Pseudallescheria angusta, A LIGNINOLYTIC MICROORGANISM FOR WOOD FIBRES BIOMODIFICATION

Gema Guisado, Maria J. López,* M. Carmen Vargas-García, Francisca Suárez-Estrella, and Joaquin Moreno

Nowadays, the discovery of lignocellulolytic microorganisms that are better adapted to operational conditions while exhibiting the strong degrading activities is highly desired for successful lignocellulose biotransformation processes. In this study, microorganisms were isolated from lignocellulose-rich composting materials by selective methods. A screening of isolates known to have lignocellulolytic abilities was performed using several tests. Seven microorganisms showed ligninolytic potential and were subjected for further analysis according to their degrading activity. The fungus *Pseudallescheriaangusta* MF4 demonstrated high decolorization rates for three aromatic dyes: Poly R-478, Poly S-119, and Remazol Brilliant Blue R. In addition, the fungus showed a high production rate of ligninolytic enzymes in the presence of inducers. This fungus achieved the highest values of growth after 21 days of incubation on sawdust without any additional nutrients. Owing to its proven ligninolytic activity and capability of growing on a lignocellulosic substrate, the application of this isolate could be of interest in different biotechnological applications, particularly in biological treatment of wood fibres in order to improve the production of wood-based composites.

Keywords: Lignocellulose; Laccase; Lignin peroxidase; Manganese peroxidase; Dye; Pseudallescheria angusta

*Contact information: Dpto. Biología Aplicada, Área de Microbiología. CITE II-B, University of Almería, La Cañada de San Urbano, 04120 Almería, Spain; * Corresponding author: mllopez@ual.es*

INTRODUCTION

Lignocellulolytic microorganisms produce cellulases, hemicellulases, pectinases, and/or ligninases that are able to hydrolyze plant cell walls. Although many different types of microorganisms are capable of degrading and utilizing cellulose and hemicellulose as carbon and energy sources, a much smaller group of microorganisms, especially fungi, have evolved the ability to break down lignin. Different groups of fungi have been reported as producers of ligninolytic enzymes (Sánchez 2009), but white-rot fungi have received extensive attention due to their powerful production of these enzymes and their unique ability to efficiently degrade lignin to CO_2 (Hofrichter 2002). Such an extent of degradation is due to the strong oxidative activity and low substrate specificity of their ligninolytic system, which is primarily comprised of laccase (Lac), lignin peroxidase (LiP), and manganese peroxidase (MnP) (Martínez et al. 2009). As a

result of their non-specific catalytic mechanisms and lignin-modifying enzymes, the ligninolytic microorganisms have huge potential in a wide range of biotechnological applications. As a matter of fact, research is currently focused on the development of low-cost and eco-friendly decontamination processes for pesticides, dyes, and many other recalcitrant pollutants (Rodríguez-Couto 2009).

Research is also focused on the biotransformation of residual lignocellulosic materials into value-added products (Sánchez 2009). Thereby, lignocellulolytic micro-organisms could be applied in the biological modification of wood fibres to make them compatible with manufacture of composites. This bioprocess can be considered an environmentally friendly alternative to chemical treatment, since the amount of harmful solvents and chemicals required for chemical treatment might be reduced by means of implementing a biotechnological step in the industrial production of wood-based composites (Hüttermann et al. 2001). Lignocellulolytic microorganisms are the most suitable source for that purpose because they posess degrading enzymes, which enable them to disrupt the lignocellulose matrix and decompose cellulose, hemicellulose, and lignin (Malherbe and Cloete 2002). Additionally, ligninolytic enzymes release a large number of reactive groups into lignin, namely, free cation radicals and carboxyl and phenolic hydroxyl groups that activate the surface of wood particles (Widsten and Kandelbauer 2008). These reactive groups are expected to improve cross-linking between lignin and adjacent fibres ,thereby enhancing the mechanical properties of fibre boards (Kharazipour et al. 1997)

. One important feature of lignin-modifying microorganisms that must be borne in mind while developing a sustainable bioprocess is that, even though the ligninolytic system is frequently produced during fungal secondary metabolism, microorganisms can produce an array of enzymes depending on cultivation conditions (Gianfreda et al. 1999). Large differences can be found in the efficiency of delignification by microorganisms according to the substrate. For example, Kadimaliev et al. (2003) found that the fungus *Panus tigrinus* grew better and consumed more readily the lignin in birch than in pine while cultivated on a solid phase. Hence, most reported processes begin with a screening of microorganisms for the specific wood modification to be performed (Unbehaun et al. 2000). Given that microorganisms can show different behaviours according to cultivation conditions, besides the determination of the desired degrading capabilities, it would be essential to check their ability to grow on the lignocellulosic substrate that is to be transformed.

