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# Survey of ectomycorrhizal, litter-degrading, and wood-degrading *Basidiomycetes* for dye decolorization and ligninolytic enzyme activity

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### Abstract

Basidiomycetes are essential in forest ecology, being deeply involved in wood and litter decomposition, humification, and mineralization of soil organic matter. The fungal oxidoreductases involved in these processes are today the focus of much attention with a view to their applications. The ecological role and potential biotechnological applications of 300 isolates of Basidiomycetes were assessed, taking into account the degradation of model dyes in different culture conditions and the production of oxidoreductase enzymes. The tested isolates belong to different ecophysiological groups (wood-degrading, litter-degrading, ectomycorrhizal, and coprophilous fungi) and represent a broad systematic and functional biodiversity among Basidiomycetes occurring in deciduous and evergreen forests of northwest Italy (Piedmont Region). The high number of species tested and the use of different culture conditions allowed the investigation of the degradation activity of several novel species, neglected to date. Oxidative enzyme activities varied widely among all ecophysiological groups and laccases were the most commonly detected enzymes. A large number of isolates (86%), belonging to all ecophysiological groups, were found to be active against at least one model dye; the wood-degrading fungi represented the most efficient group. Noteworthily, also some isolates of litter-degrading and ectomycorrhizal fungi achieved good decolorization yield. The 25 best isolates were then tested against nine industrial dyes commonly employed in textile industries. Three isolates of *Bjerkandera adusta* efficiently decolorized the dyes on all media and can be considered important candidates for application in textile wastewater treatment.

### **Keywords**

DecolorizationIndustrial dyesLigninolytic enzymesBasidiomycetes

### Introduction

The vast majority of terrestrial biomass takes the form of wood and other plant tissues. Without decay, these nutrient reserves would accumulate and eventually halt ecosystem productivity. Fungi play a pivotal role in the ecology of forests, as they are the main decomposers of lignin–cellulose and phenolic compounds, through the production of extracellular enzymes and especially oxidoreductases, such as laccases, different kind of peroxidases, and tyrosinases (Dighton 2007; Osono 2007). The monumental task of recycling the carbon sequestered in wood falls primarily to Basidiomycota species, which are deeply involved in wood and litter decomposition, humification, and mineralization of soil organic matter, which are essential processes of the terrestrial carbon cycle and soil formation (Anastasi et al. 2009).

Lignin, in particular, is a highly refractory and persistent compound that, being intimately associated with cellulose and hemicelluloses, acts as a barrier to microbial attack: lignin must be degraded before the cellulose can be consumed, and this slows down fungal decomposition of leaf litter (Berg and McClaugherty 2003; Anastasi et al. 2009). The accumulation of C and N in lignin and polyphenols has implications for the long-term development of the ecosystem and the functioning of ecosystem services (Amundson 2001; Berg and McClaugherty 2003; Lal 2005; Osono 2007; Bodeker et al. 2009).

Laccase- and peroxidase-encoding genes have been detected within different functional groups of fungi (saprotrophs, symbionts, and pathogens) but not within all species (Luis et al. 2005). Although these enzymes are known to be mainly produced by wood-decomposing fungi (WDF), they have been found also in litter-decomposing fungi (LDF) and ectomycorrhizal fungi (EMF) (Steffen et al. 2007; Cullings et al. 2008; Bodeker et al. 2009), and their patchiness and spatial distribution in soils have recently been described (Luis et al. 2004, 2005).

Moreover, these oxidoreductases possess broad substrate specificity and can also degrade other aromatic recalcitrant compounds [polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and dyes] with chemical structure similar to those produced during synthesis and degradation of lignin (Barrasa et al. 2009). As a consequence, biotechnological application of ligninolytic fungi is increasingly considered a sustainable approach to pollution problems, although great effort is still needed to select isolates belonging to taxa and/or ecophysiological groups not yet investigated (Pointing and Vrijmoed 2000). From both an ecological and an application point of view many studies have almost exclusively focused on WDF (Kirk and Fenn 1982; Hatakka 1994; Singh 2006; Anastasi et al. 2009), whereas the degradation capabilities of other ecophysiological groups, i.e., LDF and EMF, have been poorly investigated. Recently the presence of laccase- and peroxidase-coding genes has been reported across a wide taxonomic and ecological range of EMF (Bodeker et al. 2009; Luis et al. 2004) as well as their spatial distribution in the upper horizons of mixed forest cambisol (Luis et al. 2005). It has been proposed that oxidoreductase produced by EMF may be involved in several processes related to functioning of symbiotic interaction, such as lignin and polyphenol degradation, release of N from insoluble protein-tannin complexes, pigment production, neutralization of host-defense compounds, humus formation, and detoxification of the soil environment through degradation of various organopollutants (Bending and Read 1996; Bodeker et al. 2009; Burke and Cairnei 2002; Gramss et al. 1998; Luis et al. 2005; Kanunfre and Zancan 1998). Moreover, according to Northup et al. (1998), the formation of humic compounds could also play an important role by minimizing the loss of N in ecosystems, reducing toxic metal availability, and improving soil physicochemical conditions for root growth.

Screening of fungi for ligninolytic enzymes usually involves monitoring decolorization of dyes, in particular the anthraquinonic dye Poly R-478 (Leung and Pointing 2002). The ability of a fungus to decolorize this dye coincides with the onset of lignin metabolism and is regarded as predictive of its ability to degrade also recalcitrant organopollutants such as PAHs (Zheng et al. 1999; Anastasi et al. 2009; Barrasa et al. 2009).

Dye decolorization through ligninolytic enzymes has been demonstrated to depend on several factors such as carbon sources and nitrogen amount (Leung and Pointing 2002). For these reasons, an appropriate culture medium must be selected to avoid false negatives in dye decolorization screenings. Moreover, for the treatment of industrial dye-containing effluents characterized by very variable composition, it is important to select isolates that are effective against a wide range of structurally different dyes and not strictly dependent on culture conditions.

In this work the ligninolytic enzyme activities and model dye decolorization capabilities of 300 isolates of *Basidiomycetes* representing a broad systematic and functional biodiversity were assessed. The isolates belonging to different ecophysiological groups [WDF, LDF, EMF, and coprophilous fungi (CF)] correspond to 143 species, 90 genera, and 35 families. Four media, characterized by different C:N ratio, were used to assess the best conditions to allow the isolates to exhibit their degradation capabilities. Afterwards, the best isolates were tested in more restrictive conditions by using nine industrial dyes, currently used in textile industries, to evaluate their potential application in bioremediation processes.

# Materials and methods

#### Dyes

The model dyes (Table <u>1</u>), purchased from Sigma Chemical Co. (St. Louis, MO, USA), were: RBBR, a key starting molecule for the production of several polymeric dyes (Soares et al. <u>2001</u>); Poly R-478, characterized by chemical complexity similar to lignin compounds, which allows assessment of degradation of lignin and aromatic molecules (Alcalde et al. <u>2002</u>); and Poly S-119, as a representative of azo dyes, the widest and most recalcitrant class of textile dyes (Buckley and Dobson <u>1998</u>). The industrial dyes (Table <u>1</u>), kindly provided by Clariant Italia S.p.a., were selected because of their recalcitrance to biodegradation by conventional wastewater treatments (Clariant, personal communication) and their wide range of applications in the textile industries as single dyestuffs or in combination with others; besides, they are representative of the most used dyes, comprising anthraquinonic, mono-, di-, and polyazoic, and phthalocyanin chromophores.

Table 1 Model and industrial dyes used in the experiment, their commercial and color index (CI) name, chromophore, chemical group, wavelength of maximum visible absorbance ( $\lambda_{max}$ ), and dye molecule concentration

Acrony m	Commercia l name	CI name	Chromophore	Chemica l group	$\lambda_{\max}$ (nm )	Dye molecule concentratio n	Dye molecule biodegradability <sup>a</sup>
B81	Solar Blue G P 280	Direct blue 81	Tri-azoic	Direct	577	Unknown	Unknown
R80	Solar Red Ba P 150	Direct red 80	Tetra-azoic	Direct	540	30–35%	46%
B113	Nylosan Marin Blue N-Rbl P 187	Acid blue 113	Di-azoic	Acid	541	55-75%	Unknown
B225	Nylosan Blue F-2rfl P 160	Acid blue 225	Anthraquinoni c	Acid	590– 626	55–65%	20-30%
R111	Scarlet Nylosan F- 3gl 130	Acid red 111	Di-azoic	Acid	499	85–90%	60%
B41	Drimaren Turquoise X-B Cdg	Reactiv e blue 41	Phthalocyanin	Reactive	616– 666	50-60%	~ 80
R243	Drimaren Red X-6bn Cdg	Reactiv e red 243	Azoic	Reactive	517	Unknown	25%

Acrony m	Commercia l name	CI name	Chromophore	Chemica l group	$\lambda_{\max}$ (nm)	Dye molecule concentratio n	Dye molecule biodegradability <sup>a</sup>
B214	Drimaren Marine Blue X-Gn Cdg	Reactiv e blue 214	Azoic	Reactive	607	50-60%	~0
B49	Drimaren Blue P-3rln Gr	Reactiv e blue 49	Anthraquinoni c	Reactive	586– 625	Unknown	10–20%
RBBR	Remazol Brilliant Blue R	Reactiv e blue 19	Anthraquinoni c	Reactive	593	100%	Unknown
Poly R- 478	Polymeric dye R-478	_	Anthraquinoni c	Reactive	520	100%	Unknown
Poly S- 119	Polymeric dye S-119	_	Azoic	Reactive	472	100%	Unknown

#### <sup>a</sup>Method OECD 302B

Stock solutions at 20,000 ppm concentration were prepared for each dye in distilled water, sterilized using 0.2- $\mu$ m pore filters, and preserved at 4°C until use. Since reactive industrial dyes are released into textile effluents after thermal hydrolyzation of reactive groups occurring in dye-bath conditions, dyes B41, B49, B214, and R243 were hydrolyzed by 2 h treatment at 80°C in 0.1 M Na<sub>2</sub>CO<sub>3</sub> solution, and then neutralized with HCl before use.

