African Journal of Biotechnology Vol. 7 (12), pp. 1983-1990, 17 June, 2008
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB08.042
ISSN 1684–5315 © 2008 Academic Journals

Full Length Research Paper

Autochthonous white rot fungi from the tropical forest: Potential of Cuban strains for dyes and textile industrial effluents decolourisation

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Accepted 26 March, 2008

Nineteen strains of wood-inhabiting pores fungi, representing thirteen species and ten genera, collected from both natural and "anthropic" (urban) ecosystems in Cuba were tested for their ability to decolourise the industrial anthraquinonic dye Acid Blue 62 (AB 62) in laboratory conditions, in both solid and liquid media. On the basis of their decolourisation rate and growth inhibition, seven strains viz. *Tinctoporellus epimiltinus, Trametes maxima*, *Perenniporia tephropora*, *Coriolopsis rigida*, *Hexagonia tenuis*, *Pachykytospora alabamae* and *Hexagonia hydnoides* were selected for further studies. All the strains were able to decolourise partially or completely the AB62 dye added to Malt extract. Almost total decolourisation was obtained with *T. maxima*. Decolourising activity was also shown with an industrial textile effluent, containing Remazol Yellow RGB, Remazol Red RR, and Remazol Black B 133. Production of laccase, a ligninolytic enzyme possibly involved in decolourisation, was stimulated by AB 62 for all the strains tested; *T. maxima* showing the highest production. Lignin peroxidase and manganese peroxidase were not produced under the experimental conditions used. *T. maxima* could represent a potential candidate for biotechnological applications. The exploitation of local biodiversity in tropical area appears as a potentially productive approach for identifying promising microbial strains for industrial use.

Key words: Anthraquinones, dyes, bioremediation, decolourisation, laccase, textile effluent, *Trametes maxima*, white rot fungi, Acid Blue 62.

INTRODUCTION

Wastewaters from dyes and textile industries contain a variety of pollutants, including dyes which are hardly removed by conventional activated sludge treatment. A successful management of these effluents often necessitates expensive physico-chemical treatment using, for instance, ozone (Khraisheh, 2003). Microbial decolourisation has been proposed as a less expensive and less environmentally intrusive alternative (Selvam et al., 2003). In the last ten years, so-called White Rot Fungi (WRF) emerged as a promising group for biotechnological applications, especially in bioremediation (Reddy and Mathew, 2001.). The oxidative enzymes of WRF have broad substrate specificity and have been proposed for their use in the transformation and mineralization of

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organo-pollutants structurally similar to lignin (Pointing, 2001). It has also been shown that the lignin degrading system of WRF is exploitable for dye decolourisation (Chander et al., 2004; Shah and Nerud, 2002; Wesenberg et al., 2003). Among the ligninolytic enzymes produced by WRF, laccases appear as particularly active in dye degradation (Baldrian, 2004; Rodriguez et al., 1999).

Many studies dedicated to bioremediation competence of WRF assess the properties of strains deposited in public collections (Jaouani et al., 2003). There have been in contrast fewer contributions attempting to exploit directly local biodiversity, especially in African and Latin America tropical area (Levin et al., 2004; Pointing et al., 2000a; Tekere et al., 2001). However, this approach appears to be potentially productive for identifying new, promising strains for biotechnological applications (Pointing et al., 2003).

During several surveys of wood-inhabiting fungi on Cuba, in both natural and anthropic ecosystems, in different provinces, 200 strains were isolated in pure culture, and later sorted according to their type of rot (viz. brown or white rot), ecology, and habitat. On the basis of their type of rot, ecology, and habitat, nineteen strains, representing thirteen species and ten different genera (Table 1) were then selected to evaluate their ability to decolourise dyes, either as raw material or mixed in a textile effluent, in relation with laccase production. The results of this screening are presented and discussed below.

MATERIAL AND METHODS

Organisms and culture conditions

The strains used in the present study are listed in Table 1. After isolation in pure culture and identification, strains were deposited and stored at the BCCM™/MUCL and at the CRGF ("*Colleccion de Recursos Geneticos Fungicos*") at the Institute of Ecology and Systematic, Cuba. The strains were preserved by cryopreservation at – 130°C (at BCCM™/MUCL) or alternatively on agar slopes under mineral oil or water in both collections. *Pycnoporus sanguineus* (MUCL 41582) was used as a reference strain (Vanhulle et al., 2001).

