J Ind Microbiol Biotechnol (2003) 30: 536–541
DOI 10.1007/s10295-003-0077-6

ORIGINAL PAPER

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Optimization of manganese peroxidase and laccase production in the South American fungus *Fomes sclerodermeus* (Lév.) Cke

Received: 4 March 2002 / Accepted: 2 June 2003 / Published online: 5 August 2003

Abstract *Fomes sclerodermeus* produces manganese peroxidase (MnP) and laccase as part of its ligninolytic system. A Doehlert experimental design was applied in order to find the optimum conditions for MnP and laccase production. The factors studied were Cu^{2+}, Mn^{2+} and asparagine. The present model and data analysis allowed us not only to define optimal media for production of both laccase and MnP, but also to show the combined effects between the factors. MnP was strongly influenced by Mn^{2+}, which acts as an inducer. Under these conditions Cu^{2+} negatively affected MnP activity. At 13 days of growth 0.75 U ml^{-1} were produced in the optimized culture medium supplemented with 1 mM $MnSO_4$ and 4 g l^{-1} asparagine. The laccase titer under optimized conditions reached maximum values at 16 days of growth: 13.5 U ml^{-1} in the presence of 0.2 mM $CuSO_4$, 0.4 mM $MnSO_4$ and 6 g l^{-1} asparagine. Mn^{2+} promoted production of both enzymes. There were important interactions among the nutrients evaluated, the most significant being those between Cu^{2+} and asparagine.

Keywords *Fomes sclerodermeus* · Laccase · Manganese peroxidase · Statistical experimental design

Introduction

Lignin, the most abundant aromatic polymer on earth, can be depolymerized and mineralized efficiently only by wood-decomposing members of the basidiomycetes, particularly those that cause white rot in wood [18]. These organisms have a ligninase complex (LC) that is composed of at least three enzymes: lignin peroxidase (LiP) (E.C:1.11.1.14) [40], manganese-dependent peroxidase (MnP) (E.C:1.11.1.13) [10] and laccase (E.C:1.10.3.2). LiP is a heme protein with a high oxidation potential, and can oxidize phenolic and non-phenolic substrates [1,25]. MnP and LiP have been extensively studied in *Phanerochaete chrysosporium* and this organism was proposed as a model of ligninolytic fungi [34]. MnP was considered unable to oxidize non-phenolic lignin substrates, but its capacity to depolymerize synthetic [42] or natural [12] lignins in vitro, and the coupling mechanism between lipid peroxidation and destruction of a non-phenolic β-*O*-4-linked lignin model compound have been shown [16]. The laccase enzyme belongs to a family of multicopper oxidases, which preferentially oxidize phenolic lignin dimers [20]. Laccase oxidizes model lignin compounds if appropriate primary substrates such as 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), violuric acid [35] or 1-hydroxybenzotriazole are present [4]. Under these conditions laccase can oxidize substrates that are restrictive to LiP production, such as veratryl alcohol [4] or polycyclic aromatic hydrocarbons [13].

Rarely are these three enzymes present in the same organism, and different combinations of them can operate. The LC is frequently produced during secondary metabolism but different species have particular responses to nutrients. In *P. chrysosporium*, LiP and MnP activities are completely suppressed under conditions of excess nitrogen and carbon [19,30]. On the other hand, *Bjerkandera adusta* is a nitrogen-deregulated organism producing high MnP activities under high nitrogen concentrations [14]. Mn^{2+} is another metabolite implicated in LC regulation and has a triple role: (1) as an essential cofactor for the proper functioning of the MnP protein—Mn^{2+} is oxidized to Mn^{3+}, which in turn oxidizes the substrates [11]; (2) as a regulator of MnP expression by activating transcription of the *mnp* gene [5] and, (3) as an inhibitor of the production of veratryl alcohol, which

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is highly correlated with LiP titers [24]. It has been demonstrated that MnP and laccase could act synergistically in lignin degradation [9]. Therefore, organisms able to produce high amounts of both enzymes are interesting in view of their potential importance in processes like bioremediation [41], biobleaching of pulp paper [2,17], or degradation and detoxification of recalcitrant substances [31].

