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Screening of Laccase, Manganese Peroxidase, and Versatile Peroxidase Activities of the Genus *Pleurotus* in Media With Some Raw Plant Materials as Carbon Sources

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Abstract

Species of the genus *Pleurotus* are among the most efficient natural species in lignin degradation belonging to the subclass of ligninolytic organisms that produce laccase (Lac), Mn-dependent peroxidase (MnP), versatile peroxidase (VP), and the H_2O_2-generating enzyme aryl-alcohol oxidase, but not lignin peroxidases. Production of Lac and oxidation of 2,6-dimethoxyphenol (DMP) in the presence and absence of Mn^{2+} were detected both in submerged fermentation (SF) of dry ground mandarine peels and in solid-state fermentation (SSF) of grapevine sawdust in all investigated *Pleurotus* species and strains. Evidence of cultivation methods having a distinct influence on the level of enzyme activities has been demonstrated. Most of the species and strains had higher Lac activity under SSF conditions than under SF conditions. DMP oxidation in the presence and absence of Mn^{2+} was detected in all

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investigated species and strains, but was lower under SF conditions than under SSF conditions for most of them. However, relative activities of DMP oxidation in the absence of Mn^{2+}, as percentages of activity against DMP in the presence of Mn^{2+}, were higher under conditions of SF than in SSF cultures in most of the investigated species and strains. The obtained results showed that strains of different origins have different efficiently ligninolytic systems and that conditions of SSF are more favorable for ligninolytic activity than those in SF owing to their similarity to natural conditions on wood substrates.

Index Entries: *Pleurotus*; laccase; manganese peroxidase; versatile peroxidase; submerged fermentation; solid-state fermentation.

Introduction

Lignin is a natural-branched polymer and the most abundant aromatic material on Earth *(1)*. After vascular plants die or drop, lignified organic carbon has to be recycled into the earth's carbon cycle. Microorganisms and aerobic filamentous fungi, among which the Basidiomycetes lead, are good degraders. There are three types of fungal wood decay: white rot, brown rot, and soft rot *(2)*.

White-rot fungi are the most abundant degraders of wood in nature. They secrete enzymes and metabolites that cause depolymerization of the hemicellulose and cellulose, and fragmentation of the lignin *(3)*. According to Hammel *(4)* and Master and Field *(5)*, the ligninolytic enzymes are produced during secondary metabolism under limited nutrients for fungal growth; in most woods and soils, this is probably nitrogen.

Extracellular lignin-degrading enzymes, which act directly or indirectly on lignin, are: three glycosylated heme-containing peroxidases, lignin peroxidase (LiP), Mn-dependent peroxidase (MnP), versatile peroxidase (VP), copper-containing phenoloxidase, and laccase (Lac). Species of the genus *Pleurotus* are among the most efficient natural species in lignin degradation, and they belong to the subclass of ligninolytic organisms that produce Lac, MnP, VP, and the H_2O_2-generating enzyme aryl-alcohol oxidase, but not LiPs *(6)*.

Recently, there has been a growing interest in studying the lignin-modifying enzymes of a wider array of white-rot fungi, not only from the standpoint of comparative biology, but also with the expectation of finding better lignin-degrading systems for use in various biotechnological applications such as biotransformation of raw plant materials to feed and fuels; production of enzymes, antibiotics, polysaccharides, and other physiologically active compounds; biopulping; biobleaching of paper pulp; and bioremediation of soils and industrial waters polluted with toxic chemicals and dyes *(7–9)*.

The purpose of the present study was to elucidate the diversity of *Pleurotus* inter- and intraspecies in Lac, MnP, and VP activities under SF conditions of dry ground mandarine peels and under SSF conditions of grapevine sawdust.

Materials and Methods

Organisms and Growth Conditions

Investigated species and strains of the genus *Pleurotus* and their origin are presented in Table 1. Cultures are deposited on wort agar medium (wort and water in a ratio of 1:1, 0.5 g/L of peptone, 0.5 g/L of yeast extract, 0.5 g/L of $MgSO_4$, and 17 g/L of agar; pH 6.0) in the culture collection of the Institute of Evolution, University of Haifa (HAI), and documented in the *Catalogue of Cultures (10)*.

