LIGNINOLYTIC ENZYME SYSTEM IN ECOLOGICAL ADAPTATION OF LIGNICOLOUS MACROFUNGI

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Abstract. Lignicolous macrofungi are the most important group of wood decomposers. Among the enzymes involved in wood decomposition, ligninases play an important role in this process and species that produce those enzymes degrade both cellulose and lignin. In this study we hypothesized that ligninases are influencing the ecological success of lignicolous macrofungi. Three hypotheses have been tested: h1 – the species producing several types of ligninases have a broader spectrum of hosts / substrates; h2 – the obligate saprotrophic species have a greater potential in degrading lignin and related compounds than the other groups; and h3 – the frequencies of lignicolous species is related to the production of highly active enzymes. Scientific data compiled from literature and completed with our own experimental results have been statistically interpreted using XLStat and MaxLite Software. Our results confirm the first two hypotheses, proving that ligninases play a direct role in colonizing a wide range of wood types, with chemical particularities. The third hypothesis should be rejected as no correlation has been observed. The present study offers new insights into ecological meanings of ligninases, and is the first attempt to connect the ligninolytic enzyme system to host range.
Keywords: *ligninases, wood decomposers, lignin degradation, white-rot fungi, laccases*

Introduction

Lignicolous macrofungi form a large group of fungi involved in the degradation of wood, with remarkable adaptations to different ecological niches.

Saprotrophic, parasitic or sapro-parasitic lignicolous macrofungi possess different strategies for colonizing wood and using it as a substrate and a source of nutrients. Depending on their ecology and type of nutrition, these species decay the wood, decomposing cellulose, hemicelluloses and sometimes lignin, which is one of the most abundant biomacromolecule on Earth (Knežević et al., 2013). Degradation of lignin might offer fungi advantages such as eliminating the barrier for the degradation of cellulose and increasing the availability of nitrogen in a substrate with a very high C : N ratio, through degradation of other wood constituents (Deacon, 2006).

This study is aimed at testing if the number of ligninases produced is influencing the spectrum of substrate / wood type / hosts, thus having ecological meanings. For this purpose three hypothesis have been formulated: h1 – the species producing several types of ligninases are able to colonize more types of wood compared to the species that produce a smaller number of ligninases; h2 – the obligate saprotrophic species (OS)

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have a greater potential in degrading wood (by degrading lignin) than sapro-parasitic (SP) and obligate parasitic species (OP); and h3 – competitive species produce highly active enzymes. To test these hypothesis data concerning the types of substrata, ligninase production and enzyme activity, for 69 lignicolous basidiomycetes, have been collected from literature and completed with personal experimental results.

Our interpretation suggests that there is a positive correlation between the number of ligninases and the number of hosts and OS are better wood decomposers, while the third hypothesis should be rejected. These findings offer new insights over the biochemical adaptation for colonizing a substratum with particular ecological characteristics such as wood. From our knowledge, this is the first study trying to search possible directly connections between the ligninolytic enzyme system and host range of lignicolous macrofungi.

Review of Literature

Wood decay takes place trough different biochemical mechanisms, and three types of rot can occur: a – soft rot, when fungi produce cellulases and H_2O_2; b – brown rot, when a cellulolytic enzyme system is involved and the cellulose / hemicelluloses are degraded while the lignin persists in the wood giving it a characteristic brown color; and c- white rot, in this case cellulose / hemicelluloses and lignin are degraded by particular enzymes. The concept of classifying wood rot is currently in revision, as some species "mimic" the white rot production (Nagy et al., 2015).

Ligninases are enzymes that produce the breakdown of lignin during wood decay. These enzymes play an important role in the nutrition of lignicolous fungi, frequently associated and acting synergistically.

There are several types of ligninases, and a particular species of macrofungi might produce one, few or all types. Many species produce two or three isomorphs of the same enzyme. The most common type of ligninases is represented by laccases, described for a large number of lignicolous macrofungi, including the ones that produce brown rot although the synthesis of laccases have been recorded in this cases as traces.

The occurrence of laccases (EC 1.10.3.2) and their role in the ecology of lignicolous fungi have been reviewed by several authors (Baldrian, 2006; Valderrama et al., 2003). Other common ligninases are Lignin Peroxidase-LiP (E.C. 1.11.1.14), Manganese dependent Peroxidase-MnP (E.C. 1.11.1.13), Manganese Independent Peroxidase-MIP (EC 1.11.1.16), while less frequent are Aryl Alcohol Oxidase-AAO, Versatile Peroxidase-VP, Dye-degrading Peroxidase-DyP (Anastasi et al., 2010; Grąz and Jarosz-Wilkołazka, 2011; Palmieri et al., 2005). In an extensive study involving the screening of genes encoding ligninases in a broad sense, Nagy and collaborators (2015) suggest that in the degradation of lignin, a much larger group of ligninases is involved, although some classes of enzymes play a secondary role. The production of a particular ligninase vary largely from one species to another in terms of amounts and the enzyme activity and the required conditions as well.