Fomes sclerodermeus BAFC 2752 is a South American white-rot fungus that is able to produce MnP and laccase activities [27]. In the present work, the effect of Cu^{2+}, asparagine and Mn^{2+} concentrations on the production of these enzymes and their possible interactions were studied.

Materials and methods

Organism

F. sclerodermeus, strain BAFC 2752 belongs to the culture collection of the Faculty of Exact and Natural Sciences, University of Buenos Aires, Argentina.

Basal medium and culture conditions

The basal medium contained glucose, 15 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; KH_2PO_4, 0.5 g; K_2HPO_4, 0.6 g; $CuSO_4 \cdot 5H_2O$, 0.4 mg; $MnCl_2 \cdot 4H_2O$, 0.09 mg; H_3BO_3, 0.07 mg; $Na_2MoO_4 \cdot 2H_2O$, 0.02 mg; $FeCl_3$, 1 mg; $ZnCl_2$, 3.5 mg; thiamine hydrochloride, 0.1 mg; asparagine monohydrate, variable; and distilled water up to 1 l. The final pH was adjusted to 6.4. Copper and manganese basal concentrations were 1.6 μM and 4.5 μM, respectively. All chemicals were of analytical grade and were used without further purification.

Two 0.25 cm^2 agar plugs cut out from the margin of a 5-day-old colony were transferred to 250-ml Erlenmeyer flasks containing 25 ml medium. Incubation was at 28°C under stationary conditions. Cultures were harvested by vacuum filtration and mycelial dry weights were determined after drying the mycelial mats at 80°C to constant weight. The culture supernatants were used as enzyme sources.

Enzyme assays

All enzyme activities were measured spectrophotometrically at 30°C. Laccase activity was determined by using 5 mM ABTS as a substrate dissolved in 0.1 M sodium acetate buffer (pH 5) and the appropriate volume of enzyme. The products of the reaction were measured at 420 nm ($\epsilon_{420} = 36\ mM^{-1}\ cm^{-1}$) [3]. MnP was determined by using 0.01% phenol red as a substrate dissolved in 50 mM sodium dimethylsuccinate buffer (pH 4.5) and the appropriate volume of enzyme. The products of the reaction were measured at 610 nm ($\epsilon_{610} = 22\ mM^{-1}\ cm^{-1}$) [10]. The reaction was initiated by addition of hydrogen peroxide (0.1 mM final concentration). One enzyme unit was defined as 1.0 μmol product formed per minute under the assay conditions.

Experimental design

A Doehlert uniform shell design was applied to study the effects of Mn^{2+} (added as $MnSO_4$), Cu^{2+} (added as $CuSO_4$) and asparagine (N) on MnP and laccase production. The levels and sampling days were selected according to preliminary studies [23,27]. Results were analyzed using the software STATISTICA 5.1 (StatSoft, Tulsa, Okla.). To study the effect of three independent variables, Doehlert proposed an experimental design based on 13 combinations of the three variables studied. The equally spaced values of each independent variable and the combination between them were adopted and coded following the Doehlert design [7]. The coded (in brackets) and real values are shown in Table 1. Specific activity was defined as the total volumetric activity in the flask per milligram of dry mycelium. The responses measured were specific activities of MnP and laccase (U mg^{-1} mycelium) in culture supernatants at 8 and 12 days of growth. The values are the mean of triplicate cultures. The processing of data was performed by direct curvilinear regression, without any prior transformation. The observed values were fitted to a full quadratic equation model with 10 coefficients (Eq.1) [37]:

$$F = b_0 + b_1X + b_2X^2 + b_3Y + b_4Y^2 + b_5Z + b_6Z^2 + b_7XY + b_8XZ + b_9YZ \quad (1)$$

where F is the variable response and b_i the regression coefficients given by the model; X, Y and Z, are the independent coded variables.

Results and discussion

Preliminary studies of MnP and laccase activities in *F. sclerodermeus* showed that this fungus is able to produce both enzymes in medium with either high or low concentrations of asparagine. In addition, it produces a fast decolorization of Azure-B and Poly R-478 on agar plates [27]. In this work, *F. sclerodermeus* produced MnP and laccase under all the conditions studied (Table 2). The concentrations of Cu^{2+}, Mn^{2+} and N influenced growth as well as MnP and laccase activities (specific and volumetric). Only data obtained in the 12-day-old cultures were analyzed and shown because they were higher in both activities compared to values at the 8th day.

