

Isolation and selection of white-rot fungi for decolourisation of industrial dyes

Ana M. Manzano-León, Miguel Ramos-Leal, Osmel Domínguez-Guilarte, María Isabel Sánchez-López, Rocío Chinaa-Borrell, Alder Batista-Utría, Milay Cabarroi-Hernández,* Lucia Jiménez-Romero,** Magaly Alfonso-González** y Gilda Guerra-Rivera.

Laboratorio de Biotecnología, Dpto. de Microbiología y Virología, Facultad de Biología, Universidad de la Habana, Calle 25 y Calle J, El Vedado, Plaza de la Revolución, La Habana, Código Postal 10400. *Jardín Botánico Nacional, Carretera del Rocío. Kilómetro 3½, Calabazar, Boyeros, La Habana. **Hilatex. Kilómetro 2½, Carretera Aerocaribbean, Wajay, Boyeros, La Habana, Cuba. Correo electrónico: manzale@fbio.uh.cu

Received: March 1st, 2010.

Accepted: October 5th, 2010.

Palabras clave: hongos de la podredumbre blanca, colorantes textiles, decoloración, enzimas ligninolíticas.
Key words: white-rot fungi, textile dyes, decolourisation, ligninolytic enzymes.

RESUMEN. Los colorantes textiles industriales poseen estructuras aromáticas complejas que resultan tóxicas, mutagénicas, carcinogénicas y ocasionan severa contaminación en los ecosistemas donde son vertidos. Los hongos de la podredumbre blanca (HPB) pueden resultar útiles en el control de la contaminación, ya que producen oxidasas no específicas capaces de degradar la lignina y otras moléculas recalcitrantes de estructura similar tales como hidrocarburos policíclicos aromáticos, plaguicidas y colorantes. Los objetivos del trabajo consistieron en aislar y seleccionar cepas de HPB con elevada capacidad para decolorar tintes, así como analizar la producción de enzimas ligninolíticas en presencia de diferentes colorantes como inductores enzimáticos. Se colectaron treinta cepas de HPB a partir de material vegetal procedente de varios ecosistemas de La Habana y Sancti Spiritus, en el periodo del 2000 al 2004. Los aislados correspondieron a once especies diferentes de basidiomicetos ligninolíticos, de ellos, *Ganoderma* aff. *zonatum* (B-18) y *Pleurotus djamor* (B-36) fueron los más efectivos en la decoloración de once colorantes químicamente diferentes y dos efluentes textiles simulados. Como referencias en los ensayos de decoloración se utilizaron las cepas *Trametes membranacea* (B-1), *Lentinus hirtus* (B-8) y *Pleurotus djamor* (B-9) procedentes del Jardín Botánico Nacional y con probada capacidad para degradar lignina y el colorante violeta cristal. Las enzimas lacasa y manganeso peroxidasa de *G. aff. zonatum* (B-18) fueron inducidas por la mezcla de los colorantes reactivos Cibacron marino FN-B, Cibacron rojo FN-3G y Cibacron amarillo P-6GS y por un efluente textil simulado, lo que sugiere su participación en los procesos de decoloración.

ABSTRACT. Textile dyes have complex aromatic structures that result toxic, mutagenic, carcinogenic and cause serious pollution problems in the ecosystems where are released. White rot fungi (WRF) can be useful in the pollution control because they produce non-specific oxidases which are able to degrade lignin and other recalcitrant molecules of similar structure such as polycyclic aromatic hydrocarbons, pesticides and dyes. The purposes of this work were to isolate and select WRF strains with high capacity to decolourise textile dyes, as well as to analyze the production of ligninolytic enzymes in presence of different industrial dyes as enzymatic inducers. Thirty strains were collected from woody material present in several ecosystems of Havana City and Sancti Spiritus, in the period of 2000 to 2004. Isolated corresponded to eleven different species of ligninolytic basidiomycetes, from them *Ganoderma* aff. *zonatum* (B-18) and *Pleurotus djamor* (B-36) were the most effective in decolourisation of eleven chemically different dyes and two simulated textile effluents. As reference in decolourisation assays were used the strains *Trametes membranacea* (B-1), *Lentinus hirtus* (B-8) and *Pleurotus djamor* (B-9), from the National Botanical Garden and with proved capacity to degrade lignin and the dye crystal violet. The enzymes laccase and manganese peroxidase of *G. aff. zonatum* (B-18) were induced in presence of the mixture of the reactive dyes Cibacron navy FN-B, Cibacron red FN-3G and Cibacron yellow P-6GS and a simulated textile effluent, suggesting their participation in decolourisation processes.

INTRODUCTION

White rot fungi (WRF) are physiological group comprising fungi that are capable of biodegrading and mineralizing lignin. The name white rot derives from the white appearance of wood attacked by WRF, where lignin removal gives a bleached appearance. Taxonomically, WRF are mostly basidiomycetes, although few ascomycetes are also capable of white rot decay.¹ The use of this group of fungi has attracted attention as they are able to degrade a wide range of recalcitrant organic compounds, including pesticides, polycyclic aromatic hydrocarbons, dyes and textile effluents.² This ability is a result of the

non-specificity of their extracellular ligninolytic enzyme system [mainly lignin peroxidase (LiP), manganese peroxidase (MnP) and laccase],³ although in some cases additional enzymes or processes may be involved in dyes degradation.^{3,4} Ligninolytic enzymes are induced by lignin and several xenobiotic compound, many of which are aromatic or phenolic compound structurally related to lignin or lignin derivatives.⁵ Therefore, it's very interesting to study the inducer effect of different compounds mainly if they are pollutants to be treated.