Materials and Methods

The available literature has been reviewed in order to collect information regarding the host's spectrum, number of ligninases produced, frequency of species, type of nutrition and the efficiency of ligninolytic enzyme system for 69 species of lignicolous basidiomycetes. Most of the species listed in *Table 1* have also been isolated and stored

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in our laboratory collection. When data were missing from literature for particular species, the information has been completed with experimental data.

Table 1. *List of investigated species of macrofungi, their enzymatic properties, ecological features and data source (NE – number of enzymes, EA – enzyme activity, DE – dye degradation efficiency, CH – common host genera – TH, F – frequency, NT – nutrition type)*

Species	NE	EA	DE	CH	TH	F	NT	References
Abortiporus biennis	3	3	4	18	26	1	OS	Aggelis et al., 2002; ARS; Bernicchia, 2005; Casieri et al., 2010; Ryvarden and Gilbertson, 1993
Armillaria mellea	3	3	1	9	82	2	OP	Balaeș et al., 2013; Casieri et al., 2010; Diamandis and Perlerou, 2001; Matsushita and Suzuki, 2005; Stoytchev and Nerud, 2000; Otieno et al., 2003; PKB; Qin et al., 2007; Szczepkowsk, 2007
Auricularia auricula-judae	4	4	1	2	24	2	OS	ARS; Balaeș et al., 2013; Liers et al., 2010; Negrean and Anastasiu, 2004
Bjerkandera adusta	6	7	5	25	32	2	OS	Anastasi et al., 2010; Balaeș et al., 2013; Balaeș and Tănase, 2013; Bernicchia, 2005; Eichlerova et al., 2006a; Erkkilâ and Niemelâ, 1986; Guillén et al., 2011; Robinson and Nigam, 2008; Ryvarden and Gilbertson, 1993; Tinoco et al., 2007
Bjerkandera fumosa	1	1	3	15	20	1	OS	ARS; Balaeș et al., 2013; Bernicchia, 2005; Graz and Jarosz-Wilkolazka 2011; Ryvarden and Gilbertson, 1993
Cerrena unicolor	4	4	4	17	21	2	OS	Bernicchia, 2005; Elisashvili et al., 2010; Erkkilâ and Niemelâ, 1986; Hattori, 2005; Ranadive et al., 2011; Souza-Ticlo et al., 2009
Coriolopsis gallica	2	2	4	2	20	2	SP	ARS; Balaeș et al., 2013; Bernicchia, 2005; Hansent and Knudsen, 1997; Robinson et al., 2001; Ryvarden and Gilbertson, 1993
Cyathus striatus	1	1	2	1	3	2	OS	ARS; Balaeș et al., 2013; Casieri et al., 2010
Daedalea quercina	2	2	1	1	15	2	OS	Balaeș et al., 2013; Baldrian 2004; Bernicchia, 2005; Ranadive et al., 2011; Ryvarden and Gilbertson, 1993
Daedaleopsis confragosa	1	1	1	1	19	2	SP	Balaeș et al., 2013; Bernicchia, 2005; Erkkilâ and Niemelâ, 1986; Orth et al., 1993; Ranadive et al., 2011; Ryvarden and Gilbertson, 1993
Datronia caperata	1	1	3	1	3	1	SP	Abrahao et al., 2008; ARS; Gilbert and Sousa, 2002; Minter et al., 2001
Dichomitus squalens	6	5	5	2	7	1	OS	Aggelis et al., 2002; ARS; Bernicchia, 2005; Eichlerova et al., 2006c; Novotný et al., 2012; Orth et al., 1993; Ryvarden and Gilbertson, 1993; Šušla et al., 2007
Flammulina velutipes	1	1	2	2	16	2	OS	ARS; Balaeș et al., 2013; Eichlerova et al., 2006a; Gerault, 2005; Petersen et al., 1999; Szczepkowski, 2007

