Influence of Culture Conditions on Laccase Production, Growth, and Isoenzymes Patterns in Native White Rot Fungi from the Misiones Rainforest (Argentina)

María I. Fonseca,[a,*] Julia I. Fariña,[b] Noelia Irma Sanabria,[a] Laura L. Villalba,[a] and Pedro D. Zapata [a]

Many biotechnological processes pursuing sustainability aim for effective, inexpensive, and environmentally friendly alternatives to replace conventional practices. Laccase-containing lignocellulolytic systems from white rot fungi have been shown to be an efficient enzymatic tool for ecofriendly biological treatments. One objective of the biotechnological enzymes production process is to find optimum growing and secretion conditions for a selected fungus. In this work, different fungi isolated from the Misiones rainforest (*Coriolus versicolor* f. *antarcticus* BAFC-266, *Ganoderma applanatum* strain F, *Phlebia brevispora* BAFC-633, and *Pycnoporus sanguineus* BAFC-2126) were incubated at different temperatures (25, 29, 33 °C) and pH values (3.5, 4.5, 5.5) under static conditions for 7, 10, and 14 days to evaluate their growing ability and laccase (Lac) production. Results revealed specific favorable conditions for growth and protein secretion depending on the fungus under consideration, making it necessary to adjust these parameters for each particular case. The combined effect of these cultivation parameters showed a marked influence on the secreted Lac activity by *P. brevispora* BAFC 633, with the highest activity (~ 240 U/l) at 29 ºC and pH 4.5 at the 10th day of cultivation. The presence of Lac isoenzymes also depended on the pH, temperature, and time of cultivation for the different tested fungi.

Keywords: White rot fungi; Laccase; Culture conditions; Isoenzymes

*Contact information: a: Lab. de Biotecnología Molecular, Módulo de Bioquímica y Farmacia, Facultad de Ciencias Exactas Químicas y Naturales, UNaM, Ruta 12 Km 7 ½ (3300) Posadas, Misiones, Argentina; b: Lab. Biotecnología Fúngica. PROIMI-CONICET, T4001MVB, Tucumán, Argentina. e-mail: jifarina@yahoo.com; *Corresponding author: biotecmol2010@gmail.com*

INTRODUCTION

Advanced biotechnological applications developed in many countries are associated with the use of novel microbial enzymes having unusual physicochemical properties in "tailor-made processes". Description and analysis of new microorganisms from poorly explored natural environments can reveal innovative capacities for potential biotechnological applications (Fonseca *et al*. 2010; Pajot *et al*. 2008). The combination of pH and temperature for optimum growth can differently affect each fungal strain and has practical significance for the understanding of fungal physiology (Snajdr and Baldrian 2007).

White-rot fungi have the ability to break down lignin, a highly recalcitrant polymer present in plant cell walls (Polanco *et al*. 2006). Extracellular enzymes involved in lignin degradation are mainly peroxidases, such as lignin peroxidase (LiP, E.C. 1.11.1.14),

manganese peroxidase (MnP, E.C. 1.11.1.13), and laccase (Arora *et al*. 2002). Laccase (benzenediol: oxygen oxidoreductase, E.C. 1.10.3.2) is a polyphenol oxidase that catalyzes the oxidation of phenolic compounds and aromatic amines, with molecular oxygen as electron acceptor (Lundell *et al*. 1990). Laccase (Lac), which exhibits a remarkable functional diversity, and especially Lac mediator systems, have gained a lot of attention due to their broad substrate specificity, which makes them useful in different industrial applications such as pulp delignification, waste detoxification, denim decolorization, and bioconversion of chemicals (Cañas and Camarero 2010; Mäkelä *et al*. 2006; Villalba *et al*. 2010). Among the innovative applications of ligninolytic enzyme associations, the successful combination between Lac and MnP of selected white-rot fungi has been recently described as a powerful tool for endocrine disrupting chemicals degradation and estrogenic activity removal (Kum *et al*. 2011).

It has been already emphasized that a lack of systematic and comparative studies exploring the quantitative Lac production in fungi, in addition to the relevance of Lac for several biotechnological applications, has increased the need for a wider and more efficient spectrum of Lac-producing microorganisms (Arora and Gill 2001). A solid understanding of the influence of culture conditions is required to maximize Lac production by selected fungi, as production has been demonstrated to be closely dependent on medium and cultivation conditions (D`Souza-Ticlo *et al*. 2009; Kiiskinen *et al*. 2004; Vasconcelos *et al*. 2000).

Applications of Lac in biotechnological processes require high titers at relatively low costs; hence, the current focus on Lac research has been oriented towards production process optimization. Conventional optimization procedures involve varying one parameter at a time, while keeping constant the others, in order to assess the impact of a particular condition on the global process performance (Poojary and Mugeraya 2012). The present work investigated the combined effect of temperature, culture time, and pH on Lac secretion, isoenzymes profile, and biomass production for the selected wild fungi *Coriolus versicolor* f. *antarcticus* BAFC 266, *Ganoderma applanatum* strain F, *Phlebia brevispora* BAFC 633, and *Pycnoporus sanguineus* BAFC 2126, isolated from the Misiones rainforest (Argentina).

EXPERIMENTAL

Microorganisms and Culture Conditions

C. versicolor f. *antarcticus* BAFC 266, *P. brevispora* BAFC 633, and *P. sanguineus* BAFC 2126 were isolated from the Misiones rainforest (Argentina) and deposited at the Mycological Culture Collection of the Department of Biological Sciences, Faculty of Exact and Natural Sciences, University of Buenos Aires, Argentina. *Ganoderma applanatum* strain F was kindly provided by the Faculty of Forest Sciences, National University of Misiones, Argentina. Stock cultures were maintained by periodic sub-culturing on malt extract agar and kept at 4 ºC.