Most information on these fungi and their degradation abilities comes from studies with *Phanerochaete*

chryso sporium, *Trametes versicolor* and a few other species, thus representing only a small part of many hundreds species existing in nature. Recently there has been a growing interest in studying a wider variety of WRF with the expectation of finding better lignin-degrading systems for use in various biotechnological applications.⁶ The Island of Cuba has great biodiversity of microorganisms, therefore the study of native WRF for dyes degradation may be appropriate. World production of synthetic dyes is approximately 700 000 t/year, and between 10 and 15 % are discharged to the environment mainly from textile industry.⁷ These compounds have complex aromatic structures and cause pollution problems that seriously affect ecosystems, since many dyes, their precursors and degradation products are toxic, mutagenic or carcinogenic.⁸ Dyes are chemically classified as azo, anthraquinone, heterocyclic, triphenylmethane or phthalocyanine according to the chromophoric group present in the molecule. Dyes also differ in the way they interact with textile fiber during dyeing process. For example, reactive dyes are soluble in water and contain one or two functional groups capable of forming covalent bonds with active sites in cellulose of textil fibers. Direct dyes are also soluble in water and the dyeing process occur due to the high affinity of this compounds by fibers of cotton. Vat dyes are soluble only in its reduced (oxygen-free) form. This name derives from the vessel where these types of dyes were solubilized prior to dyeing process by the addition of a reducing agent.⁹ The effluents from textile industries are extremely variable and complex, containing a wide variety of dyes and dyeing additives, such as dispersants, acids, bases, salts, detergents, humectants, oxidants, etc.¹⁰ Therefore, a fungus selected for a possible application in the treatment of these wastewater should be able to degrade chemically diverse dyes, even in presence of the compounds added during dyeing processes. Thus, the purposes of this work were to isolate WRF strains, select the strains with highest capacity to decolourise textile dyes, as well as to analyze the production of ligninolytic enzymes in presence of different industrial dyes as inducers of these kinds of enzymes.

MATERIALS AND METHODS

Isolation and identification of WRF strains

Fungal strains were obtained from 10 de Octubre and Plaza de La Revolución, Havana City and Topes de Collantes, Sancti Spiritus, in the period of 2000 to 2004. The characteristic fruiting bodies of basidiomycetes were taken from trees in humid areas and from decayed woods. Identification of fungal isolated was carried out using the keys for identification of Kreisel and Pegler.^{11,12} Pure cultures were obtained as follow: the surface of each basidiocarp was ripped off with a sterile scalpel and portions of the plot of the fruiting body cultivated on malt extract agar. Purity of the cultures was checked periodically and the strains were conserved on malt extract agar slant and sterile distilled water (4 °C).¹³ Strains *Trametes membranacea* (B-1), *Lentinus hirtus* (B-8) and *Pleurotus djamor* (B-9), previously isolated in the National Botanical Garden and characterized according to their ability to degrade lignin and the dye crystal violet, were used as reference in decolourisation assays.¹⁴

Inoculation of WRF for the assays

Inocula of WRF in all the assays were made with two agar plugs (5 mm of diameter) obtained from the edge of actively growing mycelium of each test fungus, grown for seven days

at 30 °C, in Kimura medium containing 20 g · L⁻¹ glucose, 5 g · L⁻¹ bacteriological peptone, 2 g · L⁻¹ yeast extract, 1 g · L⁻¹ KH₂PO₄, 0.5 g · L⁻¹ MgSO₄ · 7 H₂O and 15 g · L⁻¹ agar y pH 5.5.

Screening and selection of the strain(s) with highest capacity to decolourise textile dyes

A screening strategy was done in three steps:

a) All the strains isolated as well as the strains employed as references, were grown (20 d, 30 °C, 120 r · min⁻¹) in Kimura liquid medium supplemented with 0.01 % (w/v) of the reactive dyes Cibacron navy FN-B, Cibacron red FN-3G and Cibacron yellow P-6GS (independently). Cultivation was done by triplicate, using biotic (without dye) and abiotic (without fungus) controls. Biomass (dry weight) and decolourisation percentages were determined at the end of the incubation period.

b) The strains selected (in a) according to their higher capacity to decolourise three reactive dyes, were cultivated in Kimura medium supplemented separately with 0.01 % (w/v) of the reactive dyes: Cibacron red FN-R, Cibacron blue FN-G, Cibacron scarlet C-6G, Cibacron violet WH-B, vat dyes: Cibacron green BF, Cibacron orange 3R, direct dyes: Solophenyl red 7BE, Solophenyl blue BFF and Solophenyl red 4GE. Biotic and abiotic controls, as were described earlier, were used.

c) Finally, the influence of dyeing auxiliary compounds on the growth and decolourisation capacity of the selected strains (in b) was determined. Cultivation was done in Kimura medium with 0.01 % of the reactive dye Cibacron red FN-3G, supplemented with the mixture of the following dyeing additives: Dekol 0.004 % (dispersing), Bublex H14 (antifoam) 0.003 %, Hispogal Jet (tensioactive) 0.01 %, Tinoclarit (H₂O₂ stabilizer) 0.001 %, Na₂SO₄ 0.9 %, in a synthetic or simulated textile effluent. As biotic control the strains were cultivated in Kimura medium and Kimura medium supplemented with dye Cibacron red FN-3G. Sterile medium of each culture variant were used as abiotic controls. The synthetic dyes and dyeing additives used in this work were kindly supplied by CIBA-GEIGY and Hilatex (a Cuban textile enterprise).

