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Determination of *Pleurotus abieticola* ligninolytic activity on Norway spruce wood

Anna Żółciak

Forest Research Institute, Department of Forest Protection, Sękocin Stary, Braci Leśnej 3, 05-090 Raszyn, Poland, phone: +48 227153822, e-mail: A.Zolciak@ibles.waw.pl

Abstract

The effect of *Phlebiopsis gigantea* treatment in control of *Heterobasidion parviporum* in Norway spruce is less effective than that in control of *Heterobasidion annosum* in pine. It is necessary to apply other fungi, for example, *Pleurotus abieticola* in Norway spruce stands. Thus, it is necessary to assess the activity of major ligninolytic enzymes, that is, laccase, lignin peroxidase (LiP), manganese peroxidase (MnP) and versatile peroxidase (VP) produced by *P. abieticola*, which may be effective in the fast degradation of Norway spruce wood. Three strains of *P. abieticola* (Pa1-3) were grown on pieces of Norway spruce sapwood and heartwood for 50 days in laboratory conditions. Enzymatic activity was determined using spectrophotometry. *Pleurotus abieticola* produced laccase, LiP, MnP and VP. The activity of laccase was low, ranging 0–3.696 and 0–0.806 mU/µg of protein in sapwood and heartwood, respectively. The highest activity in Pa1 = 3.696 mU/µg of protein in sapwood and in Pa3 = 0.806 mU/µg of protein in heartwood was observed after 30 and 50 days of incubation, respectively. The activity of LiP was also low, ranging 0–0.188 and 0–0.271 mU/µg of protein in sapwood and heartwood, respectively. The highest activity in Pa1 = 0.271 mU/µg of protein in sapwood and in Pa2 = 0.188 mU/µg of protein in heartwood was observed after 40 and 20 days of incubation, respectively. The activity of MnP ranged 0–17.618 and 0–12.203 mU/µg of protein in sapwood and heartwood, respectively. This enzymatic activity peaked at the 50th day of culture on sapwood for the Pa3 strain (17.618 mU/µg of protein) and at the 20th day of culture on heartwood for the Pa1 strain (12.203 mU/µg of protein). The activity of VP with manganese-oxidising properties was found to be high in all strains of *P. abieticola*, ranging 0–39.19 and 0–59.153 mU/µg of protein in sapwood and heartwood, respectively, whereas the activity of VP with guaiacol-oxidising properties was very low for all *P. abieticola* strains, ranging 0–0.248 and 0–0.225 mU/µg of protein in sapwood and heartwood, respectively. The values of released hydroxyphenols in *P. abieticola* strains ranged 24.915–139.766 and 25.19–84.562 µg of protocatechuic acid/ml in sapwood and heartwood, respectively. The values of released methoxyphenols for the evaluated strains of *P. abieticola* ranged 7.225–23.789 and 1.953–20.651 µg of vanillic acid/ml in sapwood and heartwood, respectively. Further studies with a higher number of strains of this species as well as an optimisation of conditions for the measurement of ligninolytic activity are needed.

Key words

laccase, lignin peroxidase, manganese peroxidase, versatile peroxidase, spruce, white rot fungus, wood

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Introduction

The root and butt rot caused by *Heterobasidion annosum* (Fr.) Bref. *sensu lato* is considered one of the most important and destructive diseases affecting conifers in the north temperate regions of the world, particularly in Europe where annual loses are estimated at least 790 million euros per year (Woodward et al. 1998; Greig et al. 2001; Korhonen and Holdenrieder 2005; Garbelotto and Gonthier 2013).

In 2018, the disease occurred on 85,125 ha of Polish National Forest [2,782 and 82,343 ha in the first-class age (to 20 years old) and in older tree stands, respectively] (Małecka 2019). Three species of *H. annosum s.l.* are recorded in Poland: *H. annosum* (Fr.) Bref. *sensu stricto*, which affects mostly Scots pine (*Pinus sylvestris* L.); *H. parviporum* Niemelä et Korhonen, which affects Norway spruce (*Picea abies* (L.) H. Karst.; and *H. abietinum* Niemelä et Korhonen, which affects fir (*Abies*), rarely spruce and sporadically larch (*Larix*) (Sierota 2001; Łakomy and Werner 2003).

Heterobasidion annosum s.l. infects with basidiospores, which germinate on fresh stump surfaces after tree cut (Stenlid and Redfern 1998). It then spreads via contacts of diseased and healthy roots of surrounding trees (Rishbeth 1951a, b; 1957; Stenlid and Redfern 1998). One method of control is the application of saprotrophic *Phlebiopsis gigantea* (Fr.: Fr.) Jülich – a competing fungus that produces the mycelium and fruiting bodies on the surface of the stump surface. The method is successfully applied in many countries. However, the treatment of spruce is not fully satisfactory, amongst others because of too slow wood colonisation process by *P. gigantea* (Korhonen et al. 1994; Holdenrieder et al. 1998; Nicolotti et al. 1999; Berglund and Rönnberg 2004; Nicolotti and Gonthier 2005; Berglund et al. 2005; Rönnberg et al. 2006; Drenkhan et al. 2008; Gunulf et al. 2012; Rönnberg and Cleary 2012). Thus, the more effective competing fungi should be used in the protection of spruce.

Species of *Pleurotus* may be taken into consideration because of (i) saprotrophic occurrence of *Pleurotus ostreatus* (Jacq.: Fr.) Kummer on wood of deciduous trees and of *Pleurotus abieticola* Petersen and Hughes on wood of Norway spruce (Schwarze et al. 2000; Kodrik 2001; Albertó et al. 2002; Żółciak 2002; Szczepkowski and Piętka 2008), (ii) potent ligninolytic enzyme system that enables successful degradation of lignin and various aromatic compounds in wood (Cohen et al. 2002; Stajić et al. 2004; Camassola et al. 2013).

Extremely rare *P. abieticola* has been first described from Far East Russia (Petersen and Hughes 1997) and then reported from China (Albertó et al. 2002; Li et al. 2014; Liu et al. 2015). It can grow on Norway spruce, fir, alder (*Alnus*) and willow (*Salix*). In Poland, it has been found in the Białowieża Primeval Forest on Norway spruce (Gierczyk et al. 2015a, b, 2017, 2018; Kujawa et al. 2017).