Influence of Incubation Temperature and pH on Fungal Growth and Lac Production

To prepare the respective inocula, each fungus was grown for 5 to 7 days in malt extract agar plates (20 g/L agar, 12.7 g/L malt extract). From these plates, one agar plug (36 mm^2) covered with mycelium per fungus was inoculated into 50 mL of ME medium

(12.7 g/L malt extract, 5 g/L corn steep liquor) dispensed in 250-mL Erlenmeyer flasks. Cultures were incubated under static conditions (unstirred) at 25, 29, or 33 °C for 7, 10, and 14 days. To study the influence of initial pH, ME culture medium pH was adjusted to 3.5, 4.5, or 5.5 with 0.1 N HCl or 0.1 N NaOH, as required. Experiments were made in triplicate

Biomass Determination

Biomass growth was determined by measuring mycelium dry weight. Spent culture medium was filtered in a Büchner funnel using pre-weighed fiberglass filters (GF/C), and filtrate was kept at -20 °C until enzyme quantification. Biomass dry weight was determined as the difference in weight of biomass-containing fiberglass filters (GF/C) after drying at 80 ºC until constant weight. Biomass determinations were performed in triplicate for each of the tested fungi.

Enzyme Assays

Cell-free filtrates were used to determine Lac activity with 5 mM 2,6-dimethoxyphenol (DMP) as substrate in 0.1 M sodium acetate buffer (pH 3.6) at 30 ºC (Moreira *et al*. 2004). The absorbance increase of the assay mixture was monitored at 469 nm (E_{469} = 27.5 mM^{-1} cm^{-1}) in a Shimadzu UV-3600 spectrophotometer. Enzyme activities were expressed in International Units (U), defined as the amount of enzyme required to produce 1 µmol of product/min at 30 ºC. Enzymatic determinations were performed in triplicate for each sample.

Polyacrylamide Gel Electrophoresis

Cell-free filtrates were subjected to native polyacrylamide gel electrophoresis (ND-PAGE, 7.5% w/v). After proteins separation, the gel was incubated in 0.1 M sodium acetate buffer containing 5 mM DMP for Lac activity detection (Fonseca *et al*. 2010). After 5-min incubation, the dye solution was discarded and the gel was immediately scanned with a Scanner HP Deskjet F300 All-in-One series. In order to determine Lac isoenzymes MW, an electrophoretic separation by SDS-PAGE (7.5% w/v), followed by a subsequent renaturation and detection technique was performed as previously described in the literature (Murugesan *et al*. 2007; Fonseca *et al*. 2010) and compared to a MW marker (Kaleidoscope, BioRad).

Statistics Analysis

Two-way ANOVA with Bonferroni post test was performed using GraphPad Prism 4.00 for Windows (GraphPad Software, San Diego, CA, USA).

RESULTS AND DISCUSSION

Biomass production and Lac secretion did not follow a common pattern with the studied culture conditions, thus impeding any kind of generalization and ruling out the possibility to predict a given fungal behavior. The trials were carried out under static conditions at different pH and temperature. The results of the present work will be the starting point for future approaches to biotechnological applications, such as biopulping and laccase production at higher level. Both systems are highly affected by agitation, as reported by other authors (Hess *et al.* 2002; Mohorčič *et al.* 2004; Tavares *et al.* 2006).

For *C. versicolor* f. *antarcticus* BAFC 266, fungal growth was highest at 25 ºC and pH 5.5 at the 10th day of culture ($p<0.05$), whilst at higher incubation temperatures (29 °C and 33 °C) mycelium growth was favored at pH 4.5. Concerning Lac activity, the highest titers occurred at 29 ºC and initial pH 5.5 on the 10th day ($p<0.05$) (Fig. 1). Two Lac isoenzymes were observed at 25 °C at different initial pH values, as well as at 29 ºC and pH 3.5. Under optimal Lac production conditions (*i.e.* 29 °C and pH 5.5) a single enzymatic fraction was detected, with a similar qualitative response at 33 °C (Fig. 1).