The inocula were prepared by growing fungi on a rotary shaker at 180 rpm in 250-mL flasks containing 100 mL of synthetic medium (10.0 g/L of glucose, 2.0 g/L of NH_4NO_3, 0.8 g/L of KH_2PO_4, 0.75 g/L of $Na_2HPO_4 \cdot 7H_2O$, 0.5 g/L of $MgSO_4 \cdot 7H_2O$, 2.0 g/L of yeast extract). The initial pH was adjusted to 6.0 prior to sterilization by adding 4% HCl. After 7 d of cultivation, mycelial pellets were harvested and homogenized using a laboratory homogenizer at 10,000 rpm.

Submerged Fermentation of Dry Ground Mandarine Peels

SF was carried out at room temperature in 250-mL flasks containing 2 g of mandarine peels and 50 mL of synthetic medium without glucose and with microelements (0.002 g/L of $ZnSO_4 \cdot 7H_2O$, 0.005 g/L of $FeSO_4 \cdot 7H_2O$, 0.06 g/L of $CaCl_2$, 0.02 g/L of $CuSO_4 \cdot 7H_2O$, 0.05 g/L of $MnSO_4 \cdot H_2O$) on a rotary shaker at 180 rpm. The initial pH was adjusted to 9.8 prior to sterilization by adding 4% NaOH and then brought to 6.0 after sterilization. Suspensions of 5 mL after inocula homogenization were used per flask. Biomasses were separated by centrifuging at 4°C and 5000 rpm for 20 min after 5 and 7 d of cultivation. Clean supernatants were used to estimate enzymatic activity.

Solid-State Fermentation of Grapevine Sawdust

SSF was carried out at 25°C in 100-mL flasks containing 4 g of grapevine sawdust and 12 mL of synthetic medium without glucose, with microelements, and supplemented with 0.4% NH_4NO_3. The initial pH of the synthetic medium was adjusted to 6.0 prior to sterilization by adding 4% HCl. Samples from flasks were harvested after 7 and 10 d of cultivation, and extracellular enzymes were extracted three times with 20 mL of distilled water (total volume of 60 mL). Solids were separated by centrifuging (4°C, 5000 rpm, 10 min), and culture filtrates were used to measure enzymatic activities.

Enzyme Activity Assays

Lac activity was assayed using syringaldazine (4-hydroxy-3,5-dimethoxybenzaldehyde azine) as a substrate and by measuring the increase in absorbance at 525 nm ($\varepsilon = 65{,}000\ M^{-1}cm^{-1}$) for 60 s. The mixture contained 0.1 *M* acetic buffer (pH 5.0), 1 m*M* syringaldazine (dissolved in 96% ethanol), and enzyme preparation (V_{tot} = 1 mL).

Table 1
Investigated Species and Strains of the Genus *Pleurotus*

Scientific name of species	HAI no. of strain	Origin of strain
P. cornucopiae Paul.:Fr.	32	Cultivated strain, Belgium
P. cystidiosus O.K. Miller	95	Israel, Haifa, park, on *Schinus terebinthifolius* Raddi
P. citrinopileatus Singer	602	Cultivated strain, England
P. djamor (Rumph.:Fr.) Boedijn	485	Hawaii, Nextlab
P. eryngii (DC.:Fr.) Quél. var. *eryngii*	193	Ukrainian SSR, Kherson region, Chaplinka district, Askania-Nova, on *Stipa* sp.
	201	Israel, Menahemya, on *Ferula* sp.
	356	Israel, Gevaot Merar, near Gedera, on *Ferula* sp.
	507	Hawaii, Nextlab
	616	Israel, Tabor, on *Ferula* sp.
	711	Israel, Tel Hazor, on *Ferula* sp.
	716	Israel, Gilboa, on *Ferula* sp.
	728	Israel, Lahav, on *Ferula* sp.
P. eryngii var. *tingitanus* Lewinsohn et al.	555	Israel, Sataf, on *Ferula tingitana* L.
P. ostreatus (Jacq.:Fr.) Kumm.	207	Ukraine, Transcarpathian region, Beregovo district, village Ivanovka, on *Quercus robur* L.
	221	Israel, Atlit, park, on *Salix* sp.
	234	Cultivated strain, Hungary
	290	Cultivated strain, England
	387	Yugoslavia, HK-35
	493	Hawaii, Nextlab
	494	Hawaii, Nextlab
	495	Hawaii, Nextlab
	592	KW, A. S. Buchalo
P. ostreatus var. *florida* Eger, nom. nudum	393	Cultivated strain, England
P. pulmonarius (Fr.) Quél.	509	Hawaii, Nextlab
	572	KW, A. S. Buchalo (194); CCBAS, M. Semerdzieva (478)
	573	KW, A. S. Buchalo (111); VKM (F-2006), Coll. Russia, Sochi
P. salignus (Fr.) Kumm.	328	Israel, Dan Natural Reserve, Tel Dan, on *Salix* sp.
P. salmoneo stramineu L. Vass.	77	Belgium (M2700)
P. smithii Guzmán	138	Haifa, Carmel park, on *Morus alba* L.
	140	Haifa, Carmel, Moria St. park, on *Morus alba*
	141	Jerusalem, on *S. terebinthifolius*