The strains were grown first on 2% (w/v) malt extract agar (MA2) incubated at 25°C for 7 days. One plug of agar was sampled at the margin of the colony with a hollow-punch of 4 mm diameter to serve as inoculum for subsequent MA2 plates or MA2 plates added with 1.75 mM Acid Blue 62. The diameter of growth and decolourisation were measured each day. Averages of radial growth rates (mm/day) were calculated.

For growing in liquid medium, seventeen plugs of agar were sampled with a hollow-punch of 4 mm diameter at the margin of the colony of a MA2 7 day's old pre-culture to serve as inoculums. Erlenmeyer of 250 ml contained either 100 ml of a 2% (w/v) malt extract broth (ML2), ML2 supplemented with 1.75 mM Acid Blue 62, or ML2 supplemented with 0.5% (v/v) of the industrial dye effluent. The cultures were incubated a 25°C for 14 days in a rotary shaker at 125 rpm [stroke 50 mm, and shaking plate dimensions 760 x 600 (W x D) mm], with daily sampling. Acid Blue 62 was courteously supplied by Yorkshire Europe (Tertre, Belgium).

The industrial effluent was obtained as an anonymous sample from a cotton dyer company. It contained *inter alia* acid and reactive dyes, salts and intermediates.

Decolourisation in liquid medium

Dye decolourisation was determined spectrophotometrically by monitoring of the absorbance at 500 and 595 nm for the dye Acid Blue 62. For the industrial effluent a spectrophotometric integrative method (SIM) was adapted from a method described by the "American Public Health Association" (APHA). APHA describes the determination of the colour standard in Hazen units, wherein 1 mg/l Pt equals one Hazen. The stock solution contained 2.49 g K_2PtCl_6 and 2.02 g $CoCl_2 \cdot 6H_2O$ dissolved in 200 ml of concentrated analytical grade HCl (d =1.19) and diluted to 1 litre with distilled water. A_{445} of this solution represented 1000 Hazen. This method was able to measure coloration of the water in the visible spectrum. The spectrophotometric integrative method (SIM) used here monitored the absorbance of the sample in the visible region from 380 to 740 nm. The integration of the curve absorbance/wavelength gave a numerical result in area units. To convert this result in colour units (APHA), five dilutions of the stock solution of the standard (containing Pt) described above were measured by both APHA and SIM methods. A graph area/hazen unit was constructed for the standard. A conversion factor was calculated, and the result obtained for the coloured sample was converted in colour units (APHA).

Biomass

The culture broth was filtered and biomass was washed and dried at 105°C for 2 days. The fungal biomass was determined as dry mycelial weight per litre of culture broth.

Laccase activity

Laccase activity was determined by monitoring the A_{414} change related to the rate of oxidation of 25 mM 2,2′-azino-bis-[3-ethylbenzothiazoline-6-sulfonic acid] (ABTS) in 100 mM Na-tartrate buffer (pH 4.5) (Lonergan and Baker, 1995). The value used for molar extinction coefficient of ABTS (ε_{414}) was 34450 M^{-1} cm^{-1}. Assays were performed in 1-ml cuvette at ambient temperature (22-25°C) with 50 µl of adequately diluted culture liquid. One unit activity (U) was defined as the amount of enzyme, which oxidized 1 µmol of ABTS per minute and the activities were expressed in U l^{-1}.

Statistics

All the experiments were performed at least two times using three replicates. The data presented correspond to mean values with a standard error less than 10%.

Cluster analysis

Cluster analysis of hierarchical order, based on Euclidian distances, using the percentages of decolourisation of the dye and the industrial effluent reached by the different strains, was performed by UPGMA (Unweighted Pair-Group Method, Arithmetic Average) method, using the statistical package NTSys-pc version 2.02i (1998), which presented the more adequate cophenetic values. The robustness of the tree topology was assessed by 2000 times resampling using a bootstrap analysis (Yap and Nelson, 1996).

Table 1. Origin of the fungi strains and growth parameters on solid medium.