Influence of dye mixture and a simulated textile effluent on *G. aff. zonatum* B-18 growth and laccase and MnP production

After the screening, a time course analysis was carried out with the best strain in the following culture medium variants: Kimura medium, Kimura medium supplemented with the mixture of the dyes of Cibacron navy FN-B, Cibacron red FN-3G and Cibacron yellow P-6GS, (0.003 % of each one) and Kimura medium with these dyes and dyeing additives mix. Incubation was done during 20 d, 30 °C, 120 r · min⁻¹. Samples were taken every four days to determine biomass dry weight, decolourisation percentages, laccase and MnP enzyme activities.

Decolourisation percentages determination

Decolourisation percentages were determined by the difference in absorbance between sample filtrates and abiotic control at the corresponding maximum wavelength for each dye.

$$\text{Decolourisation (\%)} = 100 - 100 \cdot \frac{\text{OD}_{\text{culture filtrate}}}{\text{OD}_{\text{abiotic control}}}$$

Biomass dry weight determination

For mycelia dry weight determination, cultures were filtered through Whatman No.1 filter paper and

the mycelia were maintained during 24 h at 80 °C until they had constant weight. The weight of the biomass was gravimetrically determined.

Enzyme assays

Laccase activity was measured with 10 mmol/L of 2,2'-azino-bis(3-ethylbenzothiazoline)-6-sulphonate (ABTS) as substrate.¹⁵ MnP activity was estimated by the formation of Mn³⁺-tartrate complex ($\epsilon_{238} = 6\ 500\ \text{mol}^{-1} \cdot \text{cm}^{-1}$) during the oxidation of 0.1 mmol · L⁻¹ Mn²⁺ (MnSO₄) in 100 mmol/L sodium tartrate buffer (pH 5), in presence of 0.1 mmol · L⁻¹ H₂O₂. Enzyme activities were carried out at room temperature (25 °C). One unit of enzyme activity was defined as the amount of enzyme oxidizing 1 μmol of substrate · min⁻¹.

Statistical analysis

Results are presented as the average of three replicates. Normality and variance homogeneity were investigated prior to carrying out the statistical analyses by means of the Kolmogorov-Smirnov test and the Bartlett test, respectively. Where data met these criteria, an analysis of variance of simple classification and a parametric Student-Newman-Keuls test were used. If these preliminary criteria were not met, the Kruskal-Wallis and SNK tests were used. Spearman correlation test was used to analyze the possible existence of direct relationship between biomass growth and decolourisation percentages. All data were processed with the statistical package Statistic 6.0.

RESULTS

Collection of white-rot fungi

As a result of the collect, 30 Cuban native strains of white-rot fungi, belonging to 11 species were isolated. The species more represented in our collection were *Lentinus hirtus*, with seven strains (B-27 to B-33) and *Ganoderma* aff. *zonatum*, with six strains (B-18 to B-23). Strains B-15, B-16 and B-17 were identified as *Hexagona hydnooides*. B-24 and B-25 were classified as *Pycnoporus sanguineus*. Strains B-26, B-34, B-35, B-36 belong to the species *Lentinus tigrinus*, *Panus crinitus*, *Pleurotus smithii* and *Pleurotus djamor* respectively. The specie *Coriolopsis floccosa* was represented in our collection by three strains (B-37, B-38 and B-39). B-40 and B-41 were classified as *Oudemansiella canarii* while B-42, B-43 and B-44 were identified as *Lenzites elegans*.

Decolourisation of the industrial dyes Cibacron Navy FN-B, C. Red FN-3G and C. Yellow P-6GS

All strains of basidiomycetes studied were able to grow in the presence of the reactive dyes Cibacron Navy FN-B, C. Red FN-3G and C. Yellow P-6GS (Fig. 1 a, b and c respectively). The mycelial biomass of 16 of them showed a grey-blue colour after 20 d of incubation in presence of Cibacron Navy FN-B. With the exception of *H. hydnooides* B-16, the biomass of all strains presented a reddish colour after 20 d of incubation in Kimura medium with Cibacron Red FN-3G. All the strains were coloured of yellow in presence of Cibacron Yellow P-6GS.

There were significant differences ($p < 0.05$) among the decolourisation percentages of Cibacron navy FN-B, Cibacron red FN-3G and Cibacron yellow P-6GS, obtained by the strains studied (Fig. 1a, b and c respectively). In the cultures supplemented with Cibacron Navy FN-B (Fig. 1a), *P. sanguineus* (B-25) showed the highest decolourisation capacity with 94.9 %, followed

by the strains *G. aff. zonatum* (B-21, B-22), with 92.2 and 91.7 % respectively.