Different combinations of Cu^{2+}, Mn^{2+} and N affected growth. Similar results have been reported in *Pleurotus sajor-caju* and *Lentinula edodes*, where Mn^{2+} at concentrations of 0.55 mM and above had an inhibitory effect on fungal growth, which was 100% and 58%

Table 1 The real and coded (in parentheses) values adopted for the independent variables

Variable	Values
$CuSO_4$ (mM)	0 (−1), 0.05 (−0.5), 0.1 (0), 0.15 (0.5), 0.2 (1)
$MnSO_4$(mM)	0.4 (−0.866), 0.5 (−0.577), 0.6 (−0.289), 0.7 (0), 0.8 (0.289), 0.9 (0.577), 1 (0.866)
Asparagine monohydrate (g l^{-1})	4 (−0.816), 5(0), 6 (0.816)

Table 2 Volumetric and specific (*sp*) activities and mycelial dry weight in *Fomes sclerodermeus* at 12 days of growth in liquid static cultures. *N* Asparagine, *MnP* manganese-dependent peroxidase

Run	N g l^{-1}	Mn^{2+} mM	Cu^{2+} mM	MnP mU ml^{-1}	Laccase mU ml^{-1}	Dry weight mg	MnP (sp) mU mg^{-1}	Laccase (sp) mU mg^{-1}
1	5	0.7	0.1	85.20	4,640	72.7	29.30	1,595.75
2	5	0.7	0.2	81.11	6,150	72.65	27.91	2,116.63
3	5	1	0.15	113.19	5,436	62.4	45.35	2,177.80
4	6	0.8	0.15	214.87	9,489	79.7	67.40	2,976.68
5	5	0.7	0	212.60	2,508	84.5	62.89	742.25
6	5	0.4	0.05	19.14	3,295	82.5	5.80	998.67
7	5	1	0.05	147.70	6,748	67.2	54.94	2,510.25
8	5	0.4	0.15	20.88	5,986	67.8	7.69	2,207.28
9	4	0.6	0.05	99.98	3,867	88.95	28.10	1,086.88
10	6	0.8	0.05	137.71	2,581	90.05	38.23	716.45
11	4	0.6	0.15	24.68	5,343	67.8	9.10	1,970.28
12	6	0.5	0.1	77.79	5,752	73.95	26.29	1,323.30
13	4	0.9	0.1	98.96	7,402	71.3	34.69	2,595.38

respectively, compared to unsupplemented cultures [6,8]. For *F. sclerodermeus* only some mild negative effects were recorded. The increase from 0.4 to 0.8 mM Mn^{2+} (runs 8 and 10) produced a higher dry weight (25%), although an increase from 0.4 to 1 mM Mn^{2+} but in a different nutrient combination (runs 6 and 7) caused an inhibitory effect (18%). These results showed that Mn^{2+} alone did not cause an inhibitory or enhanced effect on growth, but that there might be interactions between the nutrients that account for the differences in dry weight. When the effect on MnP and laccase was analyzed, the responses (negative or positive effect) were more variable, and greater than those observed on growth. The volumetric MnP activity was enhanced with high Mn^{2+} concentrations. Runs 6 and 7 showed that an increase of Mn^{2+} from 0.4 to 1 mM corresponded to an 8-fold increase in the MnP production, these values representing the lowest and the highest production respectively. The highest laccase production was found at the highest Cu^{2+} concentrations.

The second-order polynomial equation

Because of the physiological effect that the nutrients had on growth, and to avoid interference of possible biomass-dependent activities, it was necessary to calculate the activities per milligram of mycelium as the response variables. It has been demonstrated in *Telephora terrestris* that secretion of laccase is biomass dependent [15].

The uniform design of the experiment includes different concentrations and combinations of the nutrients to be tested. To account for the effect of each nutrient, the specific activity data were fitted to a full quadratic equation (Eq. 1) including the variables and combinations thereof, which could explain the effects observed. This was the general form of the polynomial model for three factors [37].