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Fomes fomentarius	2	2	2	3	18	2	SP	Balaeș et al., 2013; Bernicchia, 2005; Casieri et al., 2010; Elisashvili et al., 2009; Erkkilâ and Niemelâ, 1986; Ryvarden and Gilbertson, 1993
Fomitopsis pinicola	1	1	1	5	23	2	SP	Balaeș et al., 2013; Casieri et al., 2010; Erkkilâ and Niemelâ, 1986; Hattori, 2005; Ryvarden and Gilbertson, 1993
Ganoderma adspersum	1	1	3	1	9	2	OP	ARS; Balaeș et al., 2013; De Simone and Annesi, 2012; Elisashvili and Kachlishvili 2009
Ganoderma applanatum	2	2	2	9	53	2	SP	Balaeș et al., 2013; Bernicchia, 2005; Casieri et al., 2010; Elisashvili and Kachlishvili 2009; Elisashvili et al., 2009; Erkkilâ and Niemelâ, 1986; Levin et al., 2004; Ryvarden and Gilbertson, 1993; Sankaran et al., 2005; SIPMP; Szczepkowski, 2007
Ganoderma lucidum	4	4	2	13	45	2	OS	Asgher et al., 2010; Balaeș et al., 2013; Bernicchia, 2005; Elisashvili and Kachlishvili 2009; Erkkilâ and Niemelâ, 1986; Orth et al., 1993; Ranadive et al., 2011; Ryvarden and Gilbertson, 1993; Sankaran et al., 2005; SIPMP
Ganoderma resinaceum	1	1	3	1	16	2	SP	Balaeș et al., 2013; Bernicchia, 2005; Casieri et al., 2010; Ranadive et al., 2011; Ryvarden and Gilbertson, 1993
Gloeophyllum odoratum	3	3	3	1	4	2	SP	Anastasi et al., 2010, Casieri et al., 2010; Bernicchia, 2005; Erkkilâ and Niemelâ, 1986; Ryvarden and Gilbertson, 1993
*Grifola frondosa**	2	1	1	1	7	1	SP	Bernicchia, 2005; Hansent and Knudsen, 1997; Orth et al., 1993; Ryvarden and Gilbertson, 1993
Gymnopilus junonius	2	2	2	4	14	1	OS	ARS; Balaeș et al., 2013; Rees and Strid, 2001; Valentin et al., 2009
Hemipholiota populnea	0	0	1	1	1	1	OP	ARS; Balaeș et al., 2013; Szczepkowski, 2007
*Hymenopelis radicata**	1	1	2	1	4	2	OS	ARS; Balaraju et al., 2010
Hypholoma fasciculare	2	2	2	2	7	2	OS	Abrahao et al., 2008; Angelini et al., 2012; Balaeș et al., 2013; Casieri et al., 2010; Gramss et al., 1999
Inonotus hispidus	3	3	1	3	23	2	OP	Aggelis et al., 2002; ARS; Balaeș et al., 2013; Bernicchia, 2005; Casieri et al., 2010; Gerault, 2006; Nerud and Mišurcova 1996; Ryvarden and Gilbertson, 1993
Irpex lacteus	6	6	5	14	20	2	OS	Aggelis et al., 2002; Balaeș et al., 2013; Bernicchia and Gorjón, 2010; Bernicchia, 2005; Hattori, 2005; Levin et al., 2004; Novotný et al., 2009; Ryvarden and Gilbertson, 1993
Kuehneromyces mutabilis	1	1	1	1	3	2	OS	Balaeș et al., 2013; Hansent and Knudsen, 1992; Steffen et al., 2007
*Laetiporus sulphureus**	1	1	2	3	23	2	SP	ARS; Bernicchia, 2005; Casieri et al., 2010; Erkkilâ and Niemelâ, 1986; Hansent and Knudsen, 1997; Hattori, 2005; Ryvarden and Gilbertson, 1993

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Lentinula edodes	3	3	4	3	12	2	OS	ARS; Bisen et al., 2010; Boer et al., 2004; Kalmiş et al., 2008; Orth et al., 1993
*Lentinus neostrigosus**	2	2	3	3	20	2	OS	ARS; Hansent and Knudsen, 1992; Vaithanomsat et al., 2012
*Lentinus tigrinus**	3	3	4	3	12	2	OS	Aggelis et al., 2002; ARS; Hansent and Knudsen, 1992; Moreira et al., 2000
Lenzites betulina	5	5	5	8	21	2	OS	Anastasi et al., 2010; Balaeș et al., 2013; Balaeș et al., 2014; Bernicchia, 2005; Erkkilâ and Niemelâ, 1986; Guillén et al., 2011; Hansent and Knudsen, 1997; Hattori, 2005; Moturi et al., 2009; Ryvarden and Gilbertson, 1993
Lycoperdon pyriforme	1	1	1	1	10	2	OS	Angelini et al., 2012; ARS; Balaeș et al., 2013; Casieri et al., 2010; Pegler et al., 1995
*Megacollybia platyphylla**	1	1	1	1	2	2	OS	ARS; Casieri et al., 2010
*Meripilus giganteus**	2	2	1	2	24	2	SP	ARS; Bernicchia, 2005; Kalmiş et al., 2008; Ryvarden and Gilbertson, 1994
Merulius tremellosus	2	2	1	1	18	2	OS	ARS; Balaeș et al., 2013; Bernicchia and Gorjón, 2010; Kum et al., 2011; Szczepkowski, 2007
*Oudemansiella mucida**	2	1	1	2	5	2	OS	ARS; Daniel et al., 1994; Gerault, 2005
*Panellus stypticus**	3	3	3	4	17	2	OS	Aggelis et al., 2002; ARS; Bernicchia and Gorjón, 2010; Casieri et al., 2010; Hansent and Knudsen, 1997; Nerud and Mišurcova 1996
*Peniophora quercina**	1	1	2	2	14	2	OS	ARS; Balaeș et al., 2013; Bernicchia, 2005; Erkkilâ and Niemelâ, 1986; Hansent and Knudsen, 1997Ryvarden and Gilbertson, 1994
Phellinus igniarius	1	1	2	3	39	2	SP	ARS; Balaeș et al., 2013; Bernicchia, 2005; Casieri et al., 2010; Erkkilâ and Niemelâ, 1986; Hansent and Knudsen, 1997; Ryvarden and Gilbertson, 1994
Phellinus pomaceus	1	1	2	1	24	2	OP	ARS; Balaeș et al., 2013; Bernicchia, 2005; Casieri et al., 2010; Ranadive et al., 2011; Ryvarden and Gilbertson, 1994
Phellinus torulosus	1	1	4	28	64	2	OP	Casieri et al., 2010; Nakasone and Burdsall, 1995
*Phlebia floridensis**	3	3	4	2	6	2	OS	Arora and Gill, 2005; ARS; Bernicchia and Gorjón, 2010; Szczepkowski, 2007
*Phlebia radiata**	6	7	5	2	15	2	OS	Anastasi et al., 2010; ARS; Casieri et al., 2010; Hilden et al., 2005; Mäkelä et al., 2006; Smith and Hesler, 1968
Pholiota aurivella	2	2	1	3	13	2	OP	Balaeș et al., 2013; Eichlerova et al., 2006a
*Piptoporus betulinus**	1	1	1	1	1	2	SP	Bernicchia, 2005; Casieri et al., 2010; Erkkilâ and Niemelâ, 1986; Gerault, 2006; Hansent and Knudsen, 1997; Ryvarden and Gilbertson, 1994
Pleurotus florida	1	1	1	1	1	2	OS	ARS; Gbolagade et al., 2006; Pant and Adholeya, 2009
Pleurotus dryinus	2	2	2	10	18	1	OS	ARS; Eichlerova et al., 2006b; EOL
Pleurotus	7	7	2	11	43	2	OS	Aggelis et al., 2002; Anastasi et al., 2010;