Strains (*)(**)	**Origin**	**Growth rate MA2 (mm/d)**	**Growth rate Acid Blue 62 (mm/d)**	**Decolourisation rate (mm/d)**	**Yield (***) (mm decolourisation/ mm growth)**	**Inhibition (%) (****)**
*Coriolopsis rigida MUCL44156 (*CRGF*121)*	dead branch, Prov. Pinar del Río	5.3	3.3	3.7	1.13	38.2
Coriolopsis sp MUCL 41639	dead trunk, Prov. Cienfuegos,	4.2	3.2	2.8	0.88	23.7
*Flavodon flavus MUCL 44097(*CRGF *175)*	dead wood, Prov. Pinar del Río	5.1	2.6	2.6	1.00	48.8
*Hexagonia hydnoides MUCL 41636 (*CRGF *241)*	dead branch, Prov. Cienfuegos	4.6	2.0	2.9	1.4	56.3
*Hexagonia hydnoides MUCL 43543 (*CRGF *242)*	dead wood, Prov. Pinar del Río	3.4	1.8	2.5	1.4	46.5
*Hexagonia tenuis MUCL 43553 (*CRGF *248)*	dead branch, Prov. Pinar del Río	3.2	2.4	2.5	1.06	25.9
Hexagonia tenuis MUCL *43554 (CRGF 251)*	dead branch, Prov. Pinar del Río	2.8	2.4	2.7	1.10	12.1
Lentinus crinitus MUCL 41620 (CRGF *273)*	dead wood, Prov. Cienfuegos	5.5	3.3	3.5	1.08	40.3
Megasporoporia setulosa MUCL 43550 (CRGF *268)*	dead hanging branch, Prov. Pinar del Río	1.8	0.9	0.9	1.00	50.0
Pachykytospora alabamae MUCL 44100 (CRGF *296)*	dead branch, Prov. Pinar del Río	2.1	1.2	2.4	1.96	43.3
*Pachykytospora alabamae MUCL 44126 (*CRGF *298)*	dead branch, Prov. Pinar del Río	2.4	1.2	2.3	1.88	50.5
*Perenniporia cubensis MUCL 41656 (*CRGF *313)*	dead stump, Prov. Cienfuegos	3.4	1.8	2.2	1.22	47.9
*Perenniporia cubensis MUCL 43209 (*CRGF *314)*	dead stump, Prov. Cienfuegos	3.5	1.9	2.0	1.09	46.6
*Perenniporia tephropora MUCL 43535 (*CRGF *323)*	dead wood, Prov. Pinar del Río	5.1	3.4	3.6	1.05	33.5
*Perenniporia tephropora MUCL 43536 (*CRGF *325)*	dead wood, Prov. Pinar del Río	5.4	3.1	3.3	1.07	43.7
*Pycnoporus sanguineus MUCL 41627 (*CRGF *437)*	dead trunk, Prov. Cienfuegos	6.0	3.1	3.3	1.06	48.0
*Tinctoporellus epimiltinus MUCL 43523 (*CRGF *465)*	decaying stump, Prov. Pinar del Río	6.0	6.0	6.0	1.00	31.1
*Trametes maxima MUCL 44155 (*CRGF*467)*	dead stump, Prov. Pinar del Río	6.0	4.0	4.1	1.02	34.1
*Trametes maxima MUCL 44167(*CRGF *468)*	dead trunk, Prov. Cienfuegos	3.8	2.8	3.1	1.12	26.4
Pycnoporus sanguineus MUCL 41582	Reference strain	5.7	2.9	3.0	1.04	48.5

(*)The strains were identified by their deposit number at the Belgian Coordinated Collections of Microorganisms/Mycothèque de l'Université Catholique de Louvain (BCCM/MUCL).
(**) In brackets, the strains deposit number at the CRGF ("*Colleccion de Recursos Geneticos Fungicos*") at the Institute of Ecology and Systematic.
(***) The decolourisation yield was expressed as the ratio between the diameter of decolourisation and diameter of growth
(****)Percentage of growth inhibition was calculated as follows: % Inhibition = 100 X (Diameter of growth on MA2 - Diameter of growth on MA2 added with AB 62)/ Diameter of growth on MA2.

Table 2. Maximal biomass and laccase activity obtained in medium ML2 without and with the dye Acid Blue 62.

