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ORIGINAL PAPER

Phenotypical and genetic characterization of *Trichosporon* sp. HP-2023. A yeast isolate from Las Yungas rainforest (Tucumán, Argentina) with dye-decolorizing ability

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Abstract A basidiomycetous yeast isolated from Las Yungas rainforest (Tucumán, Argentina) and arbitrarily named HP-2023 was selected based on its outstanding textile dye decolorizing ability. Complete decolorization of Vilmafix® Red 7B-HE and Vilmafix® Blue RR-BB (200 mg/l) was achieved after 16 h of cultivation. Yeast characterization was accomplished by means of both traditional and molecular methods. Results concerning molecular fingerprinting and phenotypic characterization led to identify it as *Trichosporon* sp., closely related to the *T. multisporum–T. laibachii* complex. The present work represents the first description of a *Trichosporon* yeast involved in reactive textile dye decolorization processes.

Keywords Decolorization · Las Yungas · Textile dyes · *Trichosporon* sp. HP-2023 · Yeast

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Introduction

Reactive dyes are the most commonly used dyes in textile, paper, printing and cosmetic industries. They are considered as xenobiotic compounds and highly recalcitrant against biodegradative processes. Effluents of dyeing industries are markedly colored and the disposal of these wastes into receiving waters causes damage to the environment. Since color reduces light penetration, they may significantly affect photosynthetic activity in aquatic life. In addition, due to their own toxicity as well as because of the presence of metals, chlorides, etc. they are harmful to aquatic life and also to living organisms drinking from these waters. Nevertheless, despite its xenobiotic nature, during the last few years it has been demonstrated that several microorganisms are able to transform azo dyes to non-colored products or even mineralize them (Stolz 2001).

Current non-biological methods for effluent decolorization include coagulation/flocculation, oxidation or adsorption, electro-chemical destruction and photocatalysis. Such methods may successfully accomplish dye removal but could be very expensive because of the high chemical usage, costly infrastructure and high operating expenses. Moreover, accumulation of concentrated sludge becomes a new disposal problem (Nigam et al. 2000).

In order to find an eco-friendly alternative, fungal biodecolorization ability has been widely reported and it is commonly associated to lignin-degrading

exo-enzymes such as lignin-peroxidase (EC 1.11.1.14), Mn-peroxidase (EC 1.11.1.13) or laccase (EC 1.10.3.2). The non-specific nature of these enzymes makes them able to transform, and eventually mineralize, a variety of persistent environmental pollutants, including dyes. However, growth of filamentous fungi is slow compared with most single-cell microorganisms. In fact, they are poorly adapted to wastewater treatments and they tend to show an exuberant growth that aggravates disposal problems (Assas et al. 2000).

Compared to bacteria and filamentous fungi, yeasts exhibit attractive features. Though not as fast as bacteria, yeasts can grow faster than most filamentous fungi, and like them, they have the ability to resist unfavorable environments. Up to present, however, the use of yeast strains in dye wastewater treatments has been limited (Yu and Wen 2005).

In a previous work we have reported the isolation of yeasts from Las Yungas rainforest (Pajot et al. 2007). The region of LasYungas Andinas is a humid forest located in mountainous sectors linked to the Andes. It discontinuously extends from Venezuela, through Ecuador, crossing Peru and Bolivia, reaching the northwest of Argentina with extreme remnants in the provinces of Salta, Jujuy, Tucumán and Catamarca. This ecoregion represents one of the most valuable biodiversity reservoirs in Argentina.

Our yeast isolation campaign took place in the mountainous forests (between 600 and 1200 m a.s.l.), in Tucumán, Argentina. This stratum plays a key ecological role not only because of its biodiversity but also, as it serves as refuge for many other species arriving from different altitudinal strata during seasonal migrations. Despite the Yungas flora and fauna biodiversity have received great attention during last years, microbial diversity has been scarcely explored.

The present work is focused on the systematic characterization of a selected yeast displaying the greatest decolorizing ability, by means of both traditional and molecular methods. Although some *Trichosporon* species assimilate many phenolic and recalcitrant compounds (Middelhoven 1993), this is the first description of a *Trichosporon* yeast involved in reactive textile dye decolorization processes.

Materials and methods

Yeast

Trichosporon sp. HP-2023 was selected among 63 yeast isolates evaluated on their ability for textile dye removal, based on its decolorization potential (Pajot et al. 2007). It is currently maintained in the American Type Culture Collection as ATCC MYA-4129 and in the Centraalbureau voor Schimmelcultures as CBS 10550. For routine work at lab scale, the selected yeast is maintained on YM agar slants at 4°C and sub cultured at 15-day regular intervals.

