Ligninolytic Activity of *Phlebiopsis gigantea* Strains in Cultivation on Norway Spruce Wood

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Abstract

As a white-rot basidiomycetous and wood-decaying fungus, *Phlebiopsis gigantea* (Fr.: Fr.) Jülich is able to degrade lignin, cellulose and hemicellulose with a complex set of extracellular enzymes. Enzyme activity of this fungus has not been sufficiently explored.

The aim of this study was to assess the activity of laccase and peroxidases as well as the level of micromolecular compounds in *P. gigantea* strains, grown on pieces of Norway spruce wood (sapwood and heartwood) over 50 days of incubation under laboratory conditions. Enzymatic activity was determined using spectrophotometry. *Phlebiopsis gigantea* strains showed laccase (Lacc), manganese peroxidase (MnP), lignin peroxidase (LiP) and versatile peroxidase (VP) activity. Hydroxy– and methoxyphenols were released during this process as well. High levels of MnP activity (from 5.5 to 107.847 mU/µg of protein in cultures on sapwood and from 7.585 to 229.055 mU/µg of protein in cultures on heartwood) were observed in *P. gigantea* strains, as well as high activity of VP with manganese-oxidizing properties (from 3.36 to 61.708 mU/ġg of protein on sapwood and from 1.7 to 254.479 mU/µg of protein on heartwood) compared with the other examined extracellular enzymes. Laccase and LiP activity were found to be low in all strains of *P. gigantea* as well as the activity of VP in terms of guaiacol-oxidizing properties (both on sapwood and heartwood samples).

Keywords: white-rot fungus, *Phlebiopsis gigantea,* laccase, manganese peroxidase, lignin peroxidase, versatile peroxidase, hydroxyphenols, methoxyphenols

Introduction

Root and butt rot caused by the pathogen *Heterobasidion annosum* (Fr.) Bref. *sensu lato* is one of the most devastating conifer diseases in the boreal and temperate zones of the Northern Hemisphere, particularly in Europe (Bendz-Hellgren et al. 1998, Garbelotto and Gonthier 2013). Losses due to this disease in European commercial forest stands were estimated at about EUR 800 million annually (Woodward et al. 1998). Hence, controlling actions have been undertaken in many countries to contain the disease, including stump treatment using a saprotrophic white-rot fungus *Phlebiopsis gigantea* (Fr.: Fr.) Jülich. This is an effective biocontrol method to reduce spore infections on freshly-cut, healthy stumps (Rishbeth 1963, Korhonen et al. 1994, Thor and Stenlid 2005). Fungus *P. gigantea* is used commercially as a biological treatment for conifer stump protection (Holdenrieder and Greig 1998, Pratt et al. 2000, Sierota 2003, Thor 2003), but at the same time the effectiveness of its application is currently under evaluation (Sierota 1995, Nicolotti et al. 1999, Łakomy 2001, La Porta et al. 2003, Berglung and Rönnberg 2004, Vasiliauskas et al. 2004, Vasiliauskas et al. 2005, Berglung et al. 2005, Nicolotti and Gonthier 2005, Rönnberg et al. 2006, Drenkhan et al. 2008, Samils et al. 2008, Gunulf et al. 2012, Keča and Keča 2012, Rönnberg and Cleary 2012, Kenigsvalde et al. 2017). Research shows that the efficacy of this treatment varies considerably. Some studies have shown quite low control efficacy of *P. gigantea* treatment in Norway spruce stands (Berglund and Rönnberg 2004, Berglund et al. 2005, Gunulf et al. 2012). According to Pratt and Thor (2001) the control efficacy depends on the query coverage. Satisfactory results might be also obtained by increasing the spore concentrations. Rönnberg et al. (2006) noticed that the different strains of *P. gigantea* showed variable efficacy against *H. annosum* s.l. spore infections under different environmental conditions. Strains that are used in production of Rotstop showed to be one of the most effective compared to other *P. gigantea* strains, however there were found local strains that showed even higher efficacy (Berglund et al. 2005, Sun et al. 2009, Kenigsvalde et al. 2016). According to Vasiliauskas et al. (2005) large-