#### Fungal species

Three hundred isolates of *Basidiomycetes* (Table <u>2</u>), ascribable to different ecophysiological groups (161 WDF, 75 LDF, 57 EMF, and 7 CF), were screened for their decolorization capabilities. The difference between the number of isolates for each group depends in part on the difficulty encountered in isolation and maintenance in pure culture (particularly for EMF). Moreover, they represent a local survey of basidiomycetous biodiversity in deciduous and evergreen forests of Piedmont Region (northwest Italy). All isolates are preserved on malt extract agar (MEA) at 4°C at the *Mycotheca Universitatis Taurinensis* (MUT, University of Turin, Department of Plant Biology).

Table 2 Fungal growth, degradation of model dyes, and enzymes produced for each isolate

<b>N // T</b> T		<b>F</b>	Growth			Decolorization			Fnzym
MU T	Fungi	Ecogro up	RBBR	Poly R- 478	Poly S- 119	RBBR	Poly R- 478	Poly S- 119	Enzym es
	Agaricaceae								
231 1	Agaricus augustus	LDF	+(g)	-(g,G) +(p)	-(M,g,G ) +(p)	++(M,g,G )	++(g) +(G)	_	L
239 9	Agaricus benesii	LDF	-(M) +(g)	-(G,p) +(g)	-(G) +(p)	++(M,g,G )	++(g) +(G)	_	L
223 7	Agaricus bitorquis	LDF				++(M,g,G )	++(g,G)	+(M)	LTP
275 6	Agaricus bitorquis	LDF	+(g)	-(G,p)	-(p) +(g)	++(M,g,G )	+(g)	_	LTP

MIT		Fcogro	Growth		Decolorization			Enzvm	
T	Fungi	up	RBBR	Poly R- 478	Poly S- 119	RBBR	Poly R- 478	Poly S- 119	es
275 7	Agaricus bitorquis	LDF				_	_	_	LTP
128 2	Agaricus moelleri	LDF	-(g) +(G,p)	-(M) +(p)	-(G) +(p)	++(M,g,G )	++(g) +(G)	_	LP
297 5	Agaricus osecanus	LDF	-(M,g,G ,p)		-(g,G)	++(M,g,G )	_	_	L
250 5	Agaricus silvicola var. silvicola	LDF	-(M,G,p ) +(g)	-(M) +(g,G)	-(M) +(g)	++(M,g,G ,p)	++(M,G) +(g)	_	LP
297 3	Agaricus silvicola var. silvicola	LDF	-(M,g,G ,p)	-(g) +(G)	-(g) +(M)	++(M,g,G )	++(g,G)	_	ТР
270 7	Agaricus xantodermus	LDF	-(G) +(p)	+(p)	+(g,G,p)	++(M,g,G ,p)	++(G)	_	ТР
109 7	Bovista plumbea	LDF			+(M)	-	_	_	LT
294 9	Bovista plumbea	LDF				++(M)	++(M)	-	_
295 0	Bovista plumbea	LDF			-(M) +(G)	_	+(M)	_	LT
223 3	Coprinus comatus	LDF	+(p)	+(p)	+(p)	++(M,g,G )	++(M)	_	LTP
223 2	Coprinus comatus	LDF	-(p)	-(p)	-(p)	++(M,g,G )	+( <b>M</b> , <b>p</b> )	_	LP
246 9	Crucibulum laeve	WDF	-(p)			++(M,g,G ,p)	++(g,G,p)	++(g) +(G,p)	LP
112 2	Cyathus stercoreus	WDF		-(p)	-(p)	++(M,g,G ,p)	++(M,g,G ,p)	++(g,p)	L T P
217 1	Cyathus stercoreus	WDF	-(p)	-(p)	-(p)	++(M,g,G ,p)	++(M,g,G ,p)	++(g) +(p)	LTP
228 9	Cyathus striatus	WDF	-(p)	-(p)	-(p)	++(M,g,G ,p)	++(G) +(p)	++(g) +(p)	LP
131 0	Lepiota cristata	LDF	-(g,G,p)	-(M,g,G ) +(p)	-(g,G) +(M)	++(g,p) +(M)	+(g)	_	L
240 3	Leucoagaricu s americanus	LDF		-(M,p)	+(p)	++(M,g,G ) +(p)	++(g)	_	LP
298 0	<i>Leucoagaricu</i> <i>s leucothites</i>	LDF		-(g,G,p)	-(p)	++(M,g,G ) +(p)	_	_	L
120 3	Lycoperdon fragile	LDF	-(g)		+(g)	++(M,g,G )	+(g)	_	L
127 1	Lycoperdon perlatum	LDF		-(p)		++(G)	+(p)	_	L

MI		Fcogro	Growth		Decolorization			Enzvm	
T	Fungi	up	RBBR	Poly R- 478	Poly S- 119	RBBR	Poly R- 478	Poly S- 119	es
265 1	Lycoperdon perlatum	LDF		-(p)		++(M,g,G ) +(p)	_	++(G,p)	L
295 8	Lycoperdon perlatum	LDF		-(p)	-(p)	_	_	_	_
129 5	Lycoperdon utriforme	LDF			+(M)	++(M) +(G)	++(G) +(M)	+(G)	LP
293 9	Macrolepiota mastoidea	LDF	-(g) +(p)		-(p)	++(M,g,G )	++(g)	_	LTP
129 2	Macrolepiota procera var. procera	LDF	-(p)		-(p) +(M,G)	++(g)	+(M)	_	L
166 0	Macrolepiota procera var. procera	LDF	-(g,p)	+(g)	-(p) +(g)	++(M,g,G )	_	-	LTP
246 6	Macrolepiota procera var. procera	LDF	-(g)			++(M,g,G )	++(M)	-	LTP
250 3	Macrolepiota procera var. procera	LDF	-(g) +(p)	+(p)	-(p) +(G)	++(M,g,G ) +(p)	++(M,G)	-	LTP
294 6	Macrolepiota procera var. procera	LDF	-(g)	-(g)	-(g) +(G)	++(M,g,G )	++(g) +(G)	_	LTP
294 7	Macrolepiota procera var. procera	LDF	-(g,p)		+(p)	++(M,g,G ,p)	++(g) +(g)	_	L T P
294 8	Macrolepiota procera var. procera	LDF	-(g)		-(g) +(M,p)	++(M,g,G )	++(M,g,G )	_	LTP
	Amanitaceae								
113 4	Amanita excelsa var. spissa	EMF	-(M,g) +(G,p)	-(M,G)	-(M) +(G)	++(g,G,p)	++(g)	_	LP
239 7	Amanita excelsa var. spissa	EMF	-(M) +(G,p)	+(M,g,G )	-(G) +(M,g)	++(M,g,G ,p)	++(g) +(M,G)	_	LP
107 6	Amanita muscaria var. muscaria	EMF	-(M) +(g,G,p)	-(p) +(M,g,G )	-(M) +(g,G,p)	_	_	_	_
182 9	Amanita muscaria var. muscaria	EMF	-(G,p) +(g)	-(M,G,p ) +(g)	-(G,p) +(M,g)	_	_	_	_

MIT		Foogro	Growth		Decolorization		on	Enzvm	
T	Fungi	up	RBBR	Poly R- 478	Poly S- 119	RBBR	Poly R- 478	Poly S- 119	es
183 4	Amanita muscaria var. muscaria	EMF	-(M) +(G,p)	+(M)	-(M) +(g)	_	_	_	_
183 7	Amanita muscaria var. muscaria	EMF	-(M,G,p ) +(g)	-(M,G) +(g)	-(M,g,G )	_	_	_	_
290 3	Amanita muscaria var. muscaria	EMF	-(M)	+(M)	-(M,G) +(g)	-	_	-	_
246 7	Amanita muscaria var. muscaria	EMF	-(M,G,p ) +(g)	-(M,G) +(g,p)	-(M,g,G )	-	_	-	_
102 9	Amanita rubescens var. rubescens	EMF	-(M) +(g)	-(M,g) +(G)	-(M) +(g)	_	_	_	_
293 1	Amanita rubescens var. rubescens	EMF	-(M) +(g)	-(M) +(g)	-(M) +(g)	_	_	_	_
	Boletaceae								
132 1	Boletus pseudoregius	EMF	-(p)	-(p)	-(p)	++(M,G) +(p)	_	_	Р
106 4	Boletus reticulatus	EMF	-(g)		+(G)	++(M)	-	_	Р
103 1	Leccinellum lepidum	EMF				_	++(G)	_	L
124 6	Leccinellum lepidum	EMF	+(G,p)	+(G,p)	-(M,p) +(g)	++(g) +(p)	++(g) +(p)	_	L
141 8	Leccinellum lepidum	EMF	+(g)	-(M,) +(g,p)	+(G,p)	+(p)	+(p)	_	L
	Bondarzewiac	eae							
113 7	Heterobasidi on annosum	WDF				++(g,G,p)	++(g,p)	_	LP
114 6	Heterobasidi on annosum	WDF				++(g,G,p)	+(M)	_	LP
125 1	Heterobasidi on annosum	WDF		-(p)		++(M,g,G ,p)	+(g)	_	LP
216 0	Heterobasidi on annosum	WDF			-(p)	++(M,g,G )	++(g)	_	LP
216 1	Heterobasidi on annosum	WDF		-(p)	-(G)	++(g,G)	+(g)	++(g)	L

