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Lignocellulolytic Enzyme Activity of New Corticoid and Poroid Basidiomycetes Isolated from Latvian Cultural Monuments

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Abstract

Eleven corticoid and three poroid fungi have been isolated from Latvian cultural monuments and evaluated for their potential to produce wood-degrading enzymes. In submerged fermentation of mandarin peels, notable intergeneric and intrageneric differences were revealed with regard to the extent of hydrolase and oxidase activity. The xylanase activity of the tested basidiomycetes varied from 0.1 to 2.4 U/mL depending on the fungus species, while that among the strains of *Athelia neuhoffii* varied from 0.1 to 1.3 U/mL. Five strains of white-rot fungi produced neither laccase nor manganese peroxidases. Cellulase and xylanase of *Hyphoderma praetermissum* and *Tubulicrinis glebulosus* appeared to be inducible enzymes. Lignocellulosic substrates strongly affected the enzyme production, because the laccase of *H. praetermissum* and *T. glebulosus* was secreted only in the submerged fermentation on mandarin peels and wheat bran, whereas the production of manganese peroxidase by *Hypochnicium punctulatum* and *Phellinus chrisoloma* was observed only in the solid-state fermentation of wheat bran and ethanol production residue.

Keywords: Corticoid and poroid fungi, fermentation, cellulase, xylanase, laccase, manganese peroxidase.

Introduction

Wood-rotting basidiomycetes represent a taxonomically, ecologically, and physiologically extremely diverse group of saprotrophic fungi, capable of decomposing all major components of wood due to their ability to produce a variety of hydrolytic and oxidative enzymes (Eriksson et al. 1990; Baldrian and Valaskova 2008). Their major hydrolytic enzymes are endo-1,4-ß-D-glucanase (EC 3.2.1.4), exo-1,4-ß-D-glucanase (EC 3.2.1.91), and xylanase (EC 3.2.1.8). White-rot fungi (WRF) secrete one or more of the three extracellular enzymes that are essential for lignin degradation: lignin peroxidase (EC 1.11.1.14), manganese-dependent peroxidase (EC 1.11.1.13), and laccase (EC 1.10.3.2). WRF degrade all cell wall components and cause the characteristic bleaching of wood. Brown-rot fungi cause rapid depolymerization of cellulose and degradation of cell wall carbohydrates, leaving behind a lignin-rich brown colored wood.
Recently, many species of wood-rotting fungi have been studied from both basic and an applied viewpoint, some of them having shown high potential for the production of individual groups of hydrolytic and oxidative enzymes, especially in submerged (SF) and solid-state (SSF) fermentation of plant raw materials (Kachlishvili et al. 2006; Papinutti and Forchiassin 2007; Winquist et al. 2008; Bonugli et al. 2010; Quiroz-Castañeda et al. 2011). However, in contrast to lignin-degrading enzymes, the information on the hydrolases produced by poroid and corticoid basidiomycetes is still scarce. Moreover, only few reports are concerned with the simultaneous production of hydrolytic and oxidative enzymes by WRF. Taking into account the fact that cellulose and hemicelluloses are the main carbon and energy sources for the wood-rotting basidiomycetes, it is clear that hydrolytic enzymes play a decisive role in the steady supply of nutrients to the growing fungi.

The aim of this study was to characterize the lignocellulolytic systems of the new poroid and corticoid basidiomycete strains isolated from the Latvian heritage sites. To our knowledge, such fungi have not been studied for the production of lignocellu-

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lose-degrading enzymes vis-a-vis their destructive properties on materials of cultural heritage that may have lignocellulosic materials. We supposed that these fungi may possess, due to their ability to survive in this unique ecological niche, novel enzymes with improved stability and a specific activity that could be exploited for industrial applications. Moreover, by assessing the enzymatic systems of these fungi and understanding their modes of wood attack, we will able to design ways and means to prevent. Endoglucanase, xylanase, laccase and MnP activities were evaluated as representatives of lignocellulose-degrading enzymes in the SF and SSF of different lignocellulosic materials.