scale use of single genotype of *P. gigantea* for a long time can negatively affect local populations of fungi in forest ecosystems, although Sun et al. (2013) and Terhonene et al. (2013) did not find obvious adverse effect on bacterial diversity and mycobiota on the long-term use of this fungus. It seems however, that more consistent and effective genotypes of *P. gigantea* could benefit forests as well as reduce the ecological risks associated with using a single strain for treatment of conifer stumps. As reported by Schardl and Craven (2003), fungal organisms may hybridize and, at the time of adaptation to new ecological niches, alter their enzymatic activity. Therefore, it is of great importance to know the more about enzymatic activity of various strains of *P. gigantea*. Ligninolytic and cellulolytic activity of *P. gigantea* strains can be useful in the selection of strains of this fungus that are characterized by high enzymatic activity and consequently, high potential to decompose stump wood.

The white-rot fungi are able to degrade lignin, cellulose and hemicellulose with a complex set of extracellular enzymes (Hatakka 1994, 2001, Schmidt 2006). The basidiomycetes can degrade lignin substantially, mineralizing it to carbon dioxide (Hatakka 1994). During selective delignification, at the early stage of decay, lignin is broken down more than hemicellulose or cellulose (Schwarze et al. 2000). In order to degrade lignin, the white-rot fungi have developed an unspecific ligninolytic system consisting of peroxidases and laccases, which degrade lignin in an oxidative process (Hatakka 1994).

The aim of this study was to determine the ligninolytic properties of three *P. gigantea* strains. Additionally, the study was designed to quantify the laccase activity, peroxidase activity and the levels of micromolecular compounds in *P. gigantea* strains grown on spruce wood (on sapwood and heartwood). The results of this work can contribute to the overall understanding of enzymes regulation in white rot fungus *P. gigantea*.

Material and Methods

Three strains of *P. gigantea* (Pg1, Pg2, and Pg3) were used in the experiment. A fruit body of Polish *P. gigantea* was collected from a Norway spruce stump (Pg1, heterokaryotic strain, collected from – N 49º29'29"; E 19º00'17"). Sample portions were transferred to Petri dishes containing 2% malt extract agar (MEA), then incubated in darkness at 24°C for up to 20 days. Two strains of *P. gigantea* (Pg2, VRA 1984 – strain isolated from Norway spruce stumps; Råberg near Uppsala, Sweden; and Pg3, VRA 1835 strain isolated from Norway spruce log; Loppi, Finland; used in Finnish commercial preparation) were obtained as pure cultures from Dr. Marina Brandtberg (from Lallemand Plant Care, Verdera Oy, Finland). Pure cultures of all *P. gigantea* strains were cultivated on 2% MEA at 24°C. After three weeks of incubation, they were checked under a microscope to confirm the presence of *P. gigantea* oidia.

Enzymatic production of research strains was conducted on Petri dishes, on 2% MEA. The source of carbon (C) and energy for strains were wood pieces of Norway spruce (sapwood and heartwood) of size 5×8×40 mm^3. Sterilized wood pieces were placed in dishes containing pure cultures of appropriate strains and incubated for 50 days at 24 °C. Enzyme activity was established in the surface layer of spruce wood overgrown by mycelia of *P. gigantea*. The samples (powdered sapwood and heartwood, together with the mycelium), were ground at a concentration of 1:1 with distilled water, with a small addition of corundum (Al_2O_3). The homogenate was filtered through Miracloth filtration paper and centrifuged at 6,000 r.p.m. for 5 min. The resulting supernatants were used to measure enzyme activities in a spectrophotometer periodically (after 10, 20, 30, 40 and 50 days; in three-time repetitions, for all three strains). For all evaluated enzymes, one activity unit was defined as the amount of enzyme necessary to oxidize 1 µmol of substrate per minute. All activities measured in this study were expressed in units of specific activity (mU/µg of protein). Laccase (Lacc) estimation was performed according to methods by Leonowicz and Grzywnowicz (1981), using syringaldazine as a substrate. Manganese peroxidase (MnP) activity was assayed according to methods by Wariishi et al. (1992), with veratryl alcohol as the substrate, and lignin peroxidase (LiP) according to methods by Tien and Kirk (1988), modified by Matuszewska (2005). The activity of versatile peroxidase (VP) in oxidation of manganese ions and guaiacol was also assayed using methods by Sugano et al. (2006). Concentrations of methoxy- and hydroxyphenolic substances were evaluated with diazosulphanilamide in the DASA test (Leonowicz et al. 1968) as modified by Malarczyk (1984). Detailed methodology is presented in previous publications (Żółciak et al. 2008, Żółciak et al. 2012, Żółciak and Bohacz 2016).