ЛЛТТ		Facaro		Growth	Growth		Decolorization		
T	Fungi	up	RBBR	Poly R- 478	Poly S- 119	RBBR	Poly R- 478	Poly S- 119	es
216 2	Heterobasidi on annosum	WDF		-(p)		++(M,g,G )	+(g)	_	LP
216 3	Heterobasidi on annosum	WDF		-(p)	-(p)	++(M,g,G ,p)	++(g)	++(g)	L
216 4	Heterobasidi on annosum	WDF		-(p)	-(p)	++(M,g,G )	++(g)	++(g)	LP
216 5	Heterobasidi on annosum	WDF		-(p)	-(p)	_	_	_	_
216 6	Heterobasidi on annosum	WDF		+(p)	-(p)	++(M,g,G ,p)	++(g)	++(g)	L
216 7	Heterobasidi on annosum	WDF		-(p)	-(M) +(p)	++(M,g,G )	_	_	L
216 8	Heterobasidi on annosum	WDF			-(p)	++(g,G,p)	++(p)	_	LP
216 9	Heterobasidi on annosum	WDF	-(M)	-(M,p)	-(M,p)	++(M,g,G )	_	_	L
217 0	Heterobasidi on annosum	WDF	-(M)	-(M,p)	-(M)	++(M,g,G ) +(p)	++(M,g,G )	++(g)	LP
307 6	Heterobasidi on insulare	WDF	-(M)	-(p)	-(M,p)	++(g,G)	_	_	LP
	Ceratobasidia	ceae							
723	Abortiporus biennis	WDF				++(M,g,G ,p)	++(M,g,G ,p)	+(G)	LTP
688	Thanatephoru s cucumeris	WDF		+(p)	+(p)	_	_	_	_
738	Thanatephoru s cucumeris	WDF		-(p)	-(p)	-	_	_	_
124 3	Thanatephoru s cucumeris	WDF		+(p)	+(p)	-	_	_	_
266 5	Thanatephoru s cucumeris	WDF		+(p)	+(p)	-	_	_	_
266 6	Thanatephoru s cucumeris	WDF		+(p)	+(p)	-	_	_	-
	Coniophorace	ae							
104 6	Coniophora puteana	WDF				++(M,g,p )	++(M) +(g,G,p)	++(p) +(M)	_
229 8	Coniophora puteana	WDF		+(p)	+(p)	++(M) +(G,p)	+( <b>M</b> ,p)	_	_
247 1	Coniophora puteana	WDF	-(p)	-(p)	+(p)	++(M,g)	++(M)	+(p)	_
	Cortinariacea	e							

MIT		Foogra	Growth			Decolorization			Enzym
T	Fungi	up	RBBR	Poly R- 478	Poly S- 119	RBBR	Poly R- 478	Poly S- 119	es
112 7	Cortinarius violaceus	EMF				++(M) +(g)	++(M)	-	_
	Cyphellaceae								
374	Chondrostere um purpureum	WDF	+(p)	+(p)	+(g,p)	++(M,g,G ,p)	++(g,p) +(M)	+(g,p)	LP
307 5	Chondrostere um purpureum	WDF				++(M,g,G ,p)	++(M,g,G ,p)	++(M,g,G ,p)	L
	Dacrymycetac	eae							
104 8	Calocera viscosa	WDF	-(p)	+(g) -(p)	-(M) +(G)	-	_	-	L
	Entolomatace	ae							
166 1	Clitopilus prunulus	LDF		+(p)	-(G)	++(M,g)	+(G)	-	_
563	Clitopilus sp.	LDF				++(M)	++(M)	++(M)	-
214 5	Entoloma sinuatum	EMF		-(p)		_	++(G,p)	++(g,G)	L
	Fistulinaceae								
303 9	Fistulina hepatica	WDF	+(M,g,G ,p)	+(M,g)	+(M,g,G )	-	_	_	_
	Fomitopsidac	eae							
431	Antrodia sinuosa	WDF		+(p)	+(p)	_	+(p)	++(G)	_
432	Antrodia sinuosa	WDF		+(p)		_	+(p)	++(G)	L
104 2	Fomitopsis pinicola	WDF		-(p)		++(M)	_	-	_
158 4	Fomitopsis pinicola	WDF				++(M)	_	+(G)	-
229 9	Fomitopsis pinicola	WDF				_	_	-	_
290 7	Fomitopsis pinicola	WDF				++(p)	++(p)	_	_
304 1	Fomitopsis pinicola	WDF				++(M,p)	++(p)	_	L
106 1	Ischnoderma benzoinum	WDF	-(g,p)	+(g,p)	-(G) +(g,p)	++(M,g,G ) +(p)	++(M) +(p)	+(p)	LP

МЛТТ		Ecogro	Growth		Decolorization			Enzvm	
T	Fungi	up	RBBR	Poly R- 478	Poly S- 119	RBBR	Poly R- 478	Poly S- 119	es
104 0	Laetiporus sulphureus	WDF				++(M,G,p )	+(M)	_	L
223 6	Laetiporus sulphureus	WDF			+(G)	_	-	-	LP
290 9	Laetiporus sulphureus	WDF	+(g)	+(g)		++(M)	_	+	_
296 2	Laetiporus sulphureus	WDF				++(M)	+(M)	_	Р
305 0	Laetiporus sulphureus	WDF				++(M)	+(M,g)	_	_
308 1	Laetiporus sulphureus	WDF				++(M)	++(M)	-	_
304 0	Laricifomes officinalis	WDF		-(g) +(G)		++(M)	_	-	L
128 7	Phaeolus schweinitzii	WDF	-(M)			_	+(M,g)	_	LTP
214 4	Phaeolus schweinitzii	WDF	-(G)			++(M) +(g,G,p)	+(M,G,p)	-	LP
214 6	Phaeolus schweinitzii	WDF				++(M,p) +(g)	_	_	LT
294 3	Phaeolus schweinitzii	WDF				++(M)	++(M,G)	_	L T P
294 4	Phaeolus schweinitzii	WDF				++(M,g,G ,p)	++(p) +(g,G)	_	L
294 5	Phaeolus schweinitzii	WDF	+(p)	-(p)	+(p)	++(M)	++(M) +(G)	_	ТР
308 0	Phaeolus schweinitzii	WDF		+(p)	-(p)	++(M,p) +(g)	++(g) +(M,p)	-	L
205 3	Piptoporus betulinus	WDF	-(p)	-(p)	-(p)	++(M)	-	_	L
106 7	Postia stiptica	WDF	-(M,G) +(p)	-(M,G,p )	-(M,G,p )	_	-	-	L T P
	Ganodermata	ceae							
105 2	Ganoderma applanatum	WDF				++(M,g,G ,p)	++(p) +(g)	++(p) +(g,G)	LP
230 1	Ganoderma applanatum	WDF				++(M,g,G ,p)	++(M,G,p ) +(g)	_	LP
247 6	Ganoderma applanatum	WDF				++(M,g,G ,p)	++(M,g,G ,p)	++(p)	LTP
251 2	Ganoderma applanatum	WDF				++(M,g,G ,p)	+( <b>M</b> , <b>p</b> )	++(M) +(p)	LP

МТТ		Foogra	Growth			Decolorization			Enzvm
T	Fungi	up	RBBR	Poly R- 478	Poly S- 119	RBBR	Poly R- 478	Poly S- 119	es
304 4	Ganoderma applanatum	WDF				++(M,g,G ,p)	++(M,p)	++(p)	LP
304 6	Ganoderma applanatum	WDF				++(M,g,G ,p)	++(M,p) +(g)	_	LP
307 9	Ganoderma applanatum	WDF				++(M,g,G )	++(M,p) +(g,G)	+(M,g,G)	LP
304 7	Ganoderma applanatum	WDF	-(p)	-(p)	-(p)	++(M,g,G ,p)	++(M,g,p )	++(p)	LP
229 2	Ganoderma lucidum	WDF				++(M,g,G ,p)	++(M,p) +(g)	++(g,p)	LP
104 5	Ganoderma pfeifferi	WDF				++(p)	++(p)	_	L
229 1	Ganoderma pfeifferi	WDF				++(M,g,G ,p)	++(M,p)	_	L
106 3	Ganoderma resinaceum	WDF		+(p)		++(g,p)	++(p)	++(p)	_
247 4	Ganoderma resinaceum	WDF				++(M,g,G ,p)	++(g,p)	++(p)	L
304 2	Ganoderma resinaceum	WDF				++(M,g,G ,p)	++(g,G,p)	++(p)	L
304 3	Ganoderma resinaceum	WDF				++(M,g,G ,p)	++(G,p)	++(p)	L
	Gloeophyllale	S							
229 4	Gloeophyllum odoratum	WDF		-(p)		++(M,g,G ,p)	++(M,g,G ,p)	++(M,g,G ,p)	L
240 1	Gloeophyllum sepiarium	WDF		+(p)	+(p)	++(M,g,G ,p)	++(M,g,G )	++(p)	_
265 4	Gloeophyllum sepiarium	WDF	+(G,p)	-(p) +(G)	+(p)	++(M,p)	++(p)	++(p)	L
247 2	Gloeophyllum trabeum	WDF		-(p)	-(p)	++(M)	++(M)	_	L
	Hydnangiacea	ie							
109 6	Laccaria laccata	EMF	-(g,G,p) +(M)	-(M) +(g)	+(M,g)	++(M)	_	_	LP
112 9	Laccaria laccata	EMF	-(G,p) +(M)	-(M,g) +(p)	+(M,g,p)	+(p)	_	_	LP
	Hygrophorace	eae							
557	Hygrophorus pseudodiscoi deus	EMF	+(p)	+(p)	-(p)	++(M,g)	+(g)	_	LP