Materials and Methods

Lignocellulosic Substrates

Wheat bran, a residue that remains after the ethanol production from wheat grains (REP), mandarin and banana peels, walnut pericarp, and beech wood sawdust, were used as growth substrates. All residues were dried at 60°C and milled (<1 mm) for use in SF, while for SSF they were chopped into pieces of 1-5 mm.

Organisms and Inoculum Preparation

The fungal strains isolated in pure cultures from the samples taken from Latvian cultural monuments are listed in Table 1. They had been deposited in the culture collection of the Latvian State Institute of Wood Chemistry and the Durmishidze Institute of Biochemistry and Biotechnology. Fungal inocula were prepared by cultivation in 750-mL flasks containing 100 mL liquid medium on a rotary shaker (150 rpm) at 27°C. The liquid medium contained (g per liter): glucose 10, $(NH_4)_2SO_4$ 2, KH_2PO_4 0.8, K_2HPO_4 0.2, $MgSO_4$•$7H_2O$ 0.5, yeast extract 2. The medium was adjusted to pH 6.0 and sterilized at 121oC for 20 min before use. After 7 days of fungal cultivation, mycelial pellets were harvested and homogenized with a Waring laboratory blender.

Cultivation Conditions

The SF of lignocellulosic substrates was carried out on a modified version of the medium described above. The medium was prepared without glucose, but to each 50 mL of the medium in 250-mL flasks was added test lignocelluose materials (40 g/L) and 0.5 mM $CuSO_4$ $5H_2O$. To study the effect of the carbon source on the target enzyme production, glucose, glycerol, and sodium gluconate were supplemented (10 g/L) to the medium to serve as a carbon source. The initial pH of the media was adjusted to 5.5 prior to sterilization. Five mL of mycelial homogenate was used to inoculate each flask. After 5, 8, and 11 days of growth, when the cultures existed in the middle-end of logarithmic and stationary phases of growth, respectively, samples (1 mL each) were taken from the flasks and the solids were separated by centrifugation at 10,000 g for 5 minutes at 4°C.

The SSF of the selected residues was carried out at 27°C in 100-mL flasks containing 4 g of lignocellulosic substrates moistened with 12 ml of the above standard medium without glucose but supplemented with a 4 g/L yeast extract and 0.5 mM $CuSO_4$ $5H_2O$. The initial pH of the media was adjusted to 5.5 ± 0.1 prior to sterilization. Three ml of the homogenized mycelium was used to inoculate the flasks containing media with lignocellulosic substrates. After 7 and 14 days of fungal growth, when

Table 1. Taxonomy, location and decay type of basidiomycetes, isolated from wooden objects in the Latvian Ethnographic Open-Air Museum (OAM) and some churches.

Fungal isolate	Order/Family	Location	Decay type [Brown (B) /White (W)]
Aleurodiscus fennicus 28	*Russulales, Stereaceae*	OAM; fence	W
Athelia neuhoffii 2	*Atheliales, Atheliaceae*	Liksna Catholic Church; tower board	W
Athelia neuhoffii 11-1	*Atheliales, Atheliaceae*	Ruduski Old believers Church; roof shingles	W
Athelia neuhoffii 33	*Atheliales, Atheliaceae*	OAM; wall, roof boards	W
Ceraceomyces sublaevis 11-5	*Boletales, Amylocorticiaceae*	Ruduski Old believers Church; roof shingles	W
Dacryobolus sudans 38	*Polyporales, Fomitopsidaceae*	OAM; fence	W
Gloeophyllum abietinum 46	*Gloeophyllales, Gloeophyllaceae*	OAM; fence	B
Hyphoderma praetermissum 47	*Polyporales, Meruliaceae*	OAM; log wall	W
Hypochnicium punctulatum 30	*Polyporales, Meruliaceae*	OAM; roof construction	W
Oligoporus tephroleucus 56	*Polyporales, Fomitopsidaceae*	OAM; beehive	B
Phellinus chrisoloma 19	*Hymenochaetales, Hymenochaetaceae*	Berzhi Catholic Chapel; walls	W
Phlebiopsis gigantea 17	*Polyporales, Phanerochaetaceae*	OAM; ceiling beam	W
Phlebiopsis gigantea 35	*Polyporales, Phanerochaetaceae*	OAM; log wall	W
Tubulicrinis glebulosus 26	*Polyporales, Tubulicrinaceae*	OAM; pole	W

Table 2. Basidiomycetes enzyme activity in submerged fermentation of mandarin peels.