The 13 responses for each activity were adjusted to the equation, and the coefficients are shown in Table 3.

Table 3 Regression coefficients given by the model and the r^2 values for each equation

Coefficient	MnP[a]	Laccase[b]
C	26.44	1,830.25
Mn^{2+}	24.23	495.49
$(Mn^{2+})^2$	−4.36	383.11
Cu^{2+}	−8.32	846.06
$(Cu^{2+})^2$	19.21	−420.37
N	12.12	19.35
N^2	7.32	−252.25
N Mn^{2+}	−1.10	417.19
N Cu^{2+}	41.60	1,158.75
Mn^{2+} Cu^{2+}	−6.63	−889.75
r^2	0.91	0.86

[a]Specific activity of MnP $= 26.44 + 24.23\ Mn^{2+} - 4.36\ (Mn^{2+})^2 - 8.32Cu^{2+} + 19.21\ (Cu^{2+})^2 + 12.12\ N + 7.32\ N^2 - 1.1NMn^{2+} + 41.6NCu^{2+} - 6.63Mn^{2+}Cu^{2+}$

[b]Specific activity of laccase $= 1{,}830.25 + 495.49\ Mn^{2+} + 383.11\ (Mn^{2+})^2 + 846.06\ Cu^{2+} - 420.37(Cu^{2+})^2 + 19.35\ N - 252.25\ N^2 + 417.19NMn^{2+} + 1{,}158.75\ NCu^{2+} - 889.75\ Mn^{2+}\ Cu^{2+}$

A large estimate (either positive or negative) indicates that a factor has a large impact on titer, while an estimate close to zero means that a factor has little or no effect [37].

The high values of r^2, together with the significant effects, provide strong evidence that the model accurately reflects the process. The values of r^2 for MnP and laccase were 0.91 and 0.86, respectively. This means that 91% and 86% of the variability in specific activity observed can be accounted for by the second-order polynomial equation prediction. It was taken as evidence of the applicability of the model within the range of variables included in this work. The present model and data analysis allowed us not only to define optimal media composition for both specific activity of MnP and laccase production, but it also showed the combined effects among the three factors studied. These enzymes could be related in their regulation as MnP and laccase are both implicated in the process of lignin degradation. It was shown that xenobiotics regulate *mnp* and laccase genes in *Ceriporiopsis subvermispora* [39] and *P. sajor-caju*

[21,36], respectively. However, it remains to be determined if there is coordinate regulation of both enzymes within the same organism.

Manganese peroxidase

Coefficients for the regression equation are shown in Table 3. The large value for Mn^{2+} concentration in the linear term (24.23) illustrates the significant, positive effect of this mineral. This positive linear coefficient indicates that specific activity of MnP increased with increasing concentration of Mn^{2+}, whereas the negative quadratic coefficient of −4.36 indicates that the specific activity of MnP had reached a maximum and from this point the activity decreased. This showed that Mn^{2+} (from 0.4 to 1 mM) strongly increased the production and registered a weak inhibitory or neutral zone. The known white-rot fungus *Phanerochaete sordida* produced MnP only in the presence of Mn^{2+} and showed the highest activity at 0.2 mM. Addition of higher concentrations of Mn^{2+} to the culture medium did not produce a further increase in MnP levels [32]. Conversely, the MnP from *Pleurotus eryngii* was completely repressed by Mn^{2+} [22]. In all flasks, a darkening of the mycelial pellets was observed, which was probably due to precipitation of MnO_2 in cultures with MnP activity, as observed in *P. chrysosporium* [28]. The biodegradation of wood in nature is often accompanied by black depositions. Nitrogen had a positive linear effect, indicating an increase in specific activity with N concentration. An inhibitory effect by Cu^{2+} was suggested by its negative linear effect. The positive quadratic term indicates the existence of a minimum for this specific activity. The more important interactions were between Cu^{2+} and N; the positive value indicated the synergistic effect. A negative or antagonistic interaction between Cu^{2+} and Mn^{2+} was observed.