In this study, ligninolytic microorganisms were isolated from a lignocellulose-rich environment (a composting pile), and different tests were carried out to assess their potential on the modification of lignocellulose, especially for their application during manufacture of wood-based composites. In order to select microorganisms that could be useful for disrupting the lignocellulosic matrix and for modifying the lignin surface, diverse lignocellulolytic qualitative assays were applied. Further selection was accomplished by measuring dye-decolorizing potential, ligninolytic enzyme production, and growth capacity on wood fibres with low nutrient content.

EXPERIMENTAL

Isolation of Lignocellulolytic Microorganisms

Selective techniques for enrichment and isolation of mesophilic and thermo-tolerant lignocellulolytic microorganisms from lignocellulose-rich compost piles were developed. A sequential enrichment was performed on liquid media consisting of Mineral Basal Medium (MBM) (Janshekar et al. 1982) supplemented with the adequate enzyme substrate as carbon source: 0.5% (w/v) microcrystalline cellulose, 0.5% (w/v) oat spelt xylan, and 0.1% (w/v) alkaline kraft lignin or 0.06% (w/v) ferulic acid for cellulolytic, hemicellulolytic, ligninolytic, and ferulic degrading microorganisms, respectively. Cultures were shaken at 120 rpm at 30 or 40º C for mesophilic or thermotolerant microorganisms. After seven days, 5 mL of each culture was transferred into fresh medium. This process was repeated two times using an incubation period of 14 days and, subsequently, microorganisms from enriched cultures were isolated on several specific solid media according to their degrading capability. Cellulolytic and ligninolytic microorganisms showed decolorization around growth zones on cellulose- (Kauri and Kushner 1988) and Poly R-478- (Freitag and Morrell 1992) containing agar plates, respectively, whereas a halo around colonies demonstrated xylanolytic activity on xylan-containing medium (AMX: 5 g of oat spelt xylan and 20 g of bacteriological agar per L of MBM). Ferulic degrading microorganisms were those strains capable of growing on ferulic-containing agar plates (0.6 g ferulic acid and 20 g bacteriological agar per L of MBM).

Selection of Microorganisms that are Useful for Modifying Lignocellulosic Fibres

Isolates obtained using the previously indicated protocol were subjected to different tests with the aim of selecting microorganisms able to modify lignin in wood, preferentially. These tests included the following:

Qualitative tests related to lignocellulose biotransformation

Several qualitative tests related to lignocellulose biotransformation were applied to determine whether isolates were able to degrade cellulose and/or hemicellulose, instead of lignin. Microorganisms were tested for xylanolytic activity on AMX plates, while ligninolytic and cellulolytic activities were detected by the Cellulose Azure test (Thorn 1993). Additionally, the Sundman and Näse test (Sundman and Näse 1971) for ligninolytic ability and different plate assays for rapid detection of several enzymes related to lignin degradation (laccase, peroxidase, tyrosinase, and extracellular oxidase) (Rayner and Boddy 1988) were performed.

Decolorization of aromatic industrial dyes

Dye decolorization by isolated microorganisms was tested in 250 mL Erlenmeyer flasks containing 25 mL of DECOL medium composed of (L^{-1}) 10 g glucose, 5 g peptone, 2 g yeast extract, and 70 mL of trace element solution (Kirk et al. 1986) supplemented with 200 mg/L of Poly R-478, Poly S-119, or Remazol Brilliant Blue R (RBBR). These media were inoculated with a 1 cm^2 agar plug from a fungal culture in

Nutrient Agar incubated for 72-96 h, or with 0.25 mL of a 24-h-incubated bacterial culture in Nutrient Broth (Cultimed, PanreacQuímica S.A., Barcelona, Spain). Controls consisting of uninoculated flasks were also prepared for comparison. Cultures were incubated for seven days shaken at 120 rpm and at a temperature of 30 or 40º C for mesophile or thermotolerant strains, respectively. Decolorization of each dye was followed by monitoring changes in the absorption spectrum of supernatants (obtained after centrifuging cultures at 13,000 g for 5 min at 4º C) at 520, 473, and 595 nm for Poly R-478, Poly S-119, and RBBR, respectively, using a Shimadzu UV-160A spectrophotometer (Shimadzu Corporation, Kyoto, Japan). After monitoring, the results were compared to those of the respective controls. Triplicate flasks were used for controls and for each isolate and dye.