Fungi	CMCase (U/mL)	Xylanase (U/mL)	FPA (U/mL)	Laccase (U/L)	MnP (U/L)
A. fennicus 28	0.3±0.04$^{(5)}$	0.3±0.05$^{(5)}$	0.01±0$^{(8)}$	0	0
A. neuhoffii 2	0.5±0.07$^{(8)}$	1.3±0.18$^{(8)}$	0.05±0.01$^{(8)}$	0	0
A. neuhoffii 11-1	0.8±0.11$^{(8)}$	0.7±0.09$^{(5)}$	0.15±0.02$^{(5)}$	0	0
A. neuhoffii 33	0.2±0.03$^{(8)}$	0.1±0.02$^{(8)}$	0.03±0.01$^{(8)}$	50±6$^{(5)}$	0
C. sublaevis 11-5	0.6±0.08$^{(8)}$	0.4±0.05$^{(5)}$	0.08±0.01$^{(5)}$	0	0
D. sudans 38	0.7±0.10$^{(5)}$	0.3±0.02$^{(8)}$	0.08±0.01$^{(8)}$	180±28$^{(5)}$	40±7$^{(8)}$
G. abietinum 46	0.6±0.05$^{(8)}$	2.4±0.19$^{(5)}$	0.06±0.01$^{(5)}$	0	0
H. praetermissum 47	0.8±0.07$^{(8)}$	2.1±0.25$^{(8)}$	0.24±0.04$^{(8)}$	920±153$^{(5)}$	0
H. punctulatum 30	0.9±013$^{(5)}$	0.2±0.03$^{(5)}$	0.09±0.01$^{(5)}$	290±36$^{(8)}$	0
O. *tephroleucus* 56	0.5±0.08$^{(8)}$	1.1±0.15$^{(8)}$	0.07±0.01$^{(8)}$	0	0
P. chrisoloma 19	0.2±0.03$^{(5)}$	0.2±0.03$^{(8)}$	0.01±0$^{(5)}$	0	0
P. gigantea 17	0.5±0.03$^{(8)}$	0.2±0.02$^{(5)}$	0.01±0$^{(5)}$	190±20$^{(5)}$	0
P. gigantea 35	0.4±0.05$^{(8)}$	1.6±0.15$^{(8)}$	0.16±0.02$^{(8)}$	610±88$^{(5)}$	140±17$^{(5)}$
T. glebulosus 26	0.9±0.12$^{(8)}$	1.3±0.17$^{(8)}$	0.12±0.02$^{(8)}$	840±83$^{(5)}$	0

The numbers in parentheses indicate the day of peak activity.

the cultures existed in the logarithmic and stationary phases of growth, respectively, the extracellular enzymes were extracted twice with 25 mL of distilled water (total volume 50 mL) after agitation at 150 rpm for 30 min at room temperature in an orbital shaker. The solids were separated by filtration through nylon cloth followed by centrifugation at 10,000 g for 15 minutes at 4°C.

Enzyme Assays

The supernatants obtained after biomass separation were analyzed for enzyme activity. Endoglucanase (EC 3.2.1.4)/carboxymethyl cellulase (CMCase) activity was assayed according to IUPAC recommendations using low-viscosity carboxymethyl cellulose (1% w/v) in 50 mM citrate buffer (pH 5.0) at 50°C for 10 min (Ghose 1987). Xylanase (EC 3.2.1.8) activity was determined using birch wood xylan (Roth 7500) (1% w/v) in 50 mM citrate buffer (pH 5.0) at 50°C for 10 min (Bailey et al.1992). Glucose and xylose standard curves were used to calculate cellulase and xylanase activities. In all assays, the release of reducing sugars was measured by the dinitrosalicylic acid reagent method (Miller 1959). One unit of enzyme activity was defined as the amount of the enzyme, releasing 1 μmol of reducing sugars per minute.