Strain	Medium ML2		Medium ML2 + Acid Blue 62	
	Biomass at 14 days (g/l)	Maximal Laccase activity (U/L)	Biomass at 14 days (g/l)	Maximal Laccase activity (U/L)
Coriolopsis rigida 44156	7.987	42.4 (11)*	4.270	261.6 (10)
Hexagonia hydnoides 41636	4.803	70.7 (8)	3.580	600.4 (11)
Pycnoporus sanguineus 41582	6.067	28.3 (9)	4.067	43.6 (9)
Pachykytospora alabamae 44100	8.000	267.5 (11)	6.313	592.5 (11)
Tinctoporellus epimiltinus 43523	5.060	26.6 (8)	3.513	451.7 (7)
Perenniporia tephropora 43535	5.070	68.4 (8)	4.073	134.9 (8)
Hexagonia tenuis 43554	7.450	17.8 (11)	6.127	541.4 (10)
Trametes maxima 44155	7.420	68.4 (8)	5.813	758.1 (8)

*Values reported in parentheses are cultivation time (days) at which maximal laccase activity was obtained.

RESULTS

Growth and decolourisation capacity on solid medium

The growth inhibition in presence of AB 62 and the capacity to decolourise the dye on solid medium were chosen as criteria for a preliminary sorting of fungal strains. Growth and decolourisation rate attained for the nineteen selected strains and the *P.sanguineus* reference strain are shown in Table 1.

Addition of AB 62 to the media affected the growth rate of all the strains tested, but to different extent. *Hexagonia tenuis* (MUCL 43554) was the less affected, with a growth reduction of 12% (Table 1) while *Hexagonia hydnoides* (MUCL 41636), *Megasporoporia setulosa* (MUCL 43550), and *Pachykytospora alabamae* (MUCL 44126) were the most severely affected, with a growth rate reduction attaining about 50%.

Dye decolourisation was observed for all the strains, including the reference *P. sanguineus* (MUCL 41582). *Tinctoporellus epimiltinus* (MUCL 43523), *T. maxima* (MUCL 44155), *P. tephropora* (MUCL 43535) and *C. rigida* (MUCL 44156) were found to have the highest decolourization rate in the presence of the AB 62 dye incorporated in MA2. However, in terms of decolourisation yield (Table 1), the isolates of *P. alabamae* and of *H. hydnoides* appeared as the most performing.

Taking into account all these results, seven strains, namely, *T. epimiltinus* (MUCL 43523), *T. maxima* (MUCL 44155), *P. tephropora* (MUCL 43535), *C. rigida* (MUCL 44156), *H. tenuis* (MUCL 43554), *P. alabamae* (MUCL 44100) and *H. hydnoides* (MUCL 41636), were selected for further studies in liquid medium.

Growth and decolourisation in liquid medium

Biomass growth as well as decolourisation was attained for all strains after about 14 days of cultivation in liquid medium (Table 2). As for cultivation in solid media, addition of AB 62 to the medium affected the growth of the fungi, and consequently the biomass yield. For all the strains tested, the percentage of growth inhibition was smaller than 50%. Strains of *H. tenuis*, *P. tephropora* and *P. alabamae* were the less affected, although linear growth of *P. alabamae* on the solid medium was the most severely reduced (Table 1). In the absence of dye, biomass ranged from 4.7 – 8.0 g/l. In the presence of AB 62, biomass production was reduced to 3.5 - 6 g/l depending on the strain studied.

All the selected fungi were able to decolourise, partially or completely, the dye AB 62. The $A_{595\ nm}$ value decreased to a large extent for all the strains during the first five days of growth (Figure 1A). This reduction corresponded to a shift in the coloration of the medium from blue to red. The appearance of the red colour resulted from an increase of A_{500nm} (Figure 1 B).

Later the red colour disappeared gradually, resulting for example into almost total decolourisation by *T. maxima* after 18 days of culture (not shown).

Except for *H. tenuis* and *T. epimiltinus*, production of laccase in medium ML2 (without dye) was higher in the tested strains than in the reference *P. sanguineus* MUCL 41582 (Table 2). The higher level of activity in our cultivation conditions was obtained by *P. alabamae* (267.5 U/L), followed by *H. hydnoides* MUCL 41636 (70.7 U/L), *P. tephropora* MUCL 43535 (68.4 U/L) and *T. maxima* (68.4 U/L).