Production of ligninolytic enzymes

Ligninolytic enzyme production was evaluated in DECOL medium dosed with either 1 mM ABTS, 2.5 mM veratryl alcohol, 2.2 mM ferulic acid, 3.4 mM guaiacol, 0.05 % (w/v) Tween 80, or 0.1% (w/v) alkali lignin to act as inducers of ligninolytic activity. Inoculation and culture conditions were the same as those indicated for the decolorization tests. Ligninolytic enzyme production was analyzed in supernatants obtained after centrifugation for 5 min at 4ºC to yield 13,000 g. Triplicate flasks were used for controls (without any inducer) and for each isolate and inducer.

The ligninolytic enzymes analyzed were LiP, MnP, and Lac. LiP was determined by the peroxide-dependent oxidation of 2 mM veratryl alcohol to veratraldehyde (ε_{310} 9.3 mM^{-1} cm^{-1}) in 25 mM tartrate buffer, pH 2.5 with 0.4 mM H_2O_2 (Orth et al. 1993). MnP was assayed by oxidation of Mn^{2+} to Mn^{3+} and formation of a Mn^{3+}–tartrate complex (ε_{238} 6.5 mM^{-1} cm^{-1}) from 0.1 mM $MnSO_4$ in 100 mM sodium tartrate buffer at pH 5 with 0.1 mM H_2O_2 (Camarero et al. 1999). Lac was analyzed by monitoring the oxidation of 0.02 mM syringaldazine (ε_{525} 65 mM^{-1} cm^{-1}) in 100 mM citrate–phosphate buffer at pH 5.2, a method modified from Fakoussa and Frost (1999).

Growth on wood fibre

Selected microorganisms were incubated in non-sterile solid substrate cultures of sawdust (from pine, *Pinus pinaster*, and jatoba *Hymenaea courbaril*). Initial moisture of this substrate was adjusted to 60% (w/v) and no additional nutrients were added. Flasks (500 mL) with 50 g of sawdust were inoculated with the microbial biomass suspended in enough distilled water to reach the desired water content. Microbial biomass for inoculation was obtained from 7-day-old cultures on Potato Dextrose Broth (Scharlab S.L., Barcelona, Spain) and 48 h cultures on Nutrient Broth for fungi and bacteria, respectively. Cultures were incubated at 30º C for a maximum of 21 days. Growth of the inoculated microorganism was monitored at 0, 15, and 21 days of incubation by plate colony counting. Triplicate flasks were used for each isolate.

Identification of Selected Microorganisms

The selected fungi were identified by molecular (analysis of 26S rDNA with NL1 and NL2 primers) (Kurtman and Robnett 1998) and physiological and morphological

techniques (Domsch et al. 1980). The bacteria were identified by molecular method (16S rDNA) (White and Cotta 2001).

Chemicals

All chemicals used for culture media, determination of different enzymes, and qualitative tests were purchased from Sigma-Aldrich Co. (St. Louis, Missouri, USA).

RESULTS AND DISCUSSION

Selective enrichment and isolation techniques led to the isolation of 269 ligno-cellulolytic mesophile and thermotolerant microorganisms. Amongst the collection, a total of 38 microorganisms, mostly fungal strains, demonstrated ligninolytic activity in Cellulose Azure and/or in the Sundman and Näse test. Regarding rapid plate assays, most of these strains showed extracellular oxidase (O) or peroxidase (P) activity of 84 and 89%, respectively, but tyrosinase (T) or laccase (L1 and L2) were only detected in 16% and 55% of the isolates, respectively. These rapid tests were applied in addition to the Cellulose Azure and Sundman and Näse tests to ensure the detection of ligninolytic activity, owing to the lack of a specific physiological or biochemical marker exclusively related to lignin degradation (Falcón et al. 1995). Moreover, 84% of these ligninolytic isolates additionally showed cellulolytic and/or hemicellulolytic ability, which is a common feature of white and soft rot fungi (Thorn 1993).