Enzyme activity data were not normally distributed. As a result, data were analyzed with Kruskal-Wallis and Mann-Whitney U tests. Statistical analyses were performed using Statistica 10 software package (StatSoft 2010).

Results

Strains of *P. gigantea* (Pg1, Pg2 and Pg3) exhibited laccase, lignin peroxidase, manganese peroxidase and versatile peroxidase (oxidizing both manganese ions and guaiacol), all the enzymes which play major roles in lignin degradation. During the degradation process, hydroxyphenols and methoxyphenols were released. Enzymes

and degradation outputs were observed for mycelium growing on both sapwood and heartwood of Norway spruce wood.

Laccase activity was low for all strains of *P. gigantea*, ranging from 0 to 0.731 mU/µg of protein on sapwood, and from 0 to 0.216 mU/µg of protein in cultures on heartwood (a value of 0 was obtained for Pg1 at the 40th *day* of culture on both sapwood and heartwood, and for Pg3 at the 40th day of culture on sapwood and at the 10th day of culture on heartwood) (Table 1). The highest laccase activity in the Pg3 strain (0.731 mU/µg of protein) was found within 20 days on sapwood samples, whereas in the Pg1 strain (0.216 mU/µg of protein) it occurred within 30 days of culture on heartwood samples.

Table 1. Dynamics of measured enzymes and levels of secreted hydroxy-and methoxyphenols in cultures of *P. gigantea* strains (Pg1, Pg2, Pg3) on Norway spruce wood (sapwood – S and heartwood – H)

Strain	Days 10 S	10 H	20 S	20 H	30 S	30 H	40 S	40 H	50 S	50 H
Laccase activity (mU/µg of protein)										
Pg1	0.042	0.052	0.048	0.113	0.096	0.216	0	0	0.017	0.082
Pg2	0.050	0.041	0.112	0.059	0.237	0.195	0.115	0.119	0.023	0.077
Pg3	0.076	0	0.731	0.081	0.329	0.165	0	0.144	0.013	0.131
Manganese peroxidase activity (mU/µg of protein)										
Pg1	7.273	46.374	5.500	78.960	107.847	81.953	28.535	134.717	11.745	196.58
Pg2	6.019	7.585	30.611	69.609	34.941	63.904	60.977	104.498	46.716	112.79
Pg3	7.331	35.755	25.290	140.656	27.615	119.49	81.334	229.055	11.919	179.22
Lignin peroxidase activity (mU/µg of protein)										
Pg1	0.321	0.957	0.334	0.173	0.623	0.114	0.091	0.303	0.108	0.679
Pg2	0.243	0.353	0.148	0.207	0.814	0.199	0.579	0.226	0.164	0.261
Pg3	0.025	0.128	0.593	0.614	0.171	0.409	0.279	1.566	0.088	0.060
Versatile peroxidase activity oxidizing manganese ions (mU/µg of protein)										
Pg1	61.78	6.965	17.724	10.180	21.742	18.057	3.460	175.035	3.735	23.447
Pg2	35.867	1.700	18.763	12.652	6.202	18.018	6.202	93.426	17.483	11.928
Pg3	47.061	10.448	6.552	19.299	3.635	31.327	10.013	254.479	3.367	11.792
Versatile peroxidase activity oxidizing guaiacol (mU/µg of protein)										
Pg1	0.528	0.120	1.126	0.501	0.532	0.909	0.254	0.381	0.016	0.152
Pg2	1.432	0.135	0.946	1.238	0.270	0.174	0.405	0.079	0.064	0.127
Pg3	0.579	0.134	0.671	1.178	0.179	0.286	0.228	0.574	0.074	0.042
Hydroxyphenols (µg of protocatechuic acid/ml)										
Pg1	54.395	32.090	97.630	36.166	126.713	37.708	68.562	80.699	105.874	78.394
Pg2	39.204	23.098	102.210	35.021	84.18	40.655	67.616	48.471	107.248	82.195
Pg3	50.746	41.311	102.897	83.630	129.156	48.212	82.775	52.395	108.24	80.638
Methoxyphenols (µg of vanillin acid/ml)										
Pg1	8.874	14.153	18.961	15.951	19.580	16.631	15.183	35.592	21.371	34.575
Pg2	7.500	10.187	18.893	15.446	16.301	17.930	15.284	21.378	20.011	36.252
Pg3	10.295	18.220	19.304	36.885	22.987	21.263	18.705	23.108	22.529	35.565