However, for all strains tested, the production of laccase was stimulated by the presence of the dye (Table 2). In presence of the dye, the greatest levels of laccase activity were obtained by *T. maxima* (758.1 U/L), followed by *H. hydnoides* (600.4 U/L), *P. alabamae* (592.5 U/L) and *H. tenuis* (541.4 U/L).

Decolourisation of the textile effluent

The industrial effluent used contained mainly three dyes, Remazol Yellow RGB, Remazol Red RR and Remazol Black B 133, at a total concentration of about 70 g/l. The

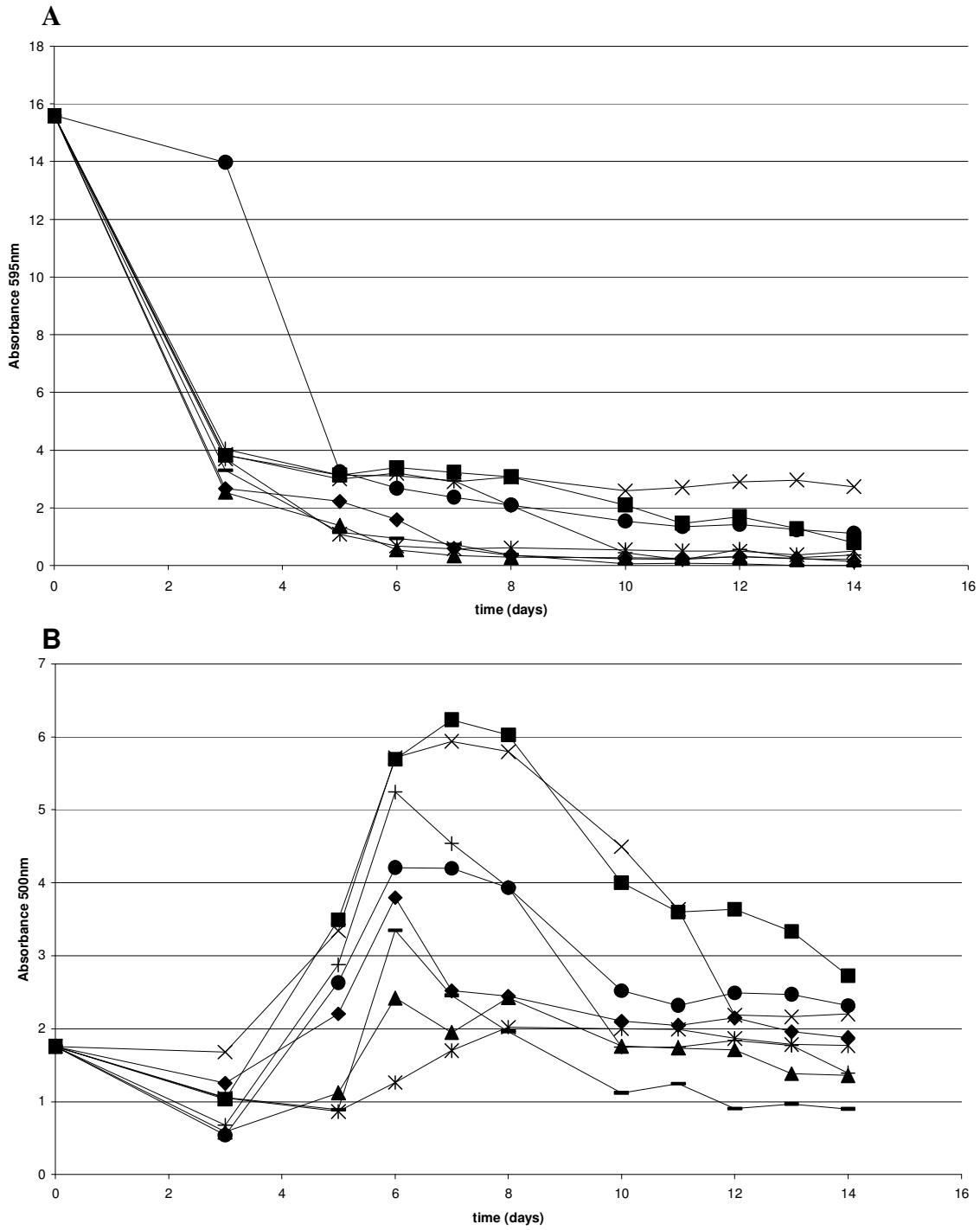

Figure 1. Time course of decolourisation of the ML2 medium supplemented with Acid Blue 62. **(A)** values of A_{595nm} and **(B)** values of A_{500nm}. (♦) *Coriolopsis rigida* 44156; (■) *Hexagonia hydnoides* 41636; (▲) *Pycnoporus sanguineus* 41582; (×) *Pachykytospora alabamae* 44100; (·) *Tinctoporellus epimiltinus* 43523; (●) *Perenniporia tephropora* 43535; (+) *Hexagonia tenuis* 43554 and (—) *Trametes maxima* 44155.