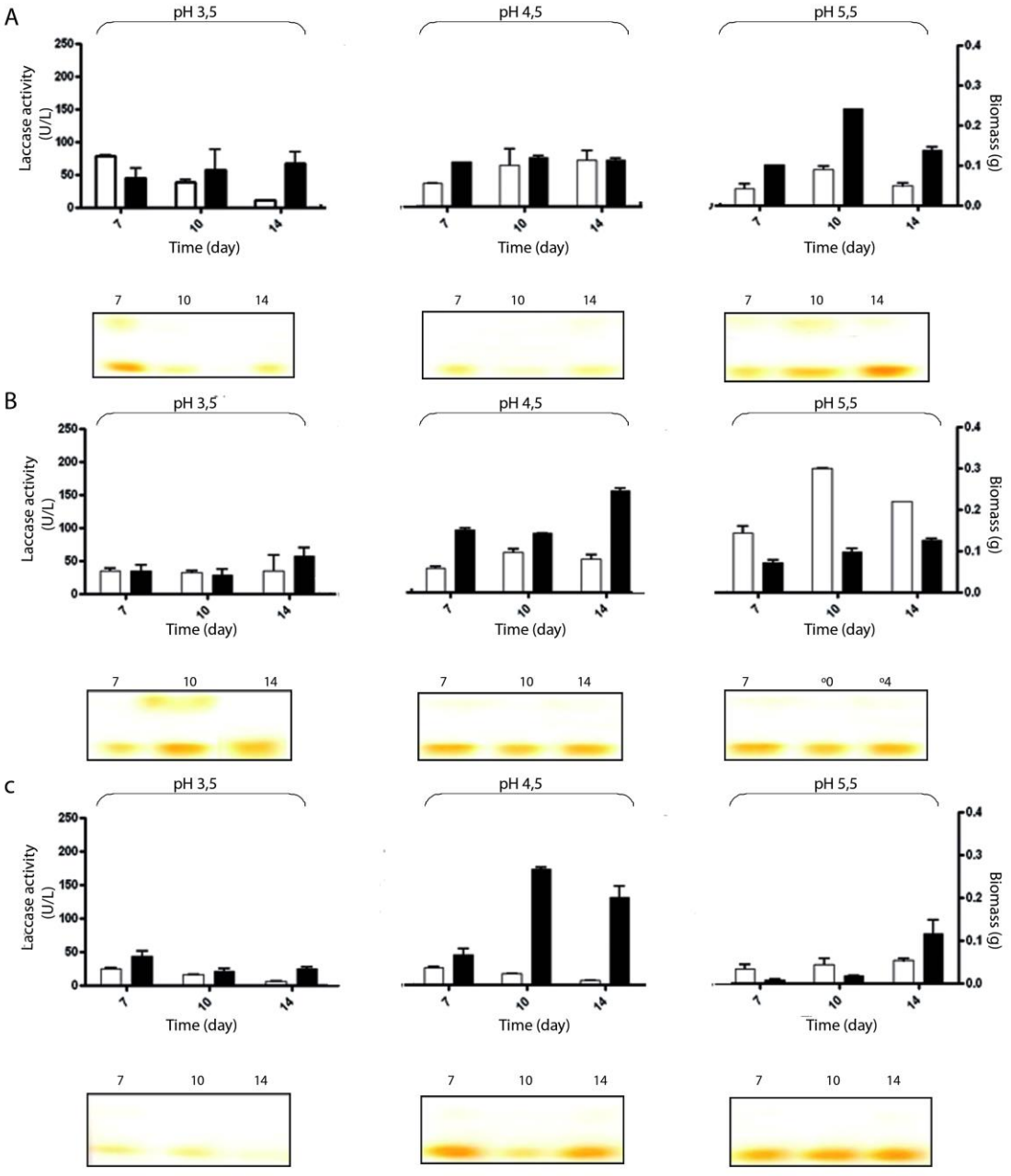

Fig. 1. Effect of pH and incubation temperature on the biomass production (■) and Lac activity (□) of *C. versicolor* f. *antarcticus* BAFC 266 at 25 °C (A), 29 °C (B), and 33 °C (C). Respective zymograms from each culture supernatant (20 µg of proteins per lane) were performed after ND-PAGE (7.5% w/v). Data are representative of three independent experiments. Biomass dry weight corresponds to 50 mL of culture.

The *G. applanatum* strain F showed maximal mycelial growth at 29 °C and initial pH values of 3.5 and 4.5 ($p<0.05$), while the highest levels of Lac activity could be obtained at the same temperature but pH 5.5 ($p <0.05$). The highest Lac activity corresponded to 14 days of culture with the presence of one enzymatic fraction on zymograms (Fig. 2). At 25 and 33 °C of incubation, neither significant growth nor quantifiable enzymatic activity could be measured.

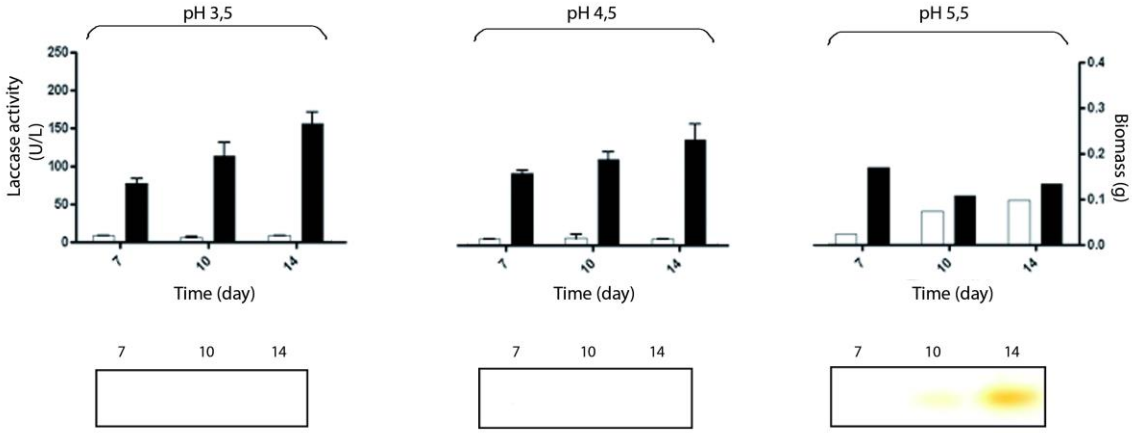

Fig. 2. Effect of pH and incubation temperature on the biomass production (■) and Lac activity (□) of *G. applanatum* strain F at 29 °C. Respective zymograms from each culture supernatant (20 µg of proteins per lane) were performed after ND-PAGE (7.5% w/v). Data are representative of three independent experiments. Biomass dry weight corresponds to 50 mL of culture.

Biomass production of *P. brevispora* BAFC 633 was comparatively higher at initial pH 3.5 and 4.5 for the different incubation temperatures tested (25, 29, and 33 °C) ($p <0.05$), generally showing a progressive increase as fungal growth proceeded. Lower biomass values were commonly observed at pH 5.5 at all temperatures ($p <0.05$). The highest Lac activity (~ 240 U/l) was recorded at pH 4.5 and 29 °C at the 10th day of culture ($p<0.05$) (Fig. 3). Regarding zymograms analysis, bands of different mobility were visualized depending on the incubation temperature and initial pH; that is to say, we observed increased mobility at pH 3.5 and lower mobility at pH 4.5 and 5.5 (Fig. 3).