The equation found for MnP was plotted as a function of Mn^{2+} and Cu^{2+}; the N factor was fixed at concentrations of 4, 5 and 6 g l^{-1}. At 4 g l^{-1} N, MnP reached maximum values and the contour plot is shown in Fig. 1a. At the other two concentrations production was lower and the plots are not shown. The zone with the highest MnP production is shadowed on the figure. It was evident that Mn^{2+} was necessary to obtain high MnP production, Cu^{2+} being an inhibitor under these conditions. The highest titers obtained were 82.5 mU mg^{-1}.

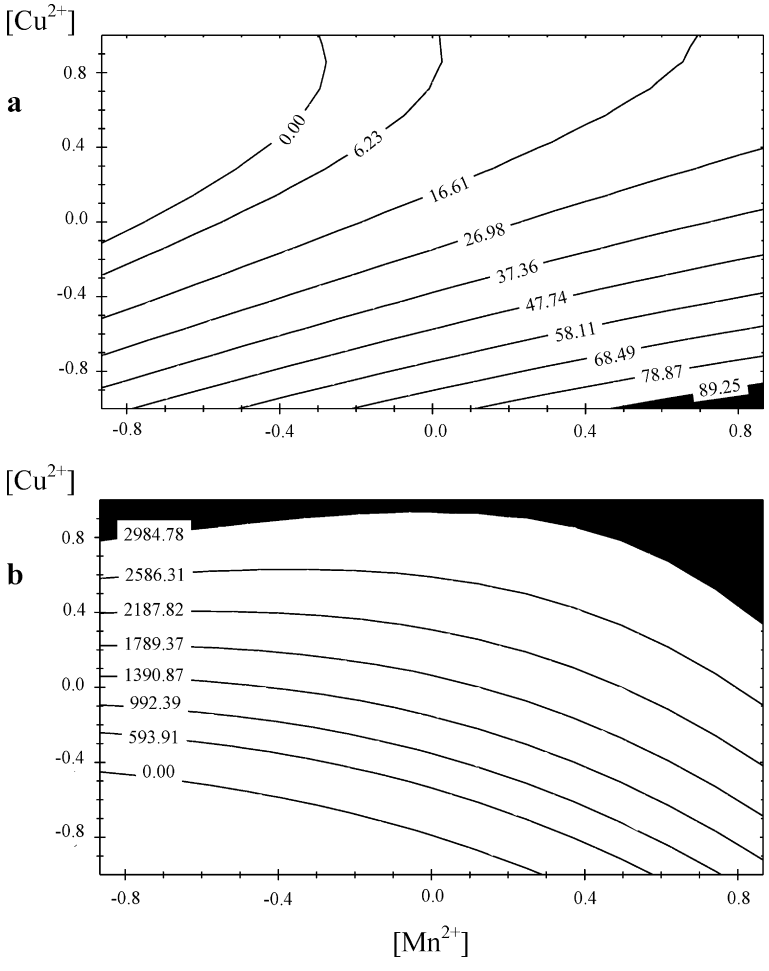

Fig. 1 Contour plots based on the equations shown in Table 3 of specific activities of manganese-dependent peroxidase (MnP) (**a**) and laccase (**b**). Cu^{2+} and Mn^{2+} concentrations are coded according to Table 1. *Shadowed zones* determine the conditions that optimized the production of each activity

Laccase

Coefficients for the regression equation are shown in Table 3. The large value for Cu^{2+} concentration in the linear term (846.06) indicated the very significant effect of this mineral. This showed that the specific activity increased as Cu^{2+} was increased. The negative quadratic term showed the existence of a maximum of laccase activity as function of Cu^{2+} concentration. Following this point, Cu^{2+} had an inhibitory effect. Compounds employed for laccase induction in other fungi, like xylidine, and other aromatic compounds did not increase the activity as Cu^{2+} did in this work [26,29]. The linear coefficient for N is close to zero; the quadratic term is negative, so there was a maximum for this nutrient. These results showed that N had a small effect on laccase activity. A similar result was shown in *P. sajor-caju* [8]. The Mn^{2+} concentration had a positive linear effect, the specific activity of laccase increased as Mn^{2+} increased. Similar results have been reported for other white-rot fungi [33,38]. Mn^{2+} is present in virtually all woods, therefore the positive regulation by Mn^{2+} of both ligninolytic enzymes could be a strategy for lignin degradation in vivo, suggesting a link in the regulation of both enzymes. There were important interactions among the three nutrients evaluated, the highest value corresponded to the pair N and Cu^{2+} (1158.75); this indicated that these nutrients acted synergistically to increase the specific activity.

