Influence of cultivation temperature on the ligninolytic activity of selected fungal strains

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Abstract: -. Many reports have shown that extracellular peroxidases and laccases oxidize persistent organic pollutants. In this study, the ligninolitic activities of selected strains were determined in a nitrogen-sufficient liquid medium. In order to determine the effect of temperature on ligninolytic activities, selected white rot fungi were incubated at different temperatures (15, 20, 25 and 30°C). The activities of manganese peroxidase and laccase were determined but no lignin peroxidase was detected for all strains. Significant differences were revealed among strains of the same species. The maximum laccase and manganese peroxidase activity was obtained by *Pleurotus ostreatus sp.3* attaining 27.8 and 26 U/l after 11 and 13 days of cultivation at 30°C.

Key-Words: Ligninolytic enzymes, White rot fungi, Peroxidases, Lignin peroxidase, Manganese peroxidase, Laccase.

1 Introduction

White rot fungi are filamentous fungi that inhabit the wood of dead or dying trees and most are members of the Basidiomycota. The characteristic feature of white rot fungi is their ability to degrade lignin. Lignin peroxidase (LiP), manganese peroxidase (MnP) and laccase (Lac) constitute the basic ligninolytic system of white rot fungi. Ligninolytic enzymes are produced during the secondary metabolism in nutrient-limited cultures and are capable of degrading persistent pollutants, such as chlorinated pesticides, nitroaromatic explosives, polyaromatic hydrocarbons and synthetic dyes [1].

White rot fungi differ significantly in the occurrence of these enzymes [2]. The fungal enzymes that are used to degrade are nonspecific with respect to substrate. They function mainly by the production of free radicals that are able to attack a wide range of organic molecules. Peroxidases using H_2O_2 and laccases (polyphenol oxidases) using molecular oxygen are the enzymes responsible for attack on lignin. Various fungi produce laccase and manganese peroxidase but apparently no lignin peroxidase, implying that they degrade lignin in a different way compared to *Phanerochaete chrysosporium* [3]. According to Nerud and Misurcova [4], fungi were categorized on the basis of their enzyme types under nitrogen-limiting conditions, in the six following groups: manganese peroxidase-laccase, manganese peroxidase, laccase, lignin peroxidase, lignin peroxidase-manganese peroxidase-laccase. Apart from these enzymes, a number of other peroxidases are produced such as glyoxal oxidase and non-specific enzymes which are correlated with the degradation of lignocellulose [5].

Lignin peroxidase is an extracellular glycoprotein with a molecular weight of 40 kDa. It contains one protoporphyrin IX prosthetic group per molecule and is dependent on H_2O_2 for catalysis [6]. The catalytic cycle of LiP starts with the oxidation of the enzyme by H_2O_2 to two-electron oxidized intermediate (LiP I). LiP I returns to its native state by catalyzing two one-electron oxidations in the presence of appropriate reducing substrates such as veratryl alcohol via LiP II state (Fig. 1) [6, 7].

MnP is an extracellular glycosylated heme protein and is dependent on H_2O_2 as LiP for the oxidation of Mn^{+2} to a Mn^{+3}–chelate. Mn^{+3} is highly reactive and chelates with organic acids such as oxalic and malonic acid, which are secondary metabolites of fungi and are secreted contemporaneously as MnP [8]. Mn^{+3}-chelate possess an important degradative potential because it oxidizes a very broad range of organic substrates such as monomeric or dimeric phenols, phenolic lignin dimmers, carboxyl acids, thioles and unsaturated fatty acids to the relative radicals [9].

Laccase belongs to the group of blue multicopper oxidases. They are glycoproteins that contain 4 atoms of copper and catalyze one electron oxidation of several substrates reducing dioxygen to water. The molecular weight ranges from 50 to 300 kDa [10].