Fungi Iymenochaeta Iymenochaet rubiginosa nonotus ispidus	up aceae WDF	RBBR	Poly R- 478	Poly S- 119	RBBR	Poly R- 478	Poly S- 119	es
<b>lymenochaet</b> lymenochaet rubiginosa nonotus ispidus	<i>aceae</i> WDF							
Iymenochaet rubiginosa nonotus ispidus	WDF							
10notus ispidus 10notus				-(M)	++(M)	_	_	_
nonotus	WDF	+(p)	+(p)	-(p)	++(M)	_	-	LP
ispidus	WDF	+(g)	-(g)	-(g)	++(M,g,G )	++(M) +(g,G,p)	_	LTP
10notus bliquus	WDF	-(p) +(g)	-(p) +(g)	-(M,g,p)	_	_	-	-
nonotus amaricis	WDF				++(M,g,G )	++(M,G)	-	-
hellinus ivus	WDF			-(p)	_	_	_	_
Phellinus gniarius	WDF			+(p)	_	_	_	L
Phellinus pini	WDF				++(M,g,G ,p)	_	_	LT
Phellinus omaceus	WDF				++(M,g,G ) +(p)	++(M,g,p )	_	LP
Phellinus omaceus	WDF		-(p)	-(p)	++(M,g,G ) +(p)	++(M,g)	_	LP
Phellinus prulosus	WDF				++(M,g,G ,p)	++(M,g,p )	_	L
Pseudoinonot s dryadeus	WDF				++(M)	++(M) +(p)	_	LP
Pseudoinonot s dryadeus	WDF	-(g,p)	-(p)	-(p)	++(M,g,G ,p)	++(M) +(p)	++(g)	LP
ncertae sedis								
Panaeolus emiovatus ar. emiovatus	CF	-(g)		-(g) +(M)	++(M)	_	_	_
Panaeolus emiovatus ar. emiovatus	CF			-(M)	++(M)	++(M)	_	-
yophyllaceae	?							
Calocybe arnea	LDF	-(M,g,G )	-(M,g) +(G)	-(g) +(M,G)	++(g,G)	+(M,g,G)	-	LP
Calocybe ambosa	LDF	-(M,g,G )	-(g)	-(g,G) +(M)	_	++(g) +(G)	_	Р
	oliquus onotus maricis maricis 'hellinus vus 'hellinus niarius 'hellinus pmaceus 'hellinus pmaceus 'hellinus rulosus seudoinonot dryadeus seudoinonot seudoinonot dryadeus seudoinonot seu	NUPonotus maricisWDFinariusInariusiniovatusCFiniovatusCFiniovatusLDFiniovasLDFiniovasLDF	wDF+(g)onotus maricisWDFinaricisWDFinellinus vusWDFinellinus paceusWDFinellinus paceusWDFinellinus paceusWDFinellinus paceusWDFinellinus paceusWDFinellinus paceusWDFinellinus paceusWDFinellinus paceusWDFinellinus paceusWDFinellinus paceusWDFinaceus paceusWDFinaceolus miovatus panaeolus miovatusCFiniovatus panaeolus miovatus panaeolus miovatusCFiniovatus panaeolus miovatus <b< td=""><td>bliquus<math>WDF</math><math>+(g)</math><math>+(g)</math>onotus<math>WDF</math>maricis<math>WDF</math>hellinus<math>WDF</math>hellinus<math>WDF</math>hellinus<math>WDF</math>hellinus<math>WDF</math>hellinus<math>WDF</math>hellinus<math>WDF</math>hellinus<math>WDF</math>hellinus<math>WDF</math>hellinus<math>WDF</math>hellinus<math>WDF</math>hellinus<math>WDF</math>hellinus<math>WDF</math>hellinus<math>WDF</math>hellinus<math>WDF</math>hellinus<math>WDF</math>hellinus<math>WDF</math>hellinus<math>WDF</math>hellinus<math>WDF</math>hellinus<math>WDF</math>hellinus<math>WDF</math>hellinus<math>WDF</math>hellinus<math>WDF</math>hellinus<math>WDF</math>hellinus<math>WDF</math>hellinus<math>WDF</math>hellinus<math>WDF</math>hellinus<math>WDF</math>hellinus<math>WDF</math>hellinus<math>WDF</math>hellinus<math>WDF</math>hellinus<math>WDF</math>hellinus<math>WDF</math>hellinus<math>WDF</math>hellinus<math>WDF</math>hellinus<math>WDF</math>hellinus<math>WDF</math>hellinus<math>WDF</math>hellinus<math>WDF</math>hellinus<math>WDF</math>hellinus<math>WDF</math>hellinus<math>WDF</math>hellinus<math>HOF</math>hellinus<math>HOF</math>hellinus<math>HOF</math>hellinus<math>HOF</math>hellinus<math>HOF</math>hellinus<math>HOF</math>hel</td><td>biliquus<math>WDF</math><math>+(g)</math><math>+(g)</math><math>-(M,g,p)</math>onotus<math>WDF</math><math>+(g)</math><math>-(M,g,p)</math>maricis<math>WDF</math><math>-(p)</math>frellinus<math>WDF</math><math>+(p)</math>frellinus<math>WDF</math><math>+(p)</math>frellinus<math>WDF</math><math>-(p)</math>frellinus<math>WDF</math><math>-(p)</math>frellinus<math>WDF</math><math>-(p)</math>frellinus<math>WDF</math><math>-(p)</math>frellinus<math>WDF</math><math>-(p)</math>frellinus<math>WDF</math><math>-(p)</math>frellinus<math>WDF</math><math>-(p)</math>frellinus<math>WDF</math><math>-(p)</math>frellinus<math>WDF</math><math>-(p)</math>frellinus<math>WDF</math><math>-(p)</math>frellinus<math>WDF</math><math>-(p)</math>frellinus<math>WDF</math><math>-(p)</math>frellinus<math>WDF</math><math>-(p)</math>frellinus<math>WDF</math><math>-(p)</math>frellinus<math>WDF</math><math>-(p)</math>frellinus<math>WDF</math><math>-(p)</math>frellinus<math>WDF</math><math>-(p)</math>frellinus<math>WDF</math><math>-(p)</math>frellinus<math>WDF</math><math>-(p)</math>seudoinonot<math>WDF</math><math>-(p)</math>seudoinonot<math>WDF</math><math>-(p)</math>frellinus<math>CF</math><math>-(g)</math>miovatus<math>(p)</math><math>+(M)</math>miovatus<math>(p)</math><math>-(p)</math>miovatus<math>(p)</math><math>+(G)</math>frellinue<math>LDF</math><math>-(M,g,G)</math>frellinus<math>(p)</math><math>+(M)</math></td><td>bilquus<math>WDF + (g) + (g) + (g) - (M,g,g) - (P) </math></td><td><math display="block">\begin{array}{cccccccccccccccccccccccccccccccccccc</math></td><td>ofiquus       WDF       <math>+(g)</math> <math>+(g)</math> <math>-(M,g,p)   -</math>         onotus       WDF       <math>+(g)</math> <math>+(g)</math> <math>  -</math>         omotus       WDF       <math>-(p)</math> <math>  -</math>         itellinus       WDF       <math>-(p)</math> <math>  -</math>         itellinus       WDF       <math>-(p)</math> <math>  -</math>         itellinus       WDF       <math>+(p)</math> <math>  -</math>         itellinus       WDF       <math>+(p)</math> <math>  -</math>         itellinus       WDF       <math>-(p)</math> <math>-(p)</math> <math>+(M,g,G)</math> <math>-</math>         invaceus       WDF       <math>-(p)</math> <math>-(p)</math> <math>+(M,g,G)</math> <math>-</math>         invacus       WDF       <math>-(p)</math> <math>-(p)</math> <math>+(M,g,G)</math> <math>-</math>         invalues       WDF       <math>-(p)</math> <math>-(p)</math> <math>+(M,g,G)</math> <math>-</math>         invalues       WDF       <math>-(p)</math> <math>-(p)</math> <math>+(M,g,G)</math> <math>-</math>         invalues       WDF       <math>-(p)</math> <math>-(p)</math> <math>+(M,g,G)</math> <math>-</math>         invalous       WDF       <math>-(g,p)</math> <math>-</math></td></b<>	bliquus $WDF$ $+(g)$ $+(g)$ onotus $WDF$ maricis $WDF$ hellinus $HOF$ hellinus $HOF$ hellinus $HOF$ hellinus $HOF$ hellinus $HOF$ hellinus $HOF$ hel	biliquus $WDF$ $+(g)$ $+(g)$ $-(M,g,p)$ onotus $WDF$ $+(g)$ $-(M,g,p)$ maricis $WDF$ $-(p)$ frellinus $WDF$ $+(p)$ frellinus $WDF$ $+(p)$ frellinus $WDF$ $-(p)$ seudoinonot $WDF$ $-(p)$ seudoinonot $WDF$ $-(p)$ frellinus $CF$ $-(g)$ miovatus $(p)$ $+(M)$ miovatus $(p)$ $-(p)$ miovatus $(p)$ $+(G)$ frellinue $LDF$ $-(M,g,G)$ frellinus $(p)$ $+(M)$	bilquus $WDF + (g) + (g) + (g) - (M,g,g) - (P) $	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	ofiquus       WDF $+(g)$ $+(g)$ $-(M,g,p)   -$ onotus       WDF $+(g)$ $+(g)$ $  -$ omotus       WDF $-(p)$ $  -$ itellinus       WDF $-(p)$ $  -$ itellinus       WDF $-(p)$ $  -$ itellinus       WDF $+(p)$ $  -$ itellinus       WDF $+(p)$ $  -$ itellinus       WDF $-(p)$ $-(p)$ $+(M,g,G)$ $-$ invaceus       WDF $-(p)$ $-(p)$ $+(M,g,G)$ $-$ invacus       WDF $-(p)$ $-(p)$ $+(M,g,G)$ $-$ invalues       WDF $-(p)$ $-(p)$ $+(M,g,G)$ $-$ invalues       WDF $-(p)$ $-(p)$ $+(M,g,G)$ $-$ invalues       WDF $-(p)$ $-(p)$ $+(M,g,G)$ $-$ invalous       WDF $-(g,p)$ $-$