Table 1. Qualitative Tests for Lignocellulolytic Activities*

Isolates**	Identification***	Cellulose-Azure		S&N (L)	Rapid plate assays					AMX
		Lig	Cel		O	L1	L2	T	P	
MB3	*Comamonas acidovorans* (AF181575, 99%)	-	-	+	+	-	+	+	+	-
MF4	*Pseudallescheria angusta* (AF275538, 99%)	+	-	+	+	+	-	-	+	+
MF14	*Aspergillus versicolor* (AF433108, 100%)	-	-	+	+	+	-	-	+	+
MF19	*Scopulariopsis brevicaulis*	-	-	+	-	-	-	-	+	+
MF20	*Aspergillus flavus*	-	-	+	+	+	-	-	-	-
MF22	*Torulomyces/Scopulariopsis*	-	-	+	+	-	-	-	+	+
MF23	*Penicillium chrysogenum*	+	-	+	+	+	+	+	+	+
MF25	*Penicillium*sp.[e]	+	-	+	-	-	-	-	-	+
* AMX, xylanolytic activity; Cellulose Azure test, ligninolytic (Lig) and cellulolytic (Cel) activity; Sundman and Näse test (S&N), ligninolytic (L) activity; rapid plate assays, laccase (L1 and L2), peroxidase (P), tyrosinase (T) and extracellular oxidase (O). **+:** detected; **-:** not detected										
** M: Mesophile; B: Bacterium; F: Fungus										
***Identification: By molecular techniques (accession number and percentage of similarity), By physiological and morphological techniques										

Microorganisms showing ligninolytic but not cellulolytic activity were selected for further experiments and identified (Table 1). These included a set of seven mesophilic fungi and one mesophilic bacterium exhibiting the reported activity.

Ligninases are useful tools for the decolorization of dye contaminated effluents and also for bioremediation of other certain xenobiotics. Their non-specific attack mechanisms allow them to react with a wide range of molecules (Raghukumar et al. 2008). Because of the high correlation between ligninase activity and decolorization, the latter has been often used to quantify the ligninolytic efficacy of microorganisms (Wesenberg et al. 2003). Decolorization ability of the eight selected isolates is summarized in Fig. 1. *Pseudallescheria angusta* (MF4), *Aspergillus flavus*(MF20), and *Penicillium chrysogenum* (MF23) achieved a decolorization efficiency higher than 90% for polymeric dyes Poly R-478 and Poly S-119, and higher than 45% for RBBR (azo-type) (Fig. 1). Decolorization levels developed by these mesophilic fungal strains were, in most of the cases, similar or even better than those obtained employing other fungi and incubating for longer periods of time. In fact, Bending et al. (2002) reported*in vivo* decolorization efficiencies between 77% and 95% for Poly R-478 on 42 day-old cultures of diverse white rot Basidiomycetes. On the other hand, *Coprinellus xanthothrix* demonstrated only 28% decolorization of Poly R-478 after 25 days of incubation (Dritsa et al. 2007). The ligninolytic bacterial strain *Comamonas acidovorans* (MB3) was able to perform more than 50% decolorization in case of Poly S-119 (Fig. 1). A similar extent of decolorization was achieved in seven days for Poly R-478 by the ligninolytic marine cyanobacterium *Oscillatoria willei* BDU 130511 (Saha et al. 2010).