effluent contained also other additives like molan, fumexol among others. Figure 2 shows the absorbance spectrum of the ML2 medium added with the textile effluent after 14 days of incubation with the fungal strains. All the strains were able to decolourise to more or less extent the industrial effluent. A noticeable collapse of the whole visible spectrum, as compared to the spectrum of the uninoculated control, was obtained with five strains; *T. maxima* being the most effective with a 94% value of decolourisation. The decolourisation performance of *P.*

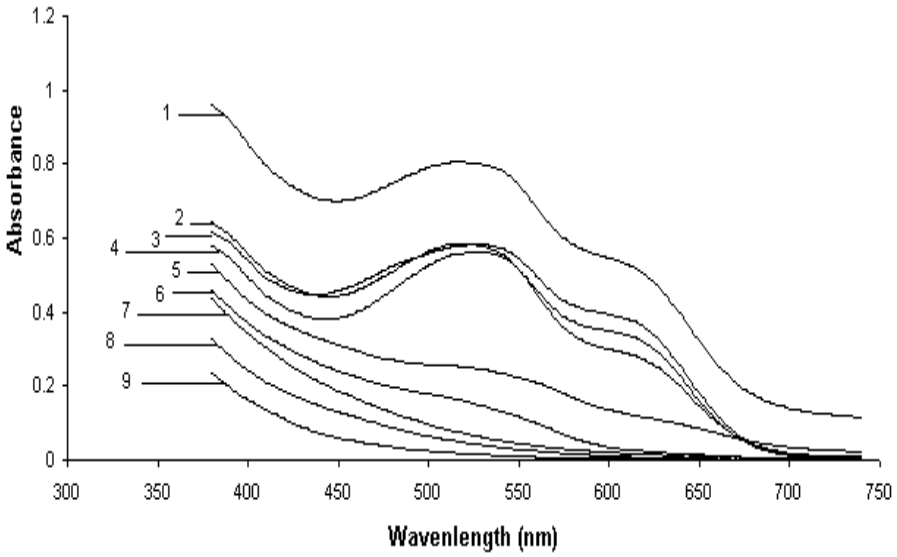

Figure 2. Absorbance spectra in the visible range of the medium ML2 supplemented with 0.5 % (v/v) of the industrial textile effluent. Data were recorded after 14 days incubation in the presence of the different WRF strains. Decolourisation values for each strain, reported as percentage in bracket, was calculated using the integrated values of the absorbance between 380 – 740 nm. (1) Uninoculated control; (2) *Perenniporia tephropora* [41]; (3) *Pachykytospora alabamae* [32]; (4) *Tinctoporellus epimiltinus* [43]; (5) *Pycnoporus sanguineus* [67]; (6) *Hexagonia tenuis* [78]; (7) *Hexagonia hydnoides* [83]; (8) *Coriolopsis rigida* [88] and (9) *Trametes maxima* [94].

sanguineus was lower than for *C. rigida, H. hydnoides, H. tenuis* and *T. maxima*.

Laccase activity was detected in all cultures; *P. alabamae* and *T. maxima* being the best enzyme producers.

