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Original Scientific Paper

Functional differentiation of two autochthonous cohabiting strains of *Pleurotus ostreatus* and *Cyclocybe aegerita* from Serbia in lignin compound degradation

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ABSTRACT:
In nature, woody substrates are usually degraded by entire communities of microorganisms, which are nowadays jeopardised by anthropogenic influence, making it important to define the functional specificity of every species. Two strains of autochthonous fungi from Serbian lowland forests (*Pleurotus ostreatus* Ser1 and *Cyclocybe aegerita* Ser1) have been investigated for their ability to degrade lignin substrates [oak sawdust, oak isolated cell walls, and synthetic dehydrogenative polymer (DHP)]. Measuring the activities of the enzymes involved in lignin degradation was coupled with detecting the HPLC profile of the phenolics in the fungal growth media, and the lignin loss. While *Pleurotus ostreatus* Ser1 appeared highly effective within a very short time span, *Cyclocybe aegerita* Ser1 failed to degrade lignin. This situation was supported by very high enzyme activities and the low presence of phenolics in the media of *Pleurotus ostreatus* Ser1, compared to very low enzyme activity and the high presence of phenolics in the media with *Cyclocybe aegerita* Ser1.

Keywords:
wood degradation, wood decaying fungi, laccase, Mn peroxidase, lignin peroxidase

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INTRODUCTION

Lignin, the second most abundant biopolymer on Earth after cellulose (Lewis & Sarkanen 1998), is synthesised in the plant cell and incorporated as the main armature in the cell wall, enabling appropriate structure and protection from biotic and abiotic stresses. It can be considered a branched polymer chain of monomeric, dimeric, and oligomeric phenylpropane units which are linked in an alkyl-aryl ether bond formation (Funaoka 2013). Due to the complexity and heterogeneity of the lignin structure, synthetic lignin model compounds (e.g. dehydrogenative polymer, DHP) are commonly used to provide a better insight into its enzymatic conversion.

Unlike cellulose, which is readily decomposed by numerous microorganisms (Pérez *et al*. 2002), lignin is one of the most recalcitrant natural substances. Its degradation occurs in two stages: (I) non-specific, extracellular depolymerisation to aryl and biaryl compounds; and (II) the mineralisation of these by specific catabolic enzymes. Only white rot fungi (WRF) belonging to aerobic Basidiomycota mineralise it completely to CO_2 and H_2O (Dashtban *et al*. 2010). Lignin-modifying enzymes are classified as phenol oxidase (laccases, Lac and heme-containing peroxidases), namely lignin (LiP), manganese peroxidase (MnP), and multifunctional or versatile (VP) peroxidase. Lacs are able to oxidise a wide range of substrates including polyphenols, methoxy-substituted

phenols and diamines (Thurston 1994). MnPs can not only cleave β-O-4 linkages in phenolic structures, but also C_{α}-C_{β} and β-aryl ether bonds in non-phenolic ones (Kapich *et al.* 1999). Due to their high redox potential, LiPs are known to oxidise different phenolic aromatic compounds and a variety of non-phenolic lignin model compounds along with many other organic molecules (Sigoillot *et al.* 2012). VPs, combining the molecular architecture of LiP and MnP, can cleave both phenolic and non-phenolic compounds (Fisher & Fong 2014). The enzymatic oxidation of lignin has been shown to result in its depolymerisation and the release of aromatics (Gall *et al.* 2018).

Nowadays science is exploring the possibility of using specific wood-degrading fungal strains as an inoculum to re-establish the ecosystem services of disturbed and dysfunctional forests (Cohen *et al.* 2002). Also, naturally occurring WRF could be used to degrade plant industrial waste or artificial recalcitrant polymers introduced into forest ecosystems with the goal of producing bio-friendly outcomes. Since we are not aware of the existence of such practices in Serbia, we decided to explore the potential of two cohabiting locally collected strains of WRF [*Pleurotus ostreatus* (Jacq.) P. Kumm. here named as strain *P. ostreatus* Ser1 and *Cyclocybe aegerita* (V. Brig.) Vizzini here named as strain *C. aegerita* Ser1], to perform the degradation of lignin originating from the locally abundant tree species (e.g. *Quercus cerris* L.). The aims of the study were to evaluate the influence of substrate type on the activities of the enzymes involved in lignin degradation, to compare the product diversity profile in the liquid medium, and to detect any differences in efficiency and strategies in the early stages of wood degradation.

MATERIALS AND METHODS

Substrates. The sawdust of oak *Q. cerris* (Q) was obtained from a living tree in the local forest. Wood chips (app. size of 1 × 0.5 cm) were dried at 60°C and then used as substrates or as the start material for the isolation of extractive free cell wall material (W). 400 mg of powdered sawdust of *Q. cerris* were homogenised for 5–10 min in 10 mL 80% methanol, stirred for 1 h at room temperature and centrifuged for 5 min at 1500 *g*. The pellet was re-extracted twice with 10 ml of 80% methanol, and subjected to the washing steps according to Strack *et al.* (1988) and Chen *et al.* (2000). The remaining material was dried and used as a substrate or for an acetyl bromide test.