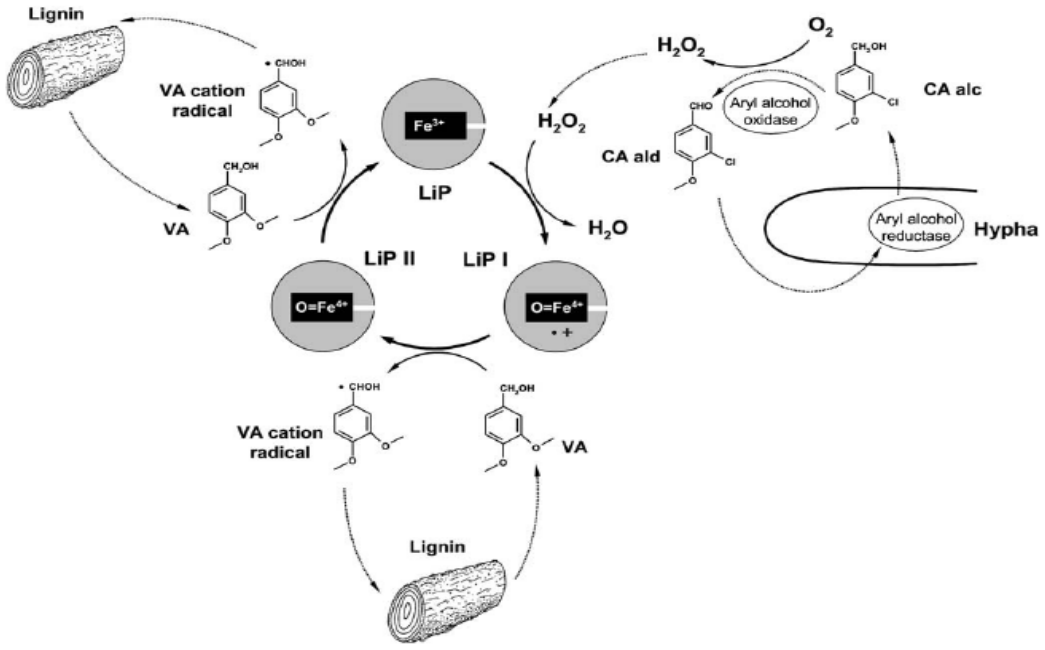

Fig 1. Reaction cycle of lignin peroxidase [12].

Laccase catalyzes the one electron oxidation of phenolic compounds to cation radicals. The radicals being generally unstable undergo spontaneous or enzyme-catalyzed reactions [11, 16].

The physiological requirements of white rot fungi are variable. Many studies have shown that the ligninolytic system is affected significantly by the nutrients of the medium, such as aromatic compounds, nitrogen or manganese [17, 18, 19]. Nevertheless, there are fungi whose production of ligninolytic enzymes is not affected by carbon or nitrogen [16]. Specifically, it has been proved that *P. chrysosporium* is regulated by nitrogen whereas strains of *Bjekandera* are not affected [17].

In contrast to the medium composition, the influence of incubation temperature has not been studied extensively. According to Vyas et al. [18], the incubation temperature affected the appearance, the enzymes patterns, the maximum value and the ratio of LiP and MnP in cultures of *P. chrysosporium*. For the same strain, Asther et al. [19] and Liebeskind et al. [20] obtained high productivity of LiP at lower temperatures than the optimum one (37^{o}C) found by most other researchers.

The macromycetes in the forests of Greece constitute an untapped biological resource and they are promising candidates for exploitation in biotechnological applications. In our laboratory over a hundred wood inhabiting Basidiomycetes were collected and isolated in pure culture [21, 22]. Due to the correlation of the ligninolytic degradation and the decolourisation of the polymeric dye Poly R-478 [2, 23], subsequent screening on plates containing the dye, led to the selection of the strains for further studies based on their rapid growth rate and extensive decolourisation on solid media. The most efficient strains, *Ganoderma australe* and *Pleurotus ostreatus sp.4* were studied in agitated liquid cultures for the biodegradation of lindane, an organochlorine pesticide, using Central Composite Design [24, 25]. The investigated factors were initial concentration of lindane, nitrogen content, incubation time and temperature.

This work aims at evaluating the ligninolytic potential of the preselected white rot fungi collected in Greece. The selection of a strain with high ligninolytic enzyme activities and the conditions promoting maximum enzyme synthesis and secretion are important factors for the development of the bioremediation technology. Since white rot fungi are mesophiles, the effect of temperature and the enzyme production levels were determined at 15, 20, 25 and 30^{o}C. Among the tested fungi, differences were revealed for strains of the same species. The cultures were cultivated in a nitrogen-sufficient medium, which is appropriate for the secretion of the enzymes.

2 Materials and Methods

2.1 Microorganisms

The following strains of basidiomycetes were used in this study: *Pleurotus ostreatus sp.3*, *Pleurotus ostreatus sp.4*, *Pleurotus pulmonarius, Polyporus ciliatus*, *Polyporus brumalis*, *Polyporus sp.2* and

Polyporus sp.3, and *Ganoderma australe*. The strains *Pleurotus ostreatus sp.3* and *Pleurotus pulmonarius* were kindly offered by the ATHUM Culture Collection of Fungi, Department of Biology, at the University of Athens, Greece. Fruiting bodies of basidiomycetes were collected from tree stumps or twigs in Greece. The strains were maintained on PDA (Potato Dextrose Agar) at 4°C.

2.2 Culture media

Cultures were maintained on Potato Dextrose Agar (PDA), composed of (g/l): peeled potatoes 200, dextrose 20, and agar 15. The liquid media for the cultivation was Malt Extract Broth (MEB) containing (g/l) malt extract 17, and mycological peptone 3. The liquid medium used for enzyme activities, was Kirk's media composed of KH_2PO_4 0.20, $CaCl_2$ 0.01, $MgSO4.7H_2O$ 0.05, ammonium tartrate 0.22, 2,2-dimethylsuccinic acid 2.90, glucose 5, thiamine 0.1, Tween 80 0.10% v/v, veratryl alcohol 1.5 mM, trace elements (10ml). The trace elements composed of (mg/l): $MnSO_4$ 33, $Fe_2(SO_4)_3$ 50, $ZnSO_4.7H_2O$ 43, $CuSO_4.7H_2O$ 80, H_2MoO_4 50 [26]. Media were sterilized by autoclaving at 121°C for 20 minutes.