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ostreatus								Balaeș et al., 2013; Casieri et al., 2008; Eichlerova et al., 2006a; Hansent and Knudsen, 1997; Palmieri et al., 2005; Pozdnyakova et al., 2010
Pleurotus pulmonarius	3	3	3	5	7	2	OS	ARS; Bernicchia, 2005; Eichlerova et al., 2006c; Hattori, 2005; Orth et al., 1993; Rigas et Dritsa, 2006; Ryvarden and Gilbertson, 1994
*Polyporus alveolaris**	2	2	5	9	20	1	OS	Barassa et al., 2009; Bernicchia, 2005; Erkkilâ and Niemelâ, 1986; Lee et al., 2010; Ryvarden and Gilbertson, 1994
Polyporus brumalis	3	3	2	22	27	2	OS	ARS; Bernicchia, 2005; Erkkilâ and Niemelâ, 1986; Kum et al., 2011; Rigas si Dritsa, 2006; Ryu et al., 2008; Ryvarden and Gilbertson, 1994
Polyporus squamosus	2	2	1	25	28	2	SP	ARS; Casieri et al., 2010; Eichlerova et al., 2006a
Postia caesia	1	1	1	7	30	2	OS	ARS; Balaeș et al., 2013; Eichlerova et al., 2006a; Gerault, 2006
Postia stiptica	1	1	1	3	22	2	OS	ARS; Balaeș et al., 2013; Bernicchia, 2005; Casieri et al., 2010; Hansent and Knudsen, 1997; Ryvarden and Gilbertson, 1994
Pycnoporus cinnabarinus	2	2	4	12	28	2	OS	ARS; Bernicchia, 2005; Casieri et al., 2010; Fedrova et al., 2013; Hansent and Knudsen, 1997; Orth et al., 1993; Ryvarden and Gilbertson, 1994
Trametes gibbosa	3	3	5	24	34	2	OS	ARS; Balaeș et al., 2013; Balaeș et al., 2014; Bernicchia, 2005; Elisashvili and Kachlishvili 2009; Elisashvili et al., 2009; Fedrova et al., 2013; Hansent and Knudsen, 1997; Orth et al., 1993; Ryvarden and Gilbertson, 1994; Szczepkowski, 2007
Trametes hirsuta	5	5	5	38	52	2	OS	Aggelis et al., 2002; Balaeș et al., 2013; Bernicchia, 2005; Casieri et al., 2010; Erkkilâ and Niemelâ, 1986; Haibo et al., 2009; Hansent and Knudsen, 1997; Hattori, 2005; Orth et al., 1993; Ryvarden and Gilbertson, 1994; Szczepkowski, 2007; Tomšovsky and Homolka 2003
Trametes ochracea	3	3	5	19	23	2	OS	ARS; Balaeș et al., 2013; Bernicchia, 2005; Casieri et al., 2010; Elisashvili and Kachlishvili 2009; Gerault, 2006; Ryvarden and Gilbertson, 1994; Tomšovsky and Homolka 2003
Trametes pubescens	6	7	5	15	20	2	OS	Anastasi et al., 2010; ARS; Balaeș et al., 2013; Bernicchia, 2005; Casieri et al., 2008, 2010; Erkkilâ and Niemelâ, 1986; Nikitina et al., 2005; Ryvarden and Gilbertson, 1994
Trametes suaveolens	2	2	4	2	9	2	SP	ARS; Balaeș et al., 2013; Bernicchia, 2005; Erkkilâ and Niemelâ, 1986; Hansent and Knudsen, 1997; Knežević et al., 2013; Ryvarden and Gilbertson, 1994
*Trametes trogii**	6	6	5	2	9	2	OS	ARS; Bernicchia, 2005; Dhouib et al., 2005; Levin et al., 2002, 2004; Ryvarden