MIT		Foogra		Growth		D	ecolorizati	on	Enzvm
T	Fungi	up	RBBR	Poly R- 478	Poly S- 119	RBBR	Poly R- 478	Poly S- 119	es
295 4	Calocybe gambosa	LDF	+( <b>M</b> , <b>p</b> )	-(G) +(M,p)	+( <b>M</b> , <b>p</b> )	+(g,G)	+(g,G)	+(G)	_
314 9	Calocybe gambosa	LDF		+(g)	+(G)	_	++(G)	_	_
986	Lyophyllum decaste	EMF		-(p) +(g)	+(g,G)	++(M,G) +(p)	_	_	L
292 8	Lyophyllum decastes	EMF	-(g,G,p)	-(p)	+(g,G)	++(M,G) +(p)	_	_	L
293 5	Lyophyllum decastes	EMF		-(p)	+(p)	++(M,G) +(p)	_	_	LP
131 9	Lyophyllum fumosum	EMF				++(M,g,G ,p)	_	_	Т
205 2	Lyophyllum semitale	EMF		-(p)	-(p)	++(M,g,G ) +(p)	++(M)	_	L
	Marasmiacea	е							
246 5	Gymnopus dryophilus	WDF	+(p)	-(g) +(p)	-(g) +(p)	++(M,g,G ,p)	++(M) +(p)	_	LP
250 4	Gymnopus erythropus	WDF	-(p)	-(p)	-(p)	++(M,g,G )	++(M,g,G )	_	_
264 8	Gymnopus erythropus	WDF	-(g)	-(g)	-(G)	++(M,g,G ,p)	++(M,g,G ) +(p)	+(g,p)	LTP
296 9	Marasmius oreades	LDF				++(M,g)	+(M)	_	LP
314 8	Marasmius oreades	LDF				++(M) +(G)	+(g)	_	LP
130 8	Omphalotus olearius	WDF	-(g,p)	+(p)	+(p)	++(M,G)	+(M)	_	L
113 3	Rhodocollybi a butyracea f. butyracea	EMF	+(g,p)	-(g) +(p)	-(g,G)	++(M,g,G ) +(p)	+(G,p)	_	LP
129 6	Rhodocollybi a butyracea f. butyracea	EMF	+(g,p)	+(g)	-(G) +(M)	++(M,g,G ) +(p)	+(g,G)	+(G)	L
250 9	Rhodocollybi a butyracea f. butyracea	EMF	-(p) +(g)	+(g,p)	+(g,G,p)	++(M,G) +(p)	++(g,p) +(M)	+(g,p)	LP
264 9	Rhodocollybi a butyracea f. butyracea	EMF			+(M,p)	++(M,g,G ,p)	++(g,G) +(M,p)	++(g)	LTP
295 5	Rhodocollybi a butyracea f. butyracea	EMF	-(G) +(g)	-(M,G,p ) +(g)	-(M,G)	++(M,g,G ,p)	++(g) +(G)	+(p)	LP

NIT		Foogra		Growth		D	ecolorizati	on	Fnaum
T	Fungi	up	RBBR	Poly R- 478	Poly S- 119	RBBR	Poly R- 478	Poly S- 119	es
295 6	Rhodocollybi a butyracea f. butyracea	EMF		+(g,p)	-(M) +(G)	++(M,G,p )	+(G)	_	LP
296 1	Rhodocollybi a butyracea f. butyracea	EMF	+(g)	+(g)	+(G)	++(M,g,G ) +(p)	+(g,G)	+(g)	LP
	Meripilaceae								
247 9	Meripilus giganteus	WDF	-(g,p)	+(g)		++(M,g,G ,p)	+(g,p)	_	LTP
230 0	Phlebia radiata	WDF				++(M,g,G ,p)	++(M,G,p )	++(M,g,G ,p)	L T P
305 8	Rigidoporus ulmarius	WDF	+(g,p)	-(g,p)	+(g,p)	++(M,g,G ,p)	_	_	L T P
	Meruliaceae								
229 5	Bjerkandera adusta	WDF				++(M,g,G ,p)	++(M,g,G ,p)	++(M,p) +(G)	LP
284 3	Bjerkandera adusta	WDF				++(M,g,G ,p)	++(M,g,G ,p)	++(M,p,G ,g)	LP
306 0	Bjerkandera adusta	WDF				++(M,g,G ,p)	++(M,g,G ,p)	++(M,p,G ) +(g)	LP
	Mycenaceae								
273 0	Mycena galericulata	WDF	-(g,p)	+(p)	-(M,g) +(p)	++(g,G) +(p)	_	_	LP
131 7	Panellus stipticus	WDF	-(p)	+(p)	+(p)	++(M,g,G ,p)	++(g) +(p)	_	LTP
	Paxillaceae								
112 8	<i>Melanogaster</i> sp.	EMF	-(g)	-(g)	-(g) +(G)	_	_	_	_
	Phallaceaea								
230 5	Phallus impudicus var. impudicus	LDF	+(g)	+(g)	+(M)	++(g)	+(g)	_	Т
	Phanerochaet	aceae							
181 0	Ceriporia metamorphos a	WDF				++(M,g,G ,p)	++(M,p)	++(M)	_
166 9	Phanerochaet e	WDF	-(g)	-(g)		_	_	_	LP

MII		Foogra		Growth		D	ecolorizati	on	Fnzum
T	Fungi	up	RBBR	Poly R- 478	Poly S- 119	RBBR	Poly R- 478	Poly S- 119	es
	chrysosporiu m								
	Phanerochaet								
169 1	e chrysosporiu m	WDF				++(M,G)	_	_	LP
	Phanerochaet								
180 4	e chrysosporiu m	WDF				-	++(p)	_	_
	Phanerochaet								
239 4	e chrysosporiu m	WDF				++(M)	++(M,p)	++(M)	L
	Phanerochaet								
266 0	e chrysosporiu m	WDF				++(M,g)	++(M,p)	++(M)	_
105 0	Phlebiopsis gigantea	WDF		-(p)	-(p)	++(M,g,G ,p)	++(g,G,p)	+(p)	LT
107 2	Phlebiopsis gigantea	WDF				++(M,g,G ,p)	++(g,G,p)	+(p)	L
158 5	Porostereum spadiceum	WDF				++(M,g,G )	++(M,g,G ) +(p)	++(M,g,G ) +(p)	LP
	Physalacriace	ae							
105 9	Armillaria mellea	WDF	-(M) +(g,G,p)	+(M,g,G )	-(g) +(G,p)	++(M,g,G )	++(M,G)	_	_
303 6	Armillaria mellea	WDF	-(g)	-(M,g,G )	-(G) +(g)	++(M,G)	_	_	L
303 7	Armillaria mellea	WDF	-(G,p) +(g,)	-(G) +(g)	-(M) +(G)	++(M) +(G)	++(M)	_	L
303 8	Armillaria mellea	WDF	-(G,p) +(g,)	-(M) +(g,G)	+(g,G,)	++(M,G)	_	_	L
113 9	Armillaria tabescens	WDF	-(g) +(M,G,p )	-(p) +(M,g)	+(M. g,G,)	++(M,g,p )	++(M,p)	++(M)	LT
132 7	Armillaria tabescens	WDF	-(M,G,p ) +(g)		-(g) +(M)	++(g,G)	_	_	L T P
166 5	Armillaria tabescens	WDF	+(G,p)	-(g) +(p)	-(G) +(M,g)	_	_	_	_
105 7	Flammulina velutipes var. velutipes	WDF		+(p)	+(p)	++(g,p)	_	_	LP

MIT		Foogra		Growth		D	ecolorizati	on	Enzym
T	Fungi	up	RBBR	Poly R- 478	Poly S- 119	RBBR	Poly R- 478	Poly S- 119	es
264 1	Flammulina velutipes var. velutipes	WDF		-(p)		_	++(M)	_	L
	Pleurotaceae								
215 0	Pleurotus ostreatus	WDF		-(p)	-(p)	++(M,g,G ,p)	++(M,g,G ,p)	++(p)	LP
247 0	Pleurotus ostreatus	WDF				++(M,g,G ,p)	++(M,g,G ,p)	_	LP
297 6	Pleurotus ostreatus	WDF				++(M,g,G ,p)	++(M,g,p )	++(p)	LP
297 7	Pleurotus ostreatus	WDF				++(M,g,G ,p)	++(M,g,G ,p)	++(p)	LP
297 8	Pleurotus ostreatus	WDF				++(M,g,G ,p)	++(M,g,G ,p)	++(g,p)	LP
297 9	Pleurotus ostreatus	WDF				++(M,g,G ,p)	++(M,g,G ,p)	++(g,p) +(M)	LP
	Polyporaceae								
247 8	Daedaleopsis confragosa	WDF		+(p)	+(p)	++(M,g,G ) +(p)	++(g,G)	++(g) +(G)	LP
103 8	Fomes fomentarius	WDF		–(G,p)		++(M,g,p )	++(M) +(p)	_	LP
141 6	<i>Hapalopilus</i> sp.	WDF				++(G) +(g)	++(M) +(g)	+(g)	L
245 1	Lenzites betulina	WDF				++(M,g,G ,p)	++(M,g,G ,p)	++(M,g,p )	L
296 5	Neolentinus lepideus	WDF	-(p)	-(p)	-(p)	++(M)	++(M)	_	LT
188 5	Panus conchatus	WDF	-(p)	-(p)	-(p)	++(M,g,G ,p)	++(M,g,G ) +(p)	++(M,g,G ) +(p)	LP
247 7	Perenniporia fraxinea	WDF		-(p)	-(p)	++(M,g,G )	_	_	LT
305 1	Perenniporia fraxinea	WDF		-(p)		++(M,g,G ,p)	_	_	LT
305 2	Perenniporia fraxinea	WDF		+(p)		++(M,g,G ,p)	++(p)	_	LT
305 3	Perenniporia fraxinea	WDF		+(p)	+(p)	++(M,g,G ,p)	+(p)	_	LT
305 4	Perenniporia fraxinea	WDF		+(p)	+(p)	++(M,g,G ,p)	_	_	LT
308 2	Polyporus ciliatus	WDF				++(M,g,G ,p)	++(M,p)	++( <b>M</b> , <b>p</b> )	L