2.3 Enzyme assays

Inocula (1-cm diameter) were cut from grown PDA cultures and transferred in 2% malt extract broth (50ml in 125 ml Erlenmeyer flasks). The flasks were incubated for 7 days at 25°C under static conditions. The cultures were homogenized using a blender and 10-ml aliquots were added to 90 ml Kirk' s media (in 250 ml Erlenmeyer flasks). The cultures were further incubated in four temperatures, 15, 20, 25 and 30°C. Samples (1 ml) were aseptically removed from the liquid cultures at various times (expression days) and tested for enzyme activities. Three replicates were performed for each strain. The culture filtrates were used for the estimation of manganese peroxidase, lignin peroxidase and laccase.

Lignin peroxidase activity was determined according to the method of Tien and Kirk [26]. Laccase activity was determined spectro-photometrically as described by Szklarz et al. [27]. The method is based on the formation of the quinone form of syrigaldazine at 25°C when incubated with the phenoloxidase preparation. Lignin peroxidase catalyses the oxidation of veratryl alcohol to veratraldehyde by H_2O_2.

Manganese peroxidase activity was measured by the method of Paszczynski et al. [28] based on the formation of quinone of syrigaldazine at 525 nm. The enzymatic activities were expressed in U/L.

2.4 Statistical validation

The data were statistically treated with the SPSS package for one-way ANOVA and if any significant difference was observed between the treatment groups, further ranking of the group was performed with the Tukey test. The statistical level of significance for all treatments was 5%.

3 Results and Discussion

3.1 Growth Rate

Apart from the already known factors that restrict the degradation of xenobiotic compounds by white rot fungi in liquid media and soil (e.g. limited bioavailability, hydrophobic properties, and production of H_2O_2), the levels of ligninolytic enzymes proved to be an important factor when aiming at higher biodegradation rates. Therefore, the expression and production of the ligninolytic enzymes should be taken into consideration in the development of bioremediation techniques. It has been shown that enzyme activities are sensitive to aeration and agitation [29, 30]. So, the cultures were incubated statically in order to be evaluated at their optimum values.

Manganese peroxidase and laccase activities were determined as a function of incubation time. No lignin peroxidase was expressed. The maximum activities and the day of their expression for all strains are listed in Table 1. Values are reported as means and standard deviations of three replicates.

Laccase activities varied with the isolate at 15°C. As it is shown in Figure 2, the highest laccase activity under the tested conditions was obtained by *P. Pulmonarius* and *P. ostreatus sp.4*, attaining 7.8 and 7.2 U/l after eleven and twelve days of incubation, respectively. The strains *P. ciliatus* and *Polyporus sp.3* had the lowest laccase activity, 3.53 and 3.84 U/l, respectively. The rest of the fungi showed no significant difference with the three former strains. The statistical analysis gave a p-value equal to 0.387 in the case of grouped with *P. pulmonarius* and 0.095 when grouped with *P. ciliatus* and *Polyporus sp.3*.

Manganese peroxidase activities were higher compared to the laccase ones under the same conditions. After the treatment with multiple comparisons, the fungi did not show any significant differences apart from *P. ciliatus* (Fig.1), which was individuated and had the highest production of MnP, 21.5 U/l after 10-day incubation. The other strains were grouped together (p-value = 0.266), where *G. australe* and *Polyporus sp.2* indicated the lowest and the highest levels of MnP, 12.4 U/l and 15.7 U/l, respectively. The production of laccase was highest at 20°C for all strains. The highest levels of laccase

were achieved by *P. pulmonarius*, *P. ostreatus sp.3* and *G. australe*, without showing any significant differences (p-value = 0.062), producing 20.7, 21.6 and 24.6 U/l, respectively (Fig. 3). The other five strains, whereof four belonged to the *Polyporus* species, were categorized in the same subset (p-value = 0.164) according to Tukey test.

Although there was no difference in the expression of laccase among the strains *P. ostreatus sp.3* and *sp.4* at 15°C (6.2 and 7.2 U/l, respectively), the increase of incubation temperature affected their activity, doubling their difference (21.6 and 10.2 U/l, respectively).

As shown in Figure 3, manganese peroxidase ranged in lower levels with regard to laccase. *P. ostreatus sp.4* showed the lowest MnP activity, 6.6 U/l at the 4th day of growth. Similar activities were achieved by *Polyporus sp.2* and *P. pulmonarius*, 9.7 and 11.6 U/l, respectively, and as a result they grouped in the same subset (p-value= 0.073). The second group contained the former two strains and additionally *P. brumalis*, *G. australe*, *P. ostreatus* sp.3 (p-value=0.376). *P. ciliatus* and *G. australe*, *P. ostreatus sp.3* constituted the third group (p-value= 0.062). Finally, the group with the highest MnP activities was represented by *P. ciliatus* and *Polyporus sp.3*, 18.2 and 17.4 U/l, respectively (p-value=0.999).