For *P. sanguineus* BAFC 2126 cultures, biomass production at 25 °C was moderately increased at initial pH 5.5 ($p <0.05$), whilst at 29 °C the same feature occurred at pH 3.5 ($p <0.05$). Concerning Lac activity, maximal values could be preferentially reached at lower initial pH values (3.5 and 4.5) both at 25 and 29 °C, with a discreetly higher production at 29 °C at the 14th day of cultivation ($p <0.05$). On the other hand, the highest enzymatic activity at 33 °C could be obtained at initial pH 4.5, but at 10 days of culture ($p> 0.05$) (Fig. 4). Zymograms showed two fractions with enzyme activity; one fraction existing at all culture conditions and the other one only at 25 and 29 °C, at pH 4.5 (Fig. 4).

Biomass production and Lac enzyme activity have already been described as highly dependent on the culture conditions (Heinzkill *et al*. 1998; Patel *et al*. 2009). In accordance to these statements, optimal combinations of pH and temperature for both growth and Lac activity showed relevant variations depending on the tested fungi in this study. These fluctuations were expected since the conditions of cultivation commonly affect fungal growth rate and physiology (Levin *et al*. 2002).

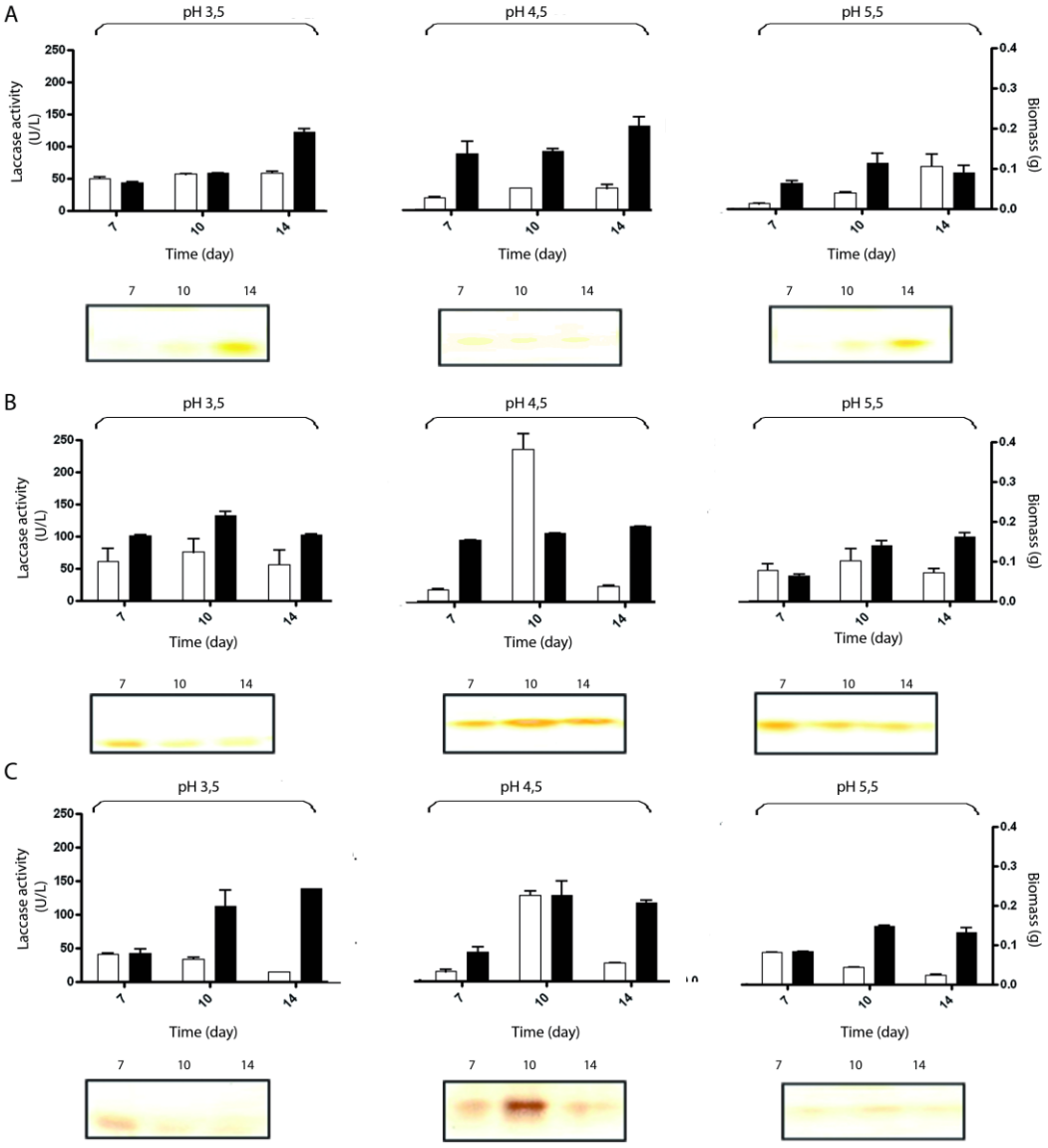

Fig. 3. Effect of pH and incubation temperature on the biomass production (■) and Lac activity (□) of *P. brevispora* BAFC 633 at 25 °C (A), 29 °C (B), and 33 °C (C). Respective zymograms from each culture supernatant (20 µg of proteins per lane) were performed after ND-PAGE (7.5% w/v). Data are representative of three independent experiments. Biomass dry weight corresponds to 50 mL of culture.

No clear pattern could be defined between biomass levels and Lac activity production, although some correlation was detected in specific cases. For instance, increasing growth with declining Lac activity (e.g. *C. versicolor* f. *antarcticus* BAFC 266 at 25 °C and pH 3.5, *P. brevispora* BAFC 633 at 33 °C at both pH 5.5 and 3.5, and *P. sanguineus* BAFC 2126 at 29 °C and pH 3.5) may be due to the presence of an isoenzyme that progressively vanished with cultivation time, probably as a consequence of low enzyme stability, protease action, or cessation of secretion (Janusz *et al*. 2006). The sensitivity of a protein to denaturation at different combinations of temperature and pH may change significantly from enzyme to enzyme (Pclczar *et al*. 2004).