The aim of this study was to assess the ligninolytic activity of *P. abieticola* strains growing on Norway spruce wood blocks of sapwood and heartwood under laboratory conditions. The results will contribute to the better understanding of metabolic processes of the fungus before its potential application for the control of *Heterobasidion*.

Material and methods

Three strains of *P. abieticola* (Pa1, Pa2 and Pa3) obtained from basidiospores collected from basidiomata found on the stem of three Norway spruces in the Białowieża Primeval Forest were used in the study. Basidiospores were placed on 2% malt extract agar (MEA) in Petri dishes. Spores were germinated and mycelium grew at 24°C in darkness. After 21 days, the 2-cm diameter discs with mycelium were transferred to sterile 2% MEA in Petri dishes and incubated at 24°C in darkness for another 21 days. Then, the sterile Norway spruce wood pieces of sapwood and heartwood (80 mm × 40 mm × 4 mm) were placed on *P. abieticola* mycelium and incubated at 28°C in 12/12 hours day/night rhythm for 50 days. The enzymatic activity was analysed in the surface layer of wood covered by mycelium of *P. abieticola*. Samples were collected after 10, 20, 30, 40, and 50 days. Both sapwood and heartwood were ground together and enzyme broth was extracted. Enzyme activities were measured spectrophotometrically (three measurements) in supernatant after centrifuging (6,000 rpm. for 5 min at 4ºC). The amount of enzyme necessary to oxidise 1 µmol of substrate per minute was defined as one activity unit. All activities were expressed in units of specific activity (mU/µg of protein). The activity of laccase was determined with syringaldazine as a substrate (Leonowicz and Grzywno-

wicz 1981). The activities of lignin peroxidase (LiP) and manganese peroxidase (MnP) were determined using the method of Wariishi et al. (1992) and Tien and Kirk (1988) modified by Matuszewska (2005), respectively.

The activity of versatile peroxidase (VP) in the oxidation of manganese ions and guaiacol was assayed using the method of Sugano et al. (2006). Concentrations of methoxyphenolic and hydroxyphenolic substances were evaluated using diazosulphanilamide in the DASA test (Leonowicz et al. 1968) modified by Malarczyk (1984). Detailed methodology is presented in publications of Żółciak et al. (2008; 2012) and Żółciak and Bohacz (2016).

The data on the enzymatic activity were analysed using the Kruskal–Wallis and Mann–Whitney U tests. Statistical analyses were performed using Statistica 10 (StatSoft, Inc.).

Results

Three strains of *P. abieticola* (Pa1, Pa2, Pa3) whilst growing on both sapwood and heartwood of Norway spruce produced lignin-modifying enzymes, that is, laccase, LiP, MnP and VP (oxidising both manganese ions and guaiacol). During the degradation process, hydroxyphenolic and methoxyphenolic substances were released.

The activity of laccase was low, ranging 0–3.696 and 0–0.806 mU/µg of protein in sapwood and heartwood, respectively. The highest activity in Pa1 = 3.696 mU/µg of protein in sapwood and in Pa3 = 0.806 mU/µg of protein in heartwood were recorded after 30 and 50 days of incubation, respectively. More often, no activity was observed in heartwood than in sapwood, particularly in case of Pa1 (Tab. 1). The activity of laccase in three *P. abieticola* strains and at five periods of incubation (after 10, 20, 30, 40 and 50 days) did not differ significantly. Only the median laccase activity in sapwood and heartwood was significantly different (Fig. 1).

The activity of LiP was also low, ranging 0–0.188 and 0–0.271 mU/µg of protein in sapwood and heartwood, respectively (Tab. 1). The highest activity in Pa1 = 0.271 mU/µg of protein in sapwood and in Pa2 = 0.188 mU/µg of protein in heartwood were recorded after 40 and 20 days of incubation, respectively. The activity of LiP in Pa1 and Pa3 was significantly different (Fig. 2A). The median LiP activity in sapwood and heartwood and at five periods of incubation (after 10, 20, 30, 40 and 50 days) was not significantly different (Fig. 2A).

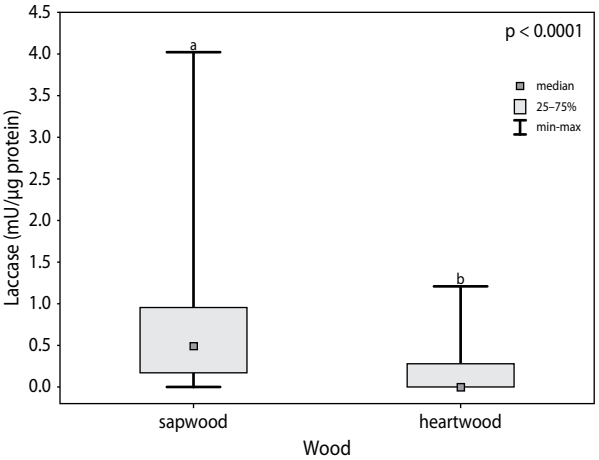

Figure 1. Laccase activity in *P. abieticola* strains cultured on sapwood versus heartwood (different letters denote statistically significant differences at $p \leq 0.05$)

The activity of MnP ranged from 0 to 17.618 mU/µg of protein in cultures on sapwood and from 0 to 12.203 mU/µg of protein in cultures on heartwood (Tab. 1). The enzymatic activity peaked at the 50th day of culture on sapwood for the Pa3 strain (17.618 mU/µg of protein) and at the 20th day of culture on heartwood for the Pa1 strain (12.203 mU/µg of protein). Strains of *P. abieticola* Pa1 and Pa3 exhibited MnP activity in cultures on both sapwood and heartwood. In the case of Pa2, no manganese activity was noticed. Significant differences were observed between the *P. abieticola* strains Pa1 and Pa2 and Pa2 and Pa3 in values of MnP activity (Fig. 2B). On the other hand, no statistically significant differences were observed between the medians of MnP activity in sapwood and heartwood as well as in samples evaluated after days of culture of *P. abieticola*.