МЛТТ		Foogra	Growth		Decolorization			Fnzym	
T	Fungi	up	RBBR	Poly R- 478	Poly S- 119	RBBR	Poly R- 478	Poly S- 119	es
218 8	Polyporus squamosus	WDF	-(p)	-(p)	+(p)	++(M,g,G )	++(M,G) +(g)	++(M)	L
223 4	Polyporus squamosus	WDF	-(p)	-(p)	+(p)	++(M,g,G )	++(M,g,G )	++(M,G)	L
239 5	Pycnoporus cinnabarinus	WDF	+(p)			++(M,g,G ,p)	_	_	LP
130 2	Royoporus badius	WDF	+(M)	+(M)	+(M,g,G ,p)	_	+(p)	_	_
104 4	Trametes gibbosa	WDF	+(p)		+(p)	++(M,g,G ,p)	+(M)	++(M)	_
297 4	Trametes hirsuta	WDF			-(p)	++(M,g,G )	++(M,g,G ,p)	_	LP
247 5	Trametes ochracea	WDF				++(M,g,G ,p)	++(M,g,G ,p)	++(M,g) +(p)	LP
240 0	Trametes pubescens	WDF				++(M,g,G ,p)	++(M,g,G ,p)	++(M,p)	LP
105 3	Trametes versicolor –	WDF				++(M,g,G ,p)	++(M,g,G ,p)	_	L
141 5	Trametes versicolor	WDF		-(M)		++(g,G,p)	++(g,p)	_	LP
229 6	Trametes versicolor	WDF	-(M)		-(M,p)	++(M,g,G ,p)	++(g,G,p)	_	LP
247 3	Trametes versicolor	WDF				++(M,g,G ,p)	++(M,g,G ,p)	++(M)	LTP
314 4	Trametes versicolor	WDF		+(p)	+(p)	++(M,g,G ,p)	++(M,g,p )	_	L
275 2	Trametes versicolor	WDF				++(g,G,p)	++(g,G,p)	_	LP
	Psathyrellace	ae							
128 5	Lacrymaria lacrymabund a	LDF			-(p)	_	_	_	LT
128 9	Lacrymaria lacrymabund a	LDF		+(p)	+(p)	++(G)	_	_	LTP
293 8	Psathyrella piluliformis	LDF	-(p)	-(p)		++(M)	_	_	L
	Rhizopogonad	ceae							
251 1	Rhizopogon roseolus	EMF		-(p)	-(p)	++(M,g,G ,p)	++(p)	_	L
	Schizophyllac	eae							

MIT		Faamo	Growth			D	Fnzym		
T	Fungi	up	RBBR	Poly R- 478	Poly S- 119	RBBR	Poly R- 478	Poly S- 119	es
103 7	Schizophyllu m commune	WDF				++(M)	_	_	_
444	Schizophyllu m commune	WDF				++(M)	_	-	L
229 3	Schizophyllu m commune	WDF				++(M)	+(M)	_	_
307 4	Schizophyllu m commune	WDF				++(M)	++(M)	-	L
	Schizoporaced	ie							
430	Schizopora paradoxa	WDF		+(p)	-(p)	++(M,g,G ) +(p)	++(M,g,G ,p)	++(G)	LTP
	Sclerodermato	iceae							
191 6	Scleroderma verrucosum	EMF	+(g,G,p)	-(M,g,G )	+(g,G)	++(p)	_	_	ТР
	Strophariacea	e							
132 6	Agrocybe cylindracea	WDF				++(M,g,G )	+(G)	_	ТР
229 0	Agrocybe cylindracea	WDF			+(p)	++(g,G)	+(G)	-	LTP
251 0	Agrocybe cylindracea	WDF		+(p)		++(g,G)	_	_	LTP
275 3	Agrocybe cylindracea	WDF			-(p)	++(M,g,G )	_	_	LTP
275 4	Agrocybe farinacea	LDF	+(g)	-(p) +(g)	-(p) +(g)	++(M,g,G ) +(p)	++(M,g,G )	++(M,g) +(p)	LP
275 5	Agrocybe farinacea	LDF	+(g)	+(g,p)	+(g,p)	++(M,g,G ) +(p)	++(M,g,G )	++(M,g,G )	LP
296 7	Agrocybe pediades	LDF		-(p)	-(p)	++(M,g,G ) +(p)	++(M,g,G )	_	LTP
296 8	Agrocybe praecox	LDF			-(p)	++(M,g,G ) +(p)	++(M,g,G )	_	LTP
113 1	Hebeloma crustulinifor me	EMF			-(g)	++(M,g)	_	_	Р
217 6	Hebeloma cylindrosporu m	EMF	-(g)		+(G)	_	_	_	Р
217 7	Hebeloma cylindrosporu m	EMF				++(M,g)	_	_	ТР

ЛЛТТ		Faagra	Growth		D	Engum			
T	Fungi	up	RBBR	Poly R- 478	Poly S- 119	RBBR	Poly R- 478	Poly S- 119	es
103 6	Hypholoma fasciculare var. fasciculare	LDF	-(p)	-(p)	-(p) +(M)	++(M,g,G ,p)	++(g,G) +(p)	+(g)	LT
385	Hypholoma lateritium	LDF	+(p)	+(p)	+(M)	++(M,g,G ,p)	++(g,G) +(M,p)	++(M,g) +(p)	_
113 0	Pholiota gummosa	LDF		+(p)		++(M,g,G )	++(g,G)	_	LTP
105 8	Pholiota squarrosa	WDF			-(G)	++(M,g,G )	++(M) +(g)	_	LTP
132 8	Pholiota squarrosa	WDF			+(p)	++(M,g,G ) +(p)	++(M,g,G ) +(p)	++(g)	L T P
239 8	Pholiota squarrosa	WDF	-(p)	+(p)		++(M,g,G ,p)	++(M,g,G ) +(p)	++(g)	L T P
103 0	Rhodocybe truncata	EMF				_	_	_	LP
103 3	Rhodocybe truncata	EMF	+(g)	+(g)	-(g,G) +(M)	++(p) +(G)	_	_	LP
293 4	Stropharia coronilla	LDF	+(M,g)	+(M,g)	-(p) +(M,g)	_	_	_	L
124 1	Stropharia rugosoannula ta	LDF		+(p)		++(M,g,G )	++(g)	_	LTP
275 8	Stropharia rugosoannula ta	LDF	-(M)	-(M) +(p)	-(M)	++(g,G,p) +(M)	++(g,p) +M)	+(p)	L T P
246 8	Stropharia semiglobata	CF		-(M)	-(M)	++(G,p) +(M)	_	_	LP
295 2	Stropharia semiglobata	CF			-(M)	++(g,G) +(p)	+(p)	++(g,p)	LP
295 3	Stropharia semiglobata	CF	+(M)	-(M)	+(M)	++(p)	+(p)	++(p)	LP
306 8	Stropharia semiglobata	CF		-(M) +(p)	+(p)	++(M,g,G ) +(p)	++(M)	_	LP
306 9	Stropharia semiglobata	CF	-(M)	-(M)	+(p)	++(p)	+(p)	_	LP
	Suillaceae								
132 0	Suillus bovinus	EMF	-(g)	-(g)	-(M) +(G)	_	_	_	Т
888	Suillus granulatus	EMF	-(g)	+(g)	-(g) +(M)	_	_	_	-
930	Suillus granulatus	EMF	-(g)	-(g)	-(M,G) +(g)	_	++(M)	_	_

ЛЛТ		Foogra		Growth		D	ecolorizati	on	Fnaum
T	Fungi	up	RBBR	Poly R- 478	Poly S- 119	RBBR	Poly R- 478	Poly S- 119	es
124 8	Suillus grevillei	EMF	-(g)		-(g)	_	_	_	Т
127 8	Suillus grevillei	EMF		-(g)	-(G)	++(M)	_	_	Т
295 7	Suillus grevillei	EMF		-(g)	+(M)	_	_	_	-
775	Suillus grevillei	EMF	-(g)	-(g)	-(M,g,G )	_	+(G)	_	_
121 8	Suillus grevillei	EMF	-(g)	-(g)	-(M,G)	_	_	_	Т
122 7	Suillus grevillei	EMF	-(g)	-(g)	-(G)	_	_	_	L
166 6	Suillus luteus	EMF			+(M)	_	_	_	_
290 8	Suillus luteus	EMF		+(g)	-(G) +(M,g)	_	++(M) +(G)	_	_
264 7	Suillus sibiricus	EMF	-(g)		-(M)	_	_	_	L
293 2	Suillus variegatus	EMF				-	_	_	_
792	Suillus viscidus	EMF		-(g)	-(M)	-	_	_	L
123 3	Suillus viscidus	EMF	-(g)	-(g,p)	-(g)	_	_	_	L
	Tricholomata	ceae							
296 6	Clitocybe ditopa	LDF	-(g,G)	+(G)	-(M) +(G)	++(M,g,G )	+(g,G)	+(g)	LP
125 2	Clitocybe gibba	LDF	+(M,G)	-(M,G)	-(M) +(g,G)	_	_	_	Р
290 4	Clitocybe gibba	LDF	+(M)	+( <b>M</b> , <b>p</b> )	+( <b>M</b> , <b>p</b> )	++(M) +(G,p)	_	_	Р
295 9	Clitocybe gibba	LDF	+(M,g,G )	-(g) +(M,G)	+(M,g,G )	+(M,g)	+(M)	_	L T P
130 1	Clitocybe nebularis	LDF	-(M,g,G ,p)			++(G) +(g,p)	++(g)	_	L
183 1	Clitocybe nebularis	LDF	-(M,g,G ,p)	-(M,p)	-(M,p)	+(M,g,p)	++(g)	_	LT
250 6	Clitocybe nebularis	LDF	-(M,g,G ,p)			++(g)	++(g)	_	L
251 3	Clitocybe nebularis	LDF			-(p)	++(G) +(g,p)	++(g)	_	LT

<b>Т ЛТ</b> Т		<b>F</b>		Growth		D	ecolorizati	on	<b>F</b>
T T	Fungi	up	RBBR	Poly R- 478	Poly S- 119	RBBR	Poly R- 478	Poly S- 119	es es
293 3	Clitocybe phyllophila	LDF	-(M,G)	-(g) +(M)	-(M,g,G )	++(g)	_	_	L
597	Clitocybe phyllophila	LDF	+(M,g)	+(M,g,G )	+(M)	++(M) +(g,G)	++(G) +(g)	++(M)	L
131 8	Lepista densifolia	LDF	-(g,G,p) +(M)	+(M)	-(p) +(M)	+(g,p)	++(g)	+(p)	LP
293 7	Lepista flaccida	LDF	-(M,g,G ,p)	-(M,g,G ) +(p)	-(g,G) +(p)	++(M,g,G ,p)	+(M,g,p)	_	L
297 1	Lepista nuda	LDF	+(M)	+(M)	(g,G,p) +(M)	++(M,g,G ) +(p)	_	_	L
306 7	Lepista nuda	LDF	+(M,g,G ,p)	+(M,g,G )	+(M,g,G )	++(M,g,G )	_	_	L
123 8	Leucopaxillus gentianeus	LDF		-(g,G,p)	-(p)	++(M,g,G ) +(p)	_	_	L
250 7	Leucopaxillus gentianeus	LDF	+(M)	-(M,g,G ,p)	-(g,G,p)	++(M,g,G ,p)	+(G)	_	LT
250 8	Leucopaxillus gentianeus	LDF	-(M) +(g,G,p)	-(M) +(g,G)	-(M) +(g,G,p)	++(M,G,p ) +(g)	_	_	LT
292 9	Leucopaxillus gentianeus	LDF	+(g,G,p)	-(M) +(g,G)	-(M) +(g,G,p)	++(M,G,p ) +(g)	++(p)	_	L T P
296 0	Leucopaxillus gentianeus	LDF		+(p)	+(p)	++(G) +(p)	_	_	LT
293 6	Leucopaxillus gentianeus	LDF	+(M,g,G ,p)	+(M,g,G )	+(M,g,G ,p)	$^{++}(G,p)$ $^{+}(M,g)$	+(p)	_	L T P
314 3	Leucopaxillus macrocephal us	LDF		-(M,g,G ,p)	-(p)	++(M,g,G ,p)	_	_	_
307 0	Leucopaxillus paradoxus	LDF		-(M,g,G )	+(p)	++(M,g,G ,p)	_	_	Т
296 3	Ripartites tricholoma	EMF				++(G) +(M,p)	_	_	L

