GANODERMA LUCIDUM AND *G. TSUGAE* – A WELL-KNOWN LIGNIN DEGRADING SPECIES AS TRANSFORMATORS OF INSUFFICIENTLY UTILIZED LIGNOCELLULOSIC WASTE

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The present study aimed to determine the capacity of *Ganoderma lucidum* and *G. tsugae* for pretreatment of common agroforestry residues. *G. lucidum* dominated in Mn-dependent peroxidase activity (1625.00 U L^{-1}) after corn stalks fermentation, while the peak of Mn-independent peroxidase activity (2458.33 U L^{-1}) was detected on raspberry sawdust fermented by *G. tsugae*. Laccases were dominant in the ligninolytic cocktail, with the maximal activity of 42480.09 U L^{-1} synthetized by *G. lucidum* cultivated on plum sawdust. The reduction of the lignocellulosic dry matter ranged between 11.00% and 33.00% in oak sawdust and wheat straw, respectively, fermented by *G. tsugae*, while the highest extent of lignin, cellulose and hemicellulose consumption (54.78%, 40.30% and 54.91%, respectively) was obtained on corn stalks. On the other hand, the smallest cellulose removal (6.26%) was detected after raspberry sawdust fermentation by *G. lucidum*, which, besides moderate ligninolysis, induced the highest selectivity index (2.87). The obtained differences in enzyme activities and lignocellulosics degradation extent could be the consequence of the different composition of the studied residues, especially macro- and microelements content. The obtained results clearly indicated the huge potential of *G. lucidum* and *G. tsugae* enzyme cocktails for the transformation of lignocellulosic residues that present abundant sources of numerous value-added products.

Keywords: *Ganoderma* spp., lignocellulose pretreatment, Mn-oxidizing peroxidases, laccases

INTRODUCTION

The rapid growth of the world's population has induced increasing demands for food and energy, but also the accumulation of various wastes from growing agricultural production.[1,2] Despite the limitations of fossil fuels, most of the energy (even 88%) is still derived from them, which, together with other human activities, further increases serious environmental concerns and has enhanced scientific interest in renewable energy sources and sustainable development.[3-7] Agricultural, forestry, and agro-industrial lignocellulosic residues, which are produced in enormous quantities all around the world, represent a huge amount of renewable biomass with great bio-energetic potential.[8,9] Although the energy production from solid biomass increased by 134% from 1990 to 2017 in the European Union, it is still insufficient, considering the amount of re-accumulating biomass.[10] Due to its inadequate management, lignocellulosic waste is a serious environmental burden, despite the fact that it is a promising raw material for numerous value-added products, such as food, feed, paper and bioethanol.[11]

The main limiting feature of the lignocellulosic feedstock lies in the chemical inertness of lignin, which has the role of providing necessary strength to the plant tissue, as well as protecting cellulose and hemicelluloses from hydrolysis.[12] The shrinkage of holocellulose in the lignin network reduces its saccharification, *i.e.* transformation into simple sugars or ethanol. Therefore, the key step in the process of lignocellulose treatment is its efficient delignification.[2] Despite numerous physico-chemical methods, the aspiration is to find ecologically and economically justified biological methods of lignocellulose pretreatment. Biological pretreatment is an attractive alternative to conventional methods, involving organisms (primarily fungi) and their enzyme cocktails under mild and non-hazardous conditions.[13]

White-rot fungi are a unique group of organisms capable of degrading all plant polymers due to their oxidative and hydrolytic extracellular enzymes.[14] Owing to their powerful non-specific and non-stereoselective enzyme system, primarily composed of peroxidases and laccases, they are capable of

complete mineralization of lignin as the most recalcitrant natural compound.[15] Species of the genus *Ganoderma*, especially *G. lucidum*, are medicinal fungi with a high market value due to their various bioactive metabolites.[16,17] On the other hand, as the members of the white-rot group, *Ganoderma* spp. have been intensively studied for various biotechnological and environmental applications.[18-22] Although confirmed as highly selective ligninolytic agents, a small number of lignocellulosic substrates for *Ganoderma* spp. have been investigated till now. Thus, various straws and husks from crop production and processing are agro-industrial wastes reported as appropriate substrates for *G. lucidum* (Curt.: Fr.) P. Karst. cultivation.[17,20,23] On the other hand, an enormous amount of lignocellulosic residues from pomiculture activities (apple, blackberry, grape, plum and raspberry) remains unused.[24] Therefore, the present study aimed to determine the ligninolytic potential of *G. lucidum* and *G. tsugae* Murrill to ferment eight frequent but unexploited lignocellulosic residues from agriculture and forestry.