The activity of VP with manganese-oxidising properties was found to be high in all strains of *P. abieticola*, ranging 0–39.190 and 0–59.153 mU/µg of protein in sapwood and in heartwood, respectively (Tab. 1). The highest activity of the enzyme was observed for the Pa3 strain (39.19 mU/µg of protein) at the 30th day of culture on sapwood and for the Pa1

Table 1. Dynamics of measured enzymes activity and levels of secreted hydroxyphenols and methoxyphenols in cultures of *P. abieticola* strains (Pa1, Pa2 and Pa3) on Norway spruce wood (S – sapwood; H – heartwood)

Strain	Days									
	10		20		30		40		50	
	S	H	S	H	S	H	S	H	S	H
Laccase activity (mU/µg of protein)										
Pa1	0.373	0	0.828	0	3.696	0	0.176	0.340	0.179	0
Pa2	1.134	0.275	1.007	0	0.573	0.170	0.080	0.115	0.035	0
Pa3	0.768	0	0.924	0	0.620	0.346	0	0.781	0.202	0.806
Lignin peroxidase activity (mU/µg of protein)										
Pa1	0.051	0.067	0.074	0.105	0.168	0.050	0.071	0.271	0	0.090
Pa2	0.178	0.019	0.188	0.019	0.061	0.022	0.045	0.007	0.030	0
Pa3	0	0	0	0	0	0.054	0.125	0	0.105	0.087
Manganese peroxidase activity (mU/µg of protein)										
Pa1	5.421	4.155	9.432	12.203	8.648	9.470	10.072	10.476	6.617	7.494
Pa2	0	0	0	0	0	0	0	0	0	0
Pa3	0.177	9.509	10.003	8.779	8.794	5.662	8.100	6.711	17.618	2.032
Versatile peroxidase activity, oxidising manganese ions (mU/µg of protein)										
Pa1	3.863	16.249	14.143	43.057	35.680	37.949	21.376	59.153	30.020	32.898
Pa2	2.020	0	0	0.322	0	0	0	0	0	0
Pa3	2.995	27.504	29.687	44.608	39.190	31.048	31.970	32.019	38.523	9.735
Versatile peroxidase activity, oxidising guaiacol (mU/µg of protein)										
Pa1	0.079	0.217	0.229	0.168	0.264	0.155	0.182	0.225	0.160	0.093
Pa2	0.205	0.053	0.089	0.030	0.047	0	0.012	0.012	0.020	0
Pa3	0.248	0.178	0.270	0.137	0.213	0.065	0.082	0.157	0	0.195
Hydroxyphenols (µg of protocatechuic acid/ml)										
Pa1	43.968	75.158	77.936	84.562	139.766	71.295	61.372	78.181	58.884	70.181
Pa2	69.891	34.457	66.173	28.976	44.869	25.190	27.083	70.465	24.915	70.099
Pa3	71.540	80.700	89.860	83.860	83.799	63.906	45.632	74.379	77.326	56.242
Methoxyphenols (µg of vanillic acid/ml)										
Pa1	9.090	17.749	15.493	21.250	23.789	17.258	13.076	19.170	13.197	15.520
Pa2	18.321	11.743	14.477	9.191	15.514	9.191	7.871	2.242	7.225	1.953
Pa3	14.901	18.779	19.015	20.651	16.369	14.786	8.767	16.301	17.426	11.581

strain (59.153 mU/µg of protein) at the 40th day of culture on heartwood. Significant differences were observed between the *P. abieticola* strains Pa1 and Pa2 and Pa2 and Pa3 in values of MnP activity (Fig. 2C). The activity of VP manganese-oxidising properties did not differ significantly amongst cultures of *P. gigantea* strains on sapwood and heartwood or amongst the time periods of strain culture.

The activity of VP with guaiacol-oxidising properties was very low for all *P. abieticola* strains, ranging 0–0.248 and 0–0.225 mU/µg of protein in sapwood and in heartwood, respectively (Tab. 1). The activity of the enzyme peaked for the Pa3 strain (0.248 mU/µg of protein) at the 10th day of culture on sapwood and for the Pa1 strain (0.225 mU/µg of protein) at the 40th day of culture on heartwood. The activity