Neither statistically significant differences were observed between the values of Lacc activity exhibited by strains of *P. gigantea* nor between cultures on sapwood and heartwood. However, statistically significant differences were observed between the median levels of Lacc activity in samples evaluated after the 10th and the 20th, the 10th and the 30th, the 30th and the 40th, and the 30th and the 50th day of culturing *P. gigantea* strains (Figure 1a).

All the strains of *P. gigantea* exhibited a high level of manganese peroxidase activity in cultures on both sapwood and heartwood (Table 1). In cultures on sapwood, activity of the enzyme was found first to gradually increase, and then to steadily decrease, whereas in culture on heartwood the enzyme showed *increased activity* over the *time* of culturing.

Activity of MnP ranged from 5.5 to 107.847 mU/µg of protein in cultures on sapwood, and from 7.585 to 229.055 mU/µg of protein in cultures on heartwood. The enzyme activity peaked at the 30th day of culture on sapwood for the Pg1 strain (107.85 mU/µg of protein), and at the 40th day of culture on heartwood for the Pg3 strain (229.05 mU/µg of protein). No statistically significant differences were observed between the *P. gigantea* strains in values of MnP activity. On the other hand, statistically significant differences were observed between the medians of MnP activity in sapwood and heartwood (Figure 2a), as well as in samples evaluated after the 10th and the 30th; the 10th and the 40th, and the 10th and the 50th day of culture of *P. gigantea* fungus (Figure 1b).

Values of lignin peroxidase activity were found to be low in all strains of *P. gigantea*. Activity of this enzyme was estimated at a range of 0.025 - 0.593 mU/µg of protein in cultures on sapwood, and at 0.060 –1.566 mU/µg of protein in cultures on heartwood (Table 1). For the Pg3 strain, the enzyme activity peaked at the 20th day of culturing on sapwood (0.593 mU/µg of protein), and at the 40th day of culturing on heartwood (1.566 mU/µg of protein). For values of LiP activity, there were no statistically significant differences among the strains of *P. gigantea* or between cultures on sapwood and heartwood. Similarly, there were no significant differences among time periods of strain cultures.

Activity of versatile peroxidase with manganese-oxidizing properties was found to be high in all the strains of *P. gigantea*, ranging from 3.36 to 61.708 mU/µg of protein on sapwood, and from 1.7 to 254.479 mU/µg of protein on heartwood (Table 1). The highest activity of the enzyme was observed for the Pg1 strain (61.708 mU/µg of protein)