DHP synthesis. DHP was synthesised according to the procedures proposed by Freudenberg & Neish (1968) and Wayman & Obiaga (1974), as reported by Radotic *et al.* (1998). DHP was prepared from coniferyl alcohol and horseradish peroxidase as an enzymatic catalyst, by the simultaneous addition of H_2O_2 and coniferyl alcohol solutions to the peroxidase solution. The reaction mixture contained 5 mM coniferyl alcohol, 5 mM H_2O_2, and 2.5×10^{-8} M horseradish peroxidase (all from Fluka Chemical Corp., New York) in a 50 mM phosphate buffer at 25°C. The solution was shaken for 48 h until the end of the reaction. The precipitate was washed twice in deionised water and evaporated in a vacuum at 5°C.

Mycelial cultures. Fungal strains of *P. ostreatus* Ser1 and *C. aegerita* Ser1 were originally isolated from the fruit bodies collected in the local forest in Serbia (Bela Crkva). The mycelial cultures were isolated and maintained in Petri dishes on malt extract agar (MEA) at room temperature. For the experiments, a liquid mineral medium (LMM) was prepared according to Jović *et al.* (2018). 3 g of Q or W were added to 50 ml of LMM in 300 ml Erlenmeyer flasks, while 50mg of DHP was added to 25 ml of LMM in 100 ml Erlenmeyer flasks and sterilised in an autoclave at 114°C for 30 min. Sawdust and DHP-containing media were inoculated with one-week-old mycelial plugs. All the experimental treatments were incubated at 30°C in the dark for 14 days. A liquid culture of both fungal strains on the LMM was used as the control for the production of enzymes by mycelia without the presence of the lignin-containing substrate, while the substrates incubated without fungi were used as the control for the production of polyphenolic substances by substrates.

Molecular identification of the fungal strains. DNA was isolated from the mycelia using a DNAeasy Plant Mini Kit (QIAGEN) and used for PCR-based amplification with standard primers for fungi (ITS1F and ITS4,) and applying standard protocols (Gardes & Bruns 1993). The PCR products were sequenced (Sanger) by Microsynth, Vienna, and the sequences were compared with those of different geographic origins in the NCBI database. The evolutionary history was inferred by using the Maximum Likelihood method and the Tamura-Nei model (Tamura & Nei 1993). Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Tamura-Nei model, and then selecting the topology with a superior log likelihood value. This analysis involved 21 nucleotide sequences. The final dataset included a total of 762 positions. Evolutionary analyses were conducted in MEGA X (Kumar *et al.* 2018).

Sample preparation. The samples (growth media with substrate and fungal mycelia) were shaken for 30 min at 30°C at a 220 rpm, followed by separation of the liquid and solid phases by filtration on a vacuum pump via filter paper. The solid was packed and stored in a freezer at -20°C until analysis (acetyl bromide test). The liquid

phase was centrifuged for 30 min at 4185 *g*. The supernatant was used for the analysis of the ligninolytic enzyme activity and phenolic profile.

Acetyl bromide test. Prior to the acetyl bromide assay, alkaline hydrolysis of the dry cell walls was performed by suspending the cell walls in 8 ml of 1M aqueous NaOH at 80°C, followed by incubating the suspension at room temperature for 17 h. 2.5 mg of hydrolysed cell walls were mixed with 250 µL of 25% acetyl bromide in glacial acetic acid and incubated for 30 min at 70°C. The mixture was then cooled quickly on ice, and 250 µL 2 M NaOH was added and centrifuged at 15000 *g* for 5 min. The reaction mixture was made by mixing 250 µL of supernatant with 5 µL of 15 M hydroxylamine and 2495 µL of glacial acetic acid and the absorbance of the mixture was measured at 280 nm. The quantification of the lignin monomers was performed using a standard curve with coniferyl alcohol as the standard and the result was expressed as milligrams of coniferyl alcohol per gram of dry weight.

Laccase activity. The oxidation of guaiacol was used to determine the laccase activity in the isolated medium samples following the protocol of Kalra *et al.* (2013). The reaction mixture consisting of a 10 mM sodium-acetate buffer pH 5, 2 mM guaiacol, and enzyme source was incubated at 30°C for 15 min. The absorbance of oxidised guaiacol was measured at 470 nm (2501 PC Shimadzu, Kyoto, Japan) and the enzyme activity (U/mL) was calculated using the extinction coefficient for guaiacol ε = 6.74 L/(mmol × cm) as reported by Hosoya (1960).