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								and Gilbertson, 1993
Trametes versicolor	8	9	4	40	61	2	OS	Anastasi et al., 2010; Balaeș et al., 2013; Bernicchia, 2005; Casieri et al., 2010; Erkkilâ and Niemelâ, 1986; Guillén et al., 2011; Hattori, 2005; Levin et al., 2004; Liu et al., 2004; Lorenzo et al., 2006; Moturi et al., 2009; Orth et al., 1993; Ryvarden and Gilbertson, 1994; Tomšovsky and Homolka 2003; Ungureanu et al., 2015
Trichaptum abietinum	2	2	2	5	22	2	OS	ARS; Bernicchia, 2005; Eichlerova et al., 2006a; Erkkilâ and Niemelâ, 1986; Ryvarden and Gilbertson, 1994
*Trichaptum biforme**	2	2	2	7	18	2	OS	ARS; Bernicchia, 2005; Elisashvili et al., 2009; Hansent and Knudsen, 1997
Schizophyllum commune	4	3	2	6	42	2	SP	ARS; Balaeș et al., 2013 ; Bhatti et al., 2008; Hansent and Knudsen, 1997; Levin et al., 2004; Li et al., 2009; Szczepkowski, 2007
Stereum hirsutum	1	1	1	2	11	2	OS	Balaeș et al., 2013; Guillén et al., 2011; Orth et al., 1993; Szczepkowski, 2007
Xylobolus frustulatus	1	1	1	1	3	1	OS	Balaeș et al., 2013 ; Cookson, 1995; Hansent and Knudsen, 1997; Ranadive et al., 2011

*experimental results from the present research are included

Analytical procedures

For assessing the efficiency of dye degradation, the protocol described in Balaeș and collaborators (2013) has been used. Pure fungal cultures were grown on solid and liquid media and used as sources of inoculum. The presence / absence of laccase, and the enzyme activity has been highlighted through a modified protocol (Guo et al., 2011), using liquid medium supplemented with wheat bran (per L^{-1}: 6 g glucose, 3 g $(NH_4)_2SO_4$, 6 g wheat bran, 0.01 g $CuSO_4$), and inoculated with pelletized mycelium (10 mL inoculum L^{-1}). As a specific substrate, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) has been used. The reaction mixture contained 0.25 mL 50mM ABTS, 0.5 mL 0.1M acetate buffer, 0.25 mL enzyme extract. Substrate oxidation was monitored by measuring the increase of absorbance at 420 nm (ε420, 3.6•104 M^{-1} cm^{-1}), after 1 min at 25º C. A unit of laccase activity was defined as the amount of enzyme oxidizing 1 μmoL of ABTS per minute in the considered analysis conditions (Bourbonnais et al., 1995).

Data processing

Compiled dataset included 6 variables (*Table 1*): number of ligninases, enzyme activity (expressed as classes from 1– poor enzyme activity / traces, to 7 – very strong activity), dye degradation efficiency (expressed as classes from 1 – very weak degradation, few dyes or no degradation at all, to 5 – very efficient degradation of different dyes), number of common host genera, number of total host genera and frequency (expressed as classes, 1 – less frequent, 2 – frequent or very frequent). A seventh variable refers to the type of nutrition – obligate saprotrophic (OS), sapro-

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parasitic (SP) and obligate parasitic species (OP). For particular tests, values from previous datasets were grouped into three categories corresponding to the nutrition type.

Statistical interpretations

For all statistical calculations and analysis XLStat (trial version) and MaxLite (freeware) softwares have been used. As data do not follow normal distribution, non-parametric tests were used. For assessing the correlations between different variables, Spearman correlation coefficients were used, at a confidence interval of 95%. Mann-Whitney test has been used to compare variables related to the three types of fungi according to their nutrition. A Principal Component Analysis was run for a better view of correlations between all variables, considering the nutrition type as a qualitative supplementary variable.

Results and Discussions

The present investigation is an attempt to elucidate the role of ligninolytic enzyme system in ecological adaptation of lignicolous macrofungi in terms of diversity of substrata (hosts colonization). For this purpose, three hypotheses have been formulated.