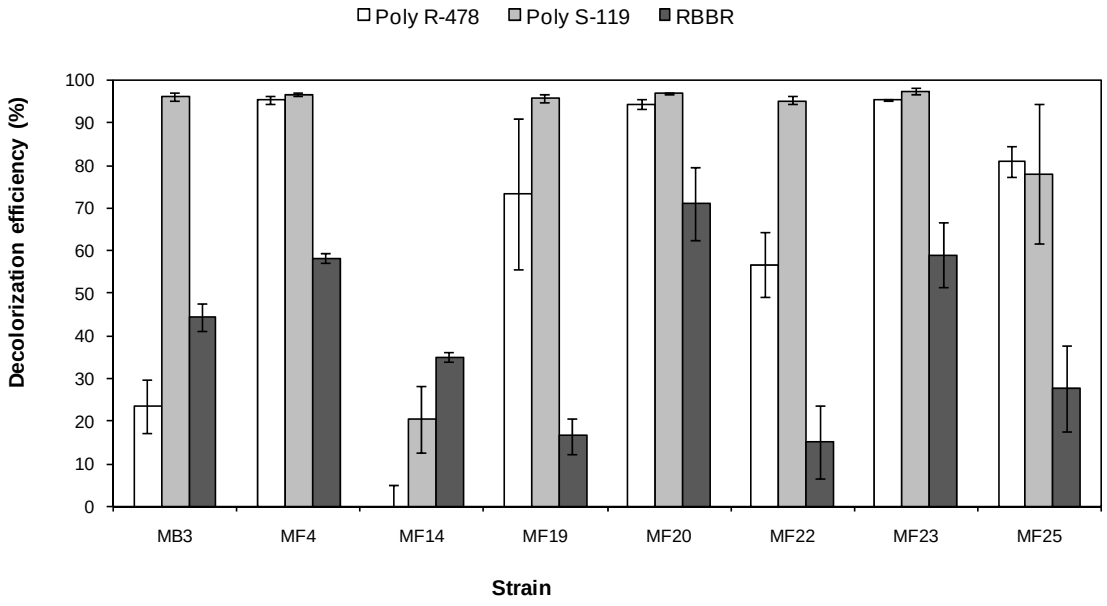

Fig. 1. Decolorization of industrial dyes Poly R-478, Poly S-119 and RBBR. Error bars represent standard deviation (n=3). MB3, *Comamonas acidovorans;* MF4, *Pseudallescheria angusta;* MF14, *Aspergillus versicolor;* MF19,*Scopulariopsisbrevicaulis;* MF20,*Aspergillus flavus;* MF22, *Torulomyces/Scopulariopsis;* MF23, *Penicillium chrysogenum;* MF25, *Penicillium* sp.

Despite the good results obtained for decolorization, only two fungi exhibited detectable levels of ligninolytic enzymes in a liquid medium. It is known that Poly R-478 decolorizing fungi can be divided into three main groups: those with strong MnP and LiP activity or those with exclusively strong MnP, or exclusively strong Lac activity (De Koker et al. 2000). Surprisingly, amongst the best Poly-R decolorizing microorganisms used in this study, only *P. angusta* MF4 and *Penicillium* sp. MF25 showed enhanced ligninolytic enzymes production when cultured on liquid media dosed with certain inducers of lignin-degrading enzymes (Table 2). Lac production was barely stimulated under the assay conditions. Although lignin model compounds have been described as strong inducers of Lac production (Koroljova-Skorobogat´ko et al. 1998), Lac activity was only slightly increased by the effect of Kraft lignin in cultures of *P. angusta* MF4. This fungal strain produced higher levels of LiP and MnP on media containing guaiacol and ABTS, respectively, compared with those obtained in the absence of inducer. Aromatic compounds such as guaiacol, ABTS, veratryl alcohol, or ferulic acid have been widely employed to improve the production of ligninolytic enzymes by several fungal species (Jaouani et al. 2006). In this study, a stimulating effect on LiP production by *Penicillium* sp. MF25 due to veratryl alcohol and, especially to ferulic acid, was observed. Moreover, Tween 80 is known to facilitate the secretion of ligninolytic enzymes (Rodríguez-Couto et al. 2004), its effect also enhanced LiP production by *Penicillium* sp. MF25 (Table 2).

Table 2. Ligninolytic Enzyme Production: Effect of Enzyme Inducers

Inducer	Enzymatic activity (U/mL: nmol/mL min)*					
	Lac		LiP		MnP	
	MF4	MF25	MF4	MF25	MF4	MF25
No inducer	2.47 c	0.00 a	0.00 a	0.00 a	0.00 a	10.02 g
ABTS	2.06 c	0.00 a	0.00 a	2420 cd	1383 b	5.02 f
Ferulic acid	0.03 a	0.00 a	0.00 a	3411 e	0.36 a	2.37 d
Guaiacol	0.00 a	0.00 a	1944 b	1841 b	4.62 a	0.60 b
Veratryl alcohol	1.43 b	0.00 a	0.00 a	2046 bc	0.00 a	0.00 a
Tween 80	3.93 d	0.00 a	0.00 a	2509 d	0.00 a	3.55 e
Alkaline kraft lignin	13.13 e	0.00 a	0.00 a	1716 b	0.62 a	1.42 c
* Values in columns with same letters (a-g) are not significantly different at 95% confidence						