The 33 strains analyzed were able to decolourise Cibacron Red FN-3G (Fig. 1b), *H. hydnooides* (B-16), *G. aff. zonatum* (B-22) and *C. floccosa* (B-37) exhibited the highest capacity to decolourise this dye with 85.7, 82.9 and 86.1 % respectively. Cibacron yellow P-6GS was the dye most recalcitrant to biological degradation, only 18 from the 33 studied strains were able to decolourise this compound (Fig. 1c), *P. smithii* (B-35) and *H. hydnooides* (B-16) presented the highest decolourisation capacity, with 48.6 and 39.6 % respectively.

It is possible to observe that the strains with better capacity to decolourise C. navy and C. red did not reach high values of decolourisation for C. yellow. *P. smithii* (B-35), differed from the rest of the WRF studied because it was the strain that reached the highest decolourisation percentages for Cibacron yellow P-6GS, but did not show a good capacity to degrade the other dyes assayed. *G. aff. zonatum* (B-18) and *P. djamor* (B-36) are the strains most effective among the 33 WRF strains tested for decolourisation of three reactive dyes.

There was not positive correlation between the growth of the strains and the decolourisation of the three analyzed dyes.

Decolourisation of chemically different industrial dyes by *G. aff. zonatum* (B-18) and *P. djamor* (B-36)

G. aff. zonatum (B-18) and *P. djamor* (B-36) were able to completely decolourise the dyes Cibacron blue FN-G, Cibacron scarlet C-6G, Cibacron violet WH-B, Cibacron green BF, Cibacron orange 3R, Solophenyl red 7BE, Solophenyl blue BFF and Solophenyl red 4GE (less than 4 % of colour remaining in culture medium). Dye Cibacron red FN-R was partially decolourised by these two fungi (B-18 and B-36 reached 76.7 and 78.9 decolourisation percentages respectively) (Fig. 2).

Influence of dyeing additive mix on *G. aff. zonatum* B-18 and *P. djamor* B-36 strains growth and their decolourisation ability

Besides dyes, other chemicals are present in textile effluent such as salts, chelating agents and surfactants.¹⁰ Thus it is important to know the effect of these dyeing additives on the microorganisms that potentially can be applied in the treatment of these wastewaters.

G. aff. zonatum (B-18) and *P. djamor* (B-36) were able to decolourise a simulated textile effluent containing dye Cibacron red FN-3G and some dyeing additives. The presence of dyeing additives was not detrimental to fungal growth (Table 1). With the addition of the dyeing additive mixture to control medium, the decolourisation ability of the strain B-18 was reduced by 14 %, however, the decolourisation ability of the strain B-36 was not affected. Strains B-18 and B-36 reached high decolourisation percentages (76.5 and 79.0 %) in this simulated textile effluent, therefore, they appear to be promising for a practical application and should be characterized in more detail.

Influence of dye mix and a simulated textile effluent on *G. aff. zonatum* B-18 growth and laccase and MnP production

Strain *G. aff. zonatum* (B-18), was chosen to investigate its physiology and the role of the most common ligninolytic enzymes, laccase and MnP, in decolourisation processes. Therefore, a time course analysis of its growth, dye decolourisation and ligninolytic enzyme production was made. When this strain was cultivated in