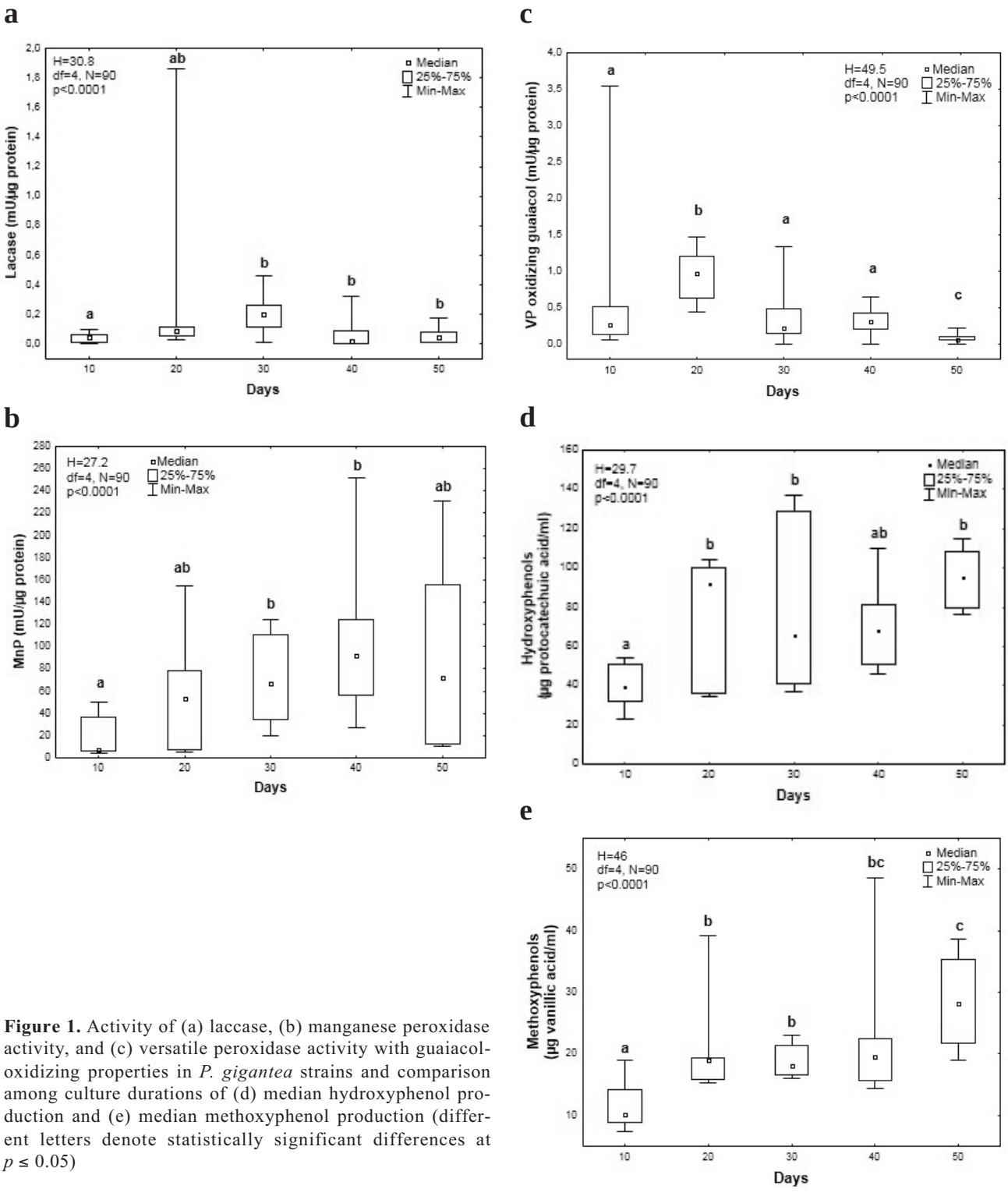

Figure 1. Activity of (a) laccase, (b) manganese peroxidase activity, and (c) versatile peroxidase activity with guaiacol-oxidizing properties in *P. gigantea* strains and comparison among culture durations of (d) median hydroxyphenol production and (e) median methoxyphenol production (different letters denote statistically significant differences at $p \leq 0.05$)

at the 10th day of culture on sapwood and for the Pg3 strain (254.479 mU/µg of protein) at the 40th day of culture on heartwood. The activity of VP manganese-oxidizing properties did not differ significantly among the strains of *P. gigantea,* as well as among the time periods of isolate culture. On the other hand, values did differ significantly between cultures on sapwood and heartwood (Figure 2b).

Activity of VP with guaiacol-oxidizing properties was very low for all *P. gigantea* strains, ranging from

