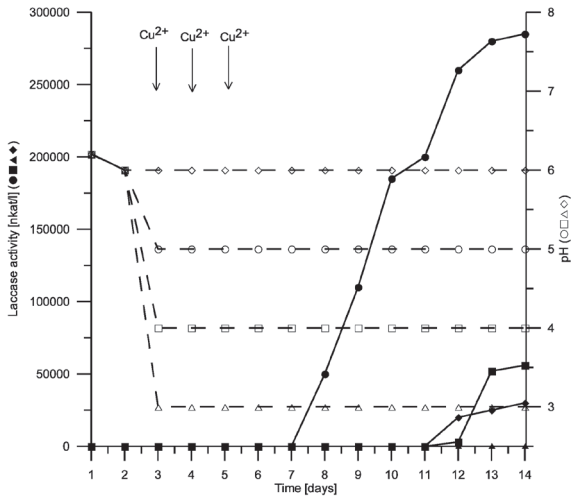

Fig. 3. Effect of stabilisation of medium pH on laccase production by *C. unicolor* in fermenter cultures on Lindenberg–Holm medium. The pH was automatically maintained at a value of 3.0 (△ ; ▲), 4.0 (□ ; ■), 5.0 (○ ; ●) and 6.0 (◇ ; ◆) after 48 h incubation (marked in the chart as horizontal dash lines). Cupric ions (10 μM) were added to the medium after 3, 4 and 5 days of cultivation (arrows show addition of Cu^{2+} into the medium). Cultures conditions: stirrer speed, 200 rev/min; aeration rate, 2 L/min. Open symbols: pH; filled symbols: laccase activities.

media containing mono and disaccharides as the only carbon sources, the fungus alkalized the medium during cultivation, resulting in a pH rise from 5.5 to about 8.0 (Janusz *et al*., 2007; Rola *et al*., 2013). In the case where potato starch was used as the only carbon source it was observed opposite tendency. Grow up of *C. unicolor* laccase activities in the medium were connected with decrease of medium pH to about 4.0 (Fig. 2). Given this, the influence of the stabilization of the medium pH after 48–h incubation on laccase activity in fermentor cultures had to be estimated (Fig. 3) The obtained data show that use of an automatic pH control set at pH 5.0 increased laccase productivity significantly (by 12 times) as compared to that obtained in the fermentor culture with a non–stabilized pH–value. Under the new conditions, the highest enzyme activity of 290,000 nkat/L was reached after 13–day incubation. The maintenance of medium pH on the level of 3.0 was completely unfavorable (no laccase activity was obtained) and that of 4.0 gave a smaller effect (65,000 nkat/L of enzyme was produced) as well as at pH 6.0 than the laccase activity reached 40,000 nkat/L. These different effect of pH stabilization on extracellular laccase activity can be resulted by differential responses to abiotic stress. Moreira *et al*. (2000) using a fermentor with pH fixed at 5.5 during the whole period of incubation obtained the highest effective production of this enzyme in *Bjerkandera sp*. Also, Nűske *et al*. (2002) using a cyclic batch system for MnP production (by *Nematoloma frowardii* and *Clitocybula dusenii*) as well as Koroleva *et al*. (2002) in the *Coriolus hirsutus* cultivation obtained the similar results.

Next the right moment of pH stabilization was deter-

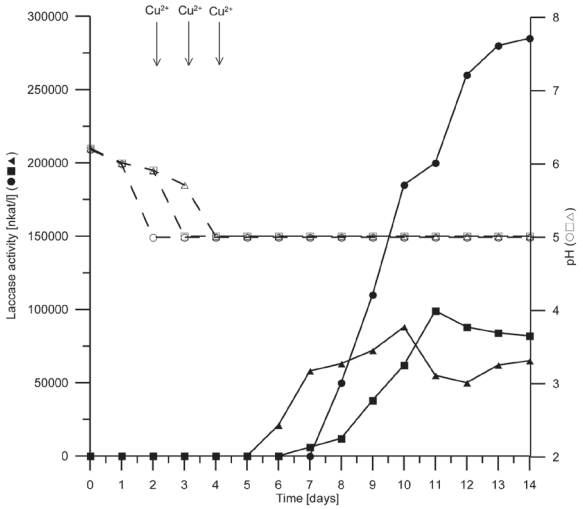

Fig. 4. Time course of laccase production (●, ■, ▲) during fermentor cultures of *C. unicolor* in the Lindeberg–Holm medium. The medium pH was automatically maintained at a value of 5.0 (marked in the chart as a solid line) after 48 (○), 72 (□) and 96 h (△) of incubation (dashed lines show the days on which the medium pH was adjusted to the adequate value). Cupric ions (10 μM) were added to the medium after 3–5 days of cultivation (arrows show addition of Cu^{2+} into the medium). Culture conditions: stirrer speed, 200 rev/min; aeration rate 2 L/min.