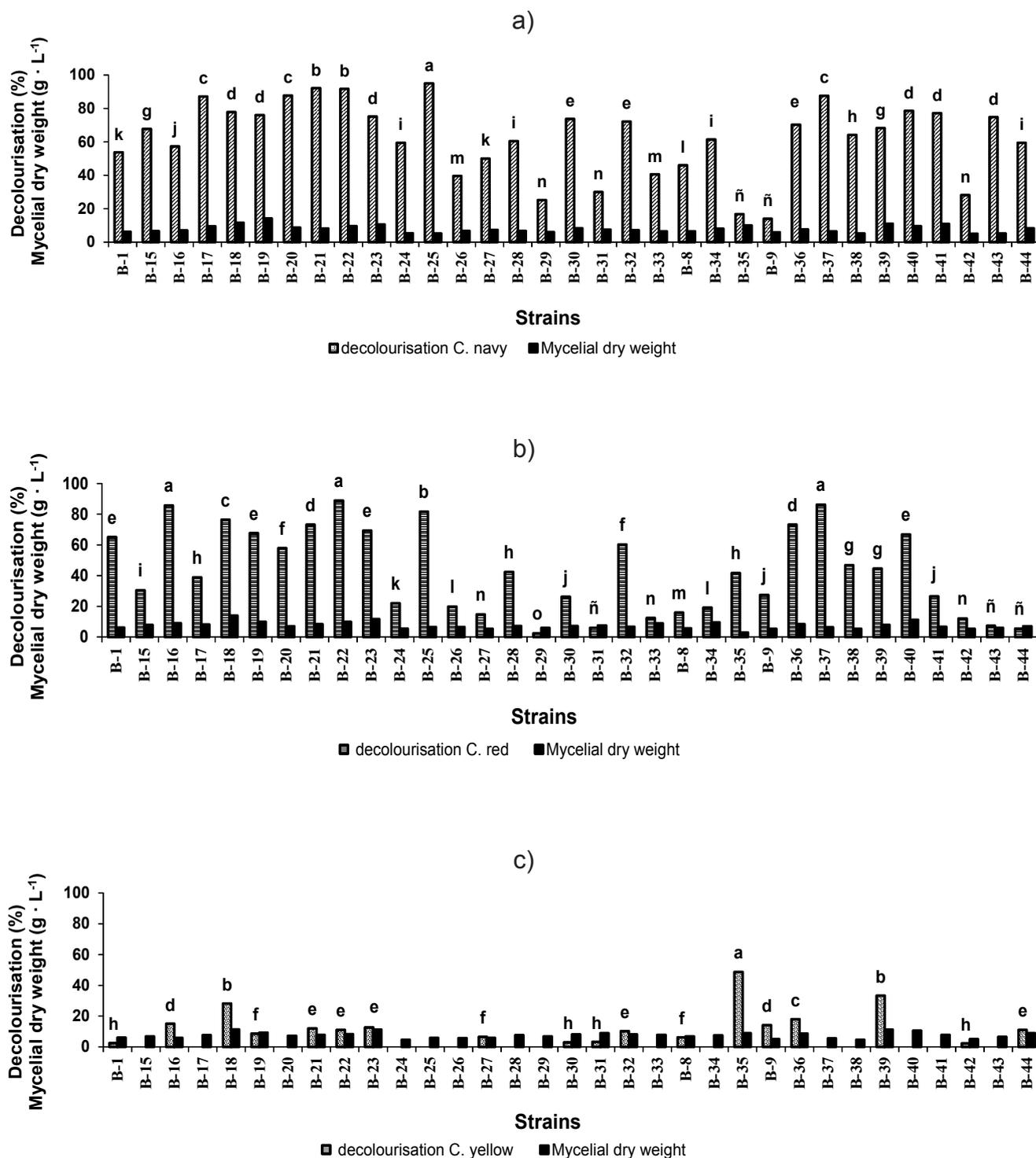


Fig. 1. Growth and decolourisation percentages reached by different strains of white-rot basidiomycetes after 20 d of incubation in Kimura culture medium supplemented with 0.01 %. a) Cibacron Navy FN-B. b) Cibacron Red FN-3G. c) Cibacron Yellow P-6GS. Results are the average of three replicates. Different letters mean significant differences ($p < 0.05$) between decolourisation percentages reached by WRF strains.

Strains	Species	Strains	Species
B-1	<i>T. membranacea</i>	B-34	<i>P. crinitus</i>
B-15 to B-17	<i>H. hydnoides</i>	B-35	<i>P. smithii</i>
B-18 to B-23	<i>G. aff. zonatum</i>	B-36, B-9	<i>P. djamor</i>
B-24, B-25	<i>P. sanguineus</i>	B-37 to B-39	<i>C. floccosa</i>
B-26	<i>L. tigrinus</i>	B-40 and B-41	<i>O. canarii</i>
B-8 and B-27 to B-33	<i>L. hirtus</i>	B-42 to B-44	<i>L. elegans</i>

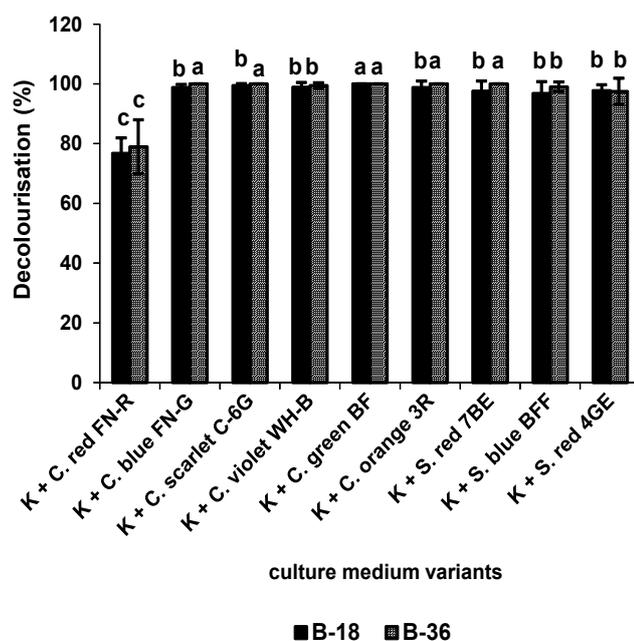


Fig. 2. Decolourisation percentages reached by strains *G. aff. zonatum* (B-18) and *P. djamor* (B-36) after 20 days of incubation in Kimura culture medium (K) supplemented with 0.01 % of the different dyes assayed. **Reactive dyes:** Cibacron red FN-R, Cibacron blue FN-G, Cibacron scarlet C-6G Cibacron violet WH-B. **Vat dyes:** Cibacron green BF, Cibacron orange 3R. **Direct dyes:** Solophenyl red 7BE, Solophenyl blue BFF and Solophenyl red 4GE. Results are the average of three replicates and error bars represent standard deviation. Different letters mean significant differences ($p < 0.05$) between decolourisation percentages reached by both strains for all the dyes assayed.

Table 1. Effect of dyeing additive mixture on the growth and decolourisation ability of *G. aff. zonatum* B-18 and *P. djamor* B-36.

Strains	Biomass ($g \cdot L^{-1}$)		Decolourisation (%)	
	KC	KC + DAM	KC	KC + DAM
<i>G. aff. zonatum</i> B-18	9.9 a	9.7 a	90.5 a'	76.5 b'
<i>P. djamor</i> B-36	8.5 a	9.7 a	79.5 b'	79.0 b'

KC Kimura medium supplemented with 0.01 % of Cibacron red FN-3G. DAM Dyeing additive mixture. Different letters mean significant differences ($p < 0.05$).