Table 1. Maximum laccase and manganese peroxidase activities for the tested strains at 15, 20, 25 and 30°C.

Strain	**Laccase (U/l) (Expression day)**				**Manganese peroxidase (U/l) (Expression day)**			
	Temperature (°C)				Temperature (°C)			
	15	20	25	30	15	20	25	30
Pleurotus pulmonarius	7.84 ± 1.84 (4)	20.69 ± 2.15 (4)	14.15 ± 2.54 (6)	11.84 ± 2.69 (7)	14.03 ± 0.7 (7)	11.58 ± 2.0 (12)	18.71 ± 1.76 (11)	13.44 ± 1.44 (13)
Pleurotus ostreatus sp.3	6.15 ± 1.41 (11)	21.61 ± 1.16 (4)	10.31 ± 0.66 (5)	27.81 ± 3.62 (11)	15.66± 2.0 (12)	13.21 ± 2.08 (12)	18.71 ± 4.10 (12)	25.96 ± 1.44 (13)
Pleurotus ostreatus sp.4	5.53 ± 1.22 (14)	10.19 ± 1.56 (9)	8.15 ± 0.7 (12)	4.31 ± 0.7 (4)	13.09 ± 2.83 (4)	6.56 ± 0.76 (4)	14.37 ± 2.89 (11)	8.18 ± 0.40 (6)
Ganoderma australe	5.69 ± 1.48 (7)	24.61 ± 0.25 (4)	9.38 ± 1.06 (5)	26.11 ± 2.29 (13)	12.39 ± 1.07 (12)	12.98 ± 1.06 (7)	14.73 ± 1.85 (6)	22.48 ± 1.74 (13)
Polyporus brumalis	6.76 ± 1.48 (4)	8.74 ± 1.18 (7)	20.31 ± 2.88 (5)	9.69 ± 0.46 (5)	13.56 ± 1.46 (7)	12.28 ± 3.28 (6)	16.37 ± 3.6 (11)	11.92 ± 1.21 (7)
Polyporus ciliatus	3.53 ± 0.96 (6)	8.76 ± 1.66 (19)	9.38 ± 1.86 (5)	4.3 ± 0.7 (6)	21.51± 2.14 (10)	18.15 ± 2.4 (6)	16.45 ± 0.4 (14)	10.99 ± 1.46 (6)
Polyporus sp.2	4.92 ± 0.96 (6)	9.08 ± 1.16 (5)	6.61 ± 2.32 (6)	6.31 ± 1.16 (11)	15.66 ± 0.4 (12)	9.73 ± 0.28 (13)	17.38 ± 1.2 (14)	9.82 ± 1.4 (6)
Polyporus sp.3	3.84 ± 0.26 (6)	6.92 ± 1.38 (11)	7.53 ± 1.62 (6)	5.07 ± 0.92 (7)	15.43 ± 0.1 (12)	17.38 ± 4.22 (5)	17.61 ± 3.5 (6)	12.39 ± 1.46 (4)

Laccase was expressed by all fungi at 25°C and the variation of activities as a function of time for four fungi is shown in Fig. 4. The maximum activity (20.3 U/l) was obtained by the fungus *P. brumalis* in a period of 5 days (group 3). *P. pulmonarius* and *P. ostreatus sp.3* showed high production of laccase, 14.2 and 10.3 U/l during the 6th and 5th day of incubation, respectively. In the same group, the strains *G. australe* and *P. ciliatus* were also assorted (p-value = 0.096). *Polyporus sp.3*, *Polyporus sp.2* and *P. ostreatus sp.4* indicated the lowest laccase activity (6.6, 7.5 and 8.2 U/l, respectively) and constituted the first group with the addition of *P. ostreatus sp.*3, *G. australe* and *P. ciliatus* (p-value = 0.302).

Manganese peroxidase in the isolates used did not differ significantly at 25°C (p-value=0.531). *P. ostreatus sp.3* and *P. pulmonarius* had the highest production of MnP (18.7 U/l) during the 12th and 6th day of growth. The lowest level was obtained by *P. ostreatus sp.4* and *G. australe* (14.4 and 14.7 U/l, respectively).

Significant differences were revealed for the strains at 30°C. The fungi *P. ciliatus*, *P. ostreatus*

sp.4, *Polyporus sp.2* and *sp.3* had the lowest activities and were grouped in the first subset, showing a p-value equal to 0.891. The second group (p-value = 0.122) contained the two former strains and *P. brumalis*, which also belonged to the third group with *P. pulmonarius* (p-value = 0.851). *G. australe* and *P. ostreatus sp.3* had the highest production of laccase, 26.1 and 27.8 U/l, respectively, after 13 and 11 days of growth at 30oC (fourth group with p-value = 0.95). The determined activities for selected strains are shown in Fig 5.