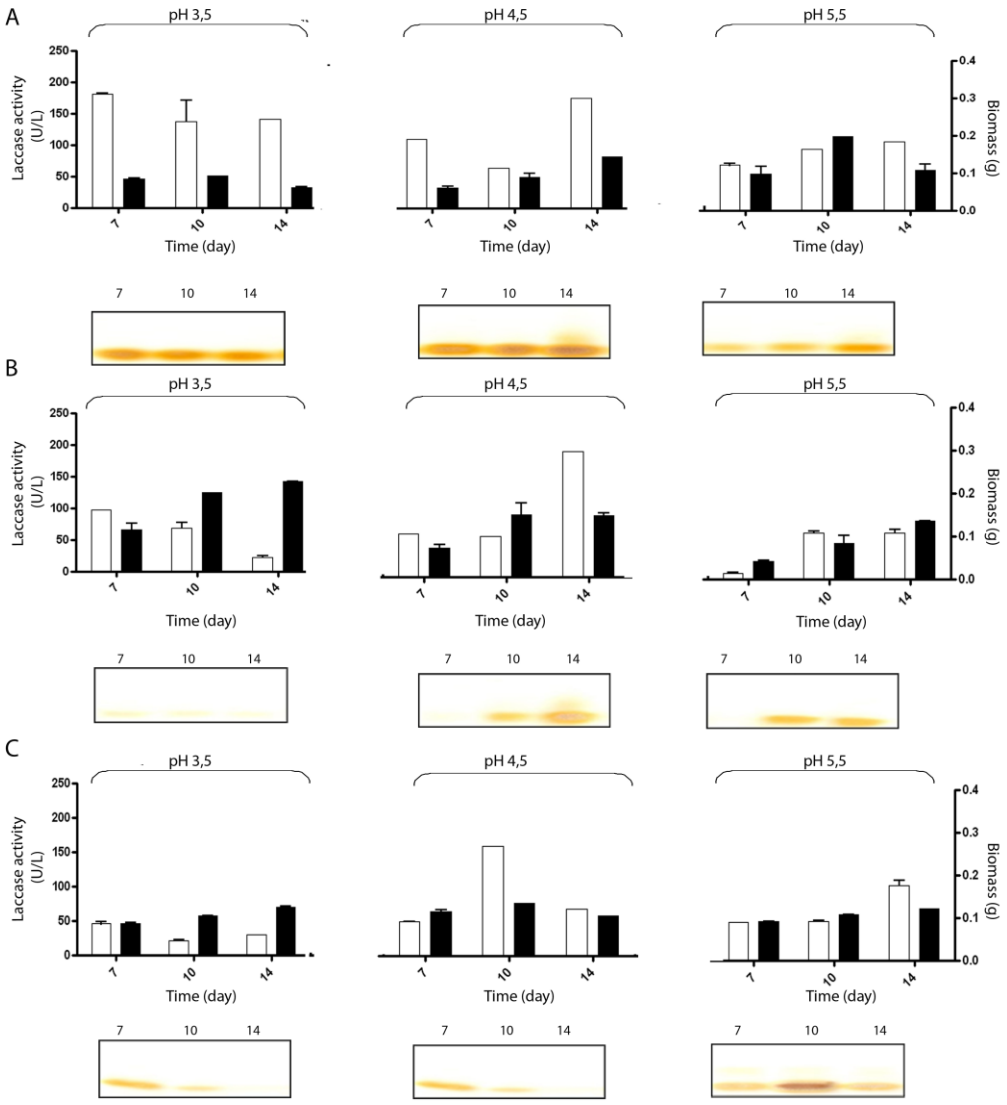

Fig. 4. Effect of pH and incubation temperature on the biomass production (■) and Lac activity (□) of *P. sanguineus* BAFC 2126 at 25 °C (A), 29 °C (B), and 33 °C (C). Respective zymograms from each culture supernatant (20 µg of proteins per lane) were performed after ND-PAGE (7.5% w/v). Data are representative of three independent experiments. Biomass dry weight corresponds to 50 mL of culture.

Concerning the culture medium used, the inclusion of malt extract, rich in aromatic amino acids such as tryptophan and tyrosine, might be beneficial for Lac production (Arora and Gill, 2001). Eggert *et al*. (1996) identified a metabolite of tryptophan derivative (3-hydroxy-2-aminobenzoate) that acted as a mediator in oxidation reactions catalyzed by Lac in the white rot fungus *Pycnoporus cinnabarinus*. It is also known that Lac reacts with 4-hydroxy-indole, a tryptophan derivative (Cai *et al*. 1993; Eggert *et al*. 1996).

Changes in the cultivation conditions during Lac production, such as the herein tested pH and incubation temperature, may modify Lac expression and/or its activity, because of alterations in the three-dimensional structure of the enzyme or in the charge of

certain amino acid groups, especially if they belong to the active site, as compared to the corresponding enzyme native form (Hossain and Anantharaman 2006; Pclczar *et al*. 2004). The finding of optimal Lac activity at pH values around 4.5 to 5.5 has been described in the literature (Mehna *et al*. 1995; Patel *et al*. 2009).

In *C. versicolor* f. *antarcticus* BAFC 266, the best conditions for Lac production were 29 °C and pH 5.5 (~ 187 U/l, 29 °C, 10 days). These conditions were also reported for *Schizophyllum commune* at 28 ºC (Adejoye and Fasidi 2010) and *Penicillium martensii* at 30 ºC (Elshafei *et al*. 2012). Similar behavior was also herein described for *G. applanatum* strain F, which was consistent with previous results by Echabarria and Gallon (2010), who found a maximum of Lac yield in *Ganoderma* sp. at pH 5 (with 0.75% bamboo sawdust and 250 mM copper). In *P. sanguineus* BAFC 2126, the maximal Lac production was obtained during steady growth at 29 °C and pH 4.5 (~ 180 U/l, 29 °C, 14 days), as also found for *P. brevispora* BAFC 633 (~ 240 U/l, 29 °C, 10 days), but this maximum required 14 days of cultivation.