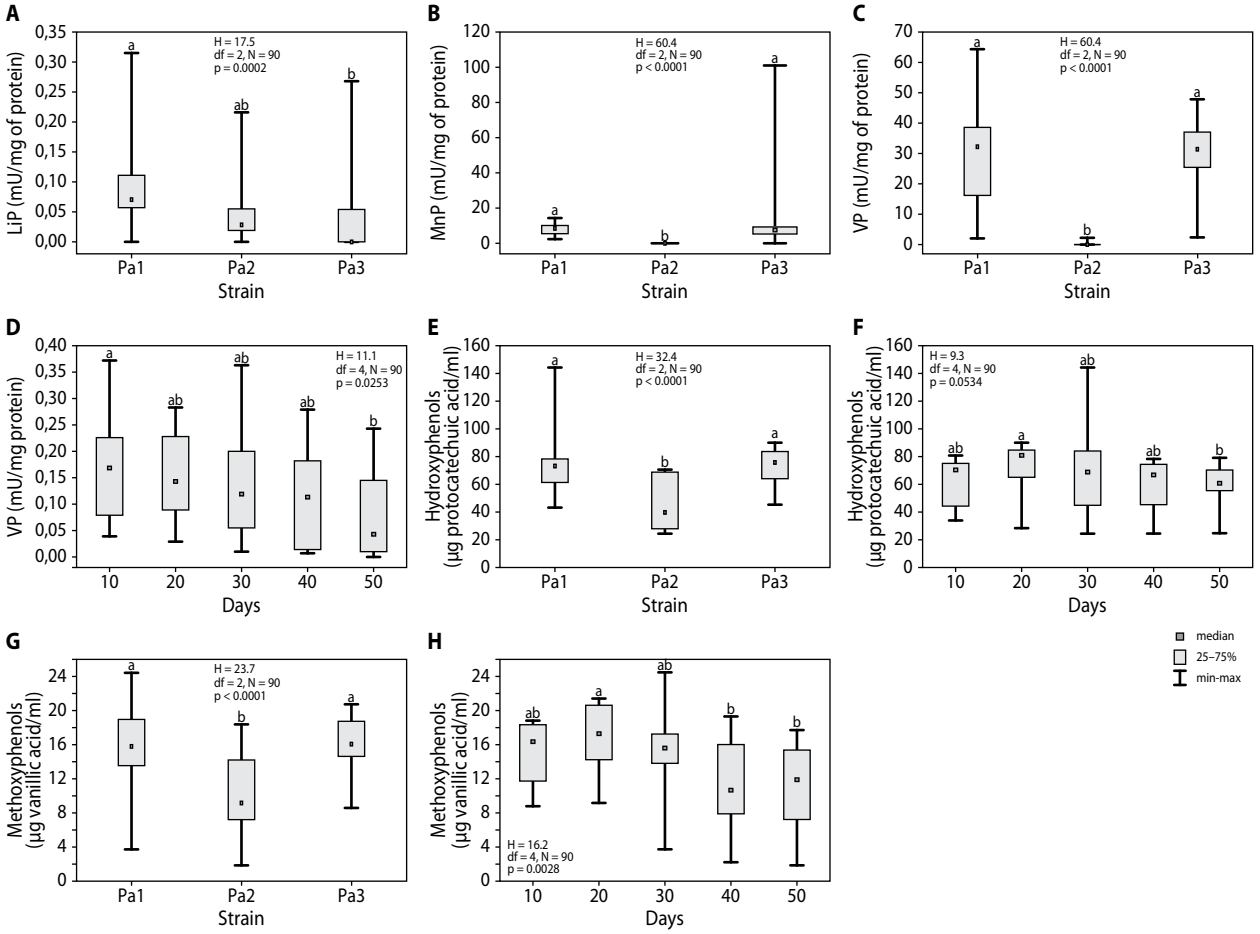

Figure 2. Activity of (A) lignin peroxidase, (B) manganese peroxidase, (C) versatile peroxidase activity oxidising manganese ions in *P. abieticola* strains, and (D) versatile-oxidising guaiacol comparison amongst culture durations and comparison of median hydroxyphenol production in *P. abieticola* strains (E) and amongst culture durations (F) and median methoxyphenol production in *P. abieticola* strains (G) and amongst culture durations (H) (different letters denote statistically significant differences at $p \leq 0.05$)

of guaiacol-oxidising VP did not differ significantly amongst the *P. abieticola* strains as well as the *P. abieticola* strains grown on sapwood and heartwood, but statistically significant differences in activity were determined amongst samples evaluated after the 10th and the 50th day of culture (Fig. 2D).

The values of released hydroxyphenols in the *P. abieticola* strains ranged from 24.915 to 139.766 µg of protocatechuic acid/ml in cultures on sapwood and from 25.19 to 84.562 µg of protocatechuic acid/ml in cultures on heartwood (Tab. 1). The highest value of released hydroxyphenols was observed for the Pa1 strain on the 30th day of culture on sapwood (139.766 µg of protocatechuic acid/ml) and on the 20th day of culture on heartwood (84.562 µg of protocatechuic acid/ml). Significant differences were observed between the *P. abieticola* strains Pa1 and Pa2 and Pa2 and Pa3 in values of released hydroxyphenols (Fig. 2E) as well as between samples evaluated after the 20th and the 50th days of culture of *P. abieticola* mycelium (Fig. 2F). No significant difference between strains of *P. abieticola* grown on sapwood and heartwood was noticed.

The values of released methoxyphenols for the evaluated strains of *P. abieticola* ranged from 7.225 to 23.789 µg of vanillic acid/ml in cultures on sapwood and from 1.953 to 20.651 µg of vanillic acid/ml in cultures on heartwood (Tab. 1). The highest values of

methoxyphenols were reported for the Pa1 strain at the 30th day of culture on sapwood (23.789 µg of vanillic acid/ml) and for Pa3 at the 20th day of culture on heartwood (20.651 µg of vanillic acid/ml). Statistically significant differences in the values of released methoxyphenols were observed between the *P. abieticola* strains Pa1 and Pa2, and Pa2 and Pa3 (Fig. 2G) as well as in samples evaluated after the 20th and the 40th, the 20th and 50th days of mycelium culture (Fig. 2H). No significant difference between strains of *P. abieticola* grown on sapwood and heartwood was noticed.

Discussion

According to Kamitsuji et al. (2004), *Pleurotus* spp. has been recognised to produce extracellular ligninolytic enzymes such as laccase and MnP. Stajić et al. (2006) showed that laccase and peroxidases production depends on the species and strains of the genus *Pleurotus*, condition of cultivation and carbon and nitrogen sources and concentrations. In this study, *P. abieticola*, similar to *P. ostreatus*, secreted laccase and three types of peroxidases, that is, LiP, MnP and VP (Leonowicz et al. 1999; Elisashvili et al. 2003; Baldrian et al. 2005; Hoegger et al. 2007).

The results obtained may indicate that *P. abieticola* belongs to groups that produce small amounts of laccase (Bollag and Leonowicz 1984; Baldrian, 2005).

In the present study, the activity of laccase was determined by the oxidation of syringaldazine (Harkin and Obst 1973; Leonowicz and Grzywnowicz 1981), but there are also other methods that may be tested for *P. abieticola* in future studies, for example, laccase production can be stimulated by the presence of a wide variety of inducing substances, particularly aromatic or phenolic compounds (Farnet et al. 1999), anilines (Fåhraeus et al. 1958; Bollag and Leonowicz 1984) or 2,5-xylidine (Fåhraeus and Reinhammar 1967; Rogalski and Leonowicz 1992; Agematu et al. 1993). According to Baldrian and Gabriel (2002), copper and cadmium increase the activity of laccase in *P. ostreatus*. Stajić et al. (2013) demonstrated a stimulatory effect of the microelements (Fe, Zn and Se) on the activity of laccase in *P. ostreatus*.

Pleurotus abieticola strains showed a very low ligninase activity. This does not seem to be related to the method of measurement as in the case of laccase. The enzymatic activity of ligninase was measured by the increase in absorbance at 310 nm under the oxidation of veratryl alcohol (Wong 2009). According to Hammel et al. (1993), veratryl alcohol enhances the action of LiP on many substrates, including lignin (Lundell et al. 1993; Schoemaker et al. 1994), by acting as a mediator (Harvey et al. 1986) or by protecting the enzyme against inactivation (Wariishi and Gold 1989). Guaiacol, acetosyringone, catechol, vanillyl alcohol and syringic acid are other phenolics susceptible to the oxidative potentials of LiP (Harvey and Palmer 1990; Wong 2009). According to Baciocchi et al. (2001), besides the oxidation of non-phenolic substrates, LiP has also shown the capability to oxidise a variety of phenolic compounds.