The maximum MnP activities were expressed by the strains *G. australe* and *P. ostreatus sp.3*, as in the case of laccase, producing 22.5 and 26 U/l, respectively, after 13 days of incubation (p-value= 0.098). *P. pulmonarius* and *Polyporus sp.3* indicated similar activities and grouped with *P. brumalis*, *P. ciliatus* and *Polyporus sp.2* (p-value=0.0767). The group with the lowest activity contained the former three strains and *P. ostreatus sp.4* (p-value= 0.063).

To determine the effect of temperature in each fungus, the results were also treated with Tukey test. Temperature had no influence in laccase production for *Polyporus sp.2*. *Polyporus sp.3* showed significant differences at 15 and 25°C whereas the activities were similar at the other two temperatures and were categorized in the former two subsets. The optimum temperature for *P. brumalis* was 25°C and no substantial change was observed in the other three temperature ranges. *P. ciliatus* had the lowest activity in the terminal temperatures, while the highest activities were found at 20 and 25°C. The lowest enzyme production of laccase for *P. ostreatus sp.3* was obtained at 15 and 25°C while the highest activity was observed at 30°C. *P. ostreatus sp.4* had the lowest level of laccase at 30°C. *G. australe* showed the highest production at 20 and 30°C. The temperature affected laccase expression for *P. pulmonarius*, presenting the highest activity at 20°C. Laccase activity at 30°C fitted to the activities at 25 and 15°C.

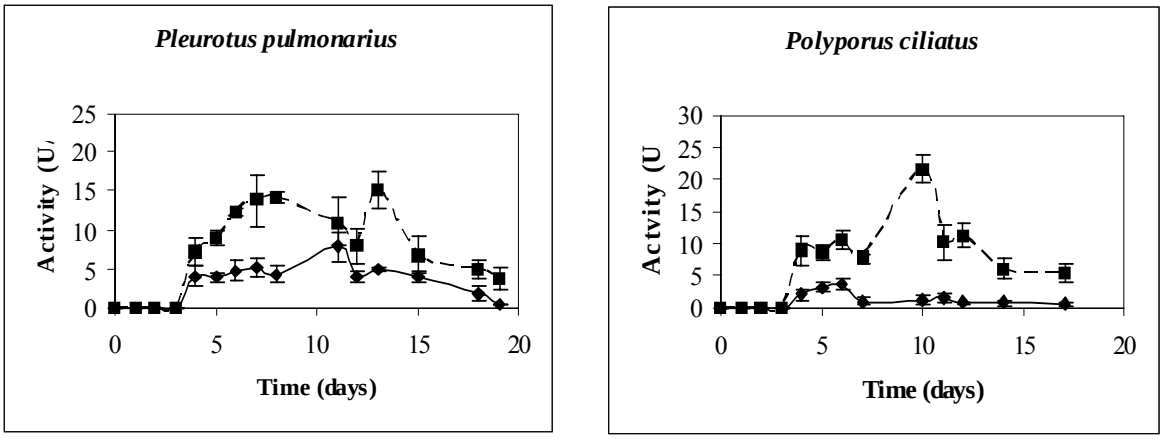

Fig. 2. Time course of laccase and manganese peroxidase production by selected strains at 15°C. (—■—) MnP activity, (—♦—) Lac activity.

Manganese peroxidase activities were not affected by the changes in incubation temperature in the case of *P. brumalis*. *P. ostreatus sp.3* and *G. australe* had similar activities at the range 15-25°C. The greatest production was observed at 30°C for both strains. A respective pattern was shown by *P. pulmonarius* with differences at the temperatures 15, 20 and 30°C while the highest MnP was observed at 25°C. In contrast to the former *Pleurotus* species, the activities for *P. ostreatus sp.4* were categorized in three subsets. The first group consisted of the activities at 20 and 30°C, the second one the activities at 30 and 15°C and the highest activities at 15 and 25°C constituted the third group. *P. ciliatus* had the lowest production at 30°C, while the production at 20°C had no significant difference with the one at 15 and 25°C. For the strain *Polyporus sp.2*, the activities were divided in two groups, the high activity group consisted of the production at 15 and 25°C and the activities at 20 and 30°C belonged to the low activity group. Temperature had a substantial influence at 30 and 20°C for *Polyporus sp.3* whereas the activities at 25 and 15°C fitted to both subsets.