***Hypothesis number 1*: *the species producing several types of ligninases are able to colonize many types of wood compared to the species that produce a smaller number of ligninases*.**

One first step of our investigation was to analyze the correlation between the number of ligninolytic enzymes and the number of hosts. In the table of correlation (*Table 2*) it can be seen that there is a positive correlation (r=0.5529, p <0.0001) between the number of enzymes and the number of common host genera for all 69 species studied, while the correlation is weaker when considering the total host genera (r=0.3738, p<0.0001). In the latter case there are also included species of trees / lignicolous substrate that are accidentally colonized by fungi and not as a normal behavior of a particular fungal species (e.g. *Stereum hirsutum* usually grows on deciduous trees, but it has been reported also on coniferous wood, although one may not expect to find *S. hirsutum* on this substrate in the field). Hence, using the number of common hosts for assessing the correlation between the number of ligninolytic enzymes and host diversity gives a better understanding of how the ligninolytic enzymes system is influencing the ecology of these fungi.

Table 2. *Correlations between ligninolytic enzyme system and number of hosts – Spearman correlation coefficients (r values in upper, and p values, two-tailed, in lower matrix; confidence interval 95%; with bold are marked the relevant results)*

	Number of enzymes	**Enzyme activity**	**Dye degradation efficiency**	**Common host genera**	**Total host genera**
Number of enzymes		0.9898	0.5712	**0.5529**	0.3738
Enzyme activity	< 0.0001		0.5902	**0.5604**	0.3883
Dye degradation efficiency	< 0.0001	< 0.0001		**0.4559**	0.182
Common host genera	**< 0.0001**	**< 0.0001**	**< 0.0001**		0.759
Total host genera	0.0016	0.001	0.1344	< 0.0001	

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We have included in our tests one other variable: the general enzyme activity, expressed as classes of efficiency (data collected from literature and completed with our own results). These data have the disadvantage of being organized as classes: the quantitative interpretation is thus affected by subjectivity to some degree. Raw quantitative data could not be used due to very different experimental conditions of various researchers. Including of this variable had the role of verifying if there is still a positive correlation between enzymes and hosts number. The correlation in this case was slightly stronger (r=0.5604, $p < 0.0001$), re-confirming h1.

A second attempt to verify this hypothesis consisted in using a new variable, derived from the first one – the efficiency of dye degradation as a measure of enzyme versatility. Ligninolytic enzymes are very versatile and attack a large spectrum of chemical compounds with structure similar to lignin. This property confers the ability to degrade different human made aromatic compounds, hydrocarbons, dyes with heterocycles (Balaeș et al., 2014), while in natural conditions these fungi degrade lignin and also other chemical compounds from wood (Deacon, 2006). Considering these facts, we hypothesize that the versatility of ligninolytic enzymes could also be a factor that influence the ecological adaptation in terms of colonizing very different types of wood (varied chemical composition). However, we found weaker correlation compared to previous variables (r=0.4559, $p < 0.0001$).

The Principal Component Analysis (PCA) is an explanatory statistical tool used for visualization of data and to a lesser extent for testing hypotheses. Running PCA lead to confirm the results from previous tests, offering, in the same time, a largely view over the connections between variable. The procedure is based on the projection of all variables (six + one in our case) onto two axes, considering the first two factors that are contributing to the variability. We have considered the first six variable as principal quantitative variables and the seventh one (*Nutrition type*) as a supplementary qualitative variable.

In *Fig. 1* we can observe on the correlation circle that the first two components explain 73% of the data variability, making the projection a trusting one. A very strong positive correlation is found between the *Number of enzymes* and *Enzyme activity*, as it would have been expected. A good positive correlation is seen between these two variables and *Common host genera* on a side and *Dye degradation efficiency* on the other side. The position of projection points at a particular distance from the circle prove that the projection should be considered with caution for these two variables.

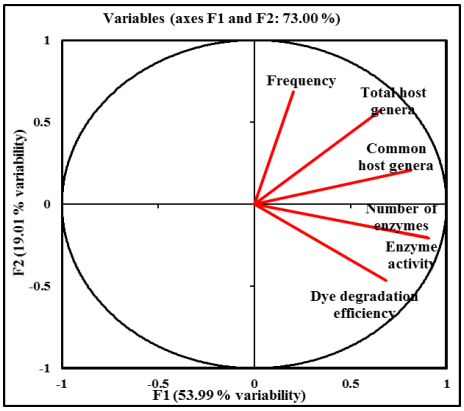

Figure 1. *Circle of correlation between factors and variables after Principal Component Analysis (numbers on axes represent the correlation coefficients)*

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As seen in *Table 3*, factor loadings for these variables have the highest values on F1 dimension and second values (close to the first ones) on F3 and F4 dimensions respectively. The *Total host genera* variable is better projected on the chart (*Table 3*) and it can be observed that it is not correlated with *Number of enzymes*. The highest values of factor loadings for *Frequency* are on the F2 and F3 dimensions and are almost equal, and therefore no explanations can be assumed based on the projection.

Table 3. *Factor loadings for the six quantitative variables in Principal Component Analysis (with bold are marked the highest values for each variable)*

Variables\Factors	F1	F2	F3	F4	F5	F6
Number of enzymes	**0.900**	-0.199	0.263	-0.273	-0.015	-0.085
Common host genera	**0.815**	0.206	**-0.443**	0.067	0.303	0.000
Frequency	0.205	**0.687**	**0.664**	0.205	0.060	0.000
Enzyme activity	**0.907**	-0.209	0.253	-0.250	-0.022	0.087
Total host genera	**0.657**	**0.574**	-0.415	-0.007	-0.259	-0.001
Dye degradation efficiency	**0.685**	-0.463	0.046	**0.554**	-0.080	-0.003

Hypothesis number 2 – OS species have a greater potential in degrading wood (by degrading lignin) than SP and OP species.