Laccase (EC 1.10.3.2) activity was determined by monitoring the absorbance change at 420 nm related to the rate of oxidation of 1 mM 2,2′-azino-bis-[3-ethylbenzthiazoline-6-sulfonate] to its cation radical in 50 mM Na-acetate buffer (pH 3.8) at room temperature (Bourbonnais and Paice 1990). MnP (EC 1.11.1.13) activity was measured at 270 nm by following the formation of Mn^{3+}-malonate-complexes (Wariishi et al. 1992). The activities in the absence of H_2O_2 were subtracted from the values obtained in its presence to establish the true peroxidase activity. One unit of laccase or MnP activity was defined as the amount of the enzyme that leads to the oxidation of 1 μmol of the substrate per minute.

To compare the enzyme activities of fungi grown in SF and SSF, all enzyme activities were expressed in international units per ml or per liter of the culture liquid. The experiments were performed at least twice using three replicates. The data presented in the tables correspond to mean values with standard deviations less than 15%.

Results

Enzyme Activity in the SF of Mandarin Peelings

At the first stage, the isolated basidiomycete strains were screened for lignocellulose-degrading enzyme activities in the SF of mandarin peels. This material is an appropriate growth substrate containing key ingredients that favour efficient production of lignocellulolytic enzymes by wood-rotting fungi from various taxonomic and ecological groups (Elisashvili et al. 2002; 2008). In addition to cellulose (21%), hemicellulose (13%), lignin (2%), and nitrogen (1.2%), mandarin peels have a significant content of free sugars and organic acids that support growth and development in many in vitro studies reported on WRF (Osma et al. 2007; Elisashvili et al. 2009). It also contains water-soluble aromatic compounds (flavones and flavonols) capable of inducing or stimulating the biosynthesis of ligninolytic enzymes (Chkhikvishvili et al 1994). Although mandarin peels promoted an excellent growth of all tested fungi in the form of pellets, enzyme production widely varied among the individual fungal strains (Table 2). The highest endoglucanase activity (0.9 U/mL) was revealed in the substrate fermentation by *H. punctulatum* 30 and *T. glebulosus* 26 followed by A. neuhoffii 11-1 and H. praetermissum 47. Many-fold lower enzyme activity appeared to be in the cultivation of *A. neuhoffii* 33 and *P. chrisoloma* 19 at the same conditions. The xylanase activity of the studied basidiomycetes varied from 0.1 to 2.4/U mL depending on the fungus strain. *G. abietinum* 46, followed by *H. praetermissum* 47, expressed the highest activity of xylanase accumulating 2.1-2.4 U/mL in the fermentation of mandarin peels. More than 10-fold lower xylanase activity was detected in strains of *A. neuhoffii* 33, *H. punctulatum* 30, *P. chrisoloma* 19, and *P. gigantea* 17. All tested fungi were capable of hydrolyzing filter paper. However, the accumulation of sugars by the culture liquids obtained from *A. fenicus* 28, *P. chrisoloma* 19, and *P. gigantea* 17 was negligible (0.01 U/mL), while *H. praetermissum* 47 secreted appreciable total cellulase activity (FPA) (0.24 U/mL).

Unexpectedly, not all white-rot basidiomycetes tested produced laccase at tested conditions (in the SF of mandarin peels). Only *H. praetermissum* 47, followed by *T. glebulosus* 26, and P. *gigantea* 17 secreted a high enzyme activity (610-920 U/L). Moreover, no MnP activity was detected in the SF of mandarin peels by the tested fungi with the exception of *D. sudans* 38 and *P. gigantea* 35, which accumulated 40 and 140 U/L MnP, respectively.