The ratio of manganese peroxidase to laccase (MnP/Lac) was calculated in order to specify the effect of temperature on the relative contribution of these enzymes. As shown in Fig. 6, the white rot fungi *G. australe*, *P. ostreatus sp.3*, *Polyporus sp.2* and *P. pulmonarius* followed the same pattern when temperature variated. The ratio MnP/Lac increased at 15 and 25°C, which indicated that the production of MnP was improved. At 20 and 30°C, the ratio decreased, and as a result the laccase production was

in favor. The production of MnP decreased with the rise of temperature for *Polyporus sp.3* and *P. brumalis*. The expression of MnP at 15°C was favorable for *P. ciliatus* but remained constant at the range 20-30°C. *P. ostreatus sp.4* produced less MnP at 20°C compared to other temperatures where the production of MnP was similar. Conclusively, the activities of managanese peroxidase and laccase are expressed differently at different incubation temperatures.

Many reports have shown that the ligninolytic enzymes are not produced by all white rot fungi. From the tested fungi, genes closely related to LiP (isozyme H8) were detected only for *G. australe*, showing that this fungus is a LiP producer, although LiP activity has not been reported elsewhere [31]. The lack of LiP activity is also confirmed in the present work, (*G. australe* expressed only laccase and manganese peroxidase) implying that the LiP gene is not expressed under the specified growth conditions. It has been also established that the fungus *Pleurotus ostreatus* produces Lac and MnP in liquid cultures [32], as confirmed in our study. The strain *Pleurotus pulmonarius* produced Lac and MnP, as expected [33, 34]. References for the strains of *Polyporus* have been found only for *P. ciliatus*, which expressed Lac and MnP [35]. This study demonstrated that among the white rot fungi tested, higher ligninolytic activities were observed compared to strains of the same species from previous studies.

The level of laccase production induced by the increase of temperature for *P. ostreatus sp.3* (up to 27.81 U/l) was higher than that induced by different concentrations of nitrogen in another strain of *P. ostreatus*, which ranged from 1.6 to 5.9 U/l in N-limited and N-rich medium relatively [21].

Manganese peroxidase activity was of the same order for the former strains under high organic N medium and at the temperature of 25°C (approximately 18.8 U/l), but further increase in MnP activity (25.9 U/l) was observed at 30°C for *P. ostreatus sp.3*. Another strain of *P. ostreatus* [36] has shown lower production of laccase under different carbon and nitrogen sources, low molecular weight aromatic compounds and other inducers (up to 0.4 U/l) compared to the strains tested in this work (*P. ostreatus sp.3* and *sp.4*). Additionally, *Ganoderma australe* secreted higher levels of Lac and MnP (24.61 and 22.48 U/l relatively) in comparison with two other strains of *Ganoderma*, which produced 0.75 - 4.9 U/l for Lac and 0.35-0.65 U/l for MnP, but in addition produced LiP [37]. With regard to *Polyporus* species, enzyme expression was also determined for *P. ciliatus*. In a previous study [38], *P. ciliatus* showed a level of 20 U/l for Lac and 14 U/l for MnP. On the contrary, the tested strain in this work showed higher levels for MnP (21.51 U/l) and lower for Lac (9.38 U/l).

The combination of manganese peroxidase and laccase was predominant for the tested strains. The negative test for lignin peroxidase entails that either these fungi do not produce Lip in significant levels or LiP production requires different conditions [39] as in the case of *Trametes versicolor*, *Bjekandera adjusta*, known as LiP producers [39, 40]. Therefore, the production depends on the strain or the cultivation conditions.

Conclusively, temperature optimization led to a substantial increase in enzyme yield compared to the findings of other researchers without using inducers or different media compositions.

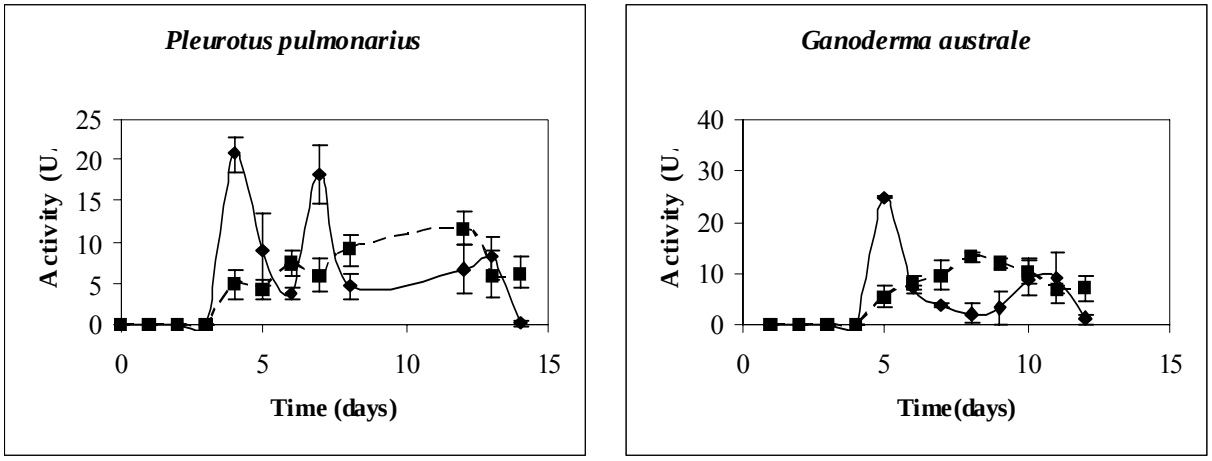

Fig. 3. Time course of laccase and manganese peroxidase production by selected strains at 20 °C. (—■—) MnP activity, (—♦—) Lac activity