EXPERIMENTAL

Organism and growth conditions

Cultures of the studied *Ganoderma* species were isolated from basidiocarp collected in Belgrade (BEOFB 435) and obtained from the Institute of Evolution, Haifa University, Israel (HAI 1032). Cultures are maintained as part of the fungal library of the Institute of Botany and Botanical Garden "Jevremovac", Faculty of Biology, University of Belgrade (BEOFB), on malt agar (MA) medium at a temperature of 4 °C.

The preparation of the inoculum involved the following steps: (*i*) inoculation of 100 mL of synthetic medium (glucose, 10.0 g L^{-1}; NH_4NO_3, 2.0 g L^{-1}; K_2HPO_4, 1.0 g L^{-1}; $NaH_2PO_4 \times H_2O$, 0.4 g L^{-1}; $MgSO_4 \times 7H_2O$ 0.5 g L^{-1}; yeast extract, 2.0 g L^{-1}; pH 6.5) with 25 mycelial discs of "mother" inoculum (Ø 0.5 cm, with 7 days-old culture on MA) in 250 mL Erlenmeyer flasks; (*ii*) incubation for 7 days at room temperature (22 ± 2 °C) on a rotary shaker (Stuart S1600C incubator, Orbital Shaker) (110 rpm); (*iii*) washing the resulting biomass 3 times with sterile distilled water (dH_2O); (*iv*) homogenization of biomass with 100 mL of dH_2O in a laboratory blender (Waring 8010 S, USA).

Solid-state cultivation was performed at 25 °C in 250 mL Erlenmeyer flasks containing 6.0 g of crushed lignocellulosic waste (apple, oak, blackberry, raspberry, plum and grapevine sawdust, wheat straw and corn stalks) as the sole carbon source and 30 mL of glucose-free synthetic medium. Homogenized inoculum (9.0 mL) was used to inoculate the thus prepared Erlenmeyer flasks. After 21 days of solid-state cultivation, the enzyme was extracted with 50 mL of dH_2O on a magnetic stirrer (Are VELP, Italy) at 4 °C for 10 minutes. The obtained extracts were centrifuged at 4 °C and 3000 rpm for 15 minutes using a Hettich Universal 32R (DE) centrifuge, and the obtained supernatants were filtered to remove any impurities that could affect the obtained values of the studied enzyme activity and total protein content.

Enzyme activity and total protein content assays

The activity of selected ligninolytic enzymes was determined spectrophotometrically (BioQuest CECIL CE2501, UK) at appropriate wavelengths, using specific substrates for each enzyme, and was expressed as U L^{-1}, where U of enzyme activity is defined as the amount of enzyme that transforms 1 μmol of the substrate in one minute. The activity of laccase (EC 1.10.3.2) was determined using 50 mM ABTS [2,2'-azino-bis [3-ethylbenzothiazoline-6-sulfonic acid, ε_{436} = 29300 $M^{-1}cm^{-1}$)] as a specific substrate in 0.1 M phosphate buffer (pH 6.0) at 35 °C.[19] The activities of Mn-dependent peroxidase (MnP; EC 1.11.1.13) and Mn-independent peroxidase (MnIP; EC 1.11.1.16) were determined using phenol red (ε_{610} = 22000 $M^{-1}cm^{-1}$) as a specific substrate in succinate buffer (pH 4.5) at 30 °C after 1.5 min of reaction stopped by 2 M NaOH.[19]

The amount of total proteins was determined using the standard curve equation obtained for 100 μL of bovine serum albumin (BSA) of known concentrations and 5 mL of Bradford reagent (Coomassie brilliant blue (CBB) G-250).[25] The reaction mixture containing the sample instead of BSA was incubated at room temperature for 5 minutes, and the absorbance was measured at 595 nm. The total protein content was expressed in mg mL^{-1} and was used to determine the specific enzymes activity (U mg^{-1}).