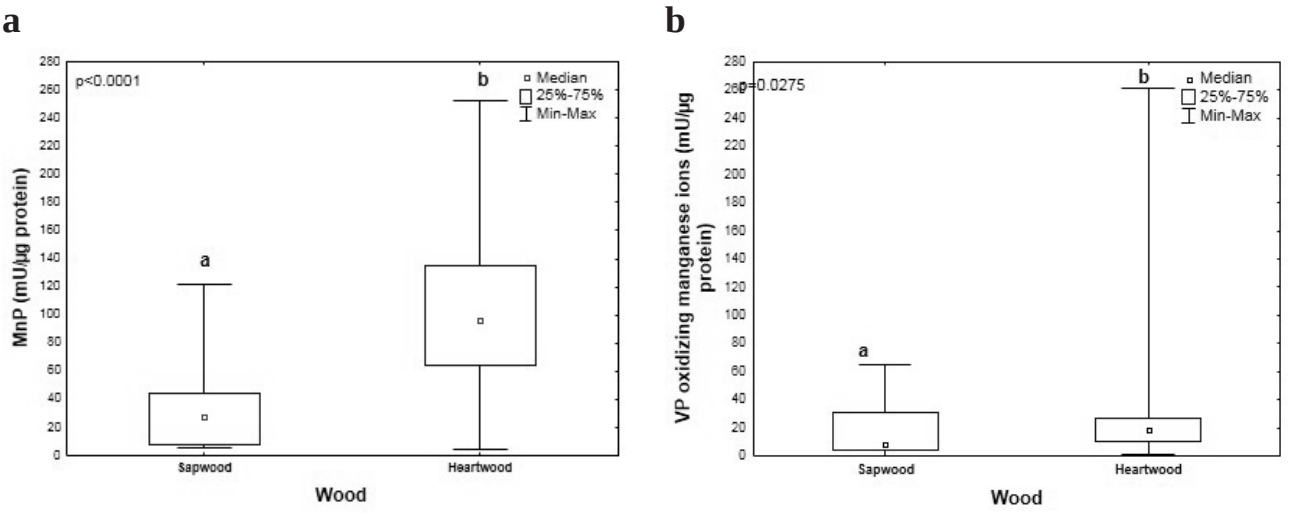

Figure 2. Activity of (a) manganese peroxidase and (b) versatile peroxidase activity with manganese-oxidizing properties in *P. gigantea* strains cultured on sapwood vs. heartwood (different letters denote statistically significant differences at $p \leq 0.05$)

0.016 to 1.432 mU/µg of protein for mycelium incubated on sapwood, and from 0.042 to 1.238 mU/µg of protein for mycelium incubated on heartwood (Table 1). The activity of the enzyme peaked for the Pg2 strain (1.432 mU/µg of protein) at the 10th day of culture on sapwood, and for the Pg2 strain (1.238 mU/µg of protein) at the 20th day of culture on heartwood. Activity of guaiacol-oxidizing VP did not differ significantly among strains of *P. gigantea* grown on sapwood and heartwood, but statistically significant differences in activity were determined between samples evaluated after the 10th and the 20th, the10th and 50th, the 20th and the 30th, 20th and 40th, the 20th and 50th, the 30th and 50th, and the 40th and 50th day of culture (Figure 1c).

The values of released hydroxyphenols in *P. gigantea* strains ranged from 39.204 to 129.156 (µg of protocatechuic acid/ml) in cultures on sapwood, and from 23.098 to 83.630 (µg of protocatechuic acid/ml) in cultures on heartwood (Table 1). The highest value of released hydroxyphenols was observed for the Pg3 strain on the 30th day of culture on sapwood (129.156 µg of protocatechuic acid/ml), and on the 20th day of culture on heartwood (83.630 µg of protocatechuic acid/ml). Values of released hydroxyphenols did not differ significantly between strains of *P. gigantea,* but there were statistically significant differences between hydroxyphenols in sapwood and heartwood as well as between samples evaluated after the10th and the 20th, the 10th and the 30th, and the 10th and 50th day of culture of *P. gigantea* mycelium (Figure 1d).

The values of released methoxyphenols for the evaluated strains of *P. gigantea* ranged from 7.5 to 22.987 (µg of vanilin acid/ml) in cultures on sapwood, and from 10.187 to 36.885 (µg of vanilin acid/ml) in cultures on heartwood (Table 1). The highest values of methoxyphenols were found for the Pg3 strain at the 30th day of culture on sapwood (22.987 µg of vanilin acid/ml), and at the 20th day of culture on heartwood (36.885 µg of vanilin acid/ml). Statistically significant differences were observed between the values of released methoxyphenols of Pg1 and Pg3 as well as between Pg2 and Pg3 in both sapwood and heartwood (Figure 4), and in samples evaluated after the 10th and the 20th, the 10th and 30th, the 10th and 40th, the 10th and the 50th, the 20th and 50th, and the 30th and 50th days of mycelium culture (Figure 1e).