Dyestuff

Four commercial reactive dyes were used: Vilmafix® Red 7B-HE (C.I. Name: Reactive Red 141), Vilmafix® Green RR-4B (C.I. Name: Reactive Green, no number in the public domain), Vilmafix® Yellow 4R-HE (C.I. Name: Reactive Yellow 84), all of them azo dyes, and Vilmafix® Blue RR-BB (C.I. Name: Reactive Blue 221), a formazan dye. They were all kindly provided by Vilmax S.A (Fig. 1).

Classical laboratory dyes including methyl orange and Congo red (azo dyes), fluorescein and methylene blue (heterocyclic), phenol red and bromophenol blue (phenolsulfonephtalein) were also used in order to evaluate the potential of *Trichosporon* sp. HP-2023 to attack different dye chemical structures.

Stock solutions were prepared by dissolving powdered dyestuff, without prior purification, in distilled water up to a concentration of 2 g l^{-1} and filter sterilized (Millipore filter, 0.22 μm, Millipore Corp., Bedford, USA).

Dye decolorizing ability

Decolorization screening on solid media was performed on Petri dishes containing 20 ml of Normal Decolorization Media (NDM), in g l^{-1}: glucose, 20; $(NH_4)_2SO_4$, 2.5; yeast extract, 2.5; KH_2PO_4, 5, $MgSO_4 \cdot 7H_2O$, 0.5; $CaCl_2$, 0.13 (Ramalho et al. 2002).

Dye stock solutions were added to culture media up to 200 mg l^{-1} (ppm) final concentration. This was the concentration systematically used in subsequent experiments. A mixture of all textile dyes (200 ppm

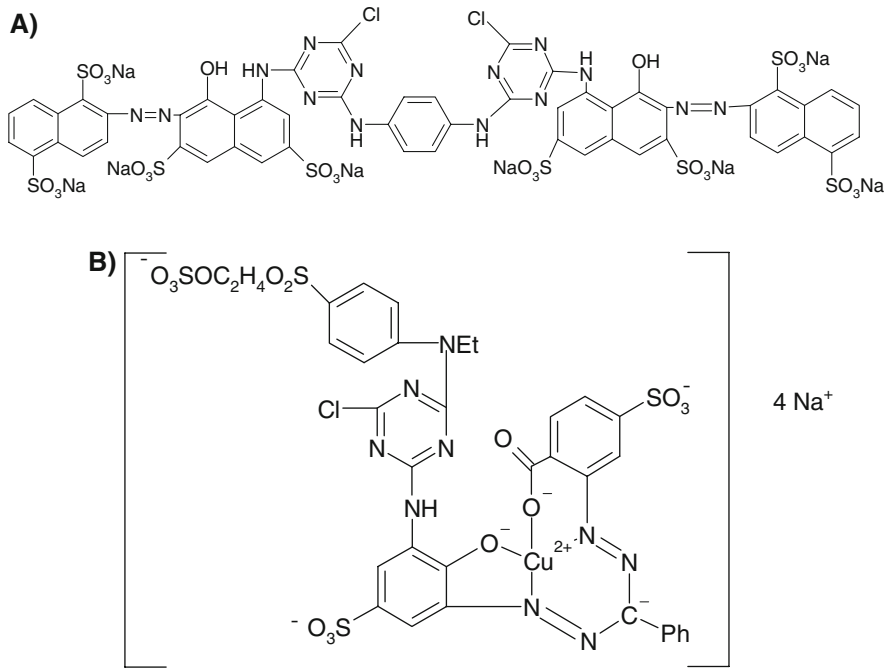

Fig. 1 Structures of Vilmafix® Red 7B-HE (**a**) and Vilmafix® Blue RR-BB (**b**)

final concentration) was used in order to simulate a textile effluent composition. All media were solidified with 15 g l^{-1} of agar.

Plates were inoculated with actively growing yeast from YM-agar, incubated at 26°C and examined for decolorization along 72 h of cultivation. As control, plates without dye were also inoculated.

Growth and decolorization in liquid cultures

Dye (Vilmafix® Blue RR-BB and Vilmafix® Red 7B-HE) decolorization kinetics was evaluated in 500 ml-Erlenmeyer flasks containing 100 ml of NDM medium. A 10-ml yeast suspension (OD_{550} = 0.8) prepared from a fresh YM broth culture was used to inoculate each flask. Incubations were carried out at 200 rpm, 26°C for 48 h. Controls (biotic or abiotic) were performed using the same medium without dyes or yeasts, respectively.