Effect of the Lignocellulosic Substrates on Fungi Enzyme Activity in their SSF

Three basidiomycetes strains have been selected for the evaluation of their lignocellulose-degrading enzyme activity in the SSF of various lignocellulosic materials. The first signs of the fungi growth were observed two days after the inoculation and complete colonization of the substrate was observed within 7-8 days of SSF. The data presented in Table 3 show that, in the cultivation of *H. punctulatum* 30, the highest CMCase activity (0.8 U/mL) appeared to be in the colonization of the lignified substrate, beech sawdust. The highest xylanase activity (0.5 U/mL) was detected in the SSF of wheat bran, whereas very low FPA was revealed in the fungus cultivation in the presence of all tested growth substrates. Mandarin peels appeared to be the best growth substrate for endoglucanase secretion and FPA expression by *P. chrisoloma* 19, whereas wheat bran promoted the xylanase accumulation by this fungus. It is interesting that the activities of hydrolytic enzymes in the SSF of all selected lignocellulosic materials, with the exception of beech wood sawdust, reached their maximum after 7 days of cultivation, whereas the activity of MnP usually peaked after two weeks of fungi cultivation. Unexpectedly, no laccase activity was detected in the SSF of the tested plant residues by the selected white-rot fungi. Nevertheless, both poroid (*P. chrisoloma* 19) and corticoid (*H. punctulatum* 30) fungi expressed MnP activity in the SSF of two lignocellulosic materials, wheat bran and the residue after the ethanol production. The activity of this enzyme reached 390-440 U/L and 120-670 U/L in cultures of H. punctulatum 30 and *P. chrisoloma* 19, respectively.

Effect of the Growth Substrate on Fungi Enzyme Activity in their Submerged Fermentation

Hence, to gain more knowledge about the possible role of carbon sources in hydrolytic and oxidative enzyme production, complex and simple compounds were assessed as growth substrates under submerged cultivation. Table 4 indicates that, among the tested lignocellulosic substrates, mandarin peels and banana peels provided the highest CMCase and xylanase production by *H. praetermissum* 47 (1.4 and 1.5 U/mL, respectively) and *T. glebulosus* 26 (1.2 and 0.9 U/mL, respectively). Under the same culture conditions, wheat bran and walnut pericarp resulted in a 2-4-fold lower endoglucanase activity. On the contrary, wheat bran along with banana peels led to the accumulation of a high xylanase activity by these fungi. All complex substrates provided a comparatively high total cellulase activity of *H. praetermissum* 47 while only banana peels promoted the FPA secretion by *T. glebulosus* 26. Finally, both fungi expressed only negligible or traces of hydrolytic enzyme activity in the nutrient medium supplementation with simple carbon sources.

The highest laccase activity was obtained in the SF of mandarin peels and wheat bran by *H. praetermissum* 47 (560 and 510 U/L, respectively) and *T. glebulosus* 26 (660 and 140 U/L1, respectively). Walnut pericarp and banana peels appeared to be very poor substrates for the laccase production by these fungi. It is worth noting that glucose, followed by glycerol, also

Table 3. Basidiomycetes enzyme activity in solid-state fermentation of lignocelluloses.

Substrates	**CMCase (U/mL)**	**Xylanase (U/mL)**	**FPA (U/mL)**	**Laccase (U/L)**	**MnP (U/L)**
Hypochnicium punctulatum 30					
Wheat bran	0.2±0.02$^{(7)}$	0.5±0.06$^{(7)}$	0.01±0$^{(7)}$	0	390±56$^{(14)}$
Mandarin peels	0.2±0.03$^{(7)}$	0.2±0.02$^{(7)}$	0.01±0$^{(7)}$	0	0
REP	0.1±0.02$^{(7)}$	0.2±0.02$^{(7)}$	0.01±0$^{(7)}$	0	440±51$^{(14)}$
Banana peels	0.1±0.01$^{(7)}$	0.1±0.01$^{(7)}$	0.01±0$^{(7)}$	0	0
Beech sawdust	0.8±0.12$^{(14)}$	0.2±0.01$^{(7)}$	0.01±0$^{(7)}$	0	0
Phellinus chrisoloma 19					
Wheat bran	0.3±0.04$^{(7)}$	3.8±0.53$^{(7)}$	0.08±0.01$^{(7)}$	0	120±16$^{(7)}$
Mandarin peels	1.3±0.30$^{(7)}$	1.1±0.09$^{(7)}$	0.13±0.02$^{(7)}$	0	0
REP	0.2±0.02$^{(7)}$	0.3±0.03$^{(7)}$	0.01±0$^{(7)}$	0	670±87$^{(14)}$
Banana peels	0.3±0.03$^{(7)}$	0.4±0.03$^{(7)}$	0.04±0.01$^{(14)}$	0	0
Beech sawdust	0.3±0.05$^{(14)}$	0.2±0.02$^{(14)}$	0.01±0$^{(14)}$	0	0