Growth columns: "+" or "-" indicates increased or reduced growth compared with controls ( $P \le 0.05$ ), respectively. Omission indicates no significant difference. Degradation columns: means of decolorization halos are categorized as follows: "-"  $\le 25$  mm; "+"  $\ge 26$  mm to  $\le 35$  mm; "++"  $\ge 36$  mm

*L* laccases, *T* tyrosinases, *P* peroxidases, *CF* coprophilous fungi, *EMF* ectomycorrhizal fungi, *WDF* wood-decomposing fungi, *LDF* litter-decomposing fungi

*M* malt extract agar (MEA), *G* GN4, *g* GN1, *p* Paterson–Bridge agar (PBA)

#### Culture media composition

The media used in the experiment were: Paterson–Bridge agar (PBA) with C:N ratio of almost 181 (Paterson and Bridge 1994); MEA with C:N ratio of almost 20; GN0.1, GN1, and GN4 with C:N ratio of 91, 10, and 2.5, respectively (modified from Leung and Pointing 2002). GN0.1, GN1, and GN4 contained per liter: 10 g glucose, 2 g KH<sub>2</sub>PO<sub>4</sub>, 0.5 g MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.1 g CaCl<sub>2</sub>·2 H<sub>2</sub>O, 18 g agar, 10 mg biotin and thiamine, 10 ml mineral stock solution, and 0.1, 1, and 4 g ammonium tartrate, respectively. The mineral stock solution contained per liter: 0.5 g MnSO<sub>4</sub>·5H<sub>2</sub>O, 1.0 g NaCl, 0.1 g FeSO<sub>4</sub>·7H<sub>2</sub>O, 0.1 g CoCl<sub>2</sub>·6H<sub>2</sub>O, 0.1 g ZnSO<sub>4</sub>·7H<sub>2</sub>O, 0.01 g CuSO<sub>4</sub>·5H<sub>2</sub>O, 0.01 g AlK(SO<sub>4</sub>)<sub>2</sub>, 0.01 g H<sub>3</sub>BO<sub>3</sub>, and 0.01 g NaMoO<sub>4</sub>·2H<sub>2</sub>O.

#### Decolorization experiments

Experiments were performed by inoculating isolates (5-mm mycelium disk, cut from the edge of a colony actively growing on MEA) on 6-cm-diameter Petri dishes containing different media (PBA, MEA, GN0.1, GN1, and GN4 for model dyes; GN0.1, GN1, and GN4 for industrial dyes) added with each dye to final concentration of 200 ppm. Plates were incubated in the dark at  $25 \pm 1$ °C, and growth (colony diameter) and the whole diameter of decolorization halos were measured regularly during 28 and 14 days for model and industrial dyes experiments, respectively. Uncolored plates for each medium were used as controls for fungal growth, while uninoculated colored plates were used as controls for dye decolorization not dependent on fungal activity (physicochemical bleaching). Each test was performed in triplicate. Significance of differences and correspondence analysis ( $P \le 0.05$ ) within and between growth and decolorization data were assessed by nonparametric Kruskal–Wallis and Friedman statistical tests, respectively (XLSTAT 7.5.2 for Windows<sup>®</sup>; Addinsoft 1995–2008).

#### Qualitative enzymatic assessment

Laccase, tyrosinase, and peroxidase activities were evaluated by means of drop tests performed in triplicate, on 7-day-old cultures on MEA by measuring color formation after 3, 24, and 72 h in presence of different substrates. In detail: for laccase, 0.1 M  $\alpha$ -naphthol, 0.1 M guaiacol, and 0.1 M 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) were used; for tyrosinase, 0.1 M *p*-cresol was used; and for peroxidases, 1% pyrogallol and 0.4% H<sub>2</sub>O<sub>2</sub> were used (Stalpers <u>1978</u>; Johannes and Majcherczyk <u>2000</u>).

### **Results and Discussion**

In this work a wide taxonomical and physiological biodiversity of *Basidiomycetes* preserved at the MUT collection was investigated. The ecological role and potential biotechnological application of the tested isolates were assessed, taking into account production of oxidoreductase enzymes and decolorization of model and industrial dyes.

The results on fungal growth, dye decolorization, and enzyme activities are reported in Table  $\underline{2}$ . Many fungi (35–55%) showed no sensitivity to the toxicity of the dyes. The growth of some isolates (23–27%), in contrast, was inhibited by the dyes on one or more media, even in the presence of dye decolorization. This apparent contradiction could be explained by the formation of highly toxic intermediates of the decolorization process, whose production may be influenced by the medium (Novotny et al. 2001). In some cases (13–23%) presence of the dye was found to stimulate growth, this phenomenon being noted particularly on PBA in presence of Poly S-119 and Poly R-478 (Fig.  $\underline{1}$ ). To the best of our knowledge,

stimulation of fungal growth by dyes has never been reported, and it could be interpreted by attributing to dyes a role as nutritional sources.



Fig. 1 Percentage of isolates not affected, stimulated, inhibited, or presenting contrasting growth effects (either stimulated, inhibited, or not affected, according to the different media) due to presence of the dyes

Concerning decolorization activity, 257 isolates (86%) were active against one or more dyes on at least one medium. No decolorization was observed in control plates. Anthraquinonic dyes were decolorized by a larger number of isolates, with 242 and 198 active isolates towards RBBR and Poly R-478, respectively; 86 isolates decolorized the azoic dye Poly S-119. It is worth noting that 52 isolates completely degraded all tested dyes on at least one medium. Figure  $\underline{2}$  shows the percentages of each ecophysiological group of fungi which efficiently (halo  $\geq$ 36 mm) decolorized the dyes on the different media.



Fig. 2 Percentages of isolates, of each ecophysiological group, able to efficiently decolorize (halo  $\geq$ 36 mm) the dyes on different media. C:N ratio of each medium in parenthesis. *CF* coprophilous fungi, *EMF* ectomycorrhizal fungi, *WDF* wood-decomposing fungi, *LDF* litter-decomposing fungi

Some isolates showed high decolorization capabilities on all media, whereas others showed it on only one or two media. In general, the greater the recalcitrance of the dye (Poly S-119 > Poly R-478 > RBBR), the more restrictive the conditions allowing its decolorization; indeed, decolorization of the azo dye was achieved mostly on one medium only (Fig.  $\underline{3}$ ).





In terms of dye decolorization capabilities and ligninolytic enzyme activities, noteworthy results were observed in all the ecophysiological groups (Fig. <u>4</u>). Of the EMF isolates, 47%, 23%, and 4% were able to decolorize RBBR, Poly R-478, and Poly S-119, respectively. Very good results were obtained using several *Rhodocollybia butyracea* isolates, which were able to decolorize both anthraquinonic and azoic dyes on several media, mostly producing laccases and peroxidases, while *Lyophyllum* spp. and *Leccinum lepidum* decolorized only the anthraquinonic dyes, showing mainly laccase activity. On the contrary, most *Amanita* and *Suillus* isolates showed almost no decolorization and ligninolytic enzyme activities, in contrast with what was previously reported by Gramss et al. (<u>1998</u>), who observed production of tyrosinases, laccases,

and peroxidases by the same genera. Moreover, Cullings et al. (2008) demonstrated activity of laccases and peroxidases in root tips of *Pinus contorta* colonized by *Suillus granulatus*.



Fig. 4 Percentages of isolates, of each ecophysiological group, showing activity for none, one, or more of the tested enzymes. *L* laccases, *T* tyrosinases, *P* peroxidases, *ND* no enzyme activity detected, *CF* coprophilous fungi, *EMF* ectomycorrhizal fungi, *WDF* wood-decomposing fungi, *LDF* litter-decomposing fungi

The species *R. butyracea*, the most active EMF in our study, has never been reported as exhibiting dye decolorization and oxidoreductase activities to date, and hence its capabilities might be exploited from a biotechnological point of view (i.e., reforestation of polluted sites).

Numerous isolates of LDF (83%) were able to decolorize RBBR, compared with 49% that could decolorize Poly R-478, and a lower proportion (9%) that could decolorize Poly S-119. Most of them showed activity of all tested enzymes (25%), laccases and peroxidases (14%), or laccases only (25%). Moreover, great variability of decolorization efficiency was found among isolates.

Interestingly, less efficient decolorization was observed on PBA, the medium characterized by the highest C:N ratio. This result could be explained by the fact that LDF, in contrast to WDF, are adapted to environments with higher N content. Actually, LDF are considered the ecophysiological group responsible for so-called white-rot of humus (Hintikka 1970), and their role in decomposition of leaf litter and forest-floor materials has already been pointed out (Osono 2007). In this screening, among the LDF fungi, the best results were obtained with isolates belonging to *Agrocybe farinacea*, *Hypholoma* spp., *Clitocybe phyllophyla*, and *Lycoperdon perlatum*, which were very effective towards both anthraquinonic and azoic dyes.