DISCUSSION

Collection of strains characterized here for their decolourisation capabilities were realized in both natural and "anthropic" ecosystems in Cuba, mostly in Pinar del Rio Province. The Sierra Del Rosario Biosphere Reserve is characterized by several vegetation types, in particular evergreen forest presenting some similarities with neotropical rain forest (Capote et al., 1988), and was reported as an important reservoir of fungal biodiversity (Minter et al., 2001). A significant part of the selected strains nevertheless came from anthropic environments, including urban areas. The presence in the urban zone of many dead fallen trunks or stump, consequence of numerous tropical storms, constitute an interesting ecosystem characterized by non buffered conditions, due to an open exposition with very high diary and seasonal variation in both surface temperature and water content. Several polypores species are commonly observed on this substrate in Cuba, as well as in the entirety of tropical areas. They include *P. sanguineus*, *H. hydnoides*, *T. maxima*, *C. rigida, P. tephropora, Earliella scabrosa,* or *H. tenuis*. These species might constitute the primary colonizers of freshly fallen dead trunk. Previous studies in other tropical area, e.g. French Guyana have also shown that polypores isolated from similar environment (freshly fallen dead trunk), produced higher enzyme activity, either linked to white or brown rotting (Decock et al., unpublished). However, the link between the ecological conditions of such substrate and the physiological properties of the fungus, of which enzyme production, remain to be obviously elucidated. Primary colonisers should have efficient enzymatic systems to penetrate and invade quickly the fresh unaltered wood in order to establish a viable colony within deeper zones of the material, less affected by drastic changes of T° and water content. They should preserve a volume of wood as large as possible from colonisation by other species, and in consequence, their enzymatic activities should also be efficient for intra or inner species competition. These species should also be able to react quickly when the conditions are optimal, although these being highly variable.

In 2003, the world textile dye consumption was 680 000 tons, with an annual turnover of 4.49 billion euros (Sunfaith China Ltd, 2004). Among these, anthraquinonic acid dyes, like Acid Blue 62, represent 166 000 tons in the World and 40 000 tons in Europe. This class forms the basis of many of the modern synthetic dyes and is reported in the *Colour Index* as one of the most important group of organic dyes (Hobson and Wales, 1998). Moreover, anthraquinonic structures have been isolated

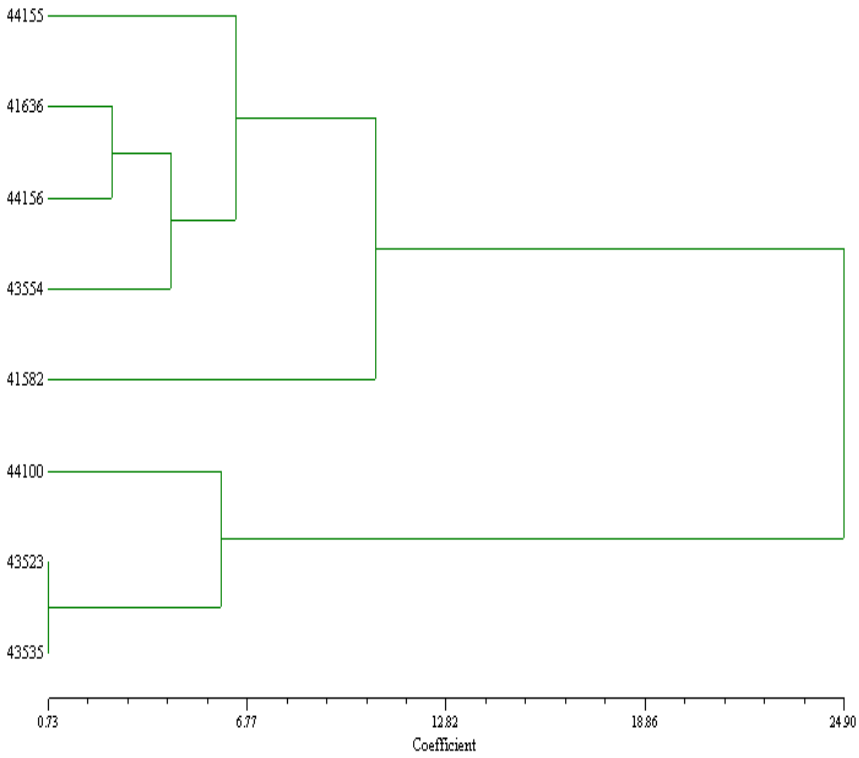

Figure 3. Cluster analysis of the white-rot fungi strains, obtained from decolourisation analysis of the dye AB 62 and the industrial effluent. Ther strains are *Coriolopsis rigida* (MUCL 44156), *Hexagonia hydnoides* (MUCL 41636), *Pycnoporus sanguineus* (MUCL 41582), *Pachykytospora alabamae* (MUCL 44100), *Tinctoporellus epimiltinus* (MUCL 43523), *Perenniporia tephropora* (MUCL 43535), *Hexagonia tenuis* (MUCL 43554) and *Trametes maxima* (MUCL 44155).