Mn peroxidase activity. The determination of manganese peroxidase activity in the isolated medium samples was based on the oxidation of ABTS, according to the protocol proposed by Casciello *et al.* (2017). The reaction mixture contained 0.5 mM ABTS and 0.16 mM $MnCl_2$ in a 40 mM sodium citrate buffer, pH 4.5. The reaction was initiated with the addition of 0.05 mM H_2O_2 to the reaction mixture. The absorbance was measured at 420 nm (2501 PC Shimadzu, Kyoto, Japan) against distilled water as the reference mixture (ε = 36,000 M^{-1} cm^{-1}). One unit of enzyme activity (U) corresponded to the amount of enzyme which oxidised 1 µmol of ABTS per min at 25°C.

Lignin peroxidase activity. The lignin peroxidase assay was performed according to the procedure of Archibald (1992) based on the oxidation of the dye azure B. The reaction mixture (final volume of 1ml) consisted of a 125 mM sodium tartrate buffer (pH 3.0), 0.16 mM azure B, and enzyme filtrate. The reaction was initiated by 2 mM H_2O_2 and the absorbance decrease was measured at 651 nm (ε = 48.8 L/mmol·cm; 2501 PC Shimadzu, Kyoto, Japan). One unit of enzyme activity (U) was expressed as ΔA of 0.1 unit per minute per ml of the culture filtrate.

The total phenolic content (TPC) in the media. The adapted Folin-Ciocalteu spectrophotometric procedure (Dragišić & Živanović 2012) was used for the analysis of TPC. The modified method is based on the chemical reduction of tungsten and molybdenum oxides forming a blue colour product which exhibits broad light absorption with the maximum at 724 nm (Multiscan® Spectrum, Thermo Electron Corporation, Vantaa, Finland) proportional to the concentration of phenols. Gallic acid (GA) was used as the calibration standard and the results were expressed as milligrams of GA equivalent per gram of fresh weight (mg GA eq × g^{-1} FW).

HPLC analysis of the low-molecular-weight phenolic compounds in the medium. Qualitative and quantitative analyses of the individual compounds were performed by reversed-phase HPLC-MS analysis. The samples were injected in a Waters HPLC system consisting of 1525 binary pumps, a thermostat, and a 717+ autosampler connected to a Waters 2996 Diode Array and EMD 1000 single quadrupole detector with ESI probe (Waters, Milford, USA). Separation was performed on a Symmetry C-18 RP column 125 × 4.6 mm in size, with 5 µm particle diameters (Waters, Milford, MA, USA) connected to the appropriate guard column. A binary gradient using mobile phases, A (0.1% formic acid) and B (acetonitrile), was used at a flow of 1 ml min^{-1} with the following gradient profile: from 10 to 20% B in the first 20 minutes; a linear rise up to 40% B in the next 10 minutes, followed by 15 minutes reverse to the initial 10% B and an additional 5 minutes of equilibration time. A post-column flow splitter (ASI, Richmond, CA, USA) with a 5/1 split ratio was used to obtain the optimal mobile phase inflow for the ESI probe. A DAD detector was used in scan mode for the detection of various classes of phenolic compounds. A negative ESI scan mode was used for the phenolic compounds with the following parameters: capillary voltage 3.0 kV, cone voltage -35 V, with the extractor and RF lens voltages at 3.0 and 0.2 V, respectively. The source and desolvation temperatures were 140°C and 400°C respectively, with N_2 gas flow of 500 l/h. The data acquisition and spectral evaluation for peak confirmation were carried out by Waters Empower 2 software (Waters, Milford, USA).

Differentiation of the low-molecular-weight phenolic compounds in the experimental medium. Principal component analysis (PCA) was used to visualise the similarities between the HPLC-analysed samples in terms of their chemical composition. This method was applied to HPLC chromatograms, where retention times from 3 to 40 min were used in the analysis. Standard normal vari-

ate preprocessing (centering and scaling each individual spectrum) was applied to remove scattering effects. Multivariate analyses were carried out by PCA using the Unscrambler ×10.4 (Camo AS, Trondheim, Norway).

Statistical analyses. Two-factor analysis of variance with a balanced design was used to examine the influence of the type of fungus and the type of substrate on the activities of Lac, MnP, and LiP, as well as on TPC and lignin content. This method was used to test the significance of the main effects of the type of fungus and type of substrate, as well as the significance of their interaction effects. Tukey's test was used for post-hoc comparisons. A significance level of 5% was used in all the tests. A two-factor ANOVA was conducted using the SPSS 25 software package (IBM Corp. in Armonk, NY).

RESULTS

The sequences of the amplified ITS regions of rDNA of the investigated strains were deposited in GenBank under accession numbers OP936994 and OP936995. The comparison of ITS sequences revealed the identity of two Serbian strains, namely OP936994 as *Pleurotus ostreatus* and OP936995 as *Cyclocybe aegerita* (Fig. 1).