Discussion

Interest by researchers in the lignolytic enzymes of white-rot fungi has been increasing, particularly due to a hope of finding better enzyme producers for use in various biotechnological applications as well as comparative biology (Wong 2009, Hatakka and Hammel 2010, Isroi et al. 2011). In this study, *P. gigantea* was shown to be capable of producing all major ligninolytic enzymes. Low levels of laccase activity were measured for three *P. gigantea* strains. According to Sierota and Miseikyte (2000), no laccase was found for *P. gigantea* on pine wood samples laid on agar medium. Żółciak et al. (2008) found laccase for two isolates of *P. gigantea* out of six isolates tested between 14 and 21 days of cultivation, but no laccase was detected in liquid cultures with lignin wastes. Mgbeahuruike et al. (2011) found high variability in laccase secretion among the tested *P. gigantea* strains. Because many white-rot fungi produce laccase in addition to lignin and manganese peroxidases and in varying combinations, three categories of fungi can be

identified as follows: (i) a lignin-manganese peroxidase group, e.g. *Phanerochaete chrysosporium* Burds. and *Phlebia radiata Fr.*, (ii) a manganese peroxidase-laccase group, e.g. *Dichomitus squalens* (P. Karst.) D.A. Reid and *Rigidoporus lignosus* (Klotzsch) Imazeki, and (iii) a lignin peroxidase-laccase group, e.g. *Phlebia ochraceofulva* (Bourdot & Galzin) Donk and *Junghuhnia separabilima* (Pouzar) Ryvarden (Hatakka 1994). According to Hatakka (2001), almost all species of white-rot fungi produce laccase to varying degrees, but the most heavily-researched fungus, *P. chrysosporium,* produces multiple isoenzymes of lignin peroxidase and manganese peroxidase but does not produce laccase (Hatakka 1994). As a slight variation on this finding, Srinivasan et al. (1995) report that laccase is produced in only certain strains of this species and under special conditions. Schwarze et al. (2000) found that laccase activity of *P. chrysosporium* is induced by the availability of phenolic compounds. According to Wong (2009), the best characterized fungus for production of laccase is *Trametes versicolor* (L.) Lloyd. Fungal laccase production is influenced by many culturing parameters such as medium composition, pH, and temperature (Niku-Paavola et al. 1990, Arora and Gill 2001). Laccase proved to be much more thermostable than manganese peroxidase and lignin peroxidase (Arora and Gill 2005). In the present study, laccase activity was measured with syringaldazine; however, laccase production can be greatly stimulated by the presence of a wide variety of inducing substances, particularly aromatic or phenolic compounds (Farnet et al. 1999). In some fungal strains, laccase can be induced by anilines (Fahraeus et al. 1958, Bollag and Leonowicz 1984). For some species, e.g. *T. versicolor*, *Fomes annosus* (Fr.) P. Karst., *Pholiota mutabilis* (Schaeff.) P. Kumm., *Pleurotus ostreatus* (Jacq.: Fr.) Kummer, *P. radiata*, laccase levels are enhanced in the presence of 2,5-xylidine (Fahraeus and Reinhammar 1967, Agematu et al. 1993, Rogalski and Leonowicz 1992). According to Bollag and Leonowicz (1984), extracellular laccases are constitutively produced in white-rot fungi in small amounts, and Baldrian (2005) confirmed this by reporting that there are many taxonomic or physiological groups of fungi that do not produce significant amounts of laccase, or for which this enzyme is only produced by a few species. Adomas et al. (2003) did not find significant differences in the ability to secrete lignin oxidases between *P. gigantea* and *H. annosum*, but *P. gigantea* produced more cellulases than *H. annosum*; this was also confirmed in studies by Żółciak et al. (2012). Although laccase activity has been demonstrated in many fungal species, the enzyme has been purified from tens of species (Baldrian 2005). The results obtained in this study may indicate that *P. gigantea,* like *H. parviporum* Niemelä et. Korhonen (Żółciak and Bohacz 2016), belongs to groups that produce small amounts of laccase. To confirm this, further analyzes should be carried out.