Samples were collected every 4 h and centrifuged for 10 min at 4,000 *g*. Pellets were washed twice with sterile distilled water and dried at 80°C to constant weight for biomass dry weight determination. Supernatants were kept for estimating dye removal, residual glucose (Miller et al. 1959), ammonia (Koroleff 1976) and pH. Total aromatic amines were measured according to Oren et al. (1991). In this work, sulfanilic acid equivalents were chosen instead of benzidine equivalents (Isik and Sponza 2007), because of the eventual release of sulfo-substituted amines from tested dyes.

Results represent the average of at least three independent assays. For the sake of simplicity, standard deviation bars were excluded from graphics.

Dye monitoring

Dye decolorization was monitored with a Beckman DU640 spectrophotometer at each dye λ_{opt} by using culture supernatants obtained as above described. Color removal was reported as percent decolorization = $(A_0-A_t)/A_0 \times 100$, where A_0 and A_t were the absorbance of dye-amended medium at the start point (0) and at a cultivation time (t), respectively. Additionally, culture supernatants were subjected to spectral scanning between 200 and 800 nm in order to analyze dye disappearance.

Enzymatic assays

Enzymatic assays were performed at room temperature and colorimetrically determined. In the case of Lignin peroxidase (LiP) assay, a Beckman DU640 spectrophotometer was used. Other enzymatic activities were determined by using a Beckman Coulter AD200

ELISA reader, following a miniaturized procedure that was setup with a 300-µl-microwell plate.

LiP (EC 1.11.1.14) assay was based on the oxidation of veratrylic alcohol by the increase in absorbance at 300 nm (Tien and Kirk 1984). Laccase, Lacc (EC 1.10.3.2) and Mn-dependent peroxidase, MnP (EC 1.11.1.13) experiments were based on the oxidation of ABTS or TMPD followed by the increase in absorbance at 420 nm or at 590 nm, respectively, as previously described (Jarosz-Wilkołazka et al. 2002; de Vrind-de Jong et al. 1990).

Morphological and physiological characterization

Morphological and physiological properties were examined in accordance to standard yeast identification methods (Yarrow et al. 1998). Utilization of carbon and nitrogen sources was examined at 26°C on solidified YNB and YCB media (Difco). pH of growth media was adjusted to 5.5, as required. Exceptionally, for galacturonic acid-, quinic acid- or potassium hemi-saccharate-containing media, pH was not adjusted in agreement with the laboratory practice of CBS Delft (Middelhoven 1997).

Assimilation of carbon compounds, other than those used in the standard species description, was evaluated in a synthetic growth medium as proposed by Middelhoven (1991). Assimilation of potentially toxic phenolic compounds was studied by the slant culture method (Middelhoven 1991). Utilization of Vilmafix® Blue RR-BB and Vilmafix® Red 7B-HE as sole carbon, energy and nitrogen sources was examined as above described using 0.2 g/l and 2 g/l of each dye. The first concentration represented the usual one in NDM medium, and the second one corresponded to a 120%–140% (blue or red dye, respectively) of the required carbon amount in standard assimilation assays.

For microscopy purposes, cells were grown in YM broth for 3 days at 26°C and 250 rpm. Hyphae formation was assayed in YM agar by the Dalmau plate method. DIC microscopy (Nikkon Eclipse E6000) and scanning electron microscopy (JEOL GSM-35CF) were performed according to routine techniques.

General molecular procedures

Ribosomal DNA regions including the D1/D2 domain of the large subunit, LSU (26S) rDNA, internal transcribed spacers (ITS1-5.8S-ITS2) region, small subunit, SSU (18S) rDNA, were sequenced at Macrogen, Korea by using procedures previously described (Fell et al. 2000; Scorzetti et al. 2002; White et al. 1990). Mitochondrial cytochrome *b* (cyt *b*) gene was partially sequenced according to the procedures reported by Biswas et al. (2001).

Sequences were edited with DNAMAN program version 5.2.2 (Lynnon BioSoft, Vandreuil, QB, Canada). Phylogenetic trees were constructed on the basis of the retrieved sequences from GenBank of other *Trichosporon* species. Sequence comparisons were performed using the basic local alignment search tool (BLAST) program within the GenBank database. The ClustalW computer program (Thompson et al. 1994) was used for alignment of multiple sequences. Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 3.1 (Kumar et al. 2004).

The sequences were registered in the GenBank Data Library under accession numbers **DQ288848** for the D1/D2 domain of LSU rDNA, ITS1-5.8S-ITS2 region, **EF427644** for the partial cyt *b* gene and **EF427643** for the partial SSU rDNA.

Determination of G + C content (by HPLC) was performed according to Mesbah et al. (1989) in DSMZ, Braunschweig, Germany.