In this study, the activity of MnP was not so high for the tested *P. abieticola* strains (except for Pa2 – no activity was noticed). Martinez et al. (1996) reported that the secretion of MnP enzymes by different white-rot fungi is strongly dependent on growth conditions. Manganese peroxidase production was observed in *P. eryngii* (DC.) Quél., *P. ostreatus, P. pulmonarius* (Fr.) Quél. and *P. sajor-caju* (Fr.) Singer in pepton medium, but it was not detected in media with other investigated nitrogen organic sources (corn-step liquor, malt extract and ammonium tartrate).

According to Giardinia et al. (2000), *P. ostreatus* produces two MnP isoenzymes when grown in solid stationary conditions on poplar sawdust, whereas a lower production of these same enzymes was observed on fir sawdust. Addition of Mn^{2+} to poplar culture resulted in a threefold increase in the MnP activity; the same addition to fir culture was able to increase 10-fold the MnP production. According to Hatakka (1994), Mn^{2+} ions are a crucial substrate for MnP. This enzyme is a heme glycoprotein that catalyses the oxidation of Mn^{2+} to Mn^{3+} in the presence of H_2O_2. However, Mn^{2+} concentration affects the MnP levels; it has been reported (Kerem and Hadar 1993, 1995; Camarero et al. 1996) that the addition of Mn^{2+} enhances the degradation of lignin during solid-state fermentation of different *Pleurotus* species, without increasing the MnP levels. On the contrary, the presence of extra Mn^{2+} strongly increases the production of MnP during solid-state fermentation on different kinds of wood sawdust (Giardinia et al. 2000).

In the present study, the activity of VP with manganese-oxidising properties for the focal strains of *P. abieticola* was found to be high in contrast to the activity of VP with guaiacol-oxidising properties. Versatile peroxidase was first reported in *P. eryngii* (Martinez et al. 1996) and then in *Bjerkandera adusta* [(Willd.) P. Karst.] and *P. ostreatus* (Sarkar et al. 1997; Heinfling et al. 1998a, b; Mester and Field 1998). Versatile peroxidases combine the properties of LiPs and MnPs, conferring the catalytic versatility inferred by their name (Camarero et al. 1999). According to Ruiz-Dueñas et al. (2001), they can oxidise Mn^{2+} to Mn^{3+} similar to MnPs but can also oxidise non-phenolic compounds in the same manner as LiPs.

These preliminary findings on the white-rot fungus *P. abieticola* are, however, not sufficient to evaluate its capabilities against root rot caused by *H. parviporum*. Further studies with a higher number of strains of this species as well as an optimisation of conditions for the measurement of ligninolytic activity are needed.

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References

Albertó, E.O., Petersen, R.H., Hughes, K.W., Lechner B. 2002. Miscellaneous notes on *Pleurotus*. *Persoonia*, 18, 55–69.

Agematu, H., Kominato, K., Shibamoto, N., Yoshioka, T. 1993. Transformation of 7-(4-hydroxyphenylacetamido)cephalosporanic acid into a new cephalosporin antibiotic, 7-(1-oxaspiro(2.5)octa-6-oxo-4,7-diene-2-carboxamido)cephalosporanic acid, by laccase. *Bioscience Biotechnology and Biochemistry*, 57 (8), 1387–1388. DOI: 10.1271/bbb.57.1387

Baciocchi, E., Gerini, M.F., Lanzalunga, O., Lapi, A., Piparo, M.G.L., Mancinelli, S. 2001. Isotope-effect profiles in the oxidative N-demethylation of N, N-dimethylanilines catalyzed by lignin peroxidase and a chemical model. *European Journal of Organic Chemistry*, 2305–2310. DOI: org/10.1002/1099-0690(200106)2001:12<2305::AID-EJOC2305>3.0.CO;2-E

Baldrian, P., Gabriel, J. 2002. Copper and cadmium increase laccase activity in *Pleurotus ostreatus*. *FEMS Microbiology Letters*, 206 (1), 69–74. DOI: org/10.1111/j.1574-6968.2002.tb10988.x

Baldrian, P. 2005. Fungal laccases occurrence and properties. *FEMS Microbiology Review*, 30, 215–242. DOI: 10.1111/j.1574−4976.2005.0 0010.x

Baldrian, P., Valášková, V., Merhautová, V., Gabriel, J. 2005. Degradation of lignocellulose by *Pleurotus ostreatus* in the presence of copper, manganese, lead and zinc. *Research in Microbiology*, 156, 670–676.

Berglung, M., Rönnberg, J. 2004. Effectiveness of treatment of Norway spruce stumps with *Phlebiopsis gigantea* at different rates of coverage for the control of *Heterobasidion*. *Forest Pathology*, 34, 233–243.

Berglung, M., Rönnberg, J., Holmer, L., Stenlid, J. 2005. Comparison of five strains of *Phlebiopsis gigantea* and two *Trichoderma* formulations for treatment against natural *Heterobasidion* spore infections on Norway spruce stumps. *Scandinavian Journal of Forest Research*, 20 (1), 12–17.

Bollag, J.M., Leonowicz, A. 1984. Comparative studies of extracellular fungal laccases. *Applied Environmental Microbiology*, 48 (4), 849–854.

Camarero, S., Bockle, B., Martinez, M.J., Martinez, A.T. 1996. Manganese-Mediated Lignin Degradation by *Pleurotus pulmonarius*. *Applied and Environmental Microbiology*, 62 (3), 1070–1072.

Camarero, S., Sarkar, S., Ruiz-Dueñas, .FJ., Martinez, M.J., Martinez, A.T. 1999. Description of a versatile peroxidase involved in the natural degradation of lignin that has both manganese peroxidase and lignin peroxidase substrate interaction sites. *Journal of Biological Chemistry*, 274 (15), 10324–10330.

Camassola, M., da Rosa, L.O., Calloni, R., Gaio, T.A., Dillon, A.J.P. 2013. Secretion of laccase and

manganese peroxidase by *Pleurotus* strains cultivate in solid-state using *Pinus* spp. sawdust. *Brazilian Journal of Microbiology*, 44 (1), 207–213. DOI: 10.1590/S1517-83822013005000006

Cohen, R., Persky, L., Hadar, Y. 2002. Biotechnological applications and potential of wood-degrading mushrooms of the genus *Pleurotus*. *Applied Microbiology and Biotechnology*, 58 (5), 582–594. DOI: 10.1007/s00253-002-0930-y

Drenkhan, T., Hanso, S., Hanso, M. 2008. Effect of the stump treatment with *Phlebiopsis gigantea* against *Heterobasidion* Root Rot in Estonia. *Baltic Forestry*, 14 (1), 16–25.