The contour plot for laccase production is shown in Fig. 1b. The criterion used for plotting laccase specific activity was the same as that for MnP specific activity. The highest production (shadowed zone) was found at high N concentration (6 g l^{-1}; ~80 mM nitrogen). In *Pycnoporus sanguineus* the optimum medium for laccase production contained 2.4 mM nitrogen [29]. In contrast, *L. edodes* showed enhancement of laccase production at high nitrogen (26 mM) [6].

Predicted optimum

Culture media optimized for laccase and MnP production were tested. For laccase production, the medium was supplemented with 0.2 mM Cu^{2+}, 6 g l^{-1} N and 0.4 mM Mn^{2+}. A maximum titer of 13.5 U ml^{-1} was reached at 16 days of growth (Fig. 2). After 12 days the specific activity was $4{,}050 \pm 375$ mU mg^{-1}, the equation given by the model predicted a value of 3,383.26 mU mg^{-1}. In the case of MnP, the medium contained 1 mM Mn^{2+}, 4 g l^{-1} N and 1.6 μM Cu^{2+}. The maximum volumetric activity (0.75 U ml^{-1}), as seen in Fig. 2, was obtained at 13 days of growth, this value was similar to that obtained in *B. adusta* (0.9–1.25 U ml^{-1}) grown under optimized conditions [23]. Taking into account biomass, MnP specific activity at 12 days of growth was 90 ± 5 mU mg^{-1}, while the predicted value was 99.63 U mg^{-1}. Therefore the model was able to describe the effect of the nutrients studied on both activities within the range of concentrations fixed for the three independent variables that were the limits of applicability of the model. It is possible to predict the response to any combination of independent variables.

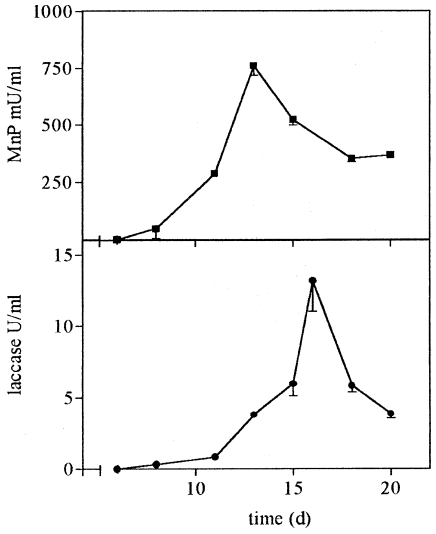

Fig. 2 Time course of MnP and laccase activities in liquid static cultures of *Fomes sclerodermeus* under the conditions optimized for each activity. Mean of culture triplicates and SEM are shown

F. sclerodermeus produced high titers of both MnP and laccase in media optimized using Doehlert experimental design.

Acknowledgements To CONICET and Universidad de Buenos Aires for financial support.