Since lignocellulolytic selected strains are expected to be useful for biological modification of wood fibres for manufacture of wood-based composites, increased capability to grow on a lignocellulosic substrate can be considered a promising feature. In this study, all selected microorganisms, with the exception of *Aspergillus flavus* MF20, were able to grow on sawdust as the sole source of nutrients. *Penicillium chrysogenum* (MF23) showed the highest growth value at 15 and 21 days of incubation. However, the highest increase of growth after 21 days was observed in cultures of *P. angusta* MF4 (Fig. 2). Although production of ligninolytic enzymes has been reported both in

submerged and in solid-state fermentation, the latter method seems to be more advantageous, since a higher enzyme yield can be achieved because of a higher biomass production and a lower proteolysis (Viniegra-González et al. 2003). The fact that no other nutrients, apart from the lignocellulosic substrate to be transformed, are required for an efficient solid-state cultivation of *P. angusta* MF4 could contribute to the development of a cost-effective biotransformation of wood fibres employing these microorganisms. Regardless of the potential that *P. angusta* MF4 has shown, further research must be done to elucidate if these fungal strains can produce high amounts of ligninolytic enzymes under solid-substrate fermentation. Their efficiency for activating the lignin surface under these conditions must also be studied.

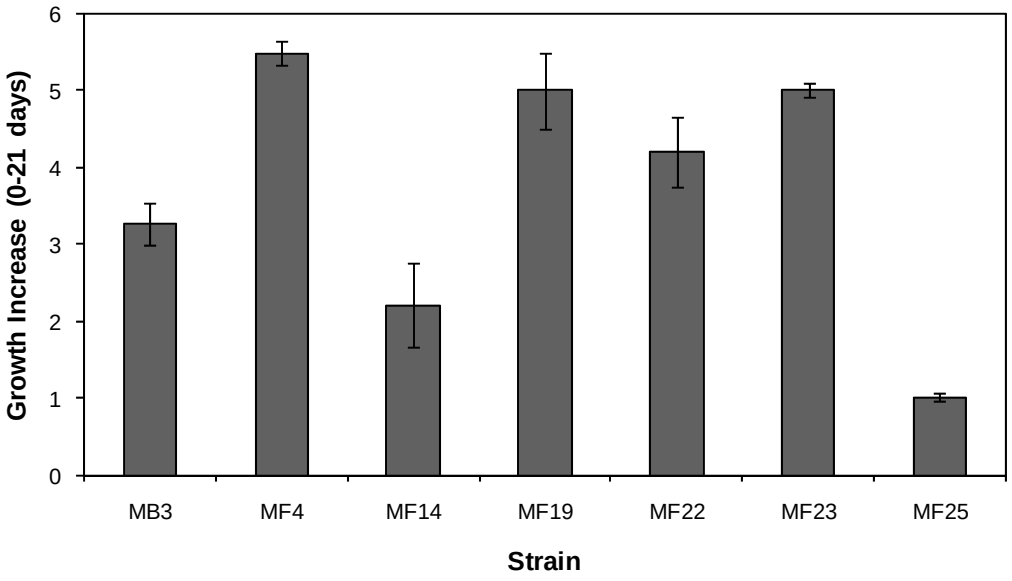

Fig. 2. Growth of selected isolates on unsterile solid substrate culture of sawdust. Increase in growth (in logarithmic units) from day 0 up to day 21 of incubation. Error bars represent standard deviation (n=3). MB3, *Comamonas acidovorans;* MF4, *Pseudallescheria angusta;* MF14, *Aspergillus versicolor;* MF19,*Scopulariopsis brevicaulis;* MF20, *Aspergillus flavus;* MF22, *Torulomyces/Scopulariopsis;* MF23, *Penicillium chrysogenum;* MF25, *Penicillium* sp.