Elisashvili, V., Kachlishvili, E., Chichua, D., Tsiklauri, N. 2003. Lignocellulolytic enzyme activity during growth and fruiting of the edible and medicinal mushroom *Pleurotus ostreatus* (Jacq.:Fr.) Kumm. (*Agaricomycetideae*). *International Journal of Medicinal Mushrooms*, 5 (2), 193–198.

Fåhraeus, G., Tullande,r V., Ljunggren, H. 1958. Production of high laccase yields in cultures of fungi. *Physiologia Plantarum*, 11, 631–643. DOI: org/10.1111/j.1399-3054.1958.tb08259.x

Fåhraeus, G., Reinhammar, B. 1967. Large scale production and purification of laccase from cultures of the fungus *Polyporus versicolor* and some properties of laccase A. Acta Chemica Scandinavica, 21 (9), 2367–2378.

Farnet, A.-M., Tagger, S., Le Petit, J. 1999. Effects of copper and aromatic inducers on the laccasses of the white rot fungus *Marasmius quercophilus*. *Compote Rendus de l'Académie des Sciences – Series III, Sciences de la Vie/Life Sciences*, 322 (6), 499–503. DOI: 10.1016/S0764-4469(99)80100-0

Garbelotto, M., Gonthier, P. 2013. Biology, epidemiology, and control of *Heterobasidion* species worldwide. *Annual Review of Phytopathology*, 51, 39–59. DOI: org/10.1146/annurev-phyto-082712-102225

Giardina, P., Palmieri, G., Fontanella, B., Rivieccio, V., Sannia, G. 2000. Manganese peroxidase isoenzymes produced by *Pleurotus ostreatus* grown on wood sawdust. *Archives of Biochemistry and Biophysics*, 376, 171–179. DOI: 10.1006/abbi.1999.1691

Gierczyk, B., Kujawa, A., Szczepkowski, A. 2015a. The 20th Jubilee Exhibition of Fungi of Białowieża Forest. Study materials on mycobiota of Białowieża Primeval Forest (in Polish with English summary). *Przegląd Przyrodniczy*, 26, 1, 11– 29.

Gierczyk, B., Kujawa, A., Szczepkowski, A. Ślusarczyk, T., Kozak, M., Mleczko, P. 2015b. The 21st Exhibition of Fungi of Białowieża Forest. Materials to the knowledge of mycobiota of the Białowieża Primeval Forest (in Polish with English summary). *Przegląd Przyrodniczy*, 26, 3, 10– 50.

Gierczyk, B., Ślusarczyk, T., Szczepkowski, A., Kujawa, A. 2017. The 22st Exhibition of Fungi of the Białowieża Forest. Materials to the knowledge of mycobiota of the Białowieża Primeval Forest (in Polish with English summary). *Przegląd Przyrodniczy*, 28 (1), 59–84.

Gierczyk, B., Ślusarczyk, T., Szczepkowski, A., Kujawa, A. 2018. The 23t h Exhibition of Fungi of the Białowieża Forest. Materials to the knowledge of mycobiota of the Białowieża Primeval Forest (in Polish with English summary). *Przegląd Przyrodniczy*, 29 (2), 9–57.

Greig, B.J.W., Gibbs, J.N., Pratt, J.E. 2001. Experiments on the susceptibility of conifers to *Heterobasidion annosum* in Great Britain. *Forest Pathology*, 31, 219–228.

Gunulf, A., Mc Carthy, R., Rönnberg, J. 2012. Control efficacy of stump treatment and influence of stump height on natural spore infection by *Heterobasidion* spp. of precommercial thinning stumps of Norway spruce and birch. *Silva Fennica,* 46 (5), 655–665.

Hammel, K.E,. Jensen, K., Mozuch, M., Landucci, L., Tien, M., Pease, E. 1993. Ligninolysis by a purified lignin peroxidase. *Journal of Biological Chemistry*, 268, 12274–12281.

Hatakka, A. 1994. Lignin-modifying enzymes from selected white-rot fungi: production and role in lignin degradation. *FEMS Microbiology Reviews*, 13, 125–135. DOI: org/10.1111/j.1574-6976.1994.tb00039.x

Heinfling, A., Martinez, M.J., Martinez, A.T., Bergbauer, M., Szewczyk, U. 1998a. Purification and characterization of peroxidases from the dye-decolorizing fungus *Bjerkandera adusta*. *FEMS Microbiology Letter*, 165 (1), 43–50.

Heinfling, A., Martinez, M.J., Martinez, A.T., Bergbauer, M., Szewczyk, U. 1998b. Transformation of industrial dyes by manganese peroxidases from *Bjerkandera adusta* and *Pleurotus eryngii* in

a manganese-independent reaction. *Applied and Environmental Microbiology*, 64 (8), 2788–2793.

Harkin, J.M., Obst, J.R. 1973. Syringaldazine, an effective reagent for detecting laccase and peroxidase in fungi. *Experientia*, 29 (4), 381–508. DOI: 10.1007/bf01926734

Harvey, P.J., Schoemaker, H.E., Palmer, J.M. 1986. Veratryl alcohol as a mediator and the role of radical cations in lignin biodegradation by *Phanerochaete chrysosporium*. *FEBS Letters*, 195, 242–246. DOI: org/10.1016/0014-5793(86)80168-5

Harvey, P.J., Palmer, J.M. 1990. Oxidation of phenolic compounds by ligninase. *Journal of Biotechnology*, 13 (2/3), 169–179. DOI: org/10.1016/0168-1656(90)90102-H

Hoegger, P.J., Majcherczyk, A., Dwivedi, R.C., Svobodová, K., Kilaru, S., Kües, U. 2007. Enzymes in wood degradation. In: Wood Production, Wood Technology and Biotechnological Impacts (ed.: U. Kües). Universitätsverlag Göttingen, Germany, 389–438.

Holdenrieder, O., Engesser, R., Sieber, T.N. 1998. Biological control of *Heterobasidion annosum* with *Phlebiopsis gigantea* on Norway spruce in Switzerland. In: Root and Butt Rots of Forest Trees. 9th International Conference on Root and Butt Rots, September 1–7, 1997 (eds.: C. Delatour, J.-J. Guillaumin, B. Lung-Escarmant, B. Marçais). Carcans-Maubuisson, France, 447.