In the present work, all tested fungi showed maximal Lac production when growing at 29 °C. The most favorable temperature for Lac production greatly differs from one strain to another (Farnet *et al*. 2000). In general, fungi are cultured at temperatures between 25 and 30 °C for optimal production (Arora *et al*. 2002; Pointing 2001). Temperatures above 30 °C showed reduced ligninolytic enzyme activity (Zadrazil *et al*. 1999), as herein particularly found for *C. versicolor* f. *antarcticus* BAFC 266.

The common lack of correlation between growth and Lac activity has been frequently observed in *C. versicolor* f. *antarcticus* BAFC 266, *G. applanatum* strain F, *P. brevispora* BAFC 633, and *P. sanguineus* BAFC 2126, suggesting a differential regulation of Lac production independent of fungal growth. Similarly, in *Trichoderma viride* and *T. longibrachiatum* grown in a basal medium at 30 °C and pH 6, there was no correlation between growth and Lac activity (Gochev and Krastanov 2007). A strong effect of pH and temperature on the laccase activity was especially evidenced in *P. brevispora* BAFC 633, with optimal values at 29 °C and pH 4.5. Comparable results were described in *Peniophora* sp. HPF-04 at 30 ºC and pH 6 (Poojary and Mugeraya 2012).

The following isoenzymes were described: 60- and 131-kDa Lacs for *C. versicolor* f. *antarcticus* BAFC 266, a unique 62.5-kDa Lac for *G. applanatum* strain F, 60- and 52.5-kDa Lacs for *P. brevispora* BAFC 633, and 54- and 71-kDa Lacs for *P. sanguineus* BAFC 2126 (Fig. 5). Particularly interesting was the finding of a higher-mobility band (52.5 kDa) in *P. brevispora* BAFC 633 culture supernatants after cultivation at initial pH 3.5 and at all temperatures tested, while the upper enzymatic band (60 kDa) was typically found in cultures at pH 4.5 and 5.5 (at all temperatures) and would be apparently the main component responsible of the Lac activity that was quantified under these conditions (Fig. 3).

P. sanguineus BAFC 2126 exhibited a higher-MW (71 kDa) enzymatic band, especially when cultured at pH 4.5 and at either 25 or 29 ºC. Its simultaneous appearance along with the lower-MW (54 kDa) isoenzyme when highest Lac activity was quantified at pH 4.5 may explain this incremental enzymatic activity (Figs. 4 and 5).

Many authors have also reported isoenzymes in several species of white rot fungi, with MWs ranging from 60 to 80 kDa (Dantan-González et al. 2008; Salas *et al*. 1995). Varied Lac isoenzyme patterns of fungal origin are obtainable according to the culture conditions used, and this enzymatic potential becomes relevant for the efficient colonization of the substrate and growth (Das *et al*. 1997; Giardina *et al*. 1999).

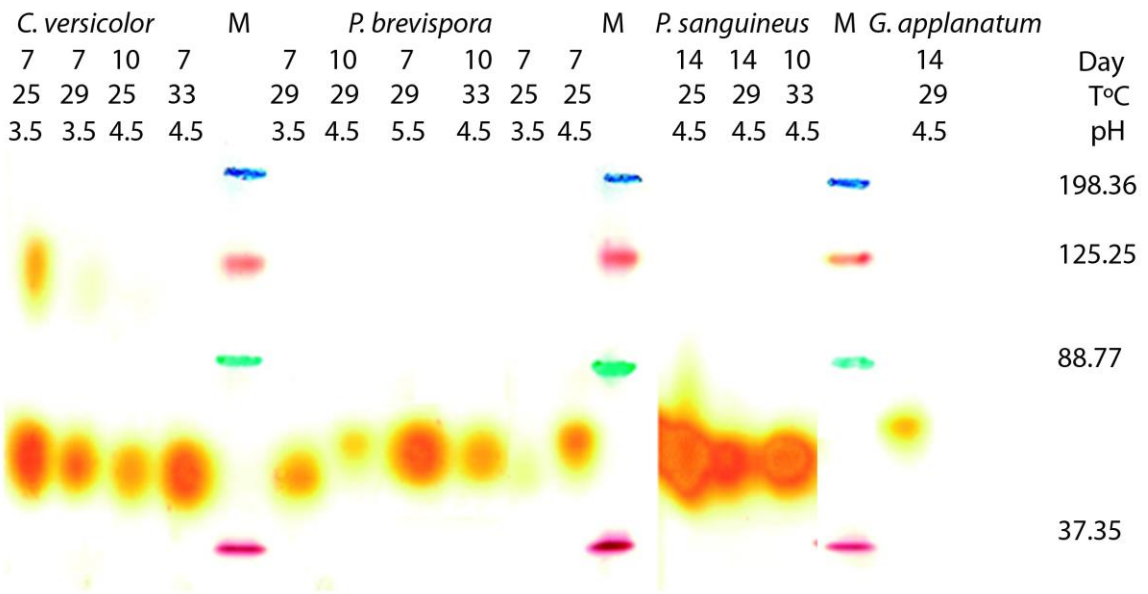

Fig. 5. Estimation of molecular weight (MW) of Lac isoforms produced by selected white rot fungi in ME culture medium at different initial pH, incubation temperature, and days of cultivation. SDS-PAGE (7.5% w/v) was carried out with 20 µg of protein per lane. Data are representative of three independent experiments. MW: Molecular weight marker.