References

1. Banci L, Ciofi-Baffoni S, Tien M (1999) Lignin and Mn peroxidase-catalyzed oxidation of phenolic lignin oligomers. Biochemistry 38:3205–3210
2. Bergbauer M, Eggert C (1994) Degradability of chlorine-free bleachery effluent lignins by two fungi: effects on lignin subunit type and on polymer molecular weight. Can J Microbiol 40:192–197
3. Bourbonnais R, Paice MG, Reid ID, Lanthier P, Yaguchi M (1995) Lignin oxidation by laccase isozymes from *Trametes versicolor* and role of the mediator 2,2′-azino-bis (ethylbenzothiazoline-6-sulfonic acid) in Kraft lignin depolymerization. Appl Environ Microbiol 61:1876–1880
4. Bourbonnais R, Paice MG, Freiermuth B, Bodie E, Borneman S (1997) Reactivities of various mediators and laccases with kraft pulp and lignin model compounds. Appl Environ Microbiol 63:4627–4632
5. Brown JA, Glenn JK, Gold MH (1990) Manganese regulates expression of manganese peroxidase by *Phanerochaete chrysosporium*. J Bacteriol 172:3125–3130
6. Buswell JA, Cai YJ, Chang Y (1995) Effect of nutrient nitrogen and manganese on manganese peroxidase and laccase production by *Lentinula* (*Lentinus*) *edodes*. FEMS Microbiol Lett 128:81–88
7. Doehlert DH (1970) Uniforms shell designs. Appl Stat 19: 231–239
8. Fu SY, Yu HS, Buswell JA (1997) Effect of nutrient nitrogen and manganese on manganese peroxidase and laccase production by *Pleurotus sajor-caju*. FEMS Microbiol Lett 147:133–137