MnP activity was measured indirectly by monitoring the Mn^{2+}-dependent oxidation of 20 m*M* DMP to coerulignone (3,3',5,5'-tetramethoxy-*p*,*p*'-diphenoquinone) spectrophotometrically at 469 nm ($\varepsilon = 27{,}500\ M^{-1}cm^{-1}$). A final volume of 1 mL of reaction mixture contained 250 m*M* Na malonate buffer (pH 4.5), 20 m*M* DMP, 20 m*M* $MnSO_4 \cdot H_2O$, 4 m*M* H_2O_2, and the enzyme preparation suitably diluted by distilled water (V_{tot} = 1 mL).

VP and Lac activities were also measured by using 20 m*M* DMP (without Mn^{2+} and without H_2O_2, respectively) in 250 m*M* Na malonate buffer (pH 3.0 and 5.0, respectively). The amount of enzyme that transforms 1 µmol of substrate/min was defined as 1 U of enzyme activity. A UV-160A Spectrophotometer (Shimaden) was used for this assay.

Results

Lac production was detected in both SF and SSF cultures of the investigated species and strains (Table 2). Most of them had higher Lac activity under SSF conditions than under SF. For example, in the case of *P. ostreatus*, strain no. 493, Lac activity was 80-fold higher under SSF conditions after 10 d of cultivation than under SF conditions on d 7 of cultivation (2144.62 ± 57.78 and 27.05 ± 0.25 U/L, respectively). During SSF, Lac activity was from 2.99 ± 0.59 U/L in *P. salmoneostramineus*, strain no. 77 on d 7 of cultivation to 2144.62 ± 57.78 U/L in *P. ostreatus*, strain no. 493 after 10 d. In some investigated strains (nos. 201, 616, 393), levels of Lac activity were higher under SF conditions than under SSF conditions, and values ranged from 266.49 ± 12.74 U/L in *P. eryngii* var. *eryngii* strain no. 201 to 999.54 ± 20.73 U/L in *P. eryngii* var. *eryngii*, strain no. 616, after 7 d of cultivation.

Under conditions of submerged fermentation of mandarine peels, levels of Lac activity were higher after 7 d of cultivation in most of the investigated strains, and only a few strains (nos. 485, 356, 507, 494, 77) had higher levels of Lac activity after 5 d, when levels of Lac started to decrease. Similar results were obtained under conditions of SSF of grapevine sawdust; activity was significantly higher on d 10 of cultivation in most of the investigated strains, and in some strains (nos. 507, 616, 555, 495, 592, 393, 509, 573, 328) activity was higher on d 7 of cultivation and then started to decrease (Table 2).

Lac has wide substrate specificity. Under SF conditions, it was shown that DMP was a more specific substrate for Lac of some investigated species and strains (nos. 602, 193, 555, 234, 290, 494, 509, 572, 573, 77, 141), some strains showed almost the same activities with both DMP and syringaldazine, and for other syringaldazine was a more specific substrate. Similar results were obtained under conditions of SSF of grapevine sawdust (Table 2).