Kamitsuji, H., Honda, Y., Watanabe, T., Kuwahara, M. 2004. Production and induction of manganese peroxidase isozymes in a white-rot fungus *Pleurotus ostreatus*. *Applied Microbiology and Biotechnology*, 65, 287–294.

Kerem, Z., Hadar, Y. 1993. Effect of manganese on lignin degradation by *Pleurotus ostreatus* during solid-state fermentation. *Applied and Environmental Microbiology*, 59 (12), 4115–4120.

Kerem, Z., Hadar, Y. 1995. Effect of manganese on preferential degradation of lignin by *Pleurotus ostreatus* during solid-state fermentation. *Applied and Environmental Microbiology*, 61 (8), 3057–3062.

Kodrik, M. 2001. Results of beech stump inoculation with antagonistic fungi. *Journal of Forest Science*, 47 (11), 505–512.

Korhonen, K., Holdenrieder, O. 2005. Recent advances in research on the root rot fungus *Heterobasidion annosum* s.l. A literature review (in German). *Forst und Holz*, 60, 206–211.

Korhonen, K. et al. 1994. Control of *Heterobasidion annosum* by stump treatment with "Rotstop", a new commercial formulation of *Phlebiopsis gigantea*. In: Proceedings of the 8th International Conference on Root and Butt Rots. Wik, Sweden and Haikko, Finland, August 9–16, 1993. IUFRO Working Party S2.06.01. (eds.: M. Johansson, J. Stenlid). Swedish University of Agricultural Sciences, Uppsala, Sweden, 662–667.

Kujawa, A., Gierczyk, B., Kozak, M., Mleczko, P., Ślusarczyk, T. 2017. Diversity of agaricoid, boletoid and hypogeous macrofungi in the Białowieża Forest – project concept and preliminary results. (in Polish with English summary). *Fragmenta Floristica et Geobotanica Polonica*, 24 (1), 119–131.

Leonowicz, A., Grzywnowicz, K. 1981. Quantitative estimation of laccase forms in some white-rot fungi using syringaldazine as a substrate. *Enzyme Microbial Technology*, 3, 55–58. DOI: org/10.1016/0141-0229(81)90036-3

Leonowicz, A., Wojtowicz, B., Trojanowski, J. 1968. Model experiment on the humification of rye roots. I. Phenolic products in the humification process. *Polish Journal of Soil Science*,1, 129–136.

Leonowicz, A., Matuszewka, A., Luterek, J., Ziegenhagen, D., Wojtas-Wasilewska, M. 1999. Biodegradation of lignin by white rot fungi. *Fungal Genetics and Biology*, 27, 175–185.

Li, Y., Liu, Y., Li, T.H. 2014. Flora fungorum sinicorum. Vol. 45. Pleurotoid-lentinoid fungi (in Chinese). Science Press, Beijing.

Liu, X.-B., Liu, J.-W., Yang, Z.-L. 2015. A new edible mushroom resource, *Pleurotus abieticola*, in southwestern China. *Mycosystema*, 34 (4), 581–588. DOI: 10.13346/j.mycosystema.150051

Lundell, T., Wever, R., Floris, R., Harvey, P., Hatakka, A., Brunow, G., Schoemaker, H. 1993. Lignin peroxidase L3 from *Phlebia radiata*. Pre-steady-state and steady-state studies with veratryl alcohol and a non-phenolic lignin model compound 1-(3,4-dimethoxyphenyl)-2-(2-methoxyphenoxy) propane-1,3-diol. *European Journal of Biochemistry*, 211, 391–402.

Łakomy, P., Werner, A. 2003. Distribution of *Heterobasidion annosum* intersterility groups in Poland. *Forest Pathology*, 33 (2), 105–112. DOI: 10.1046/j.1439-0329.2003.00309.x

Malarczyk, E. 1984. Substrate−induction of veratric acid O−demethylase in *Nocardia* sp. *Acta Biochimica Polonica*, 31 (4), 383−395.

Małecka, M. 2019. Choroby infekcyjne. In: Krótkoterminowa prognoza występowania ważniejszych szkodników i chorób infekcyjnych drzew leśnych w Polsce w 2019 r. Instytut Badawczy Leśnictwa (in Polish). *Analizy i Raporty*, 31, 71–82.

Martinez, M.J., Ruiz-Dueñas, F.J., Guillén, F., Martinez, A.T. 1996. Purification and catalytic properties of two manganese peroxidase isoenzymes from *Pleurotus eryngii*. *European Journal of Biochemistry*, 237, 424–432. DOI: org/10.1111/j.1432-1033.1996.0424k.x

Matuszewska, A. 2005. The influence of mediators on the degradation of model lignin preparations by *Trametes versicolor* (in Polish). Doctoral dissertation, UMCS Lublin.

Mester, T., Field, J.A. 1998. Characterization of a novel manganese peroxidase hybrid isozyme produced by *Bjerkandera* species strain BOS55 in the absence of manganese. *Journal of Biological Chemistry*, 273, 15412–15417. DOI: org/10.1074/jbc.273.25.15412

Mester T, Field JA. 1998. Characterization of a novel manganese peroxidase-lignin peroxidase hybrid isozyme produced by Bjerkandera species strain BOS55 in the absence of manganese. J Biol Chem 273:15412–15417

Nicolotti, G., Gonthier, P., Varese, G.C. 1999. Effectiveness of some biological and chemical treatment against *Heterobasidion annosum* on Norway spruce stumps. *European Journal of Forest Pathology*, 29, 339–346.

Nicolotti, G., Gonthier, P. 2005. Stump treatment against *Heterobasidion* with *Phlebiopsis gigantea* and some chemicals in *Picea abies* stands in the western Alps. *Forest Pathology*, 35, 365–374.

Petersen, R.H., Hughes, K.W. 1997. A new species of *Pleurotus*. *Mycologia*, 89 (1), 173–180.

Rishbeth, J. 1951a. Observations on the biology of *Fomes annosus*, with particular reference to East Anglian pine plantations. II. Spore production, stump infection, and saprophytic activity in stumps. *Annals of Botany*, 15, 1–21.