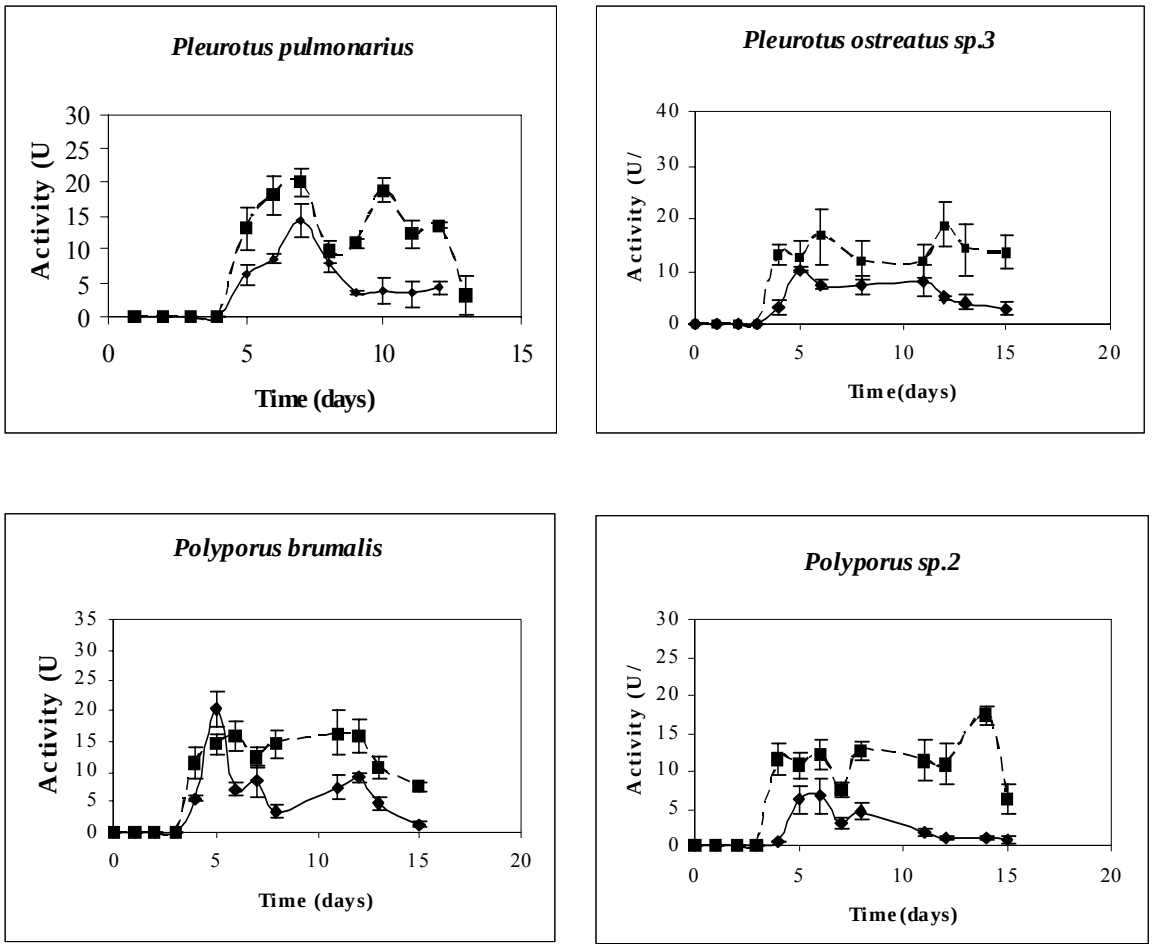

Fig. 4. Time course of laccase and manganese peroxidase production by selected strains at 25 °C. (—■—) MnP activity, (—♦—) Lac activity