Results and discussion

Dye decolorizing ability

Slight differences on decolorization levels were noted depending on the nature of tested dye. Most of them were decolorized at 72 h, except for methylene blue and fluorescein, and in the case of Congo red after 5 days of cultivation. Decolorization of dyes such as Congo red or bromophenol blue (with pH-associated color change) was positively recorded only when colorless halos surrounding the colonies were observed. *Trichosporon* sp. HP-2023 was able to decolorize the mixture of all textile dyes. This ability would be particularly valuable at industrial level, since the inapplicability to a variety of dyes has been one of the main reasons for the lack of implementation of several biological decolorization treatments.

Despite the interest on yeast decolorizing ability against dye mixtures, simulated-effluent composition

was not further used in liquid cultures because of the spectral complexity in the corresponding aqueous solutions. Similarly, Vilmafix® Green RR-4B was also excluded as its spectrum showed a combination of absorbance maxima (blue and yellow). Dyes with pH-indicator properties were also excluded from further assays in order to avoid misleading results when analyzing yeast decolorizing potential.

Two dye models, formazan dye Vilmafix® Blue RR-BB, most susceptible to decolorization, and azo dye Vilmafix® Red 7B-HE, moderately recalcitrant, were chosen for further liquid medium decolorization assays.

Decolorization in liquid cultures

Complete decolorization of Vilmafix® Red 7B-HE and Vilmafix® Blue RR-BB was achieved at 16 h of cultivation. Results from abiotic controls indicated that both tested dyes remained almost intact (99 ± 1%) throughout incubation in NDM. Accordingly, any reduction on the initial dye concentration for inoculated media could be securely assigned to yeast activity. Comparing to the growth in dye-amended medium, biotic controls showed that yeast growth was not restricted in their presence. Moreover, pH, residual glucose or ammonia profiles were not affected because of the presence of dyes in culture medium (Fig. 2).

Faster decolorization stage occurred during the yeast exponential growth phase (Fig. 2). This was not unexpected, since Ramalho et al. (2002) postulated that in some cases, decolorization produced by yeasts might depend of the actively growing cells.

Aromatic amines reaction resulted positive at the beginnings of cultivation both, for NDM control medium and dye-amended media. This fact might be related to a possible reaction with some yeast extract components, or to the presence of impurities from the manufacture of textile dyes (by- or sub-products). Nevertheless, the minimal concentrations reached at 20 h of cultivation (4 h after complete medium decolorization, Fig. 2), suggested a non-aromatic amines generating mechanism of decolorization.

Figure 2 also shows a progressive acidification of culture medium, starting from the very beginning of the exponential growth phase. Medium acidification may play an essential role in decolorization either promoting dye adsorption or affecting the activity of involved enzymes (Akzu and Dönmez 2003). Dye adsorption at cell surface may be related to a charge neutralization mechanism; tested dyes are normally negatively charged. In contrast, cells in solution tend to possess relative positive charges at low pHs exhibiting higher affinity for the dyes.

Enzymatic assays

The fact that batch cultures turned from initial dark blue or dark red to light blue or pink, respectively, and at last tended to colorless, as well as the absence of intensively colored biomass, reinforced the assumption that *Trichosporon* sp. HP-2023 would decolorize both dyes through a biodegradation mechanism.

Kalme et al. (2007) described that reactive red 140 (Vilmafix® Red 7B-HE) might be decolorized by LiP and laccase, whilst Abadulla et al. (2000) reported that reactive blue 221 (Vilmafix® Blue RR BB) might be decolorized by laccase.

We have previously obtained positive results when ligninolytic activities were tested on agar plate assays with *Trichosporon* sp. HP-2023 (Pajot et al. 2007). Positive reactions often appeared within 2–4 weeks after inoculation. Nevertheless, in the present work, none of the tested enzyme activities could be detected in liquid cultures under the conditions assayed, even when complete decolorization was achieved. Similar disagreement between decolorization and ligninolitic enzymes expression was reported by Novotny et al. (2001).

Morphological and physiological characterization of *Trichosporon* sp. HP-2023

After 3 days of growth at 25°C in a liquid medium containing glucose (1%, w/v), peptone (0.5%, w/v), yeast extract (0.3%, w/v) and malt extract (0.3%, w/v), ovate budding cells and globose single cells (5–6.5 × 6.5–8.5 μm), branched septate hyphae and fragmenting pseudomycelium were present (Fig. 3). A dense white sediment and a thin creeping pellicle were formed, which remained present after 4 weeks. Slant cultures on PDA agar after 3 days at 25°C showed a butyrous texture with a flat, white to cream-colored and dull appearance with a fringe of mycelium; their appearance did not change over 4 weeks. In slide cultures on malt extract agar after 3 days at