The growth of both strains was abundant in all the treatments. No activity of the tested enzymes (Lac, MnP, LiP) was detected in the media containing substrates only, implying that the detected enzymatic activities in the isolated medium samples originate from fungi (Fig. 2a, b & c). The two investigated WRF strains expressed considerably different patterns of enzyme activities involved in lignin degradation as presented in Fig. 2a, b & c. Lac and MnP expressed a similar pattern of activities in all the treatments, being significantly higher in *P. ostreatus* Ser1 than in *C. aegerita* Ser1 in the presence of substrates, but exhibiting the lowest activities with DHP (Fig. 2a, b & c). *Cyclocybe aegerita* Ser1 expressed no statistical differences between the activities of MnP on DHP and in the absence of a substrate. LiP activity was detected in the mediums of both strains only where Q and W were provided as the substrate, and with significantly higher activity in *P. ostreatus* Ser1. All the enzymes in *P. ostreatus* Ser1 appeared to be more active in W, while this was not the case in *C. aegerita* Ser1 (Fig. 2a, b & c). Significant lignin degradation was detected only when Q or W were added as the substrates and only with *P. ostreatus* Ser1 (Fig. 2d). The total phenolic content measurements implied high degradation of the phenolic compounds in the growth medium originating from all sources by both fungal strains (Fig. 2e). However, small amounts of phenolic compounds appeared to be present in comparable amounts in all the treatment mediums (significantly higher in *C. aegerita* Ser1 than in *P. ostreatus* Ser1).

The PCA of the HPLC-generated chromatograms of phenolic compounds (Figs. 3 & 4) differed considerably between the samples according to the added substrate – the samples containing only fungi (M, P, C) were clearly different from those where the plant material had been added (including treatments with fungi), as well as from the samples containing only plant substrates (Q, W). The Q samples were similar to those containing W, but with higher and much more diverse contents of phenolic substances of the plant origin in Q. The least diverse content of phenolics appeared in sample PW (Fig. 4).

Due to the fact that DHP is a synthetic polymer which can be obtained in small amounts, its degradation was followed in a separate experiment and the chromatograms are presented in Fig. 5. The chromatogram of DHP in the growth medium showed the clear presence of dimers and trimers. Both fungi were able to degrade such substrates. The presence of small amounts of phenolics in the medium with *C. aegerita* Ser1 may be attributed to the previously detected secretion of such compounds by mycelia, while almost no phenolic substances remained in the treatment with *P. ostreatus* Ser1.

DISCUSSION

The comparison of lignin enzyme degradation essays, accompanied by the simultaneous measurement of the remaining phenolic substances in the growth mediums and the evaluation of the lignin degradation of the two autochthonous WRF strains from Serbian lowland forests indicated strong differences between enzyme production patterns and their efficiency in lignin degradation. The detection of phenolic substances in the growth media by HPLC appeared to be an appropriate technique for following the lignin degradation process by fungi.

An investigation of the fungal succession of wood colonisation showed that Basidiomycota dominate in the earlier stages of its decomposition (Fukasawa *et al.* 2011), but the literature data showed the preference of different fungal species for a certain type of substrate (Deacon 2006). Nowadays, it is clear that plant biomass in ecosystems is degraded by entire fungal communities which work synergistically towards enabling efficient nutrient circulation (Kjøller & Struwe 2002). Describing the functional specificities of different fungal strains in this process is very important to gain a better understanding of the functional diversity of such fungal communities and their potential for substrate utilisation in specific conditions.

The high enzyme activities of *P. ostreatus* Ser1 (Fig. 3a, b & c) could be directly linked to its ability to degrade lignin polymer as well as small phenolic molecules in a very short time span, while *C. aegerita* Ser1 was able to degrade only certain small phenolic compounds (Figs. 3d, 4, 5 & 6). The enzyme activities (Fig. 3a, b & c) of *P. ostreatus* Ser1 were significantly higher when W was used as the substrate in comparison to Q, implying the potential presence of some inhibitors in Q. The total