Recently, the potential application of LDF in soil bioremediation for degradation of recalcitrant organopollutants has been demonstrated (Steffen et al. 2007). Moreover, their abilities to colonize the soil, survive there over long periods, and compete with other microorganisms should also be considered as ecological feature that can make them even more suitable for bioremediation applications compared with WDF, which usually prefer to colonize compact woods (logs, trunks, etc.) and have poor capability to grow in different niches such as soil (Sasek et al. 2003; Steffen et al. 2007).

All CF were able to decolorize the anthraquinonic dyes, in particular RBBR, while the azoic Poly S-119 was decolorized by only two *Stropharia semiglobata* isolates; the main enzyme activities detected were laccases and peroxidases. To the best of our knowledge, the CF *S. semiglobata* has never been reported before as exhibiting dye decolorization and oxidoreductase capabilities. The genus *Stropharia* encompasses also LDF species; among them *S. rugosoannulata* was able to produce all the tested enzymes and to decolorize both the anthraquinonic and azoic dyes. This species, together with *S. coronilla*, which in our study resulted ineffective, has already been reported for ligninolytic enzyme production and efficiency in degradation of PAHs (Steffen et al. 2007). Among CF, *Coprinus* spp. have recently been signaled for peroxidase activities which enable them to catalyze aromatic peroxygenation (Anh et al. 2007) and remove phenolic and other aromatic compounds from industrial wastewaters (Ikehata and Buchana 2002).

The WDF ecophysiological group was confirmed to be the most efficient in terms of dye decolorization. Of 159 isolates, 87%, 61%, and 32% extensively decolorized RBBR, Poly R-478, and Poly S-119, respectively; these degradations were generally coupled with the tested enzyme activities, alone or in combination. On the contrary, 14% of isolates from 159 WDF which were effective toward the model dyes did not show any enzyme activity.

In recent decades, a plethora of studies have been published on dye degradation by WDF (Barrasa et al. 2009; Bumpus 2004; Hernandez-Luna et al. 2008; Jarosz-Wilkolazka et al. 2002a, b; Novotny et al. 2001, 2004; Tekere et al. 2001). However, only a few species (*Phanerochaete chrysosporium*, *Pleurotus* spp., *Bjerkandera adusta*, *Irpex lacteus*, and *Trametes* spp.) have been investigated in detail to clarify the enzymes involved and the culture conditions needed for dye decolorization (Faraco et al. 2009; Forgacs et al. 2004; Gavril and Hodson 2007).

In our case, among the most effective isolates, some are ascribable to commonly investigated species, i.e., *Bjerkandera adusta*, *Phlebia radiata*, *Pleurotus ostreatus*, and *Trametes versicolor*. However, this extensive screening that embraced a wide biodiversity allowed us to highlight the remarkable decolorization capabilities of novel species that have been neglected to date, such as *Cyathus stercoreus*, *Chondrostereum purpureum*, *Ganoderma applanatum*, *Gleophyllum odoratum*, *Lenzites betulina*, *Panus conchatus*, *Poliporus ciliatus*, *P. squamosum*, and *Porostereum spadiceum*. These results emphasize the importance of extending screening for dye decolorization capabilities to as-yet-unconsidered species. Moreover, the high variability in dye decolorization capability observed between different species of the same genus, and often between different isolates of each species in screening experiments.

According to the results presented above, 25 isolates belonging to 18 species and 15 genera of WDF and LDF were selected for further experiments on industrial dye decolorization (Table <u>3</u>). The decolorization of model dyes can be considered only predictive of the applicability of fungi in bioremediation processes and, as already stressed by Lucas et al. (2008), cannot be considered without verification of effectiveness in decolorization of industrial dyes. Indeed, industrial dyes, even with apparently similar structure, differ from model dyes in terms of chromophore purity, presence of auxiliary compounds, and recalcitrance. Moreover, to determine the best conditions allowing the isolates to exhibit their decolorization capabilities, three synthetic media differing only in N amount were used, replacing PBA which proved to be unsuitable with the model dyes, with GN0.1.

Table 3 Number of industrial dyes efficiently decolorized (halo  $\geq$ 36 mm) within 14 days by the best selected isolates, and their enzyme activities

MUT	Species	GN4 (2.5)	GN1 (10)	GN0.1 (91)	Sum	Enzymes	Eco
2295	Bjerkandera adusta	9	9	9	27	LP	WDF
2843	Bjerkandera adusta	9	9	9	27	LP	WDF
3060	Bjerkandera adusta	9	9	9	27	LP	WDF
2294	Gloeophyllum odoratum	8	8	9	25	L	WDF
2451	Lenzites betulina	7	7	9	23	L	WDF
2400	Trametes pubescens	6	7	8	21	LP	WDF
2473	Trametes versicolor	7	6	8	21	LTP	WDF
2976	Pleurotus ostreatus	5	7	8	20	LP	WDF
3075	Chondrostereum purpureum	7	7	6	20	L	WDF
1585	Porostereum spadiceum	7	7	5	19	LP	WDF
2300	Phlebia radiata	4	7	7	18	LTP	WDF
2977	Pleurotus ostreatus	5	5	5	15	LP	WDF
2979	Pleurotus ostreatus	5	4	6	15	LP	WDF
2978	Pleurotus ostreatus	4	5	5	14	LP	WDF
1122	Cyathus stercoreus	4	4	5	14	L T P	WDF
2171	Cyathus stercoreus	3	3	7	13	L T P	WDF
3082	Polyporus ciliatus	4	3	4	11	L	WDF
1885	Panus conchatus	2	2	6	10	LP	WDF
2755	Agrocybe farinacea	3	3	4	10	LP	LDF
3044	Ganoderma applanatum	1	4	3	8	LP	WDF
2754	Agrocybe farinacea	2	2	3	7	LP	LDF
385	Hypholoma lateritium	0	1	5	6	_	LDF
2968	Agrocybe praecox	1	1	4	6	L T P	LDF
2234	Polyporus squamosus	0	1	1	2	L	WDF
2398	Pholiota squarrosa	0	0	0	0	LTP	WDF

*L* laccases, *T* tyrosinases, *P* peroxidases, *Eco* ecophysiological groups, *CF* coprophilous fungi, *EMF* ectomycorrhizal fungi, *WDF* wood-decomposing fungi, *LDF* litter-decomposing fungi

The three *B. adusta* isolates were able to decolorize all dyes on all media, showing high effectiveness coupled with significant physiological versatility (Table <u>3</u>). These results are consistent with the work of Nordström et al. (<u>2008</u>) showing the decolorization potential of *Bjerkandera* sp. toward several dyes regardless of N content, and this aspect is crucial with a view to application, since industrial effluents usually contain a range of different dyes and have very complex and variable composition (Lucas et al. <u>2008</u>).

With the exception of the *Bjerkandera* isolates, the medium composition strongly affected the decolorization capability of many strains, as already reported for the model dyes; GN0.1 was shown to be almost always the most suitable medium (Table <u>3</u>). Actually, it is well known that depletion of nutrients, including the N source, triggers production of ligninolytic enzymes (Kaal et al. <u>1993</u>) and, hence, the degradation potentialities of fungi.

In addition to the *Bjerkandera* isolates, remarkable degradative capabilities toward a broad spectrum of dyes were observed for the WDF *G. odoratum*, *L. betulina*, *T. pubescens*, and *T. versicolor*. On the contrary, other isolates, including also all the LDF selected, did not confirm the brilliant performances obtained with model dyes when tested against industrial dyes (i.e., *P. squamosus*, *Agrocybe* spp., *G. applanatum*, and *H. sublateritium*), and hence they might be less suitable for textile wastewater treatment.

Considering the number of isolates able to decolorize the industrial dyes efficiently (Table <u>4</u>), it was possible to establish a dye recalcitrance order, namely:

R243 > B41 > R80 > B214 > B81 > R111 > B113 > B49 = B225. In general, the azo and the phthalocyanin dyes resulted more recalcitrant than the anthraquinonic ones, confirming the results obtained against model dyes. Our results are in accordance with those reported by other authors (Chander and Arora 2007; Chander et al. 2004; Jarosz-Wilkolazka et al. 2002a; Lucas et al. 2008; Novotny et al. 2001; Pointing and Vrijmoed 2000) and might be correlated with the presence of azo-bonds in the dye molecules. Since these chemical groups are rarely present in nature, microorganisms have not often been exposed to compounds containing azo-bonds; thus selective pressure might not have been efficient enough to select microorganisms with effective pathways to degrade azoic compounds (Bumpus 2004).

Table 4 Number of isolates able to decolorize efficiently (halo  $\geq$ 36 mm) the different industrial dyes within 14 days on three media (GN4, GN1, and GN0.1) differing in C:N ratio (in parenthesis)

B49	Reactive (anthraquinonic)	22	21	25
B225	Acid (anthraquinonic)	22	22	24
B113	Acid (azoic)	20	20	24
R111	Acid (azoic)	16	15	19
<b>B</b> 81	Direct (azoic)	11	13	20
B214	Reactive (phthalocyanin)	6	10	10
R80	Reactive (azoic)	6	10	12
B41	Direct (azoic)	7	7	11
R243	Reactive (azoic)	7	6	10

#### Chemical class (chromophore) GN4 (2.5) GN1 (10) GN0.1 (91)

## Conclusions

From the results obtained in this work several conclusions can be drawn:

- Oxidative enzyme activity is widespread in all the tested ecophysiological groups, and laccases were the most commonly detected enzymes;
- The WDF ecophysiological group had the highest number of active strains in terms of dye decolorization, and the degradation activity of several genera and species of this group is reported for the first time herein;
- The most active LDF and EMF in terms of dye decolorization (*Agrocybe farinacea*, *Hypholoma* spp., *Clitocybe phyllophyla*, *Leucopaxillus perlatum*, and *Rhodocollybia butyracea*), although usually less efficient than WDF, might be potentially suitable for soil bioremediation applications, being naturally adapted to colonize soil and litter layers in forest and grassland, and to compete with other microorganisms;
- From an application point of view the capability of the three best selected isolates (*B. adusta*) to survive and grow in presence of high concentration of toxic molecules, i.e.,

dyes, is an adaptive feature that, together with their degradation capacity and physiological versatility (high degradation capability over a wide range of C:N ratio), makes them very promising candidates for application in bioremediation processes.

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