mined (Fig. 4). As discussed above, the best laccase productivity (290,000 nkat/L) was recorded when the maintenance of medium pH on the level of 5.0 was performed on the second day of cultivation. The obtained laccase activity was 10 times higher that when *C. unicolor* had been grown in the original non optimised conditions (Rogalski *et al.*, 1999). The maximal laccase activities are connected with reaching the stationary growing phase. The after culture fluid showing the highest laccase activities were taken out from the fermentor and centrifuged for removing the mycelium. The supernatants containing laccase were next concentrated by ultrafiltaration, purified by ion–exchange chromatography on DEAE–Sepharose (fast flow) and liophylized to obtain the enzyme preparation.

CONCLUSION

Summarizing, the data presented here imply that the tested *C. unicolor* strain can synthesis the high active preparation in submerged and fermentor cultures containing potato starch as only carbon source. The study also shows that an appropriate combination of culture conditions (*i.e.* a C– and N– sufficient medium) combined with supplementation with an adequate dose of Cu^{2+} ions supply on a yield of laccase production. In fermentor cultures, the maintance of medium pH at the optimized level increase laccase productivity 12 times.

ACKNOWLEDGEMENTS

This work was supported by the research program "Tailored Lipidic Mesophases as Novel Functional Nanomaterials in Bioenergetics and Biosensing" under the framework of Polish–Swiss Research Programme No. PSPB–079/2010, The National Center for Research and Development (NCBiR), grant NR05–0017–10/2010 (PBR–11) and the research program BS/UMCS.

REFERENCES

Barrett F. M. 1991 Phenoloxidases and the integument. *In* "Physiology of the insect epidermis", ed. by K. Binnington and A. Retnakaren, East Melbourne, Vic., Australia, CSIRO Publ., pp. 195–212

Bradford M. M. 1976 A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding, *Anal. Biochem.*, **72**: 248–254

Duran N., M. A. Rosa, A. D. Annibale and L. Gianfreda 2002 Applications of laccases and tyrosinases (phenoloxidases) immobilized on different supports: a review, *Enzyme Microb. Technol.*, **31**: 907–931

Fenice M., G. G. Sermanni, F. Federici and A. D'Annibale 2003 Submarged and solid–state production of laccase and Mn–peroxidase by *Panus tigrinus* on olive mill wastewater–based media. *J. Biotechnol.*, **100**: 77–85

Gianfreda L., F. Xu and J.–M. Bollag 1999 Laccases: a useful group of oxidoreductive enzymes. *Biorem. J.*, **3**: 1–25

Giardina P., V. Faraco, C. Pezzella, A. Piscitelli, S. Vanhulle and G. Sannia 2010 Laccase: a never–ending story. *Cell.Mol. Life Sci.*, **67**: 369–385

Givaudan A., A. Effosse, D. Faure, P. Portier, M.–L. Bouillant and R. Bally 1993 Polyphenol oxidase in *Azospirillum lipoferum* isolated from rice rhizosphere: Evidence for laccase activity in non–motile strains of *Azospirillum lipoferum. FEMS Microbiol. Lett.*, **108**: 205–210

Janusz G., J. Rogalski and J. Szczodrak 2007 Increased production of laccase by *Cerrena unicolor* in submerged liquid cultures. *World J. Microb. Biotechnol.*, **23**: 1459–1464

Jönsson L. J., M. Saloheimo and M. Penttilä 1997 Laccase from the white–rot fungus *Trametes versicolor*: cDNA cloning of *lcc1* and expression in *Pichia pastoris. Curr. Genet.*, **32**: 425–430

Kashlishvilli E., E. Metieveli and V. Ekisashvilli 2014 Modulation of *Cerrena unicolor* laccase and manganese peroxidase production. *Springer Plus,* **3**: 463 (doi:10.1186/2193–1801–3–463)

Kantelinen A., A. Hattaka and L. Viikari 1989 Production of lignin peroxidase and laccase by *Phlebia radiate*. *Appl. Microbiol. Biotechnol.*, **31**: 234–239