DMP oxidation in the presence of Mn^{2+} was detected in all investigated species and strains but was lower under conditions of submerged fermenta-

Table 2
Lac Activity Against Syringaldazine and DMP of *Pleurotus* Species
Under Conditions of SF of Dry Ground Mandarine Peels and SSF of Grapevine Sawdust

Species	Strain (CN)	SF of mandarine peels				SSF of grapevine sawdust			
		Activity against				Activity against			
		Syringaldazine (U/L)		DMP (U/L)		Syringaldazine (U/L)		DMP (U/L)	
		5 d	7 d	5 d	7 d	7 d	10 d	7 d	10 d
P. cornicopiae	32	0.95 ± 0.02	3.18 ± 0.44	0.88 ± 0.02	1.25 ± 0.06	16.62 ± 2.61	371.85 ± 16.83	17.93 ± 2.4	166.37 ± 1.04
P. cystidiosus	95	0.84 ± 0.33	5.86 ± 0.46	0.79 ± 0.06	0.54 ± 0.06	57.21 ± 1.37	240.77 ± 83.89	56.11 ± 0.38	76.73 ± 0.6
P. cytrinopileatus	602	0.78 ± 0.08	8.63 ± 0.19	0.38 ± 0.13	22.8 ± 0.48	37.89 ± 1.59	60.38 ± 0.82	16.99 ± 0.21	99.28 ± 3.56
P. djamor	485	12.65 ± 3.52	10.86 ± 1.6	6.67 ± 0.24	11.66 ± 0.65	133.81 ± 7.7	145.85 ± 0.25	114.54 ± 3.18	189.94 ± 9.98
P. eryngii var. *eryngii*	193	9.27 ± 0.42	14.3 ± 2.65	76.13 ± 1.17	25.37 ± 1.14	19.15 ± 0.73	72.56 ± 1.97	9.45 ± 0.33	101.1 ± 2.36
	201	22.76 ± 3.31	266.49 ± 12.74	27.17 ± 3.11	251.25 ± 36.67	24.13 ± 2.76	24.29 ± 1.29	30.1 ± 0.79	20.83 ± 1.4
	356	12.03 ± 2.31	5.45 ± 0.92	6 ± 0.03	2.35 ± 0.008	208.1 ± 3.33	244.62 ± 17.53	66.1 ± 0.37	44.31 ± 2.12
	507	3.57 ± 0.58	2.37 ± 0.15	0.84 ± 0.06	0	120 ± 3	41.97 ± 1.68	80.55 ± 1.57	61.16 ± 1.84
	616	162.31 ± 14.13	999.54 ± 20.73	443.1 ± 90.26	858.82 ± 113.05	41.82 ± 1.33	9.33 ± 0.22	22.4 ± 3.72	18.84 ± 0.89
	711	4.6 ± 2.81	9.25 ± 1.14	3.39 ± 0.14	2.24 ± 0.01	139.15 ± 4.36	220.89 ± 8.26	151.91 ± 1.85	13.07 ± 2.36
	716	1.09 ± 0.18	281.77 ± 4.34	0.43 ± 0.16	206.37 ± 4.7	96.2 ± 1.59	357.23 ± 5.27	78.1 ± 1.41	35.1 ± 2.82
	728	4 ± 0.14	6.48 ± 1.14	8.68 ± 0.13	6.77 ± 0.19	54.26 ± 16.12	177.57 ± 4.26	17.96 ± 2.38	15.06 ± 0.65
P. eryngii var. *tingitanus*	555	1.52 ± 0.29	5.72 ± 1.05	6 ± 0.27	9.32 ± 0.25	27.2 ± 3.77	11.16 ± 0.44	19.32 ± 1.91	11.32 ± 0.47