Kimura medium (control), fungal growth began rapidly and reached the stationary phase at day 12 (Fig. 3a). Laccase and MnP enzymes were both detected in the culture medium. Laccase was synthesized from the beginning of the growth and reached maximum activity at day 4 with $46 U \cdot L^{-1}$. The production of MnP began at the end of the growth phase (day 8) and reached its highest expression ($10 U \cdot L^{-1}$) in the stationary phase (day 12).

With the addition of the mixture of the dyes Cibacron navy FN-B, Cibacron red FN-3G and Cibacron yellow P-6GS to Kimura medium (Fig. 3b) the growth was not affected. However, laccase and MnP activities showed a differential behaviour in relation to the control medium. As in Kimura medium, the synthesis of laccase started since the beginning of the fermentation and reached the maximum activity at day 4. The presence of dyes mix increased the maximum laccase activity level 1.4-fold. The

presence of dyes induced the synthesis of MnP from the beginning of fungal growth, but there were only small increments in the highest MnP activity levels reached in this culture, compared to the control. This strain was able to decolourise efficiently the mixture of dyes. The maximum decolourisation values were 92.4 % for Cibacron navy FN-B at day 12, 85.1 and 56.5 % for Cibacron red FN-3G and C. yellow P-6GS at day 15.

In presence of the mixture of dyes and dyeing additives (a simulated textile effluent), growth was affected (Fig. 3c). Strain B-18 showed a prolonged lag phase (for 12 d).

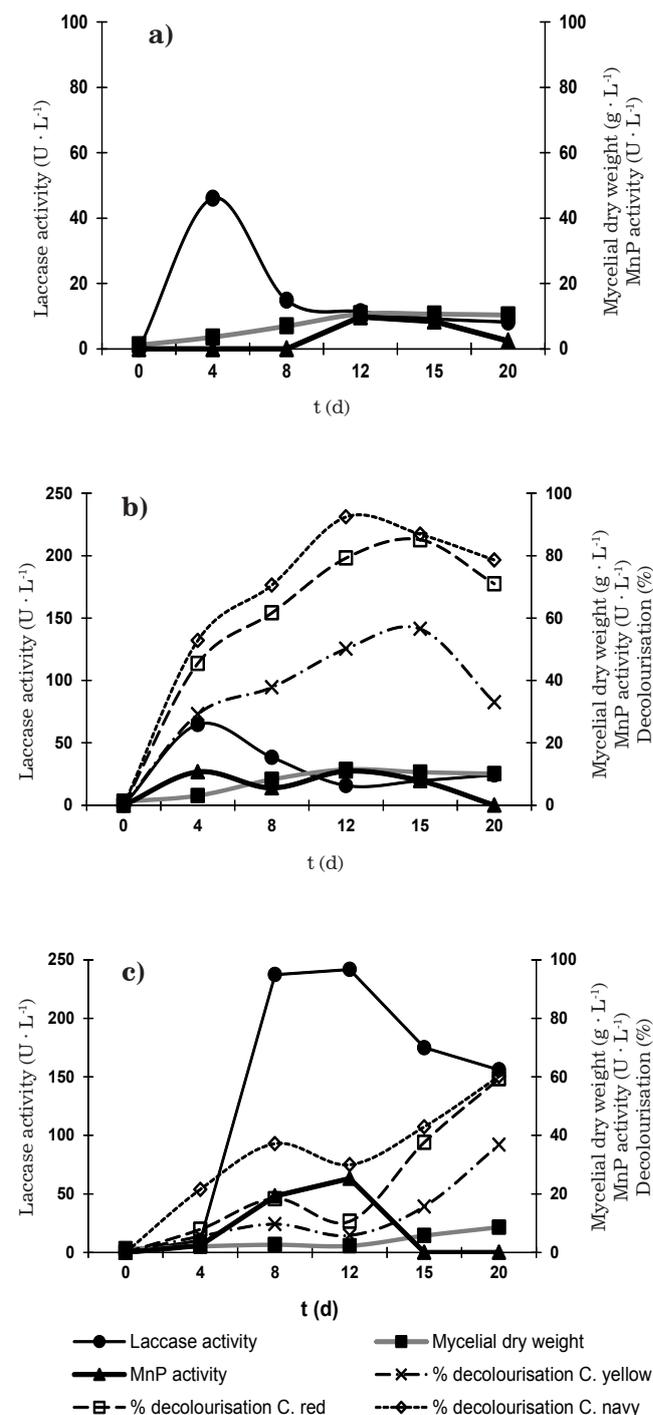


Fig. 3. Time course analysis of the growth, dyes decolourisation, laccase and MnP production by *G. aff. zonatum* B-18 on: a) Kimura medium, b) Kimura medium + dyes mix c) Kimura medium + dyes mix and textile additives (simulated textile effluent).

However, during this period, laccase and MnP activity levels were much higher than the levels obtained in the other variants of culture medium. The maximum activities measured were 5.2-fold and 2.5-fold higher for laccase and MnP respectively, when compared with control treatment. Growth began after 12 d and decolourisation of dyes was increased. The decolourisation percentages at the end of fermentation were 60.2, 59.3 and 36.8 % for Cibacron navy FN-B, Cibacron red FN-3G and Cibacron yellow P-6GS respectively, high values since decolourisation conditions had not been optimized yet.