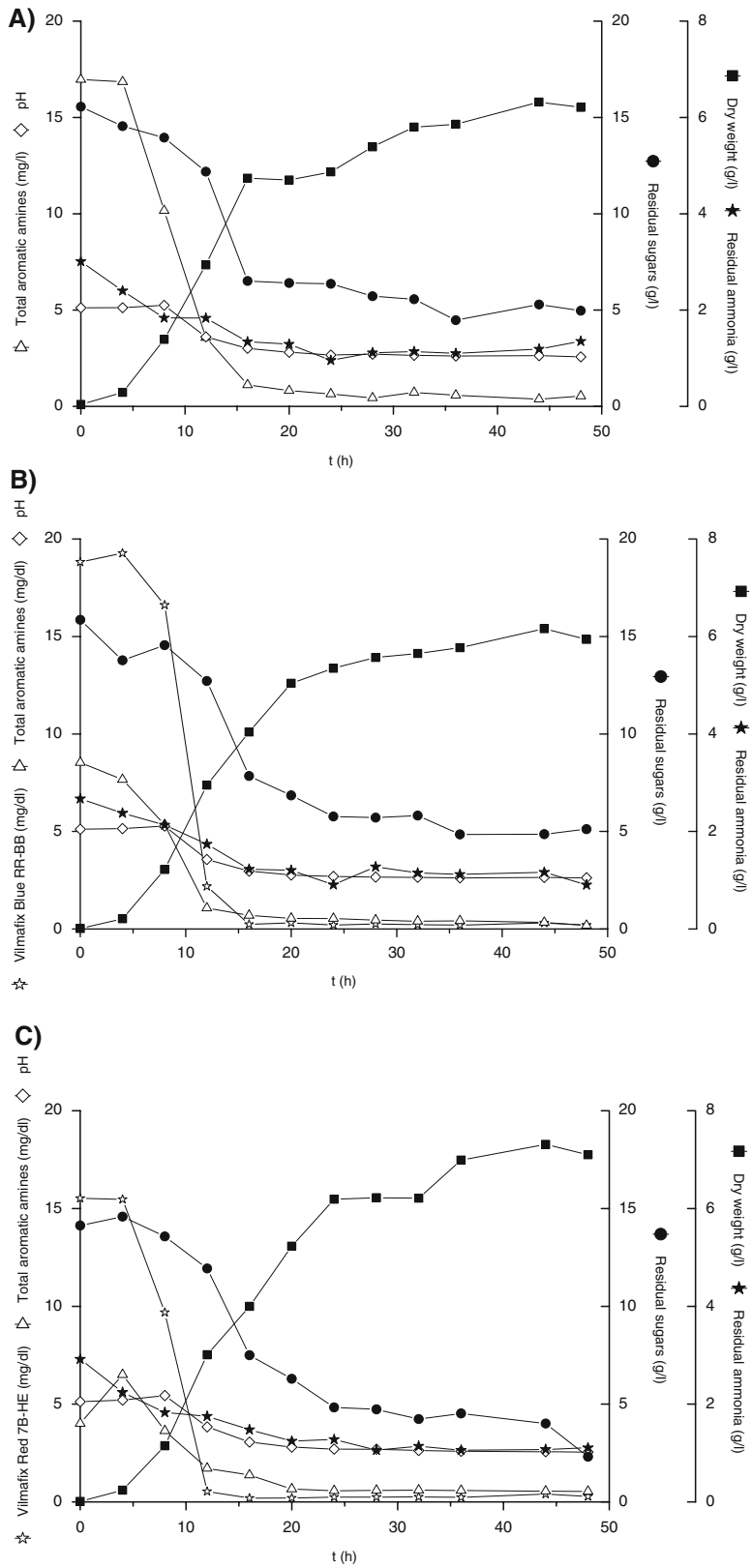

Fig. 2 Growth and decolorization kinetics in NDM medium with *Trichosporon* sp. HP-2023. (**a**) NDM Control. (**b**) NDM plus Vilmafix® Blue RR-BB. (**c**) NDM plus Vilmafix® Red 7B-HE