P. ostreatus	207	5.83 ± 0.79	10.48 ± 1.29	3.29 ± 0.15	5.49 ± 0.23	106.39 ± 21.61	232.92 ± 20.22	215.91 ± 2.6	175.82 ± 0.44
	221	8.74 ± 0.77	17.31 ± 2.47	2.53 ± 0.02	0.45 ± 0.06	300.32 ± 20.36	307.08 ± 46.25	340.72 ± 25.97	330.15 ± 26.28
	234	1.63 ± 0.09	51.9 ± 21.94	5.81 ± 0.08	87 ± 8	153.54 ± 0.65	1233.23 ± 339.66	170.1 ± 4.23	188.55 ± 19.15
	290	50.55 ± 17.5	252.04 ± 15.53	114.24 ± 32	269.21 ± 21.77	22 ± 2.89	7.91 ± 0.38	28.63 ± 1.68	19.49 ± 0.01
	387	4.32 ± 1.45	9.66 ± 3.72	13.42 ± 0.12	7.92 ± 0.48	158.81 ± 10.58	360.46 ± 42.08	163.91 ± 16.55	106.88 ± 9.86
	493	3.21 ± 0.12	27.05 ± 0.25	1.18 ± 0.08	1.95 ± 0.1	226.38 ± 14.9	2144.62 ± 57.78	185.66 ± 3.69	193.1 ± 2.97
	494	256.1 ± 18.9	6.06 ± 1.31	414.47 ± 1.31	117.68 ± 13.18	75.23 ± 15.69	160 ± 1.19	139.45 ± 0.79	185.82 ± 4.3
	495	2.85 ± 0.15	9.89 ± 1.42	0.7 ± 0.08	0.74 ± 0.08	152.95 ± 15.29	46.31 ± 10.96	90.55 ± 5.49	117.1 ± 2.82
	592	3.07 ± 0.94	274.54 ± 18.78	27.8 ± 1.51	3.8 ± 0.18	62.7 ± 0.94	24.97 ± 1.45	69.51 ± 0.52	30.69 ± 1.31
P. ostreatus var. *florida*	393	103.73 ± 9.83	415.54 ± 22.86	71.55 ± 4.67	0.09 ± 0.01	283.22 ± 10.74	209.31 ± 1.6	347.45 ± 11.76	236.8 ± 7.13
P. pulmonarius	509	7.1 ± 2.63	24.86 ± 2.61	18.96 ± 3.71	87.31 ± 3.54	61.76 ± 4.57	51.53 ± 3.1	86.76 ± 3.22	86.37 ± 2.22
	572	2.45 ± 0.08	9.03 ± 3.92	1.16 ± 0.13	38.24 ± 3.54	154 ± 0.09	148.22 ± 8.77	133.76 ± 10.45	168.46 ± 1.26
	573	0.8 ± 0.06	1.52 ± 0.16	0	2.83 ± 1.25	226.97 ± 9.81	151.16 ± 0.41	163.82 ± 5.34	140.18 ± 2.52
P. salignus	328	4.27 ± 2.35	4.58 ± 1.13	0.7 ± 0.02	2.14 ± 0.02	351.88 ± 3.9	17.49 ± 2.15	272.62 ± 11.1	91.1 ± 4.16
P. salmoneostramineus	77	5.79 ± 0.2	2.91 ± 0.22	3.87 ± 0.34	4.32 ± 0.28	2.99 ± 0.59	75.41 ± 9.07	15.71 ± 0.19	81.58 ± 12.78
P. smithii	138	0.89 ± 0.01	6.76 ± 0.27	0.65 ± 0.03	1.09 ± 0.05	13.69 ± 7.49	91.74 ± 5.94	18.09 ± 5.39	77.93 ± 3.53
	140	2.56 ± 0.13	6.86 ± 0.28	1.3 ± 0.2	0.75 ± 0.02	20.71 ± 0.59	176.54 ± 8.61	33.06 ± 0.57	56.55 ± 0.15
	141	2.8 ± 1.13	4.22 ± 1.45	9.61 ± 0.004	16.79 ± 0.13	20.11 ± 1.31	22.66 ± 3.02	78.04 ± 1.48	135.31 ± 2.58