Hemicellulose, cellulose and lignin contents determination

Total dry mass loss was calculated according to the following formula and expressed as a percentage:

$$((Mi - Mf)/Mi) \times 100 \quad (1)$$

where Mi – initial mass of tested plant residues, Mf –mass of plant residues after fungal fermentation.

The determination of the contents of hemicelluloses, cellulose and lignin was performed using the modified methods of Van Soest *et al.*[26] and Kirk and Obst.[27] The procedure for hemicellulose content measurement included the following steps: (*i*) boiling of a mixture of ground dry sample (1.0 g), neutral detergent solution

(NDS) (Ethylenediaminetetraacetic acid (EDTA), 18.6 g L^{-1}; SDS, 30.0 g L^{-1}; 2-ethoxyethanol, 10.0 mL; $NaH_2PO_4 \times H_2O$, 4.56 g L^{-1}; $Na_2B_4O_7 \times 10H_2O$, 6.81 g L^{-1}; pH 6.9-7.1), Na_2SO_3 (0.5 g) and a few drops of 1-octanol for one hour under reflux to remove soluble sugars, proteins, pectin, lipids and vitamins from the initial sample; (*ii*) filtering and rinsing the sample (three times with boiling water and twice with acetone); (*iii*) drying the sample at 105 °C for 8 hours; (*iv*) measuring the mass of the dry sample. The obtained value represents the mass of fibers after the action of a neutral detergent (NDF).

Acid detergent solution (ADS) (20 g CTAB dissolved in 1000 mL H_2O and 27.2 mL 72% H_2SO_4; pH 6.9-7.0) was used to determine the cellulose and lignin content. The mixture of NDF and ADS was heated to boiling for one hour under reflux conditions. Afterwards, the sample was filtered and washed with boiling water and acetone, dried at 105 °C overnight and weighed. The use of ADS achieved the removal of hemicelluloses from the tested sample. The mass of fibers obtained by the action of the acid detergent (ADF) was determined gravimetrically as the mass of the remaining sample after extraction. The hemicellulose content represents the difference in the mass of NDF and ADF. Fibers obtained by the action of the acidic detergent were used to determine the content of cellulose and lignin. Lignin content was determined by the Klason method or using 72% H_2SO_4.[27] The sample was transferred to an Erlenmeyer flask and 72% H_2SO_4 (1.0 mL per 100 mg of the sample) was added followed by incubation in a water bath (30 °C) for one hour with stirring every 15 minutes. After incubation, the solution was diluted by the addition of water (28 mL per 1 mL of acid), followed by secondary hydrolysis in an autoclave at 120 °C for 1 hour. The solution was filtered through pre-measured filter paper on a funnel with sintered glass, and the residues of Klason lignin were washed with dH_2O. The sample was then dried at 105 °C to constant weight and the lignin content (LC) in the sample was expressed as a percentage of the initial sample. The cellulose content was represented by the difference in mass of ADF and LC.

The key parameter for determining the ability of white-rot fungi to selectively degrade lignin is defined through the selectivity index (SI), which represents the ratio between removed lignin and cellulose.

Determination of the chemical composition of plant residues

Samples of biomass (1 g) were treated according to the EPA 3050B method,[28] with the following mixture: concentrated HNO_3:dH_2O (1:1), 10 mL; H_2O_2, 8 mL, and conc. HCl, 5 mL, in a water bath at 90 °C for 2.25 hours. The thus prepared samples were filtered into 100 mL volumetric flasks, filled up to the line, and recorded on Spectroblue TI, ICP-OES (induced-coupled plasma with optical emission spectrometer) according to the validated EPA 200.7 method.[29]

Statistical analysis

All experiments were carried out in three replicates to perform statistical analyses and the obtained results were expressed as mean ± standard error. Data were statistically processed in STATISTICA, version 6 (StatSoft, Inc. 2001). To assess the significance of the differences between the mean values, variance analysis (ANOVA) and Tukey HSD post-hoc test were performed and the differences were considered statistically significant for $p < 0.05$.