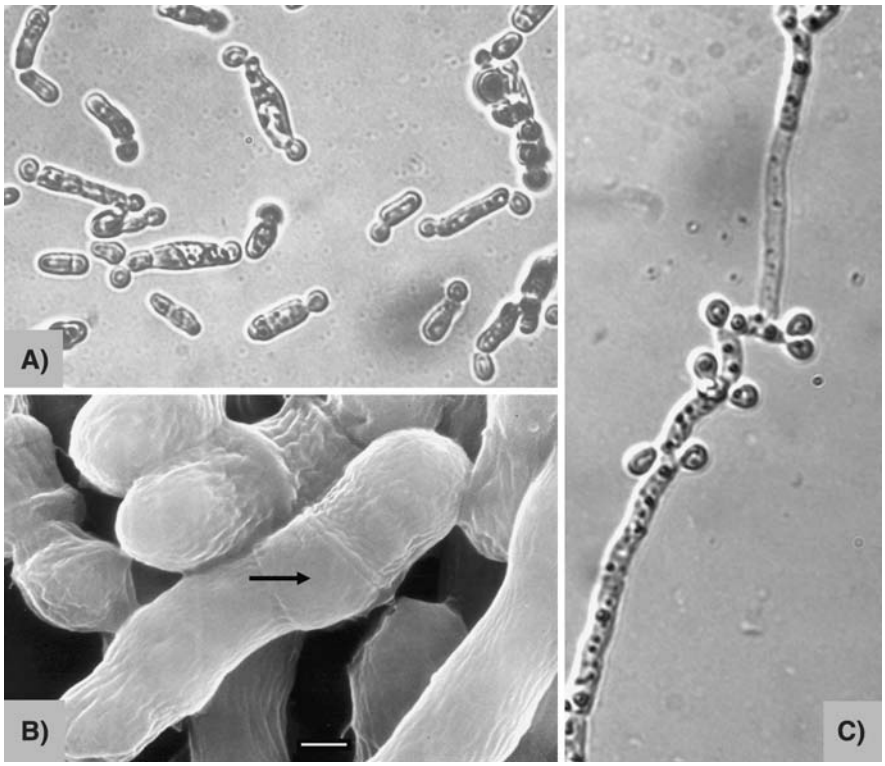

Fig. 3 (**a**) DIC micrography of *Trichosporon* sp. HP-2023 cells grown in YM broth for 2 days at 26°C and 250 rpm. (**b**) Scanning electron micrograph (SEM) of *Trichosporon* sp. HP-2023 cells grown in YM broth for 3 days at 26°C and 250 rpm. Bar = 1 μm. (**c**) Hyphae with lateral globose conidia from a Dalmau plate culture on YM agar for 3 days at 25°C

25°C, abundant, true septate, long, fragmented and branched mycelium, arthroconidia and some pseudomycelium were formed. Micromorphology of yeasts growing in YM broth is displayed in Fig. 3.

Growth responses of *Trichosporon* sp. HP-2023 on standard carbon and nitrogen compounds, and assimilation profiles of some non-conventional aliphatic and phenolic compounds are shown in Table 1. No detectable gas was formed during fermentation of glucose. Assimilation of further alternative C- and N-sources, Vilmafix® Blue RR-BB and Vilmafix® Red 7B-HE, along with some other nutritional requirements, physiological and biochemical characteristics are also displayed in Table 2.

Because of the extremely variable responses of *T. laibachii* in the numerous physiological profiles reported, only a small number of assimilation tests could be helpful when comparing *Trichosporon* sp. HP-2023, *T. laibachii* and *T. multisporum*.

Trichosporon sp. HP-2023 and *T. multisporum* could be differentiated by D-glucosamine, xylitol, D-glucono-1,5-lactone and orcinol (Table 1). *T. laibachii* can be easily identified because it is the only one able to assimilate nitrite as sole nitrogen source (Table 1).

Phylogenetic analysis

Trichosporon sp. HP-2023, as a fungal specimen, could be distinguished via D1/D2 domain sequence analysis. Nevertheless, the number of base pair differences required to separate closely related yeast species has not been yet well established, and possibly, varies between phylogenetic groups. For instance, D1/D2 domain base pair differences between the type strains of *T. mucoides* and *T. moniliiforme* are 5/633 positions (0.8%), while *T. inkin* and *T. ovoides* only differ in 0.5% bases (Middelhoven et al. 2001).

The two closest relatives of *Trichosporon* sp. HP-2023, *T. multisporum* and *T. laibachii*, differ in between at 1.1% base positions of the D1/D2 domain (Middelhoven et al. 2001). For the same LSU rDNA region, our study showed a 1% base position difference between *Trichosporon* sp. HP-2023 and *T. laibachii*, while a 2% base pair difference exists

Table 1 Physiological characteristics of *Trichosporon* sp. HP-2023 and some related species