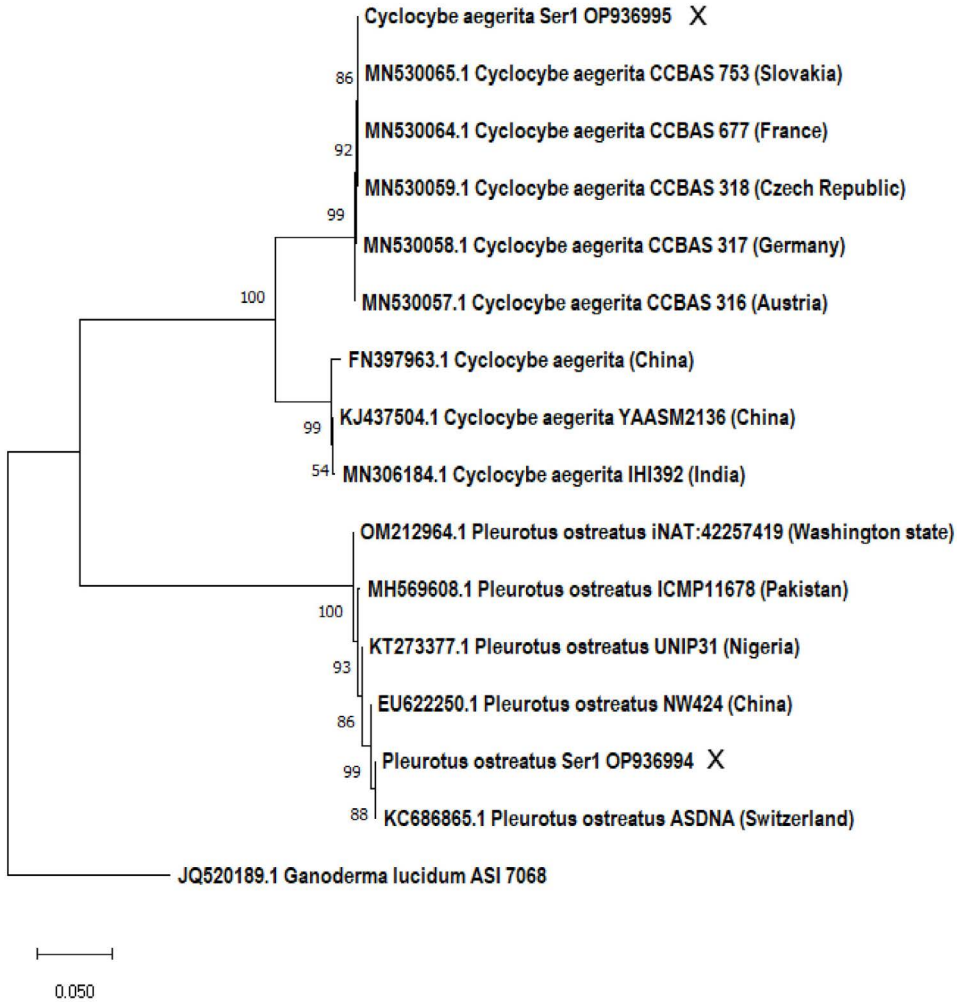

Fig. 1. The phylogenetic trees with the highest log likelihood obtained by analyses of the *ITS* sequences of *P. ostreatus* Ser1 and *C. aegerita* Ser1. The percentage of trees in which the associated taxa clustered together is shown next to the branches.

phenolic content measurements clearly reflect the presence of phenolic substances in the media with *C. aegerita* Ser1 (Fig. 3e), while the media chromatogram provides evidence that *C. aegerita* Ser1 emits fungal-characteristic phenolics, which remain in the medium even when the fungus utilises other phenolics of the plant origin (Figs. 4 & 5). However, the small amounts of phenolic substances detected in the media containing *P. ostreatus* Ser1, especially when W was used as the substrate, were very scarce (Figs. 3e, 4 & 5), indicating their rapid degradation. *Cyclocybe aegerita* Ser1 was much less effective in mineralising such substances, which can be connected to far lower activities of the measured enzymes (Fig. 2a, b & c). Lower MnP and Lac activities when DHP was added in comparison to the other two substrates imply that these enzymes were more active when the lignocellulose substrate was present, probably because of sugar utilisation by the mycelia causing an improvement in its energy status (Fig. 2a, b & c). Lignin degradation, accompanied by the strong degradation of phenolic substances in a short time span, indicates that *P. ostreatus* Ser1 may be selective in substrate utilisation, preferring aromatic polymers to carbohydrates, which has been previously postulated by Bezalel *et al.* (1996). On the other hand, *C. aegerita* Ser1 was not so effective in the degradation of either lignin or low-molecular-weight phenolics (Figs. 3 & 4) which is in accordance with the literature data showing that this fungal species shows a preference for utilising cellulose from the plant substrates (Wang *et al.* 2013). Lignin degradation by this species was detected previously, but only after 24 days of incubation, probably after the depletion of carbohydrate sources (Liers *et al.* 2011). It can be postulated that, in the experimental conditions and times applied here, *C. aegerita* Ser1 was feeding mostly on the carbohydrates present in the medium/substrate, and degraded only selected aromatic substances by the actions of Lac and MnP (Fig. 2a & b).