RESULTS AND DISCUSSION

The obtained results clearly showed the significant ligninolytic potential of *Ganoderma lucidum* and *G. tsugae* on all studied lignocellulosic residues, except blackberry sawdust, which did not induce the synthesis of Mn-dependent peroxidases, and wheat straw and corn stalks, on which laccase activity was not detected (Figs. 1, 2 and 3). The maximal MnP activity of even 1625.00 U L^{-1} was measured after the cultivation of *G. lucidum* on corn stalks, which also favored the synthesis of this enzyme in another tested species (948.23 U L^{-1}). On the other hand, grapevine sawdust suppressed the synthesis of this enzyme in *G. lucidum*, while in *G. tsugae*, it was slightly lower than the maximum MnP activity (1331.44 U L^{-1}). Significant MnP activity was measured in *G. tsugae* cultivated on raspberry sawdust and in *G. lucidum* on wheat straw, while many-fold lower activities were obtained on other substrates (Fig. 1). A considerably different picture was obtained in the profile of Mn-independent peroxidases activity since *G. tsugae* dominated over *G. lucidum* on almost all the tested substrates. It was also noted that the activities of this enzyme were almost uniform on all the substrates, except the raspberry sawdust, where the maximum activity of 2458.33 U L^{-1} was measured after its fermentation by *G. tsugae*. The lowest activity of MnIP of only 35.98 U L^{-1} was obtained after *G. lucidum* cultivation on wheat straw (Fig. 2). Among the three ligninolytic enzymes monitored in this study, laccases proved to be dominant in the enzyme cocktail, with particularly high values observed in *G. lucidum* (Fig. 3). Thus, the peak activity of even 42480.09 U L^{-1} was observed on plum sawdust fermented with *G. lucidum*, and significant values were also detected after oak, grapevine, apple and

blackberry sawdust fermentation, ranging from 15858.93 U L^{-1} to 32218.43 U L^{-1}, while other activities were many-fold lower, up to 3856.65 U L^{-1} (Fig. 3).

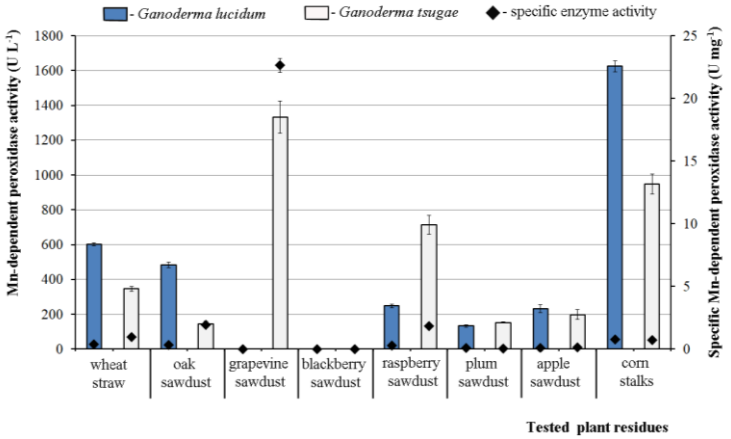

Figure 1: Effect of agro-forestry residues on activity of Mn-dependent peroxidase produced by *Ganoderma spp.* during solid-state cultivation