In this study, manganese-peroxidase activity was high for the tested *P. gigantea* strains, in contrast to values previously obtained for *H. parviporum* strains (Żółciak and Bohacz 2016). According to Schwarze et al. (2000), in fungi causing decay similar to the white rot caused by *P. chrysosporium*, the activity of manganese-dependent peroxidase is induced by the presence of manganese, hydrogen peroxide and lignin. Manganese peroxidase catalyzes the reaction depending on the presence of manganese: $2Mn\ (II) + 2H^{+} + H_2O_2$ (Wong 2009). The first manganese peroxidase was isolated from *P. chrysosporium*, where it was shown that its activity and quantities are controlled by the presence of manganese Mn (II) in the medium. Manganese Mn (II) controls the transcription of a manganese-dependent peroxidase gene, which in turn is dependent on fungal growth and manganese concentration (Wong 2009). Hofrichter (2002) provides a list of forty-four species of fungi that cause white rot and produce manganese-dependent peroxidase, including: *Armillaria mellea* (Vahl ex Fr.) Kummer, *A. ostoyae* (Romagn.) Herink, *Bjercandera adusta* (Willd. ex. Fr.) P. Karst., *H. annosum*, *Hypholoma fasciculare* (Huds.: Fr.) Kummer, *Ganoderma lucidum* (Curtis) P. Karst., *P. chrysosporium*, *Phaeolus schweinitzii* (Fr.) Pat., *P. ostreatus* and *T. versicolor*.

Phlebiopsis gigantea strains showed very low ligninase activity. This does not seem to be related to the method of measurement as in the case of laccase. According to Wong (2009), the enzymatic activity of ligninase can be easily measured by the increase in absorbance at 310 nm under oxidation of veratryl alcohol – the optimal substrate for ligninase – to veratryl aldehyde; this method was used to measure ligninase activity in this study. Although most studies on lignin degradation focus on the *P. chrysosporium* (Tien and Kirk 1983, 1988, Kirk et al. 1990), ligninase has been found in *Peniophora gigantea* (Fr.) Masse by Varela et al. (2000). Some manganese peroxidases previously isolated from *B. adusta*, *B. fumosa* (Pers.: Fr.) P. Karst., *P. eryngii* (DC.) Quél., *P. ostreatus*, and *P. pulmonarius* (Fr.) Quél. exhibit similar activity to ligninase activity on aromatic substrates (Wong 2009). This group, known as the universal oxidases, is not only manganese-specific – Mn (II) – as in manganese peroxidase, but also oxidizes phenol and non-phenol substrates that are typically oxidized by ligninase, including veratrix alcohol, methoxybenzenes and model lignin compounds in the absence of manganese. Hence, it is important to separately consider versatile peroxidase with manganese-oxidizing properties and with guaiacol-oxidizing properties. In this study, in all three strains of *P. gigantea*, the activity of versatile peroxidase with manganese-oxidizing properties was found to be high in contrast to versatile

peroxidase with guaiacol-oxidizing properties. Versatile peroxidase was reported first in *P. eryngii* (Martinez et al. 1996) and then *B. adusta* and *P. ostreatus* (Sarkar et al. 1997, Heinfling et al. 1998a, Heinfling et al. 1998b, Mester and Field 1998). Versatile peroxidases combine the properties of lignin peroxidases and manganese peroxidases, conferring the catalytic versatility inferred by their name. They can oxidize Mn 2+ to Mn 3+ like manganese peroxidases but can also oxidize non-phenolic compounds in the same manner as lignin peroxidases (Ruiz-Dueńas et al. 2001). In this study, the activity of VP with manganese-oxidizing properties for the focal strains of *P. gigantea* was found to be high in contrast to this activity detected by Żółciak and Bohacz (2016) for *H. parviporum* strains; however, the activity of VP with guaiacol-oxidizing properties was low for strains of *P. gigantea,* as well as for the previously studied *H. parviporum* strains. The values of hydroxyphenols and methoxyphenols produced by *P. gigantea* strains, by contrast, are comparable with those previously detected for *H. parviporum* strains (Żółciak and Bohacz 2016).

In summary, this study indicated that the three focal strains of *P. gigantea* secreted laccase and three types of peroxidases: LiP, MnP and VP. The research revealed micromolecular compounds for mycelium growing on both sapwood and heartwood of Norway spruce wood.

Conclusions

No significant differences were found in the ligninolytic activity of the tested fungal strains of *P. gigantea* on samples of sapwood and heartwood.

Distinguishing between the types of wood with reference to *P. gigantea* colonization is not justified.

Recommended future research would optimize the conditions for the determination of ligninolytic activity for *P. gigantea* strains and aim to select a strain with a high production of enzymes.

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