OS macrofungi use products derived from wood decomposition as sources of nutrients: cellulose and hemicelluloses are primary sources of C and energy, while lignin is degraded especially for making cellulose and other constituents of wood more accessible (Deacon, 2006). Since OS are dependent on the dead wood constituents, it was assumed that these species tend to have a highly versatile and active enzymes system compared to SP and OP macrofungi, which are using additional or mainly other strategies for nutrition. In other words, the second hypothesis assumed that OS have more active enzyme and degrade a broader spectrum of synthetic dyes (different chemical structure), being more versatile.

When applying the Mann-Whitney test for two by two datasets (*Table 4*) we observed that there is a significant superiority of dye degradation efficiency of OS compared to SP (one tailed *p* value 0.047) and to OP (one tailed *p* value 0.048), while between SP and OP there are no significant differences for this property. Concerning enzyme activity, OS produce more highly active enzymes than SP (one tailed *p* 0.008) and OP (one tailed *p* 0.046). These results are confirming our hypothesis.

Table 4. *Mann-Whitney test (right-tailed test) for comparing the enzyme activity and dye degradation efficiency for the three type of macrofungi (the Mann-Whitney's U is normalized and tested against the normal distribution)*

	Dye degradation efficiency			Enzyme activity		
	OS-SP	OS-OP	SP-OP	OS-SP	OS-OP	SP-OP
U	469.000	222.500	64.000	514.000	223.500	57.000
U (expected value)	368.000	161.000	56.000	368.000	161.000	56.000
U (variance)	3651.782	1369.084	200.316	3627.943	1381.644	193.233
Z (observed value)	1.671	1.662	0.565	2.424	1.681	0.072
Z (critical value)	1.645	1.645	1.645	1.645	1.645	1.645
One-tailed p-value	0.047	0.048	0.286	0.008	0.046	0.471
Alpha	0.05	0.05	0.05	0.05	0.05	0.05

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Lignin is considered a barrier for wood decomposition, but woody species also produce tannins, alkaloids, resins and other types of chemical compounds with defensive and/or structural role. Lignin is formed from three different monomers: *p*-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol, and their varied ratio give spatial different structures for each species. Broad leaves trees (dicotiledons) have lignine made predominantly from *p*-coumaryl, while coniferous trees contain lignin composed mainly from coniferyl alcohol. Ligninases are versatile enzymes, able to breakdown the lignin with its different structure from a species to another, as well as oxidize other compounds polyphenols and heterocyclic compounds (Elisashvili et al., 2010). For OS macrofungi ligninases play important roles in their nutrition.

The Principal Component Analysis also offered information for a better view of ecological features of the three groups of macrofungi separated based on their nutrition type. *Fig. 2* shows the projection of each observation (species characteristics) on the chart, grouped according to the nutrition type (three colors for OS, SP and OP). There is a distinct projection of the three groups, with only OS sharing some features with other two groups and with a higher dispersal on the chart, meaning there is a higher variability inside group, in terms of ligninases synthesis and host spectrum. OP group is more homogenous, but three species have particular characteristics, with *Armillaria mellea* (AM) and *Phellinus torulosus* (PT) having a very large host spectrum and producing ligninases to some degree, while *Hemipholiota populnea* (HP) has a completely distinct projection with a reduced host range (only *Populus*) and does not produce known ligninases.