	T. gracile[a]	*T. dulcitum*[a]	*T. laibachii*[a]	*T. multisporum*[a]	*T.* sp. HP-2023
Carbon compounds					
D-Glucose	+	+, D	+	+	+
D -Galactose	−	−	+	+	+
L-Sorbose	−, D	−, D	V	+	+
D-Glucosamine	D	−, D	V	+	–
D-Ribose	+	+, D	+	+	+
D-Xylose	+	+, D	+	+	+
L-Arabinose	+	+, D	+, D	+	+
D-Arabinose	−	−, D	+, D	−	−
L-Rhamnose	−	−, D	+	+	D
Sucrose	−	V	+	+	+
Maltose	D	V	+	+	+
D-Trehalose	+	V	+, D	+	+
Methyl-α- D-glucoside	−	−	+	+	+
Cellobiose	+	V	+	+	+
Salicin	+	+, D	+	+	+
Melibiose	−	−	+	+	+
Lactose	+	+, D	+	+	+
Raffinose	−	V	+	+	+
Melezitose	−	V	V	+	w, −
Inulin	−	−	−	−	−
Soluble starch	D	−	+	+	+
Glycerol	+	V	+	+	+
Erythritol	−	−, D	−	−	w
Ribitol	−	−, D	−, D	D	−
Xylitol	D	−, D	V	+	−
L-Arabinitol	−	−, D	V	+	w
D-Glucitol	D	V	V	+	D
D-Mannitol	D	V	V	D	−
Galactitol	−	−, D	+, D	+	+
myo-Inositol	+	V	+	+	+
D-Glucono-1,5-lactone	D	−	V	−	+
D-Gluconate	+	V	+	+	+
Glucuronic acid	+	+	+	+	+
D-Galacturonic acid	−	−	V	−	+
DL-Lactate	+	V	+	+	+
Succinate	+	+, D	+	+	+
Citrate	+	V	+	+	+
Methanol	−	NDA	+	NDA	−
Ethanol	−	NDA	−, D	NDA	+
Fermentation					
D-Glucose	−	−	−	−	−
Nitrogen compounds					
Ammonium sulfate	+	+	+	+	+
Nitrate	−	−	−	−	−

Table 1 continued

	T. gracile[a]	*T. dulcitum*[a]	*T. laibachii*[a]	*T. multisporum*[a]	*T.* sp. HP-2023
Nitrite	−	+	−,+	−	−
L-Lysine	+	+	+	+	+
Creatine	−	−	V	−	−
Creatinine	−	−	V	−	−
D-Glucosamine	−	−	+	+	+
Imidazole	−	−, D	−, D	−	−
Aliphatic compounds					
n-Hexadecane	−	−	−	−	D
Uric acid (CN)	−	+	+	+	D
Glycine (CN)	D	D	+, D	+	+
Xylan	−	−	V	−	D
Phenolic compounds					
Phenol	+	+	+	+	+
Hydroquinone	+	+	+	+	D
Resorcinol	+	+	+	+	+
Phloroglucinol	−	−	−	−	−
Orcinol	−	−	−	−	+

[a] Data from CBS Yeast Data Base (http://www.cbs.knaw.nl)

+: growth within 7 days; −: no growth after 21 days; D: growth after 7 days or more; w: weak growth response; V: variable; NDA: no data available. Data from the CBS Yeast Database (http://www.cbs.knaw.nl)

Table 2 Assimilation of some alternative N- and C-sources and physiological characteristics of *Trichosporon* sp. HP-2023

Alternative N-source		Polysaccharides	
Urea	+	Carboxy methyl cellulose	D
Yeast extract	+	Dextran	D
Peptone	+	Pectin	−
α-methyl-D-glucosamine	−	Pullulan	+
Vilmafix® Red 7B-HE	−		
Vilmafix® Blue RR-BB	−		
		Miscellaneous	
		Without amino acids	+
Alternative C-source		Vitamin free	−
2-aminobenzoic acid	+	Vitamin free + thiamine	w
4-aminobenzoic acid	−	0·1% cycloheximide	+
2-amino-2-methyl-1,3-propanediol	−	0·01% cycloheximide	+
Benzoate	+	Hydrolysis of urea	+
Gentiobiose	+	DBB test	+
Phenylalanine	+	Growth at 20°C	+
Salicylic acid	−	Growth at 26°C	+
Tannic acid	−	Growth at 30°C	+
Veratryl alcohol	+	Growth at 37°C	−
Vilmafix® Red 7B-HE	w	50% glucose	−
Vilmafix® Blue RR-BB	−	10% NaCl + 5% glucose	−
		GC content of nuclear DNA (mol%)	58·30%

Codes for growth responses as described in Table 1. Data from the CBS Yeast Database (http://www.cbs.knaw.nl)