The numbers in parentheses indicate the day of peak activity.

Table 4. Effect of carbon sources on enzyme synthesis in submerged cultivation of *Tubulicrinis glebulosus* and *Hyphoderma praetermissum*.

Substrates	CMCase (U/mL)	Xylanase (U/mL)	FPA (U/mL)	Laccase (U/L)
Hyphoderma praetermissum 47				
Mandarin peels	1.4±0.18$^{(8)}$	1.8±0.20$^{(5)}$	0.34±0.05$^{(8)}$	660±93$^{(5)}$
Wheat bran	0.7±0.05$^{(5)}$	3.2±0.36$^{(5)}$	0.38±0.04$^{(5)}$	140±12$^{(5)}$
Walnut pericarp	0.6±0.08$^{(5)}$	1.8±0.20$^{(5)}$	0.20±0.03$^{(5)}$	0
Banana peels	1.5±0.20$^{(8)}$	2.1±0.28$^{(5)}$	0.23±0.03$^{(8)}$	50±4$^{(8)}$
Glucose	Traces$^{(8)}$	0.2±0.03$^{(8)}$	0.01±0$^{(8)}$	300±43$^{(8)}$
Glycerol	0	0.2±0.03$^{(8)}$	0	50±6$^{(8)}$
Na-glucanate	0	0.2±0.02$^{(8)}$	0	0
Tubulicrinis glebulosus 26				
Mandarin peels	1.2±0.09$^{(8)}$	0.9±0.02$^{(5)}$	0.07±0.01$^{(5)}$	560±43$^{(5)}$
Wheat bran	0.3±0.03$^{(8)}$	1.3±0.14$^{(5)}$	0.04±0.01$^{(5)}$	510±64$^{(5)}$
Walnut pericarp	0.5±0.08$^{(8)}$	0.7±0.08$^{(5)}$	0.05±0.01$^{(5)}$	0
Banana peels	0.9±0.13$^{(8)}$	1.6±0.20$^{(8)}$	0.23±0.03$^{(8)}$	0
Glucose	0.1±0.01$^{(8)}$	0.1±0.01$^{(5)}$	0.02±0$^{(5)}$	270±34$^{(5)}$
Glycerol	0.1±0.01$^{(8)}$	0.1±0.01$^{(8)}$	0.02±0$^{(8)}$	120±13$^{(8)}$
Na-glucanate	Traces$^{(5)}$	0.1±0.01$^{(5)}$	0.02±0$^{(8)}$	0

The numbers in parentheses indicate the day of peak activity.

ensured a significant secretion of laccase by both fungi. No MnP was detected in the submerged cultivation of these fungi in the presence of complex and simple carbon sources.