Interestingly, when DHP was added as the substrate, *P. ostreatus* Ser1 expressed relatively high activities of Lac and MnP, while *C. aegerita* Ser1 expressed significant activity of only Lac (Fig. 2a & b). Laccases are copper-containing oxidases which oxidise phenolic rings to

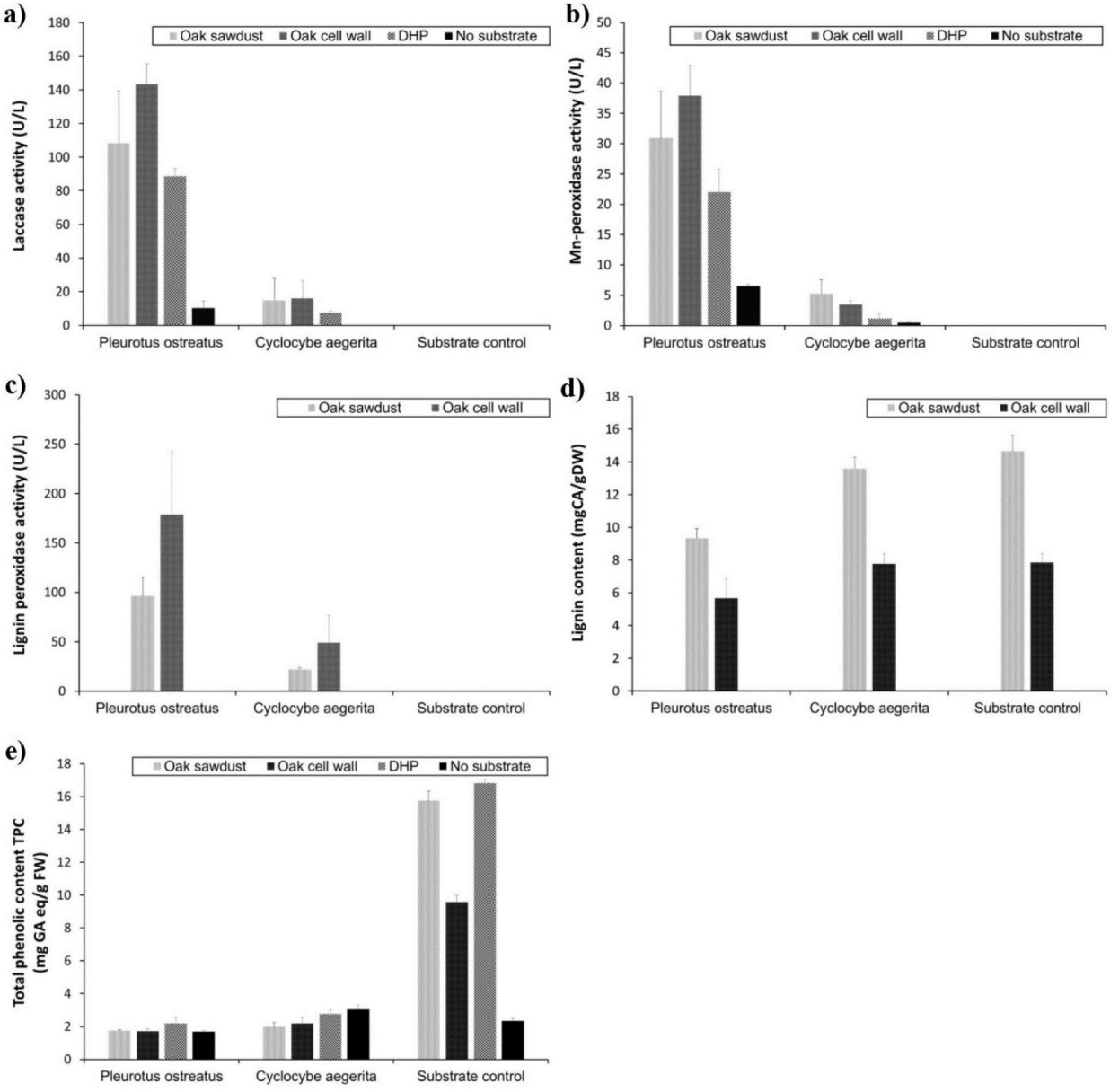

Fig. 2. The enzyme activities included in the lignin degradation measured in the media (a- Lac, b-MnP, c- LiP); d- plant substrate lignin content estimated by acetyl bromide test; d- total phenolic content

phenoxy radicals using molecular oxygen as an oxidant (Baldrian 2006). They usually have low redox potential with few exceptions, which is why they are unable to directly catalyse the oxidation of non-phenolic aromatic rings of lignin (Ruiz-Dueñas & Martínez 2009). Also, the structure of Lac is rather bulky, which is why these enzymes are unable to directly act on the lignin polymer and thus depend on the use of smaller mediator molecules (Rich *et al.* 2016). In the present experiments, both strains produced laccases, but those from *P. ostreatus* Ser1 expressed much higher activities, being active even if the lignin-containing substrate was not present in the growth medium (Fig. 2a). The reason for this high activity may be the high diversity of Lac-coding genes (12) which have previously been detected in *Pleurotus* sp. (Janusz *et al.* 2013).