Both ND-PAGE and PAGE methods applied in this work revealed the existence of isoenzymes of different MWs appearing at different conditions of pH and temperature. Interestingly, the higher-mobility isoenzyme (52.5 kDa) found in *P. brevispora* BAFC 633 cultured at initial pH 3.5 may be a pH-responsive Lac. That could be related to the previously described phenomenon for *Pleurotus ostreatus* (Diaz *et al*. 2011), where fungal metabolite-driven pH signals regulated some Lac isoenzymes expression. Nevertheless, the proper role of each type of isoenzymes in the hyphal maturation process and the adaptive ability of fungi to different environments and conditions remain to be studied.

CONCLUSIONS

1. Biomass production and Lac secretion in static conditions did not always follow a common pattern with culture conditions, thus impeding any generalization to predict a given fungal behavior.
2. The four strains showed an attractive and alternative source for laccase production influenced by combined effects of temperature and pH.
3. The highest Lac activities in supernatant cultures at 29 °C and pH 4.5, found in *P. brevispora* BAFC 633, clearly reveals the potential of this strain for laccase production at 29 °C and pH 4.5.
4. The studied fungi showed the existence of isoenzymes appearing at different physiologic states influenced by physical conditions such as pH and temperature.

ACKNOWLEDGMENTS

Authors wish to give thanks for financial support from Secretaría de Ciencia y Tecnología de la Universidad Nacional de Misiones, through grants for innovation projects (16Q446 and 16Q486). MIF have a fellowship for doctoral studies from CONICET, Argentina.

REFERENCES CITED

Adejoye, O. D., and Fasidi, I. O. (2010). "Effect of cultural conditions on biomass and laccase production in submerged medium by *Schizophyllum commune* (Fries), a Nigerian edible mushroom," *Electronic J. Environ., Agric. Food Chem.* 9, 600-609.

Arora, D. S., and Gill, P. K. (2001). "Effects of various media and supplements on laccase production by some white rot fungi," *Biores. Technol.* 77, 89-91.

Arora, D. S., Chander, M., and Gill, P. K (2002). "Involvement of lignin peroxidase, manganese peroxidase and laccase in degradation and selective ligninolysis of wheat straw," *Int. Biodet. Biodeg.* 50, 115-120.

Cai, W., Martin, R., Lemaure, B., Leuba, J. L., and Petiard, V. (1993). "Hydroxy-indoles: A new class of laccase substrates," *Plant Physiol. Biochem.* 31, 441-445.

Cañas, A. I., and Camarero, S. (2010). "Laccases and their natural mediators: Biotechnological tools for sustainable eco-friendly processes," *Biotechnol. Adv.* 28, 694-705.

D´Souza-Ticlo, D., Sharma, D., and Raghukumar, C. (2009). "A thermostable metal-tolerant laccase with bioremediation potential from a marine-derived fungus," *Mar. Biotechnol.* 11, 725-737.

Dantán-González, E., Vite-Vallejo, O., Martínez-Anaya, C., Méndez-Sánchez, M., González, M.C., Palomares, L.A., and Folch-Mallol, J. (2008). "Production of two novel laccase isoforms by a thermotolerant strain of *Pycnoporus sanguineus* isolated from an oil-polluted tropical habitat," *International Microbiol.* 11, 163-169.

Das, N., Sengupta, S., and Mukherjee, M. (1997). "Importance of laccase in vegetative growth of *Pleurotus florida*," *Appl. Environ. Microbiol.* 63, 4120-4122.

Díaz, R., Sánchez, C., Bibbins-Martínez, M. D., and Díaz-Godínez, G. (2011). "Effect of medium pH on laccase zymogram patterns produced by *Pleurotus ostreatus* in submerged fermentation," *Afr. J. Microbiol. Res.* 5, 2720-2723.

Eggert, C., Temp, U., and Eriksson, E. (1996). "The ligninolytic system of the white rot fungus *Pycnoporus cinnabarinus*: Purification and characterization of the laccase," *Appl. Environ. Microbiol.* 62, 1151-1158.

Elshafei, A. M., Hassan, M. M., Haroun, B. M., Elsayed, M. A., and Othman, A. M. (2012). "Optimization of laccase production from *Penicillium martensii* NRC 345," *Adv. Life Sci.* 2, 31-37.

Farnet, A. M., Criquet, S., Tagger, S., Gil, G., and Le Petit, J. (2000). "Purification, partial characterization, and reactivity with aromatic compounds of two laccases from *Marasmius quercophilus* strain 17," *Can. J. Microbiol.* 46, 189-194.

Fonseca, M., Shimizu, E., Villalba, L., and Zapata, P. (2010). "Laccase-producing ability and the inducing effect of copper on laccase production of white rot fungi native from Misiones (Argentina)," *Enzyme Microb. Technol.* 46, 534-539.

Giardina, P., Palmieri, G., Scaloni, A., Fontanella, B., Faraco, V., Cennamo G, and Sannia G. (1999). "Protein and gene structure of a blue laccase from *Pleurotus ostreatus*," *Biochem. J.* 341, 635-663.

Gochev, V. K., and Krastanov, A. I. (2007). "Fungal laccases," *Bulg. J. Agric. Sci.* 13, 75-83.

Heinzkill, M., Bech, L., Halkier, T., Schneider, P., and Anke, T. (1998). "Characterization of laccases and peroxidases from wood-rotting fungi (family Coprinaceae)," *Appl. Environ. Microbiol.* 64, 1601-1606.

Hess, J., Leitner, C., Galhaup C., Kulbe, K. D., Hinterstoisser, B., Steinwender, M., and Haltrich, D. (2002). "Enhanced formation of extracellular laccase activity by the white-rot fungus *Trametes multicolor*," *Appl. Biochem. Biotechnol.* 98, 229-241.