DISCUSSION

White rot fungi are considered powerful biotechnological tools to biodegrade dyes present in industrial effluents.^{1,16} To assess the decolourisation capacity of different WRF we used a screening method with reactive, vat and direct dyes to assay a wider range of chemical structures and dyes with high and low solubility. These dyes have great colour stability over a wide range of pH (3-11), thermal stability and stability under culture conditions. They are commonly used in Cuba and world wide. The use of industrial dyes in screening methods was positive, it has been reported that studies on the biodegradability of model chromophoric compounds cannot be extrapolated to those used for industrial dyeing, which be expected to be more recalcitrant.¹⁷ The use of dyeing additives in the screening strategy was practical because these are present in textile effluents, and some of these compounds can affect several WRF and their enzymes.¹⁶

In this work, 33 native Cuban strains of basidiomycetes belonging to 11 species were studied. With exception of *Pycnoporus sanguineus* and *Lentinus tigrinus*, the species assayed, have previously attracted only little or none research attention of other scientists, in relation with its ability to decolourise textile dyes. The selected strains *G. aff. zonatum* (B-18) and *P. djamor* (B-36), were effective in decolourisation of a broad spectrum of chemically different dyes, including dyes with high solubility (reactive and direct) as well as dyes with low solubility (vat) and even a simulated textile effluent. The values of decolourisation achieved by these strains are comparable to the results reported by other author using promising WRF strains for dyes degradation.^{4,9} The ability of these strains to decolourise dyes are superior to the capacity of other strains that decolourise preferentially dyes with high or low solubility.¹⁸

The inter-generic, inter-specific and intra-specific differences found in decolourisation abilities of WRF assayed (Fig.1) are in agreement with the data reported by Claus *et al.*¹⁹ This information suggests physiological differences in ligninolytic enzymes production, considering that decolourisation abilities of white-rot fungi are assumed to be mainly connected with their ligninolytic properties.²⁰ A possible explanation to the differences in decolourisation abilities detected among strains of the same species could be that they were isolated from different ecosystems where different selective pressures due to the environmental conditions in those natural habitats could provoke changes in the properties of their ligninolytic enzymes.

Fungal biomasses of many of the studied strains were coloured during decolourisation processes. This fact suggests that biosorption process also took place in dye decolourisation, according to Coulibaly *et al.*,⁶ Glenn and Gold.²⁰

The effect of dyeing additives on decolourisation ability of WRF varies among strains. These results are

consistent with those of Abadulla *et al.*,²¹ who reported that some dyeing additive compounds found in real textile effluents inhibited ligninolytic enzymes of *Polyporus* sp. and *Trametes villosa* up to 20 %, whereas lignin modifying enzymes of *Schizophyllum commune* were inhibited up to 70 %.

It has been shown by many authors that the effect of different inducers supplemented to culture media stimulates ligninolytic enzyme production. In general, the most commonly used inducers have been phenolic or aromatic compounds such as gallic acid or catechol, 2,5 xylidine, ρ -anisidine, veratryl alcohol, guaiacol, ferulic acid.^{5,22} Recently there has been a growing interest in studying the effect of dyes²³ and textile effluents²⁴ on these enzymes. Many papers suggest a key role of laccase²⁵ or MnP¹⁶ in dye decolourisation. Both ligninolytic enzymes were detected in all culture media assayed for *G. aff. zonatum* B-18 in this work. These enzymes were strongly influenced in presence of dye mix and simulated textile effluent (according to Swamy and Ramsay,² Wesenberg *et al.*²⁴) not only because of the increment of enzyme activities due to the presence of inducers, but also because MnP was synthesized since the beginning of the growth in contrast with its behaviour in control medium. These findings imply that these enzymes could be involved in decolourisation processes. However these results did not indicate a positive correlation between laccase or MnP production and dye decolourisation, which is in agreement with results reported by Eichlerová *et al.*²³ Therefore, additional enzymes or biomass associated factors could also be involved in these degradation processes. Further studies that involve laccase or MnP enzyme purification or gene cloning to get a more powerful source of these enzymes should be necessary to demonstrate their role in decolourisation *in vitro*.

CONCLUSIONS

The present findings could contribute to a better understanding of the properties of some white-rot fungi species which have not been studied in details by other authors. *G. aff. zonatum* (B-18) and *P. djamor* (B-36) were the most effective among the strains tested and also were found to be efficient in decolourisation of nine chemically different dyes and a simulated textile effluent. In addition, the fact that *G. aff. zonatum* (B-18) was able to biodegrade all these xenobiotics and that the presence of these compounds induced the synthesis of the ligninolytic enzymes needed for their degradation, making this strain or its enzymes very promising candidate(s) for developing a biotechnological treatment of industrial textile wastewaters.

ACKNOWLEDGEMENTS

This work was supported by The International Foundation for Sciences (IFS) grant F/4442-1 to A.M. Manzano and the grant DB-25 from the Cuban Agency for Science, Technology and Environment (CITMA) to M. Ramos-Leal. The authors also wish to thank PhD. Michael D. McLean, University of Guelph, Canada, due to his valuable comments about this work and to CIBA-GEIGIE and Hilatex, for supplied dyes and dyeing additives used in this research.