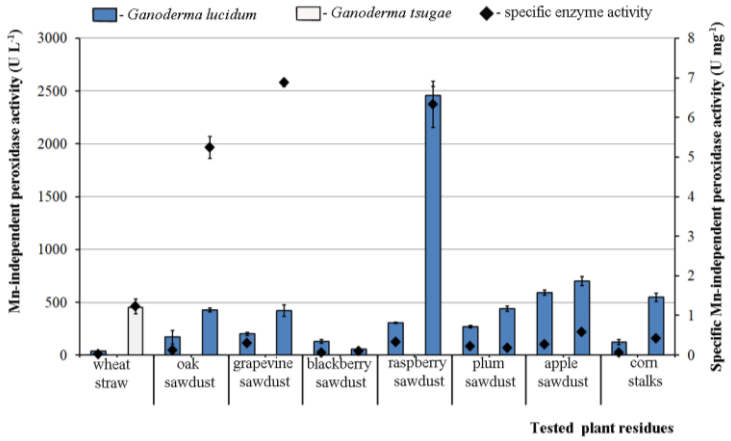

Figure 2: Effect of agro-forestry residues on activity of Mn-independent peroxidase produced by *Ganoderma spp.* during solid-state cultivation

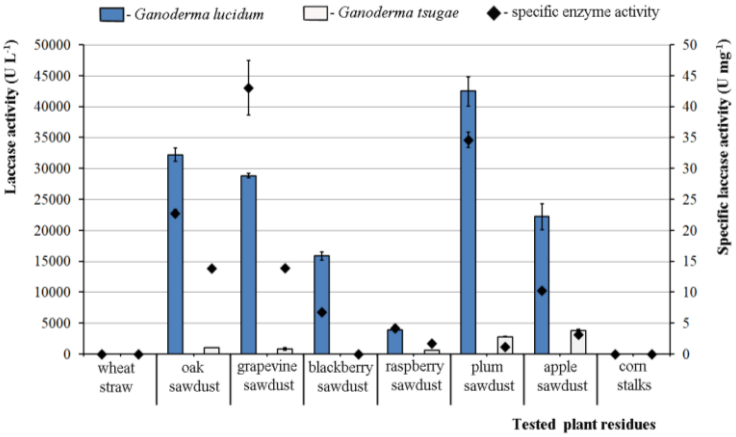

Figure 3: Effect of agro-forestry residues on activity of laccase produced by *Ganoderma spp.* during solid-state cultivation

The influence of the type and period of cultivation, as well as the composition of lignocellulosic substrate, on the activity of ligninolytic enzymes of *Ganoderma* spp. has been the subject of numerous studies till now, some of which have addressed the presence of inter- and intraspecific diversity.[18,21,30] The results of our study confirmed previous reports, which, in addition to the genetic basis of the species/strain, emphasized the composition of the lignocellulosic substrate as a key factor for the expression of ligninolytic capacity. However, the results of our research have not coincided with the others regarding the level of enzyme activity, as well as the type of substrate, that induced the highest level of enzyme activity. Thus, corn stalks did not induce laccase activity in any of the species in our study, despite the highest percentage of weight loss and delignification, while the same substrate was optimal for laccase synthesis during 7 days of fermentation by *G. lucidum* HAI 957, but many-fold lower values were obtained compared to the maximum in our study.[18] This could be explained by the fermentation period (21 days) until the laccase activity probably decreased, since the highest activity in numerous white-rot species was recorded earlier, usually from the 7th to the 14th day.[21] Despite the high degree of delignification, laccase activity was not measured on wheat straw in our study, contrary to the results reported by Asgher *et al*.,[31] who cultivated the *G. lucidum* strain on the same substrate for only 4 days and obtained as much as 340 U mL^{-1}. Differences in the levels of ligninolytic enzyme activity on the same substrate could be explained by the genetic basis, since significant differences were previously observed in different species/strains of the genus *Ganoderma*.[14,25,30,32] The genetic predisposition for laccase synthesis was also confirmed by isoelectric focusing of active isoforms of this enzyme in *G. lucidum* strains grown on the same substrate.[19,32] In addition to the substrate composition and the species/strain selection, the type of cultivation also has a significant effect on the level of ligninolytic activity. Thus, Songulashvili *et al*.[14] recorded extremely high laccase activity in *G. lucidum* HAI 447 and *G. adspersum* 845 after their submerged cultivation in mandarin peel-based medium (35980 U L^{-1} and 27380 U L^{-1}, respectively). Oak sawdust also induced the synthesis of highly active isoforms of this enzyme during submerged cultivation of *G. applanatum*,[21] which were as much as 10-fold more active than those synthesized after solid-state fermentation of the same substrate by *G. tsugae* HAI 1032. On the other hand, Ntougias *et al*.[30] obtained incomparably lower activities of this enzyme after cultivation of *G. australe* and *G. carnosum* in olive oil wastewater, ranging from 5.11 U L^{-1} to 21.30 U L^{-1}. The mentioned factors also affected the activity of Mn-oxidizing peroxidases. Namely, Ntougias *et al*.[30] observed extremely low activities of MnP and MnIP (up to 36.09 U L^{-1}) after olive oil wastewater fermentation by *G. australe* and *G. carnosum*, while Asgher *et al*.[31] reported incredible MnP activity of even 882 U mL^{-1} in *G. lucidum* cultivated on wheat straw.