tion than under SSF conditions (Table 3). Under conditions of SF, values of activity against DMP in the presence of Mn^{2+} varied from 0.56 ± 0.34 U/L (*P. salignus*, strain no. 328) to 371 ± 3.34 U/L (*P. eryngii* var. *eryngii*, strain no. 616) after 5 d, and from 1.16 ± 0.09 U/L (*P. smithii*, strain no. 138) to 705.82 ± 80.46 U/L (*P. eryngii* var. *eryngii*, strain no. 616) after 7 d of cultivation. In SSF cultures, the obtained results were from 3.58 ± 0.16 U/L in *P. eryngii* var. *eryngii*, strain no. 193, to 444.24 ± 2.4 U/L in *P. pulmonarius*, strain no. 572 on d 7 of cultivation, and from 15.1 ± 0.3 U/L in *P. eryngii* var. *tingitanus*, strain no. 555, to 458.9 ± 44.3 U/L in *P. ostreatus*, strain no. 494, on d 10 of cultivation.

All investigated species and strains showed the ability to oxidize DMP in the absence of Mn^{2+} under both conditions of SF and SSF (Table 3). Just as in the case of the oxidation ability of DMP in the presence of Mn^{2+}, the oxidation ability of DMP in the absence of Mn^{2+} was higher in SSF than in SF cultures. Relative activities of DMP oxidation in the absence of Mn^{2+}, as percentages of the activity against DMP in the presence of Mn^{2+}, under conditions of SF were higher than in SSF cultures in most of the investigated species and strains. They varied from 1.2% (strain no. 494) to 91.9% (strain no. 138) after 5 d of cultivation and from 2.1% (strain no. 616) to 82% (strain no. 207) after 7 d of cultivation. Under conditions of SSF, the obtained values were from 1.7% (strain no. 393) to 48.6% (strain no. 193) after 7 d of cultivation and from 1.2% (strain no. 77) to 27% (strain no. 328) after 10 d of cultivation.

Discussion

Analyses of the results showed that differences in extracellular enzyme production exist among *Pleurotus* species and even among strains of the same species. Cultivation conditions, which were characterized by different physico-chemical characteristics, influenced the levels of Lac, MnP, and VP activities. High Lac and MnP activities were found in *P. ostreatus*, strain no. 493, and *P. pulmonarius*, strain no. 572, respectively, under conditions of SSF of grapevine sawdust. These results correspond with the results of Martínez et al. *(11)* and Muñoz et al. *(6)*, in which Lac production in *P. eryngii* was strongly stimulated by the alkali lignin present in straw, and with the results of Elisashvili et al. *(12)*, who also found high MnP activity (184.6 nkat/mL) under conditions of SSF of grapevine sawdust in *P. ostreatus* IBB 191. The conditions of SSF were quite similar to those existing in nature on wood substrates, whereas those in SF were quite different. This could account for the low ligninolytic activity in liquid cultures.

In these experiments, substrate specificity was also determined for *Pleurotus* Lac by observing activity levels in reaction mixtures containing either syringaldazine or DMP as substrate. Our results are in agreement with those of Muñoz et al. *(6)*, which showed that phenolic compounds, which are substituted with electron-donor groups such as methoxy, methyl, amino, and hydroxy groups in *ortho* or *para* positions, were oxidized by Lac.

Table 3
Activity Against DMP in Presence and Absence of Mn^{2+} of *Pleurotus* Species and Strains Under Conditions of SF of Dry Ground Mandarine Peels and SSF of Grapevine Sawdust[a]