CONCLUSIONS

1. The fungus *Pseudallescheria angust*a MF4 is a promising microorganism for biological modification of wood because of its ligninolytic activity and ability to grow on wood.
2. Mesophilic and thermotolerant ligninolytic microorganisms were isolated from a lignocellulose-rich environment.
3. Qualitative tests related to lignocellulose biotransformation led to the selection of seven microorganisms (mostly fungal strains), which showed ligninolytic activity, directly measured by quantifying their enzymatic activity in the presence of certain ligninolytic inducers or indirectly by determining their dye-decolorizing capability.

ACKNOWLEDGMENTS

This work has been funded by a European project from the Seventh Framework Program FORBIOPLAST (KBBE-212239).

REFERENCES CITED

Bending, G.D., Friloux, M., and Walker, A. (2002). "Degradation of contrasting pesticides by white rot fungi and its relationship with ligninolytic potential," *FEMS Microbiol. Lett.* 212, 59-63.

Camarero, S., Sarkar, S., Ruiz, D. F., Martínez, M. J., and Martínez, A. T. (1999). "Description of a versatile peroxidase involved in the natural degradation of lignin that has both manganese peroxidase and lignin peroxidase substrate interaction sites," *J. Biol. Chem*. 274, 10324-10330.

De Koker, T. H., Zhao, J., Allsop, S. F., and Janse, B. J. H. (2000). "Isolation and enzymatic characterisation of South African white-rot fungi," *Mycol. Res.* 104, 820-824.

Domsch, K. H., Gams, W., and Anderson T. H. (1980). *Compendium of Soil Fungi*, Vol. 2, Academic Press, London.

Dritsa, V., Rigas, F., Natsis, K., and Marchant, R. (2007). "Characterization of a fungal strain isolated from a polyphenol polluted site," *Bioresour. Technol.*98, 1741-1747.

Fakoussa, R. M., and Frost, P. J. (1999). "*In vivo*-decolorization of coal-derived humic acids by laccase-excreting fungus *Trametes versicolor*," *Appl. Microbiol. Biotechnol.* 52, 60–65.

Falcón, M. A., Rodríguez, A., Carnicero, A., Regalado, V., Perestelo, F., Milstein, O., and de la Fuente, G. (1995). "Isolation of microorganisms with lignin transformation potential from soil of Tenerife island," *Soil Biol. Biochem.* 27, 121-126.

Freitag, M., and Morrell, J. J. (1992). "Decolorization of the polymeric dye Poly R-478 by wood-inhabiting fungi," *Can. J. Microbiol.* 38, 811-822.

Gianfreda, L., Xu, F., and Bollag, J. M. (1999). "Laccases: A useful group of oxidoreductive enzymes," *Bioremediat. J.* 3, 1-25.

Hofrichter, M. (2002). "Review: Lignin conversion by manganese peroxidase (MnP)," *Enzyme Microb. Technol.* 30, 454-466.

Hüttermann, A., Mai, C., and Kharazipour A. (2001). "Modification of lignin for the production of new compounded materials," *Appl. Microbiol. Biotechnol.* 55, 387-394.

Janshekar, H., Haltmeier, T., and Brown, C. (1982). "Fungal degradation of pine and straw alkali lignins," *Eur. J. Appl. Microbiol. Biotechnol.* 14, 174-181.

Jaouani, A., Tabka, M. G., and Penninckx, M. J. (2006). "Lignin modifying enzymes of *Coriolopsis polyzona* and their role in olive oil mill wastewaters decolourisation," *Chemosphere* 62, 1421-1430.

Kadimaliev, D. A., Revin, V. V., Atykyan, N. A., and Samuilov, V. D. (2003). "Effect of wood modification on lignin comsumption and synthesis of ligninolytic enzymes by the fungus *Panus* (*Lentinus*) *tigrinus*," *Appl. Biochem. Microbiol.* 39, 488-492.

Kharazipour, A., Huttermann, A., and Luedmann, H.D. (1997)."Enzymatic activation of wood fibers as a means for the production of wood composites," *J. Adhes. Sci. Technol*. 11, 419-427.

Kauri, T., and Kushner, D. J. (1988). "Detection of cellulolytic activity of bacteria and fungi growing on agar surfaces," *Biotechnol. Tech.* 2, 149-152.