The studied species showed different ability for degradation or selective depolymerization of the tested lignocellulosic residues (Table 1). The level of degradation of the tested lignocellulosics was rather substrate- than species-dependent, and ranged from 11% of dry matter loss in oak sawdust to 33% of weight loss in wheat straw fermented with *G. tsugae* (Table 1). On the other hand, the degree of depolymerization of the fibers was equally substrate- and species-dependent since species differed significantly in the delignification capacity of the same substrate, but also individually on each one (Table 1). The highest degree of delignification was observed after cultivation on crop residues (wheat straw and corn stalks), but species differed in substrate preference. Thus, a maximum of 54.78% of lignin removal was obtained after fermentation of corn stalks with *G. tsugae*, while *G. lucidum* delignified wheat straw to a similar extent (50.45%). *G. lucidum* and *G. tsugae* considerably delignified corn stalks and wheat straw, respectively (~39%), while significantly lower lignin removal was observed on other lignocellulosic substrates (Table 1).

Table 1
Extent of plant residue depolymerization by *Ganoderma lucidum* and *Ganoderma tsugae*

Plant residue	Studied samples	Sample weight (g)	Fibre content in samples (g)			Dry matter loss (%)	Extent of polymer degradation (%)			Selectivity index
			Lignin	Cellulose	Hemicelluloses		Lignin	Cellulose	Hemicelluloses	
Apple sawdust	Control*	6.00	1.16	2.81	1.18	/	/	/	/	/
	G. lucidum	5.01	0.84	2.17	1.00	16.50^{c}	26.96^{e}	22.64^{f}	14.88^{c}	1.19^{b}
	G. tsugae	4.98	0.92	2.17	0.82	17.00^{c}	20.84^{c}	22.82^{f}	30.10^{e}	0.91^{a}
Blackberry sawdust	Control*	6.00	1.22	2.71	1.04	/	/	/	/	/
	G. lucidum	4.99	0.78	2.09	0.82	16.83^{c}	35.72^{f}	22.95^{f}	21.21^{d}	1.56^{c}
	G. tsugae	4.82	0.87	2.27	0.79	19.67^{d}	28.74^{e}	16.26^{d}	23.81^{d}	1.77^{c}
Corn stalks	Control*	6.00	0.59	2.80	1.60	/	/	/	/	/
	G. lucidum	4.79	0.36	2.13	1.04	20.17^{d}	39.55^{f}	23.80^{g}	43.90^{g}	1.66^{c}
	G. tsugae	4.13	0.27	1.67	0.84	31.17^{g}	54.78^{g}	40.30^{j}	54.91^{i}	1.36^{c}
Grapevine sawdust	Control*	6.00	1.36	2.65	0.89	/	/	/	/	/
	G. lucidum	5.00	1.10	1.96	0.82	16.67^{c}	22.21^{c}	26.24^{h}	6.94^{a}	0.85^{a}
	G. tsugae	5.04	1.22	2.32	0.72	16.00^{c}	14.21^{a}	12.41^{c}	19.34^{d}	1.14^{b}
Oak sawdust	Control*	6.00	1.53	2.81	1.16	/	/	/	/	/
	G. lucidum	5.33	1.17	2.47	1.03	11.17^{a}	23.78^{d}	12.03^{c}	11.40^{b}	1.98^{d}
	G. tsugae	5.34	1.14	2.49	0.80	11.00^{a}	25.31^{d}	11.14^{b}	30.95^{e}	2.27^{d}
Plum sawdust	Control*	6.00	1.84	2.54	1.37	/	/	/	/	/
	G. lucidum	5.08	1.56	1.95	0.74	15.33^{b}	15.19^{a}	23.38^{g}	45.83^{h}	0.65^{a}
	G. tsugae	4.85	1.44	2.03	0.72	19.17^{d}	21.84^{c}	20.28^{e}	47.15^{h}	1.08^{b}
Raspberry sawdust	Control*	6.00	1.20	2.16	1.31	/	/	/	/	/
	G. lucidum	4.69	0.98	2.02	0.73	21.83^{e}	17.98^{b}	6.26^{a}	44.10^{g}	2.87^{e}
	G. tsugae	4.71	0.93	1.77	0.88	21.50^{e}	22.25^{c}	18.19^{d}	32.63^{e}	1.22^{b}
Wheat straw	Control*	6.00	0.67	2.42	1.69	/	/	/	/	/
	G. lucidum	4.40	0.33	1.78	1.03	26.67^{f}	50.45^{g}	26.30^{h}	38.89^{f}	1.92^{d}
	G. tsugae	4.02	0.40	1.60	0.96	33.00^{g}	39.64^{f}	33.67^{i}	42.98^{g}	1.18^{b}