		SF of mandarine peels				SSF of grapevine sawdust			
		Activity against DMP (U/L)				Activity against DMP (U/L)			
		$+Mn^{2+}$		$-Mn^{2+}$		$+Mn^{2+}$		$-Mn^{2+}$	
Species	Strain (No.)	5 d	7 d	5 d	7 d	7 d	10 d	7 d	10 d
P. cornicopiae	32	2.10 ± 0.17	1.84 ± 0.09	0.97 ± 0.02 (46.2%)	0.55 ± 0.02 (30%)	22.07 ± 2.7	229.1 ± 8.61	7.42 ± 0.3 (33.6%)	15.93 ± 0.3 (7%)
P. cystidiosus	95	1.36 ± 0.08	1.56 ± 0.19	0.83 ± 0.06 (61%)	0.7 ± 0.03 (45%)	70.91 ± 1.07	108 ± 5.94	5.28 ± 0.21 (7.4%)	8.33 ± 0.12 (7.7%)
P. cytrinopileatus	602	0.61 ± 0.02	23.05 ± 0.34	0.25 ± 0.09 (41%)	4.93 ± 0.12 (21%)	25.6 ± 0.71	161.64 ± 11.13	2.62 ± 0.18 (10.2%)	3.22 ± 0.07 (2%)
P. djamor	485	10.78 ± 0.43	15.79 ± 0.12	0.99 ± 0.35 (9.2%)	7.22 ± 0.04 (46%)	146.54 ± 3.1	232.85 ± 2.46	10 ± 0.29 (6.8%)	17.35 ± 0.09 (7.5%)
P. eryngii var. *eryngii*	193	68.55 ± 2.22	19.96 ± 1.22	4.12 ± 0.09 (6%)	0.87 ± 0.23 (4.4%)	3.58 ± 0.16	83.93 ± 0.12	1.74 ± 0.07 (48.6%)	3.84 ± 0.1 (4.6%)
	201	45.28 ± 12	344.32 ± 15.65	1.86 ± 0.16 (4%)	11.39 ± 0.64 (3.3%)	29.84 ± 1.53	27.52 ± 2.07	0.54 ± 0.09 (1.8%)	1.69 ± 0.19 (6.1%)
	356	21.1 ± 0.92	18.97 ± 0.46	10.01 ± 0.16 (47.4%)	5.55 ± 0.13 (29%)	50.36 ± 3.27	36.31 ± 0.04	7.96 ± 0.5 (15.8%)	0.69 ± 0.05 (1.9%)
	507	0.85 ± 0.06	1.03 ± 0.02	0.62 ± 0.32 (73%)	0.46 ± 0.05 (44.7%)	114.93 ± 0.22	95.56 ± 0.01	3.67 ± 0.1 (3.2%)	2.36 ± 0.05 (2.5%)
	616	371 ± 3.34	705.82 ± 80.46	41.73 ± 5.57 (11.2%)	14.77 ± 2.31 (2.1%)	16.98 ± 1.45	14.11 ± 0.71	1.17 ± 0.07 (6.9%)	1.5 ± 0.23 (10.6%)
	711	10.12 ± 1.22	9.14 ± 1.46	2.86 ± 0.1 (28.3%)	2.98 ± 0.18 (32.6%)	114.06 ± 7.69	16.22 ± 1.00	8.62 ± 0.16 (7.6%)	1.51 ± 0.19 (9.3%)
	716	2.26 ± 0.18	261.82 ± 0.51	0.69 ± 0.03 (30.5%)	39.42 ± 0.96 (15%)	90.46 ± 2.75	47.09 ± 4.3	2 ± 0.38 (2.2%)	2.48 ± 0.12 (5.3%)
	728	21.7 ± 0.61	19.14 ± 1.38	6.69 ± 0.42 (30.8%)	4.39 ± 0.008 (23%)	23.97 ± 0.8	21.31 ± 0.65	1.83 ± 0.19 (7.6%)	1.29 ± 0.11 (6%)
P. eryngii var. *tingitanus*	555	6.99 ± 0.13	10.39 ± 1.06	3.93 ± 0.01 (56.2%)	3.78 ± 0.29 (36.4%)	23.88 ± 1.22	15.13 ± 0.26	0	1.20 ± 0.11 (7.9%)