Class II peroxidases (MnP, LiP, VP) are considered lignin-modifying enzymes and the primary agents of enzymatic lignin decomposition (Kellner *et al.* 2014). However, in *C. aegerita* Ser1, MnP did not appear necessary for DHP degradation (Figs. 3b & 4). Five putative peroxidases (AA2) were previously detected in *Cylocybe cylindracea (syn. aegerita)*, four of which are MnPs (Liang *et al.* 2020), explaining the strong MnP effect on DHP. *Pleurotus ostreatus* was previously reported to produce 6 different MnPs, but LiP coding genes were

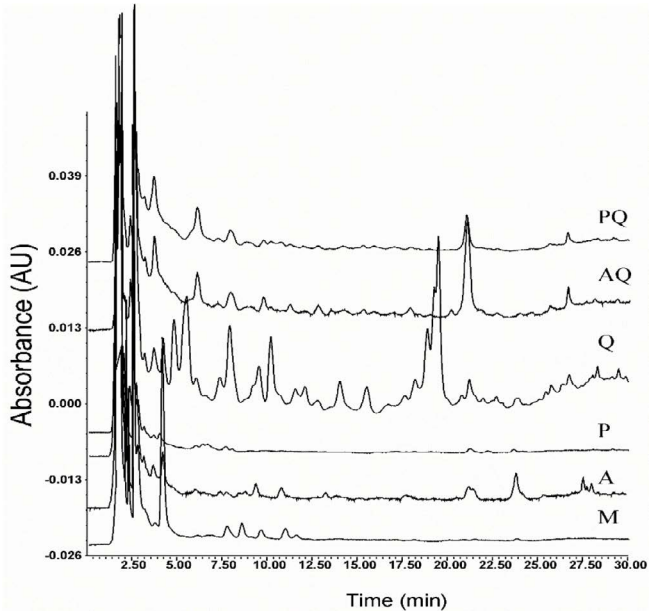

Fig. 3. HPLC chromatograms of the phenolic content in different experimental treatments: M – medium, C- *C. aegerita* Ser1 in the medium; P- *P. ostreatus* Ser1 in the medium; Q-oak sawdust in the medium; CQ – *C. aegerita* Ser1 in the medium with oak sawdust; PQ – *P. ostreatus* Ser1 in the medium with oak sawdust, W – medium with the cell wall isolated from oak dust, PW – *P. ostreatus* Ser1 in the medium with oak cell wall isolate, CW – *C. aegerita* Ser1 in the medium with oak cell wall isolate.

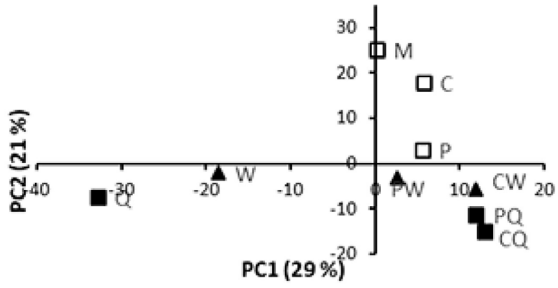

Fig. 4. The PCA score plot of the first two principal components distinguishes the chromatogram profiles of different experimental treatments; M – medium, C- *C. aegerita* Ser1 in the medium; P- *P. ostreatus* Ser1 in the medium; Q-oak sawdust in the medium; CQ – *C. aegerita* Ser1 in the medium with oak sawdust; PQ – *P. ostreatus* Ser1 in the medium with oak sawdust, W – medium with the cell wall isolated from oak dust, PW – *P. ostreatus* Ser1 in the medium with oak cell wall isolate, CW – *C. aegerita* Ser1 in the medium with oak cell wall isolate.

not detected in the genome of this species (Fernández-Fueyo *et al.* 2014). However, using the methods applied here (Mn^{2+} was present in the growth medium), we detected strong LiP activity (Fig. 2c). The explanation may

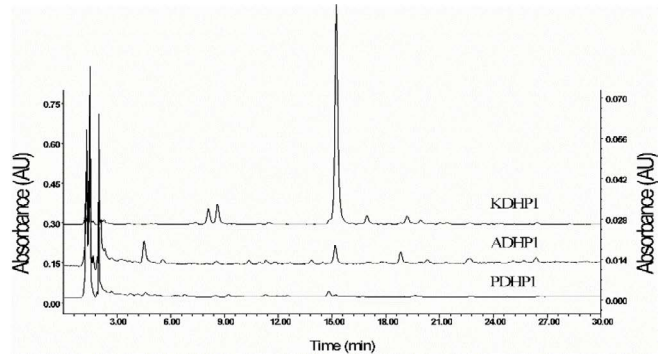

Fig. 5. HPLC chromatograms of the phenolic content in different experimental treatments with DHP: KDHP – DHP in the medium; CDHP – *C. aegerita* Ser1 in the medium with the addition of DHP; PDHP- *P. ostreatus* Ser1 in the medium with the addition of DHP. Qualitative and quantitative analyses of individual compounds were performed by reversed-phase HPLC-MS analysis.