*untreated plant residue
$^{a-j}$Values superscripted with the same letter in the same column are not significantly different ($p < 0.05$)

Table 2
Chemical composition of the tested plant residues

Plant residue	Concentration of macro- and microelements (mg kg^{-1})					
	P	Ca	Cu	Fe	Mn	Zn
Apple sawdust	797^{b}	6726^{c}	5.53^{b}	23.28^{a}	10.34^{a}	6.65^{b}
Blackberry sawdust	1337^{d}	6077^{c}	13.72^{e}	58.09^{c}	110.83^{e}	21.99^{e}
Corn stalks	1663^{e}	4328^{b}	9.43^{d}	57.94^{c}	17.12^{b}	59.27^{f}
Grapevine sawdust	2771^{f}	10227^{d}	7.79^{c}	23.09^{a}	30.07^{c}	14.79^{d}
Oak sawdust	106.21^{a}	13298^{e}	4.45^{b}	851.74^{d}	68.20^{d}	5.26^{a}
Plum sawdust	1085^{c}	6808^{c}	8.69^{d}	49.28^{b}	7.80^{a}	10.23^{c}
Raspberry sawdust	1222^{d}	4081^{b}	6.48^{c}	25.67^{a}	110.55^{e}	26.41^{e}
Wheat straw	79.05^{a}	2262^{a}	1.57^{a}	50.49^{b}	25.51^{c}	4.11^{a}

*Values superscripted with the same letter in the same column are not significantly different ($p < 0.05$)

Corn stalks were particularly susceptible to holocellulose removal, as the maximum levels of removed hemicelluloses (54.91%) and cellulose (40.30%) were obtained after fermentation of this substrate with *G. tsugae*. Besides corn stalks, hemicelluloses were significantly removed from plum and raspberry sawdust, as well as from wheat straw, after fermentation by both tested species, while the other substrates were significantly more resistant and the minimum degree of degradation (6.94%) was measured in grapevine sawdust. The percentage of decomposed cellulose was significantly lower in all other substrates, with a minimum of only 6.26% measured in raspberry sawdust fermented with *G. lucidum*, which, besides a moderate degree of delignification, induced the highest selectivity index of even 2.87. Significant levels of depolymerization selectivity (~2) were shown by both species on oak and blackberry sawdust, as well as by *G. lucidum* on wheat straw. On the other hand, *G. lucidum* proved to be less selective during the fermentation of grapevine and plum sawdust, with selectivity indices of only 0.85 and 0.65, respectively (Table 1).