P. ostreatus	207	11.63 ± 0.92	14.43 ± 1.27	1.51 ± 0.4 (13%)	11.81 ± 1.16 (82%)	369.73 ± 5.27	284.36 ± 2.38	39.18 ± 1.41 (10.6%)	13.36 ± 1.81 (4.7%)
	221	12.39 ± 0.79	4.01 ± 0.17	3.68 ± 0.6 (29.7%)	2.2 ± 0.02 (55%)	328.58 ± 23.47	374.25 ± 37.23	5.83 ± 0.16 (1.8%)	11.32 ± 0.59 (3%)
	234	16.6 ± 0.18	137.06 ± 15.41	5.68 ± 0.02 (34.2%)	19.91 ± 0.19 (14.5%)	277.82 ± 20.72	313.27 ± 36.07	11.31 ± 1.69 (4.1%)	23.17 ± 0.62 (7.4%)
	290	99.48 ± 37.93	362.16 ± 17.49	1.75 ± 0.45 (1.8%)	12.34 ± 1.06 (3.4%)	29.72 ± 1.68	24.55 ± 0.92	0	2.24 ± 0.05 (9.1%)
	387	30.11 ± 0.15	25.58 ± 0.7	21.58 ± 0.67 (71.7%)	13.81 ± 0.55 (54%)	190.91 ± 2.52	195.47 ± 4.88	10.13 ± 1.23 (5.3%)	7.45 ± 0.24 (3.8%)
	493	3.7 ± 0.05	8.42 ± 0.19	1.32 ± 0.06 (35.7%)	3.45 ± 0.09 (41%)	289.82 ± 5.64	259.64 ± 21.97	15.35 ± 0.12 (5.3%)	16.4 ± 0.09 (6.3%)
	494	328.46 ± 14.77	116.95 ± 12.29	3.85 ± 0.12 (1.2%)	3.17 ± 0.08 (2.7%)	426.67 ± 18.94	458.91 ± 44.3	14.74 ± 0.81 (3.5%)	38.42 ± 6.17 (8.4%)
	495	1.98 ± 0.3	3.94 ± 0.11	0.77 ± 0.09 (39%)	1.66 ± 0.24 (42%)	253.64 ± 2.82	381.64 ± 26.28	8.15 ± 0.48 (3.2%)	31.92 ± 2.25 (8.4%)
	592	39.46 ± 0.06	10.29 ± 0.69	7.95 ± 0.4 (20%)	3.6 ± 0.15 (35%)	38.69 ± 0.71	22.26 ± 0.24	1.55 ± 0.35 (4%)	1.28 ± 0.03 (5.8%)
P. ostreatus var. *florida*	393	97.31 ± 4.04	31.06 ± 0.48	45 ± 0.96 (46%)	18.51 ± 0.92 (59.6%)	335.46 ± 5.25	285.19 ± 22.36	5.8 ± 0.31 (1.7%)	10.98 ± 0.33 (3.9%)
P. pulmonarius	509	24.80 ± 3.36	168.62 ± 12.62	3.52 ± 0.62 (14.2%)	15.38 ± 0.69 (9%)	227.64 ± 7.42	340.64 ± 7.5	6.84 ± 0.11 (3%)	38.49 ± 0.25 (11.3%)
	572	1.50 ± 0.28	39.49 ± 2.62	1.13 ± 0.22 (75%)	2.65 ± 0.29 (6.7%)	444.24 ± 2.42	434.18 ± 32.66	12.55 ± 1.04 (2.8%)	22.16 ± 1.44 (5.1%)
	573	0.51 ± 0.14	0.8 ± 0.1	0.59 ± 0.19 (100%)	0.58 ± 0.14 (72.5%)	297.91 ± 6.31	293.82 ± 14.25	3.97 ± 0.21 (1.3%)	21.46 ± 1.48 (7.3%)
P. salignus	328	0.56 ± 0.34	1.96 ± 0.51	0	0	492.51 ± 45.49	241.09 ± 0.89	11.76 ± 0.45 (2.4%)	65.24 ± 1.9 (27%)
P. salmoneostramineus	77	4.37 ± 0.24	5.04 ± 0.21	1.99 ± 0.11 (45.5%)	2.38 ± 0.11 (47.2%)	24.84 ± 0.56	104.92 ± 15.63	2.66 ± 0.16 (10.7%)	1.30 ± 0.17 (1.2%)
P. smithii	138	1.23 ± 0.02	1.16 ± 0.09	1.13 ± 0.18 (91.9%)	0	20.84 ± 3.56	67.02 ± 4.78	3.11 ± 0.53 (14.9%)	2.02 ± 0.09 (3%)
	140	2.88 ± 0.02	3.5 ± 0.25	1.36 ± 0.07 (47%)	1.68 ± 0.16 (48%)	42.96 ± 1.81	71.27 ± 0.89	3.83 ± 0.25 (8.9%)	7.84 ± 0.52 (11%)
	141	27.42 ± 0.23	29.18 ± 2.3	8.12 ± 0.13 (29.6%)	7.82 ± 0.46 (26.8%)	82.42 ± 1.23	143.31 ± 0.33	7.11 ± 0.61 (8.6%)	12.65 ± 0.55 (8.8%)

[a]Relative activities, as percentages of the activity against DMP in the presence of Mn^{2+}, are indicated in parentheses.

Our results demonstrate the importance of studying biodiversity of the ligninolytic enzyme system of *Pleurotus*. Increasing knowledge of the physiology and biochemistry of fungi that cause lignin degradation with emphasis on the genus *Pleurotus* will also enable the selection of more promising strains for biotechnological application.

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