lie in the production of VP which has been accepted as typical for *Pleurotus* sp., including *P. ostreatus* (Ruiz-Duenas & Martínez 2010). VP combines strong Mn^{2+}-oxidising activity with moderate activities towards phenolic and nonphenolic substrates and hence represents a functional hybrid of MnP and LiP (Ruiz-Duenas & Martínez 2010). Therefore, the detected activity of LiP in the mediums of *P. ostreatus* Ser1 may belong to VP.

The enzymatic oxidation of lignin has been shown to result in its depolymerisation and the release of aromatics (Gall *et al.* 2018). Our aim was not to follow the process of decomposition by determining the products, but to distinguish between potentials for the practical usage of the investigated strains, for which the chromatogram comparison by PCA provided sufficient evidence (Fig. 4). While the analysis clearly differentiated between the chromatograms of fungal and plant origin, the chromatograms of *P. ostreatus* Ser1 and *C. aegerita* Ser1 present in the medium with lignin-containing substrates appeared to be quite closely positioned (Fig. 4), implying the production of similar lignin decomposition products. *Cyclocybe aegerita* Ser1 continuously released small phenolic compounds by itself in all the treatments, while *P. ostreatus* Ser1 utilised all the small phenolics from the media including those from the yeast extract. Interestingly, the chromatograms with *P. ostreatus* Ser1 and that where W was offered as the substrate for *P. ostreatus* Ser1 appeared to be the least diverse and abundant and are therefore positioned quite closely on the graph (Fig. 4), thus providing evidence of very efficient and non-selective phenolic degradation by this strain.

CONCLUSIONS

In the forests of Serbia, fruitbodies of *P. ostreatus* s.l. are usually detected on the decaying logs of different woody

species in highly variable conditions, while those of *C. aegerita* s.l. are usually detected in the base of living *Populus nigra* L. trees (probably following the decaying root). We may assume that this substrate specialisation of *C. aegerita* Ser1 may have been one of the reasons why it expressed such a weak capacity to degrade lignin originating from *Q. cerris*. *Pleurotus ostreatus* Ser1, on the other hand, had no preferences towards the substrate, and therefore the amazing possibility to degrade lignin and the wide variety of phenolic substrates detected here may explain its high potential in wood decomposition, as well as its worldwide distribution. The functional (and therefore genetic) specificities of these local strains may lead to discoveries of those with high potential for bioremediation (especially in local conditions), as a preferable strategy for forest restoration, as well as sustainable waste and pollutant management. *Pleurotus ostreatus* Ser1 expressed high efficiency in degrading polymer as well as in mineralising the products of polymer degradation which strongly recommends this strain for potential use in bioremediation processes including some polluters such as microplastic or PAH (Zhuo & Fan 2021).

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REZIME

Botanica SERBICA

Funkcionalna diferencijacija u degradaciji ligninskih jedinjenja dva autohtona kohabitirajuća soja gljiva *Pleurotus ostreatus* i *Cyclocybe aegerita* iz Srbije

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Razgradnju drvenastih substrata u prirodi vrše čitave zajednice mikroorganizama koje su ugrožene antropogenim uticajem, zbog čega je neophodno definisati funkcionalnu specifičnost svake vrste. Mogućnost razgradnje drvenastih substrata [piljevina hrasta, izolovani ćelijski zidovi i dehidrogenativisani polimer (DHP)] je istraživana kod dva autohtona soja gljiva iz ravničarskih šuma u Srbiji (*Pleurotus ostreatus* Ser1 i *Cyclocybe aegerita* Ser1). Aktivnosti enzima koji učestvuju u degradaciji lignina su praćene uporedo sa detekcijom HPLC profila fenolnih jedinjenja i degradacijom lingina. Dok je *Pleurotus ostreatus* Ser1 bio visoko efektivan u kratkom roku, *Cyclocybe aegerita* Ser1 nije degradirala lignin. Ovo je bilo praćeno izuzetno visokom aktivnošću enzima i malom količinom fenola u medijumu *Pleurotus ostreatus* Ser, dok je aktivnost enzima i količina fenolnih jedinjenja u medijima *Cyclocybe aegerita* Ser1 bila vrlo niska.

Ključne reči: degradacija drveta, gljive razlagači drvne mase, lakaza, Mn peroksidaza, lignin peroksidaza