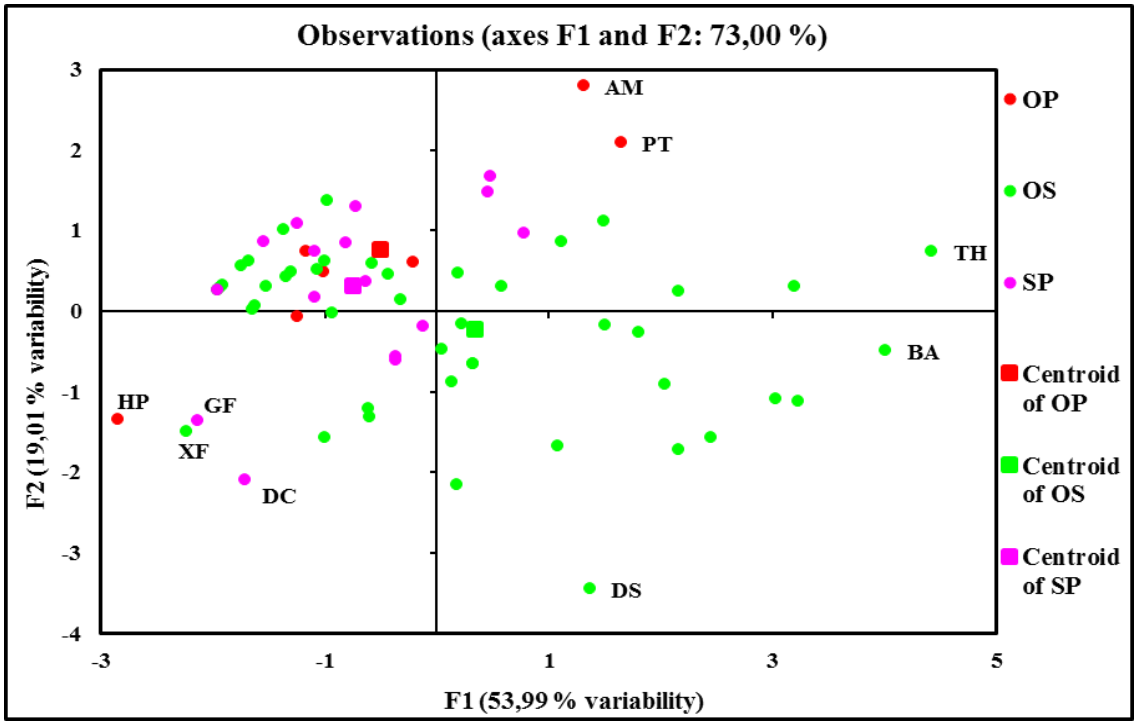

Figure 2. *The projections of observations (species characteristics) on the first two factors axes (AM – A. mellea, BA – B. adusta, DC – D. caperata, DS – D. squalens, GF – G. frondosa, HP – H. populnea, PT – P. torulosus, TH – Trametes hirsute, XF – Xylobolus frustulatus; legend: OP – obligate parasitic, OS – obligate saprotrophic, SP – sapro-parasitic macrofungi)*

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Among the SP species, *Grifola frondosa* (GF) and *Datronia caperata* (DC) possess features distinct from the other species in the group, with reduced host range, less frequent and producing only laccase in small amounts. Four OS species have characteristics different from other species in the group: *Bjerkandera adusta* (BA) and *Trametes hirsuta* (TH) produce many enzymes with strong activity and have a large host spectrum, while *Xylobolus frustulatus* (XF) produces few enzymes and has a reduced host spectrum (only *Quercus*) and *Dichomitus squalens* (DS) with strong enzyme activity but reduced host spectrum.

The projection of the species based on the nutrition type offers an interesting view of their ecological behaviors. The higher variability for OS fungi can be explained through their very different strategies for survival and development, some species growing on wood not affected by decay, while other grow on rotten wood; some species manifest antagonism against competitors, while other expand their mycelium in soil and litter, being more opportunistic. In contrast, SP and OP appear to be more specialized on their ecological niche, probably their strategies being oriented toward "fighting" their hosts.

Hypothesis number 3 – competitive species (frequent species) produce highly active enzymes.

To test this assumption, we verified if there is a positive correlation between frequency and enzyme activity / dye degradation efficiency. As it can be observed in *Table 5*, there is no correlation, at least in the case of our limited data, and we might assume that other factors are being involved in the process (hosts number, host frequency, other ecological factors). The third hypothesis has been rejected.

Table 5. *Correlations between frequencies and enzyme activity / dye degradation efficiency – Spearman correlation coefficients (r values in upper, and p values, two-tailed, in lower matrix; confidence interval 95%)*

	Enzyme activity	**Dye degradation efficiency**	**Frequency**
Enzyme activity		0.5902	0.1650
Dye degradation efficiency	< 0.0001		-0.0383
Frequency	0.1755	0.7544	

An attempt to include data for distribution of fungal species has been abandoned since the distribution is strongly dependent on their hosts and production of ligninases is irrelevant in this case. The limitations of our study consist in the reduced availability of data concerning ligninase production and activity for many species of macrofungi, forcing us to take into consideration only 69 species. Different macrofungi might produce a particular ligninase in different amounts or with a different activity, and a reasonable assumption is that the quantity and quality of ligninases also play roles in wood degradation efficiency. In these circumstances is difficult to compare the ligninolytic activity of certain species as different authors are using varied protocols and particular conditions to test the enzyme activity. However, using classes of efficiency, we overcome this limitation and tested the formulated hypotheses.

In summary, we have investigated the influence of ligninolytic enzyme system over ecological success of lignicolous fungi in terms of hosts' spectrum. We assumed that fungal species producing several types of ligninases are cosmopolitan and colonize a broader spectrum of tree hosts and we found positive correlation that sustain this

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hypothesis ($r=0.5529$, $p<0.0001$). Our analysis suggests that obligate saprotrophic fungi tend to degrade several types of wood and more efficiently due to higher enzyme activity and versatility. It appears that this group of macrofungi is more heterogeneous in terms of ligninases production and host range, probably due to other ecological factors involved in their adaptation strategies than in the case of parasitic species.

In was assumed that fungal species with a larger host spectrum produce highly active and versatile ligninases, but this hypothesis has been rejected.

Future studies might contribute further to the understanding of the ecological importance of ligninases.

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