BIBLIOGRAPHIC REFERENCES

1. Asgher M, Bhatti HN, Ashraf M and Legge RL. Recent developments in biodegradation of industrial pollutants by white rot fungi and their enzyme system. *Biodegradation*. 2008;19:771-783.

2. Swamy J and Ramsay JA. The evaluation of white rot fungi in the decoloration of textile dyes. *Enzyme Microb Technol.* 1999;24:130-137.
3. Blázquez P, Casas N, Font X, Gabarrell X, Sarrà M, Caminal G, *et al.* Mechanism of textile metal dye biotransformation by *Trametes versicolor*. *Water Research.* 2004;38:2166-2172.
4. Máximo C and Costa Ferreira M. Decolourisation of reactive textile dyes by *Irpex lacteus* and lignin modifying enzymes. *Process Biochem.* 2004;39:1475-1479.
5. Galhaup C, Wagner H, Hintzstoisser B and Haltrich D. Increased production of laccase by the wood-degrading basidiomycete *Trametes pubescens*. *Enzyme Microb Technol.* 2002;30:529-536.
6. Coulibaly L, Gourene G and Agathos NS. Utilization of fungi for biotreatment of raw wastewaters. *African J Biotech.* 2003;2:620-630.
7. Li X and Jia R. Decolorization and biosorption for Congo red by system rice hull- *Schizophyllum* sp. F17 under solid-state condition in a continuous flow packed-bed bioreactor. *Biores Technol.* 2008;99:6885-6892.
8. Vanhulle S, Trovaslet M, Enaud E, Lucas M, Sonveaux M, Decock C, *et al.* Cytotoxicity and genotoxicity evolution during decolorization of dyes by White Rot Fungi. *World J Microbiol Biotechnol.* 2008;24:337-344.
9. Asgher M, Batool S, Bhatti H, Noreen R, Rahman SU and Asad J. Laccase mediated decolorization of vat dyes by *Coriolus versicolor* IBL-04. *International Biodeterioration & Biodegradation.* 2008;62:465-470.
10. Kalyani DC, Telke AA, Dhanve RS and Jadhav JP. Eco-friendly biodegradation and detoxification of Reactive Red 2 textile dye by new isolated *Pseudomonas* sp. SUK1. *Journal of Hazardous Materials.* 2009;163(2-3):735-742.
11. Kreisel H. Clave para la identificación de los macromicetas de Cuba. *Ciencias. Serie Ciencias Biológicas.* 1971;4(16):1-100.
12. Pegler DN. Agaric Flora of the Lesser Antilles. *Kew Bulletin Additional.* 1983;Series 9:1-668.
13. Burdsall HJ and Dorworth EB. Preserving cultures of wood-decaying basidiomycotina using sterile distilled water. *Mycologia.* 1994;86:275-280.
14. Manzano AM, León T, Argüelles J, Ramos-Leal M, Chinaea R, Guerra G, *et al.* Hongos de la podredumbre blanca con capacidad ligninolítica y acción decolorante sobre el violeta cristal. *Biología.* 2004;18(2):123-127.
15. Camarero S, Ibarra D, Martínez MJ and Martínez AT. Lignin-Derived Compounds as Efficient Laccase Mediators for Decolorization of Different Types of Recalcitrant Dyes. *Appl Environ. Microbiol.* 2005;71(4):1775-1784.
16. Wesenberg D, Kyriakides I and Spiros NA. White rot fungi and their enzymes for the treatment of industrial dyes effluents. *Biotech Advances.* 2003;22:161-187.
17. Máximo C, Pessoa Amorium MT and Costa-Ferreira M. Biotransformation of industrial reactive azo dyes by *Geotrichum* sp. *Enzyme Microb Technol.* 2003;32:145-151.
18. Hardin IR. Decolorization of Textile Wastewater Using Microorganisms (Project 2001, FY00 CCACTI) Executive Summary. University of Georgia. Consultado: 14 mayo de 2003. Disponible en: <http://www.gatip.org/Decolorize%20textile%20wastewater.doc>.
19. Claus H, Faber G and König H. Redox mediated decolorization of synthetic dyes by fungal laccases. *Appl Microbiol Biotechnol.* 2002;59:672-678.
20. Glenn JK and Gold MH. Decolorization of several polymeric dyes by the lignin-degrading basidiomycete *Phanerochaete chrysosporium*. *Appl Environ Microbiol.* 1983;45:1741-1747.
21. Abadulla E, Tzanov T, Robra KH, *et al.* Decolorization and detoxification of textile dyes with a laccase from *Trametes hirsute*. *Appl Environ Microbiol.* 2000;66:3357-3362.
22. Gianfreda L, Xu F and Bollag JM. Laccase a useful group of oxidoreductive enzymes. *Biorem J.* 1999;3:1-25.
23. Eichlerová I, Homolka L and Nerud F. Ability of industrial dyes decolorization and ligninolytic enzymes production by different *Pleurotus* species with special attention on *Pleurotus calyptratus*, strain CCBAS 461. *Process Biochem.* 2006;41:941-946.
24. Wesenberg D, Buchon F and Agathos SN. Degradation of dye-containing textile effluent by the agaric white-rot fungus *Clitocybula dusenii*. *Biotechnol Lett.* 2002;24:989-993.
25. Majeau JA, Brar SK and Tyagi RD. Laccases for removal of recalcitrant and emerging pollutants. *Biores Technol.* 2010;101:2331-2350.