Numerous reports have already shown significant inter- and intra-species differences in lignocellulosic residues depolymerization capacity and selectivity.[21,33,34] In general, a positive correlation between enzyme activity and delignification was not observed in all cases in this study, *i.e.* higher enzyme activity did not mean a higher degree of depolymerization of the plant substrate, which was in accordance with the results of previous studies.[21,33-36] This can be explained by the duration of cultivation, *i.e.* the point of measurement till the activity of some ligninolytic enzymes could significantly decrease. Namely, as Kneževic *et al.*[33] pointed out that the onset of enzyme synthesis corresponds to the colonization phase associated with cell wall opening and initiation of lignin degradation by reactive oxygen species, while enzymatic delignification occurs much later. On the other hand, Salvachua *et al.*[37] suggest the presence of other mechanisms involved in lignin depolymerization, such as the involvement of low molecular weight oxidative compounds that act as radicals. However, it is evident that the degree of lignin degradation depends on the selection of species/strain and the type of lignocellulosic substrate.[21,33,38] Thus, *G. lucidum* from the study of Martínez-Patiño *et al.*[36] did not delignify olive wood after 45 days of fermentation, and poorly removed cellulose (16.3%) and hemicelluloses (7.9%). A similar picture was obtained for *G. lobatum* cultivated on corn stover, which, despite the 20% of total dry weight loss and approximately 20% of cellulose and hemicellulose removal, left the lignin network almost intact.[39]

Lignocellulosic residues used as substrates for the cultivation of *Ganoderma* spp. differed significantly in their chemical composition, *i.e.* in the concentration of macro- and microelements. The tested lignocellulosic residues differed mostly in the content of Fe and P, while the content of Ca varied the least (Table 2). Calcium was also the most present element, with a concentration ranging from 2262 mg kg^{-1} in wheat straw to 10227 mg kg^{-1} in grapevine sawdust, and even 13298 mg kg^{-1} in oak sawdust. Phosphorus, as the second macroelement tested in this study, was determined to be in the highest concentration in grapevine sawdust (2771 mg kg^{-1}), while its concentration was even 35-fold lower in wheat straw, where the least amounts of Cu and Zn (1.57 mg kg^{-1} and 4.11 mg kg^{-1}, respectively) were also detected. The most common microelement was Fe, whose concentration in oak sawdust reached 851.71 mg kg^{-1}, while in grapevine sawdust, it was even 37-fold lower. The highest concentrations of Mn, Zn and Cu were measured in blackberry sawdust and corn stalks, but they varied to a lower extent concerning the Fe content (Table 1).

Our study did not show direct connection between the concentrations of tested macro- and microelements in lignocellulosic waste and enzyme activity, as well as its depolymerization by both species. Thus, it can be noticed that grapevine sawdust as the most macroelements-rich substrate induced high MnP activity in *G. tsugae* and high laccase activity in *G. lucidum*. Likewise, oak sawdust, which contained the highest calcium amount, also induced the synthesis of highly active laccase isoforms. On the other hand, the absence of laccase activity on wheat straw could be explained by the lowest concentrations of macronutrients. The influence of individual microelements on enzyme activity was not observed, since most enzyme activities were not measured on substrates with the highest content of microelements present in their active centers. It seems that the interrelationship of microelements concentrations in the substrate is also important for enzyme activity, since it is known that, for example, laccases of some species can contain Mn, Zn and Fe, besides Cu, in their active centers.[40,41]

CONCLUSION

The results of this study clearly showed the exceptional potential of the tested species, especially *G. lucidum*, for the production of ligninolytic enzymes during the fermentation of several lignocellulosic residues, some of them having been studied for the first time, and most importantly, they showed exceptional capacity for their depolymerization. The findings of this work open up new directions for more detailed studies in order to use ligninolytic cocktails in biotechnological processes in which the pretreatment of lignocellulose is necessary, primarily in the production of bioethanol, paper, *etc*. Another important contribution of this study is the fact that hitherto unstudied but available lignocellulosic residues have proven to be promising substrates for the cultivation of basidiocarps of extraordinary medicinal potential.

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