Koroleva O. V., E. V. Stepanova, V. P. Gavrilova, N. S. Yakovleva, E. O. Landesman, I. S. Yavmetdinov and A. I. Yaropolov 2002 Laccase and Mn–peroxidase production by *Coriolus hirsutus* strain 075 in a jar fermentor. *J. Biosci. Bioeng.*, **93**: 449–455

Leonowicz A., L. Gianfreda, J. Rogalski, M. Jaszek, J. Luterek, M. Wojtaś–Wasilewska, E. Malarczyk, A. Dawidowicz, M. Fink–Boots, G. Ginalska, M. Staszczak and N–S. Cho 1997 Appearance of laccase in wood–rotting fungi and its inducibility. *J. Kor. Wood Sc., Technol.* **25**: 29–36

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

Leonowicz A., A. Matuszewska, J. Luterek, D. Ziegenhagen, M. Wojtas–Wasilewska, M. Hofrichter, J. Rogalski and N.–S. Cho 1999 White–rot fungi as lignin degraders. *Proc. Internat'l Conf. For. Sci., Chungbuk Nat. Univ., Chengju, Korea*, **27**: 175–193

Leontievsky A., N. Myasedova, B. Baskunov, N. Pozdnyakova, T. Vares, N. Kalkkinen, A. Hatakka and L. Golovieva 1999 Reaction of blue and yellow fungal laccases with lignin model compound. *Biochemistry,* **64**: 1150–1156

Lindeberg G. and G. Holm 1952 Occurrence of tyrosinase and laccase in fruit bodies and mycelia of some *Hymenomycetes Physiol. Plant,* **5**: 100–114

Mayer, A. M. and Staples,R. C. (2002) Laccase: new function for an old enzyme. *Phytochemistry,* **60**: 551–565

Minussi R. C., G. M. Pastore and N. Duran 2002 Potential applications of laccase in the food industry. *Tr. Food Sci. Technol.*, **13**: 205–216

Moreira M. T., A. Torrado, G. Feijoo and J. M. Lema 2000 Manganese peroxidase production by *Bjerkandera* sp. BOS55. 2. Operation in stirrer tank reactors. *Bioprocess Eng.*, **23**: 657–661

Nüske J., K. Scheibner, U. Dornberger, R. Ullrich and M. Hofrichter 2002 Large scale production of manganese–peroxidase using agaric white–rot fungi. *Enzyme Microbial Technol.*, **30**: 556–561

Okunev O. N., T. I. Bilai, E. G. Musich and E. L. Golovlev 1981 Obrazovanie tsellylulaz plesnevymi gribami pri roste na tsellyulozosoderzhashchikh substratakh. *Prikl. Biokhim. Mikrobiol.*, **17**: 408–413

Palmieri G., P. Giardina, C. Bianco, A. Scaloni, A. Capasso and G. Sannia 1997 A novel white laccase from *Pleurotus ostreatus. J. Biol. Chem.*, **272**: 31301–31307

Rogalski J. and G. Janusz 2010 Purification of extracellular laccase from *Cerrena unicolor. Prep. Bioch. Biotechnol.*, **40**: 242–255

Rola B., M. Karaskiewicz, D. Majdecka, I. Mazur, R. Bilewicz, J. Rogalski and S. Ohga 2013 Scale up of *Cerrena unicolor* laccase production. *J. Fac. Agr., Kyushu Univ.*, **58**: 231–238

Shleev S., J. Tkac, A. Christenson, T. Ruzgas, A.I. Yaropolov, J. W. Whittaker and L. Gorton 2005 Direct electron transfer between copper–containing proteins and electrodes. *Biosens. Bioelectron.*, **20**: 2517–2554

Solomon E. I., U. M. Sundaram and T. E. Machonkin 1996

Multicopper oxidases and oxygenases. *Chem. Rev*., **96**: 2563–2605

Tien M. and T. K. Kirk 1984 Lignin–degrading enzyme from *Phanerochaete* chrysosporium: Purification, characterization, and catalytic properties of a unique H_2O_2–requiring oxygenase. *Proc. Natl. A cad. Sci. USA,* **81**: 2280–2284

Viswanath B., M.S. Chandra, H. Pallavi and B. Rajasekhar–Reddy 2008 Screening and assessment of laccase producing fungi isolated from different environmental samples. *Afr. J. Biotechnol.*, **7**: 1129–1133

Widstein P. and A. Kandelbauer 2008 Laccase applications in the forest products industry: A review. *Enzyme Microbiol. Technol*., **42**: 293–307