Rishbeth, J. 1951b. Observation on the biology of *Fomes annosus*, with particular reference to East Anglian pine plantations. III. Natural and experimental infection of pines, and some factors affecting severity of the disease. *Annals of Botany*, 15, 221–246.

Rishbeth, J. 1957. Some further observation on *Fomes annosus* Fr. *Forestry*, 30, 69–89.

Rogalski, J., Leonowicz, A. 1992. *Phlebia radiata* laccase forms induced by veratric acid and xylidine in relation to lignin peroxidase and manganese-dependent peroxidase. *Acta Biotechnologica*, 12 (3), 213–221. DOI: 10.1002/abio.370120310

Rönnberg, J., Cleary, M.R. 2012. Presence of *Heterobasidion annosum* infections in Norway spruce stumps six years after treatment with *Phlebiopsis gigantea*. *Forest Pathology,* 42 (2), 144–149. DOI: org/10.1111/j.1439-0329.2011.00731.x

Rönnberg, J., Sidorov, E., Petrylaitė, E. 2006. Efficacy of different concentrations of Rotstop® and Rotstop® S and imperfect coverage of Rotstop® S against *Heterobasidion* spp. spore infections on Norway spruce stumps. *Forest Pathology*, 36 (6), 422–433. DOI: org/10.1111/j.1439-0329.2006.00476.x

Ruiz-Dueñas, F.J., Camarero, S., Pérez-Boada, M., Martinez, M.J., Martinez, A.T. 2001. A new versatile peroxidase from *Pleurotus*. *Biochemical Society Transactions*, 29 (2), 116–122. DOI: 10.1042/BST0290116

Sarkar, S., Martinez, A.T., Martinez, M.J. 1997. Biochemical and molecular characterization of a manganese peroxidase isoenzyme from *Pleurotus ostreatus*. *Biochimica et Biophysica Acta*, 1339, 23–30. DOI: 10.1016/S0167-4838(96)00201-4

Schwarze, F.W.M.R., Engels, J., Mattheck, C. 2000. Fungal strategies of wood decay in trees. Springer-Verlag Berlin, Heidelberg, New York.

Schoemaker, H.E., Lundell, T.K., Hatakka, A.I., Piontek, K. 1994. The oxidation of veratryl alcohol, dimeric lignin models and lignin by lignin peroxidase - the redox cycle revisited. *FEMS Microbiol Review*, 13, 321–332.

Sierota, Z. 2001. Choroby lasu (in Polish). Centrum Informacyjne Lasów Państwowych, Warszawa.

Stajić, M., Persky, L., Cohen, E., Hadar, Y., Brceski, I., Wasser, S.P., Nevo, E. 2004. Screening of the laccase, manganese peroxidase, and versatile

peroxidase activities of the genus Pleurotus in media with some raw plant materials as carbon sources. *Applied Biochemistry and Biotechnology*, 117 (3), 155–164.

Stajić, M., Persky, L., Friesem, D., Hadr, Y., Wasser, S.P., Nevo, E., Vukojević, J. 2006. Effect of different carbon and nitrogen sources on laccase and peroxidases production by selected *Pleurotus* species. *Enzyme and Microbial Technology*, 38, 65–73.

Stajić, M., Vukojević, J., Knežević, A., Milovanović, I. 2013. Influence of trace elements on ligninolytic enzyme activity of *Pleurotus ostratus* and *P. pulmonarius*. *Bioresources,* 8 (2), 3027–3037. DOI: 10.15376/biores.8.2.3027-3037

Stenlid, J., Redfern, D.B. 1998. Spread within the tree and stand. In: *Heterobasidion annosum*. Biology, ecology, impact and control (eds.: S. Woodward, J. Stenlid, R. Karjalainen, A. Hüttermann). CAB International, Wallingford, UK, 125–141.

Sugano, Y., Matsushima, Y., Shoda, M. 2006. Complete decolorization of antraquinone dye Reactive blue 5 by the concerted action of two peroxidases from *Thanatephorus cucumeris* Dec 1. *Applied and Environmental Microbiology*, 73 (4), 862–871.

Szczepkowski, A., Piętka, J. 2008. Results of inoculation of beech (*Fagus sylvatica* L.) and oak (*Quercus* sp.) stumps with *Pleurotus ostreatus* (Jacq.: Fr.) Kumm. *Folia Forestalia Polonica*, *Series A – Forestry*, 49/50, 15–25.

Tien, T., Kirk, K. 1988. Lignin peroxidase of *Phanerochaete chrysosporium*. *Methods in Enzymology*, 161, 238−249. DOI: 10.1016/0076-6879(88)61025-1

Wariishi, H., Gold, M.H. 1989. Lignin peroxidase compound III: formation, inactivation, and conversion to the native enzyme. *FEBS Letters*, 243, 165–168. DOI: org/10.1016/0014-5793(89)80122-X

Wariishi, H., Valli, K., Gold, H.M. 1992. Manganese (II) oxidation by manganese peroxidase from the Basidiomycete *Phanerochaete chrysosporium*. *Journal of Biological Chemistry*, 267, 23685−23695.

Wong, D.W.S. 2009. Structure and action mechanism of ligninolytic enzymes. *Applied Biochemistry and Biotechnology*, 157, 14−209.

Woodward, S., Stenlid, J., Karjalainen, R., Hüttermann, A. 1998. *Heterobasidion annosum*. Biology, ecology, impact and control. Preface. Cab International, Wallingford, UK.

Żółciak, A. 2002. Deciduous stump inoculation with *Pleurotus ostreatus* as biological method of *Armillaria* root rot control (in Polish with English summary). *Prace Instytutu Badawczego Leśnictwa*, *Seria A*, 944, 5–20.

Żółciak, A., Korniłłowicz-Kowalska, T.A., Sierota, Z., Iglik, H. 2008. Enzymatic activity of *Phlebiopsis gigantea* isolates. *Acta Mycologica*, 43 (1), 41–48.

Żółciak, A., Sierota, Z., Małecka, M. 2012. Characterization of some *Phlebiopsis gigantea* isolates with respect to enzymatic activity and decay of Norway spruce wood. *Biocontrol Science and Technology*, 22 (7), 777–790. DOI: org/10.1080/09583157.2012.691156

Żółciak, A., Bohacz, J. 2016. Ligninolytic activity of *Heterobasidion parviporum* isolates in cultivation on Norway spruce wood (in Polish with English summary). *Sylwan*, 160 (12), 1027−1036.