Kirk, T. K., Croan, S., Tien, M., Murtagh, K. E., and Farrell, R. L. (1986). "Production of a multiple ligninase by *Phanerochaete chrysosporium*: Effect of selected growth conditions and use of a mutant strain," *Enzyme Microb. Technol.* 8, 27-32.

Koroljova-Skorobogat´ko, O. V., Stepanova, E. V., Gavrilova, V. P., Morozova, O. V., Lubimova, N. V., Dzchafarova, A. N., Jaropolov, A. I., and Makower A. (1998). "Purification and characterization of the constitutive form of laccase from the basidiomycete*Coriolus hirsutus* and effect of inducers on laccase synthesis," *Biotechnol. Appl. Biochem.* 28, 47-54.

Kurtman, C. P., and Robnett, C. J. (1998). "Identification and phylogeny of ascomycetous yeasts from analysis of nuclear large subunit (26S) ribosomal DNA partial sequences," *Antonie van Leeuwenhoek* 73, 331-371.

Malherbe, S., and Cloete, T. E. (2002). "Lignocellulose biodegradation: Fundamentals and applications," *Rev. Environ. Sci. Biotechnol.* 1, 105-114.

Martínez, A. T., Ruiz-Dueñas, F. J., Martínez, M. J., del Río, J. C., and Gutiérrez, A. (2009). "Enzymatic delignification of plant cell wall: from nature to mill," *Curr. Opin. Biotechnol.* 20, 348-357.

Orth, A. B., Royse, D. J., and Tien, M. (1993). "Ubiquity of lignin-degrading peroxidases among various wood-degrading fungi," *Appl. Environ. Microbiol.* 59, 4017-4023.

Raghukumar, C., D'Souza-Ticlo, D., and Verma, A. K. (2008). "Treatment of colored effluents with lignin-degrading enzymes: An emerging role of marine-derived fungi," *Crit. Rev. Microbiol.*34, 189-206.

Rayner, A. D. M., and Boddy, L. (1988). *Fungal Decomposition of Wood. Its Biology and Ecology*, John Wiley, Chichester.

Rodríguez-Couto, S. (2009). "Dye removal by immobilised fungi," *Biotechnol. Adv.* 27, 227-235.

Rodríguez-Couto, S., Rosales, E., Gundín, M., and Sanromán, M. A. (2004). "Exploitation of a waste from the brewing industry for laccase production by two *Trametes*species," *J. Food Eng*. 64, 423-428.

Saha, S. K., Swaminathan, P., Raghavan, C., Uma, L., and Subramanian, G. (2010). "Ligninolytic and antioxidative enzymes of a marine cyanobacterium *Oscillatoria willei* BDU 130511 during Poly R-478 decolourization," *Bioresour. Technol.* 101, 3076-3084.

Sánchez, C. (2009). "Lignocellulosic residues: Biodegradation and bioconversion by fungi," *Biotechnol. Adv.* 27, 185-194.

Sundman, V., and Näse, L. (1971). "A simple plate test for direct visualization of biological lignin degradation," *Pap JaPuu-Pap Timber* 2, 67-71.

Thorn, R. G. (1993). "The use of cellulose azure as a crude assay of both cellulolytic and ligninolytic abilities of wood-inhabiting fungi," *Proc. Japan Acad.* 69 B, 29-34.

Unbehaun, H., Dittler, B., Kühne, G., and Wagenführ, A. (2000). "Investigation into the biotechnological modification of wood and its application in the wood-based material industry," *ActaBiotechnol.*20, 305-312.

Viniegra-González, G., Favela-Torres, E., Aguilar, C. N., Rómero-Gomez, S. J., Díaz-Godínez, G., and Augur, C. (2003). "Advantages of fungal enzyme production in solid state over liquid fermentation systems," *Biochem. Eng. J.* 13, 157-167.

Wesenberg, D., Kyriakides, I., and Agathos, S. N. (2003). "White-rot fungi and their enzymes for the treatment of industrial dye effluents," *Biotechnol. Adv.* 22, 161-187.

White, T. R., and Cotta, M. A. (2001). Characterisation and comparison of microbial populations in swine faeces and manure storage pits by 16S rDNA gene sequence analyses," *Anaerobe* 7, 181-187.

Widsten, P., and Kandelbauer, A. (2008). "Adhesion improvement of lignocellulosic products by enzymatic pre-treatment," *Biotechnol. Adv.* 26, 379-386.