Discussion

Eleven corticoid and three poroid fungi were isolated from the Latvian heritage sites to evaluate their potential to produce wood-degrading enzymes. In the SF of mandarin peels, notable intergeneric and intrageneric differences were revealed with regard to the extent of hydrolase and oxidase activity. Thus, the xylanase activity of the tested basidiomycetes varied from 0.1 to 2.4 U/mL depending on the fungus strain whereas that among the strains of *Athelia neuhoffii* varied from 0.1 to 1.3 U/mL (Table 2). In this respect, our results are in agreement with other studies reporting on species- and strain-dependent quantitative variations of enzyme activity (Mata and Savoie 1998; Elisashvili et al. 2009). In accordance with general observations (Silva et al. 2005; Papinutti and Forchiassin 2007; Levin et al. 2008; Elisashvili et al. 2008), all isolated corticoid and poroid fungi produced both cellulases and xylanases when they were grown in lignocellulose-containing media. The capacity of these basidiomycetes to produce these enzymes is of importance in supplying the growing cultures with a carbon source essential for their biosynthetic activity. However, when *H. praetermissum* 47 and *T. glebulosus* 26 were grown in media containing easily metabolizable carbon sources, only negligible or no cellulase and xylanase activity was detected (Table 4), testifying that the synthesis of these enzymes is under the control of an inducible mechanism, characteristic for all white-rot basidiomycetes (Elisashvili et al. 2002).

It is widely accepted that the expression of laccase activity in the fermentation of plant raw materials is a common feature of white-rot basidiomycetes (Hatakka 1994; Elisashvili et al., 2009). However, in this study, five strains of white-rot fungi did not produce either laccase or MnP (like two tested brown-rot fungi) even in the SF of mandarin peelsfavoring laccase secretion (Mikiashvili et al. 2005; Osma et al. 2007). Nevertheless, *H. praetermissum* 47, P. gigantea 36, and *T. glebulosus* 26 accumulated significant levels of laccase after 5 days of the SF of mandarin peels (Table 2). It is worth noting that *H. praetermissum* 47 and *T. glebulosus* 26 produced high levels of laccase activity in the SF of mandarin peels and wheat bran, and these fungi were capable of secreting this enzyme in a glucose-based synthetic medium (Table 4). This fact indicates that the presence of a soluble easily available carbon source favors the laccase expression by these fungi.

In agreement with other studies (Silva et al. 2005; Elisashvili et al. 2008; Gassara et al. 2010), our data show that the values of individual hydrolases and oxidases as well as the ratios of enzyme activities differ significantly upon the variation of the growth substrates. Lignocellulosic growth substrates particularly strongly affected the production of ligninolytic enzymes. Thus, the laccase of *H. praetermissum* 47 and *T. glebulosus* 26 was secreted in SF of only mandarin peels and wheat bran (Table 4) whereas the production of MnP by *H. punctulatum* 30 and *P. chrisoloma* 19 was observed in the SSF of only wheat bran and the residue after ethanol production (Table 3). It is not inconceivable that these lignocellulosic materials, in contrast to other tested substrates, contain some specific compounds appearing during their fermentation and triggering laccase/MnP synthesis. Earlier, D'Souza et al. (1999) have shown that the type of the wood substrate tends to determine the type and amount of the ligninolytic enzymes produced by *Ganoderma lucidum*. The authors supposed that some unique compound in poplar triggered the MnP production and that this compound was lacking in pine.

Therefore, further study is required to elucidate the reason why some complex substrates stimulate the target enzyme production.

Some reports indicate that the lignocellulose fermentation method may have a considerable effect on the enzyme production by white-rot fungi (Elisashvili et al. 2009; Sun et al. 2004). This study also emphasizes that the expression of the biosynthetic potentials of basidiomycetes depend on the method of cultivation. Indeed, the comparison of volumetric enzyme activities indicates that the SSF of mandarin peels was appropriate for the cellulase and xylanase production by *P. chrisoloma* 19 (Tables 2, 3). In SSF, the fungi grow under conditions close to their natural habitats, and as such are capable of producing more enzymes as compared with submerged cultures (Pandey et al. 1999). Moreover, the SSF of lignocellulosic materials was essential for the MnP production by the studied fungi, whereas their SF provided the production of laccase without the secretion of MnP.

Thus, both groups of the poroid and corticoid fungi isolated from the cultural monuments of Latvia displayed a feature, common to wood-degrading basidiomycetes in expressing hydrolytic enzyme activity in the fermentation of lignocellulosic materials. However, the secretion of ligninolytic enzymes by several fungi was rather unusual and requires further studies.

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