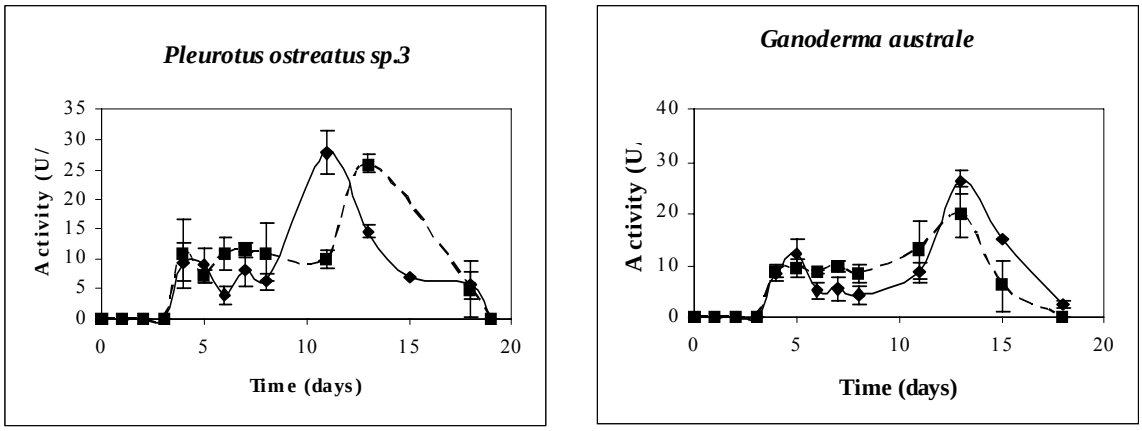

Fig. 5. Time course of laccase and manganese peroxidase production by selected strains at 30°C. (—■—) MnP activity, (—♦—) Lac activity

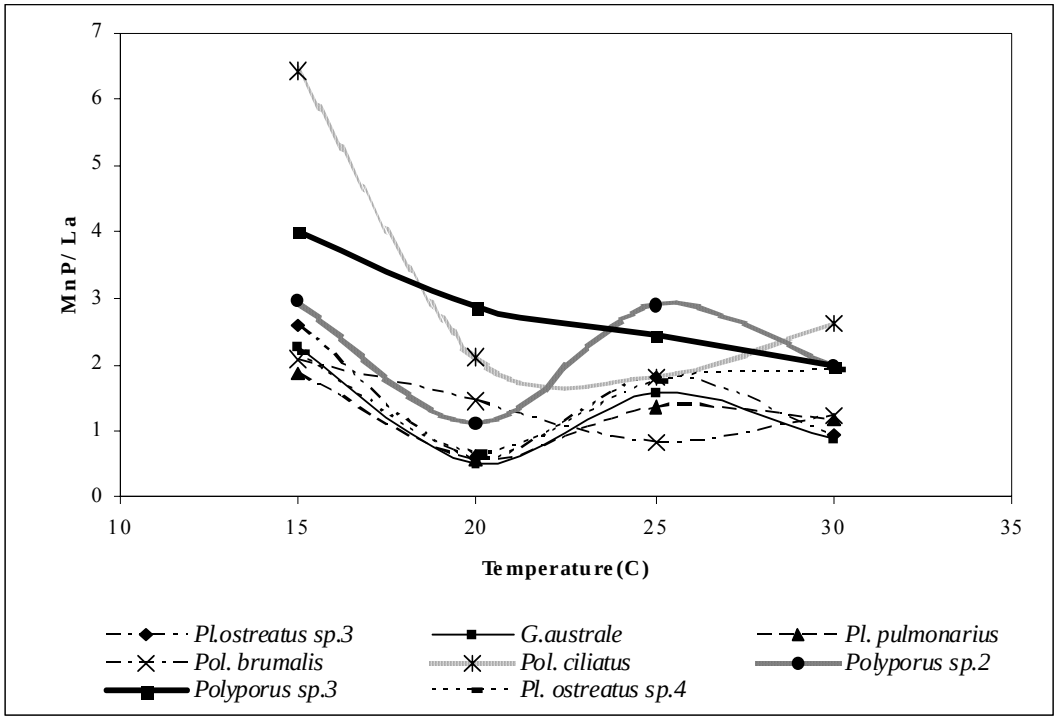

Fig. 6. The influence of temperature on the ratio MnP/Lac for all strains.

4 Conclusion

In this study, eight white rot fungi were tested in order to estimate their ligninolytic potential. The enzyme activities showed that the combination of laccase and manganese peroxidase prevailed in all strains. It was confirmed that the incubation temperature affected the expression and the pattern of the ligninolytic enzymes. Differences were revealed between laccase and manganese peroxidase. At 15°C, significant differences were not observed among the fungi with regard to laccase activities, except for *P. ciliatus* and *Polyporus sp.3* possessing the lowest laccase activity and *P. pulmonarius* and *P. ostreatus sp.4* possessing the highest laccase activity. The levels of manganese peroxidase were high for all strains and *P. ciliatus* had the higher production of this enzyme. The strains *P. pulmonarius*, *P. ostreatus sp.3* and *G. australe* showed the highest production of laccase at 20°C. The best MnP producers at the same temperature were *P. ciliatus* and *Polyporus sp.3*. The highest activity of Lac was observed by *P. brumalis*, while MnP activities ranged in the same range at 25°C. Finally, the maximum enzyme activities were expressed by *P. ostreatus sp.3* and *G. australe* at 30°C.

Significant differences revealed in the expression and the levels of the linginolytic enzymes of the same species are in agreement with previous studies, implying that the enzyme production of fungi depends not only on the strain but also on the cultivation conditions. Conclusively, temperature optimization led to a substantial increase in enzyme yield compared to the findings of other researchers without using inducers or different media compositions.

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