Hossain, S. K. M., and Anantharaman, N. (2006). "Activity enhancement of ligninolytic enzymes of *Trametes versicolor* with bagasse powder," *Afr. J. Biotechnol.* 5, 189-194.

Janusz, G., Rogalski, J., Barwińska, M., and Szczodrak, J. (2006). "Effects of culture conditions on production of extracellular laccase by *Rhizoctonia praticola*," *Pol. J. Microbiol.* 55, 309-319.

Kiiskinen, L. L., Rättö, M., and Kruus, K. (2004). "Screening for novel laccase-producing microbes," *J. Appl. Microbiol.* 97, 640-646.

Kum, H., Lee, S., Ryu, S., and Choi, H. T. (2011). "Degradation of endocrine disrupting chemicals be genetic transformants with two lignin degrading enzymes in *Phlebia tremellosa*," *J. Microbiol.* 49, 824-827.

Levin, L., Forchiassin, F., and Ramos, A. M. (2002). "Copper induction of lignin modifying enzymes in the white rot fungus *Trametes trogii*," *Mycologia* 94, 377-383.

Lundell, T., Leonowicz, A., Rogalski, J., and Hatakka, A. (1990). "Formation and action of lignin-modifying enzymes in cultures of *Phlebia radiata* supplemented with veratric acid," *Appl. Environ. Microbiol.* 56, 2623-2629.

Mäkelä, M. R., Hildén, K. S., Hakala, T. K., Hatakka, A., and Lundell, T. K. (2006). "Expression and molecular properties of a new laccase of the white rot fungus *Phlebia radiata* grown on wood," *Curr. Genet.* 50, 323-333.

Mehna, A., Bajpai, P., and Bajpai, P. K. (1995). "Studies of decolorization of effluent from a small pulp mill utilizing agriresidues with *Trametes versicolor*," *Enzyme Microb. Technol.* 17, 18-22.

Mohorčič, M., Friedrich, J., and Pavko, A. (2004). "Decolourization of the diazo reactive black 5 by immobilized *Bjerkundera adusta* in a stirred tank bioreactor," *Acta Chim. Slov.* 51, 619-628.

Moreira, M. T., Viacava, C., and Vidal, G. (2004). "Fed-batch decolorization of Poly R-478 by *Trametes versicolor*," *Braz. Arc. Biol. Technol.* 47, 179-183.

Murugesan, K., Nam, I. H., Kim, Y. M., and Chang, Y. S. (2007). "Decolorization of reactive dyes by a thermostable laccase produced by *Ganoderma lucidum* in solid culture," *Enzyme Microb. Technol.* 40, 1662-1672.

Pajot, H. F., Figueroa, L. I. C., Spencer, J. F. T., and Fariña, J. I. (2008). "Phenotypical and genetic characterization of *Trichosporon* sp. HP-2023. A yeast isolate from Las Yungas rainforest (Tucumán, Argentina) with dye-decolorizing ability," *Ant. van Leeu. J.* 94, 233-244.

Patel, H., Gupte, A., and Gupte, S. (2009). "Effect of different culture conditions and inducers on production of laccase by Basidiomycetes fungal isolate *Pleurotus ostreatus* HP-1 under solid state fermentation," *BioResources* 4, 268-284.

Pclczar, M. J., Chan, E. C. S., and Kring, N. R. (2004). *Microbiology*, 5th ed., Tata McGraw-Hill Publ. Co. Ltd., New Delhi, India.

Pointing, S. B. (2001). "Feasibility of bioremediation by white-rot fungi," *Appl. Microbiol. Biotechnol.* 57, 20-33.

Polanco, R., Canessa, P., Rivas, A., Larrondo, L. F., Lobos, S., and Vicuña, R. (2006). "Cloning and functional characterization of the gene encoding the transcription factor Ace1 in the Basidiomycete *Phanerochaete chrysosporium*," *Biol. Res.* 39, 641-648.

Poojary, H., and Mugeraya, G. (2012). "Laccase production by *Phellinus noxius* hpF17: optimization of submerged culture conditions by response surface methodology," *Res. Biotechnol.* 3, 09-20.

Salas, C., Lobos, S., Larraín, J., Salas, L., Cullen, D., and Vicuña, R. (1995). "Properties of laccase isoenzymes produced by the basidiomycete *Ceriporiopsis subvermispora*," *Biotechnol. Appl. Biochem.* 21, 323-333.

Snajdr, J., and Baldrian, P. (2007). "Temperature affects the production, activity and stability of ligninolytic enzymes in *Pleutorus ostreatus* and *Trametes versicolor*," *Folia Microbiol.* 52, 498-502.

Tavares, A. P. M., Coelho, M. A. Z., Agapito, M. S. M., Coutinho, J. A. P., and Xavier, A. M. R. B. (2006). "Optimization and modeling of laccase production by *Trametes versicolor* in a bioreactor using statistical experimental design," *Appl. Biochem. Biotechnol.* 134, 233-248.

Vasconcelos, A. F. D., Barbosa, A. M., Dekker, R. F. H., Scarmínio, I. S., and Rezende, M. I. (2000). "Optimization of laccase production by *Botryosphaeria* sp. in the presence of veratryl alcohol by the response-surface method," *Proc. Biochem.* 35, 1131-1138.

Villalba, L. L., Fonseca, M. I., Giorgio, M., and Zapata, P. D. (2010). "White rot fungi laccases for biotechnological applications," *Rec. Pat. DNA & Gene* 4, 106-112.

Zadrazil, F., Gonser, A., and Lang, E. (1999). "Influence of incubation temperature on the secretion of extracellular ligninolytic enzymes of *Pleurotus* sp. and *Dichomitus squalens* into soil," Proc Conference on Enzymes in the Environment., Granada, Spain, p 526.

Article submitted: February 11, 2013; Peer review completed: April 16, 2013; Revised version received and accepted: April 17, 2013; Published: April 24, 2013.