9. Galliano H, Gas G, Seris JL, Boudet AM (1991) Lignin degradation by *Rigidoporus lignosus* involves synergistic action of two oxidizing enzymes: Mn peroxidase and laccase. Enzyme Microb Technol 13:478–482
10. Glenn JK, Gold MH (1985) Purification and characterisation of an extracellular Mn(II)-dependent peroxidase from the lignin degrading basidiomycete *Phanerochaete chrysosporium*. Arch Biochem Biophys 242:329–341
11. Glenn JK, Akileswaran L, Gold MH (1986) Mn(II) oxidation is the principal function of the extracellular Mn- peroxidase from *Phanerochaete chrysosporium*. Arch Biochem Biophys 251:688–696
12. Hofrichter A, Lundell T, Hatakka A (2001) Conversion of milled pine wood by manganese peroxidase from *Phlebia radiata*. Appl Environ Microbiol 67:4588–4593
13. Johannes C, Majcherczyk A, Hüttermann A (1996) Degradation of anthracene by laccase of *Trametes versicolor* in the presence of different mediator compounds. Appl Microbiol Biotechnol 46:313–317
14. Kaal EE, Jong E, Field JA (1993) Stimulation of ligninolytic peroxidase activity by nitrogen nutrients in the white rot fungus *Bjerkandera* sp strain BOS 55. Appl Environ Microbiol 59:4031–4036
15. Kanunfre CC, Zancan GT (1998) Physiology of exolaccase production by *Telephora terrestris*. FEMS Microbiol Lett 16:151
16. Kapich A, Hofrichter M, Vares T, Hatakka A (1999) Coupling of manganese peroxidase-mediated lipid peroxidation with destruction of nonphenolic lignin model compounds and ^{14}C-labeled lignins. Biochem Biophys Res Commun 259:212–219
17. Karlsson S, Holmbom B, Spetz P, Mustranta A, Buchert J (2001) Reactivity of Trametes laccases with fatty and resin acids. Appl Microbiol Biotechnol 55:317–320
18. Kirk TK, Shimada M (1985) Lignin biodegradation: the microorganisms involved and the physiology and biochemistry of degradation by white-rot fungi. In: Higuchi T (ed) Biosynthesis and biodegradation of wood components. Academic Press, San Diego, pp 579–605
19. Li D, Alic M, Gold MH (1994) Nitrogen regulation of lignin peroxidase gene transcription. Appl Environ Microbiol 60:3447–3449
20. Li K, Xu F, Eriksson KE (1999) Comparison of fungal laccases and redox mediators in oxidation of a nonphenolic lignin model compound. Appl Environ Microbiol 65:2654–2660
21. Lo SC, Ho YS, Buswell JA (2001) Effect of phenolic monomers on the production of laccases by the edible mushroom *Pleurotus sajor-caju*, and partial characterization of a major laccase component. Mycologia 93:413–421
22. Martínez MJ, Ruiz-Dueñas FJ, Guillén F, Martínez AT (1996) Purification and catalytic properties of two manganese peroxidase isoenzymes from *Pleurotus eryngii*. Eur J Biochem 237:424–432
23. Mester T, Field JA (1997) Optimization of manganese peroxidase production by the white rot fungus *Bjerkandera* sp strain BOS 55. FEMS Microbiol Lett 155:161–168
24. Mester T, de Jong E, Field JA (1995) Manganese regulation of veratryl alcohol in white rot fungi and its indirect effect on lignin peroxidase. Appl Environ Microbiol 61:1881–1887
25. Mester T, Ambert-Balay K, Ciofi-Baffoni S, Banci L, Jones AD, Tien M (2001) Oxidation of a tetrameric nonphenolic lignin model compound by lignin peroxidase. J Biol Chem 276:22985–22990
26. Muñoz C, Guillén F, Martínez AT, Martínez MJ (1997) Induction and characterization of laccase in the ligninolytic fungus *Pleurotus eryngii*. Curr Microbiol 34:1–5
27. Papinutti VL, Forchiassin F (2000) Enzimas de hongos de pudrición blanca involucradas en la degradación de lignina. Rev Arg Microbiol 32:83–88
28. Pérez J, Jeffries TW (1992) Roles of manganese and organic acid chelators in regulating lignin degradation and biosynthesis of peroxidases by *Phanerochaete chrysosporium*. Appl Environ Microbiol 58:2402–2409
29. Pointing SB, Jones EBG, Vrijmoed LLP (2000) Optimization of laccase production by *Pycnoporus sanguineus* in submerged liquid cultures. Mycologia 92:139–144
30. Reddy CA, D'Souza TM (1994) Physiology and molecular biology of the lignin peroxidases of *Phanerochaete chrysosporium*. FEMS Microbiol Rev 13:137–152
31. Rodriguez E, Pickard MA, Vazquez-Duhalt R (1999) Industrial dye decolorization by laccases from ligninolytic fungi. Curr Microbiol 38:27–32
32. Ruttimann-Johnson C, Cullen D, Lamar RT (1994) Manganese peroxidases of the white rot fungus *Phanerochaete sordida*. Appl Environ Microbiol 60:599–605
33. Scheel T, Hofer M, Ludwig S, Holker U (2000) Differential expression of manganese peroxidase and laccase in white-rot fungi in the presence of manganese or aromatic compounds. Appl Microbiol Biotechnol 54:686–691
34. Schoemaker HE, Lundell TK, Hatakka AI, Piontek K (1994) The oxidation of veratryl alcohol, dimeric lignin models and lignin by lignin peroxidase—the redox cycle revisited. FEMS Microbiol Rev 13:321–332
35. Soares GM, de Amorim MT, Costa-Ferreira M (2001) Use of laccase together with redox mediators to decolourize Remazol Brilliant Blue R. J Biotechnol 89:123–129
36. Soden DM, Dobson AD (2001) Differential regulation of laccase gene expression in *Pleurotus sajor-caju*. Microbiology 147:1755–1763
37. Strobel RJ, Sullivan GR (1999) Experimental design for improvement of fermentations. In: Demain AL, Davies JE (eds) Manual of industrial microbiology and biotechnology. American Society for Microbiology, Washington, D.C., pp 80–90
38. Tekere M, Zvauya R, Read JS (2001) Ligninolytic enzyme production in selected sub-tropical white rot fungi under different culture conditions. J Basic Microbiol 41:115–129
39. Tello M, Corsini G, Larrondo LF, Salas L, Lobos S, Vicuña R (2000) Characterization of three new manganese peroxidase genes from the ligninolytic basidiomycete *Ceriporiopsis subvermispora*. Biochim Biophys Acta 1490:137–144
40. Tien M, Kirk TK (1983) Lignin-degrading enzyme from the hymenomycete *Phanerochaete chrysosporium* Burds. Science 221:661–663
41. Van Aken B, Hofrichter M, Scheibner K, Hatakka AI, Naveau H, Agathos SN (1999) Transformation and mineralization of 2,4,6-trinitrotoluene (TNT) by manganese peroxidase from the white-rot basidiomycete *Phlebia radiata*. Biodegradation 10:83–91
42. Wariishi H, Valli K, Gold MH (1991) In vitro depolymerization of lignin by manganese peroxidase of *Phanerochaete chrysosporium*. Biochem Biophys Res Commun 176:269–275