from a number of fungi including *Drechslera* S. Ito, *Trichoderma* Pers., *Aspergillus* Link and *Curvularia* Boedijn strains, and could serve as basis for development of a future generation of new natural and/or semi-synthetic dyes (Alvi and Rabenstein, 2004). Acid blue 62 was chosen as a model for evaluating the Cuban strains and all strains studied were able to decolourise this dye. The action of the seven selected strains toward other types of colorants (e.g. azo dyes), remain to be evaluated. However, the noteworthy decolourisation activity of the strain towards a textile effluent containing different dyes and some additives indicate that they could potentially have a positive action against complex pollution situations.

Considering decolourisation analysis of the dye AB 62 at two different optical densities (500 and 595 nm) and the industrial effluent, it is possible to determine several highly related strains (Figure 3). The four strains of subgroup A (*T. maxima* MUCL 44155, *C. rigida* MUCL 44156, *H. hydnoides* MUCL 41636 and *H. tenuis* MUCL 43554) which produced the highest decolourisation, behave quite similarly to the strain *P. sanguineus* MUCL 41582, used as reference for dye decolourisation [Van Hulle et al., 2001]. A strain of *P. sanguineus* was also described as a very efficient laccase producer (Pointing et al., 2000b).

No correlation was found between the levels of laccase activity and the decolourisation rate in the strains tested. *T. maxima* MUCL 44155, *H. hydnoides* MUCL 41636 and *H. tenuis* MUCL 43554 demonstrated high level of both laccase activity and decolourisation. *C. rigida* MUCL 44156 obtained a high level of decolourisation with a lower level of laccase. *P. alabamae* produced one of the highest levels of laccase activity but notwithstanding, the lowest level of decolourisation. Nevertheless, for all the strains, the laccase activity was stimulated by the presence of dye. *T. maxima* MUCL 44155 showed the highest decolourisation capacity and also the highest laccase activity (Table 2) in the culture conditions tested. For that reason we consider this strain as very promising for future studies. Members of the genus *Trametes*, in particular *T. versicolor*, have been reported as very efficient in dye decolourisation (Amaral et al., 2004; Libra et al., 2003; Liu et al., 2004). The genus *Trametes* emerged also as a potent decolouriser from recent fungal screening of natural environment, for example from the Chirinda and Chimanimani sub-tropical hardwood forests in Zimbabwe (Tekere et al., 2001). Several members of

the genus *Trametes*, in particular *T. versicolor* (L.: Fr.) Pilát, *T. hirsuta* (Fr.) Pilát and *T. ochracea* (Pers.) Gilb. and Ryvarden were reported as potent laccase producers (Tomsovsky and Homolka, 2003). However, to our knowledge, this is the first report of decolourisation and laccase production by *T. maxima*. Laccase has been identified as the principal enzyme active in dye decolourisation by several WRF strains (Baldrian, 2004; Levin et al., 2004; Liu et al., 2004; Wesenberg et al., 2003) although manganese peroxidase (Boer et al., 2004) or other intracellular enzymes were reported as the main activities in some strains (Blanquez et al., 2004). Lignin and Mn peroxidases were not detected in the cultivation conditions we used in the present investigation (data not shown). This indicated a plausible involvement of laccase in decolourisation by *T. maxima*. The noticeable increase in laccase activity in the presence of AB 62 or the textile effluent supported also this hypothesis.

In conclusion, *T. maxima* seems thus to constitute a promising strain for biotechnological applications. Its description highlighted the interest of screening of environments still little exploited. This type of approach which has clear connexions with white biotechnology (Frazzetto, 2003) could also constitute a good incentive for better protection of fungal biodiversity.

ACKNOWLEDGMENTS

This work was supported by the “Elite Internationale” (CUBACHAM) and “FIRST Objectif 3” (CHAMBOIS) programmes from the Walloon Region, the CIUF (*Conseil Inter-Universitaire de la Communauté Française de Belgique*) -CUD (*Commission Universitaire pour le Développement*) cooperation programme (project CIUF-CUD-MUCL-Cuba) and the EU Sixth Framework Program (NMP2-CT2004-505899 - SOPHIED). Cony Decock gratefully acknowledges the financial support received from the Belgian Federal Science Policy Officeontract BCCM C3/10/003).

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