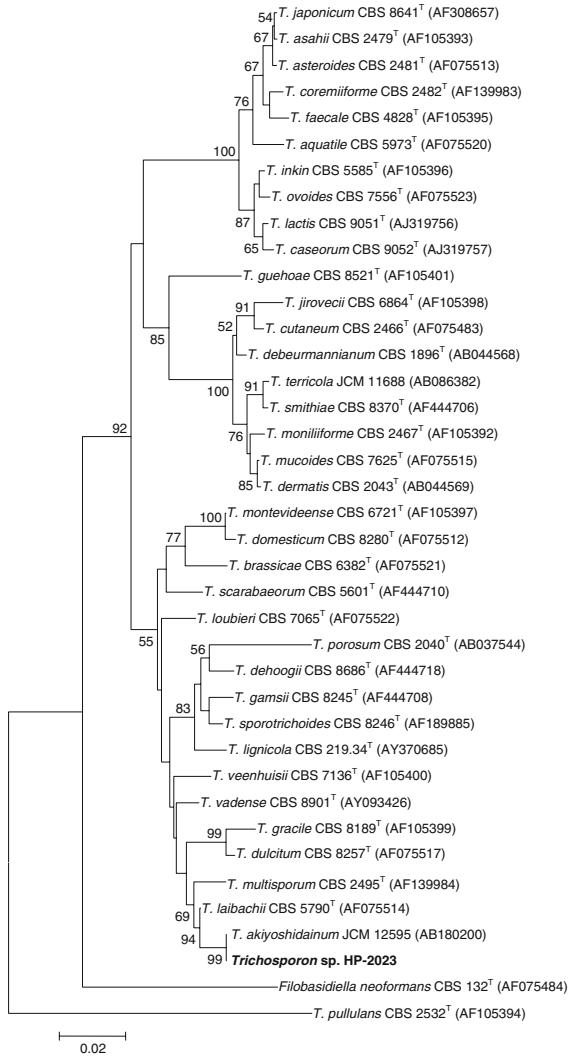

Fig. 4 Phylogenetic tree based on 26S rDNA D1/D2 domain sequences for species of the genus *Trichosporon*. The tree was constructed by the neighbor-joining method and the Jukes-Cantor model. Numerals represent the confidence level from 1,000 replicate bootstrap samplings (frequencies less than 50% are not indicated). Bar indicates the distance corresponding to two-bases change per hundred nucleotide positions. *Filobasidiella neoformans* var. *neoformans* and *Trichosporon pullulans* were used as outgroup

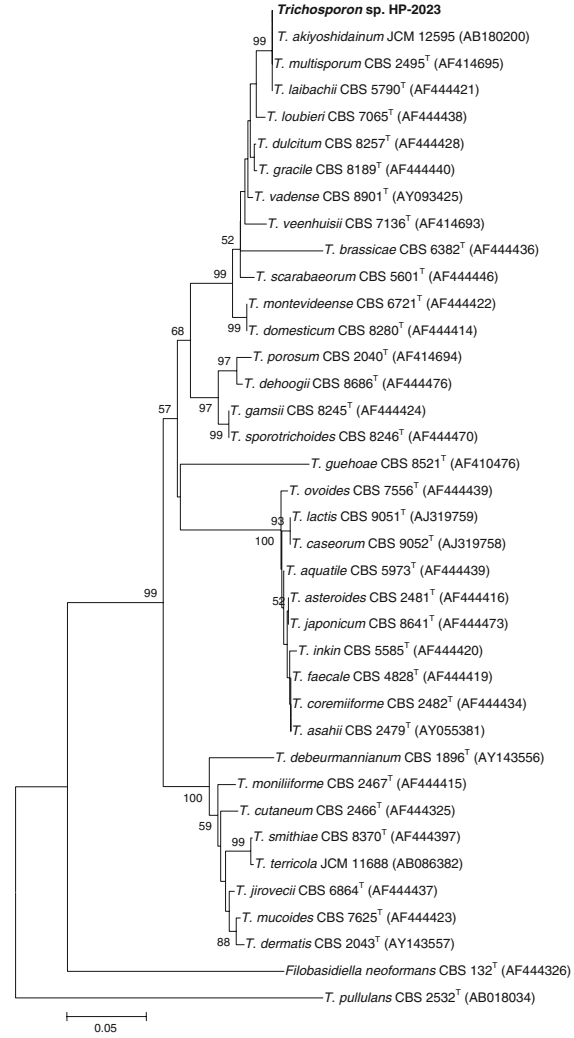

Fig. 5 Phylogenetic tree based on rDNA ITS1-5.8S-ITS2 sequences for species of the genus *Trichosporon*. The tree was constructed by the neighbor-joining method and the Jukes-Cantor model. Numerals represent the confidence level from 1,000 replicate bootstrap samplings (frequencies less than 50% are not indicated). Bar indicates the distance corresponding to five-bases change per hundred nucleotide positions. *Filobasidiella neoformans* var. *neoformans* and *Trichosporon pullulans* were used as outgroup

when compared to *T. multisporum*. These divergence values indicate a closer relationship between *Trichosporon* sp. HP-2023 and *T. laibachii*, and base position differences found in the present work (1–2%) would be then within the range for species separation (Middelhoven et al. 2000).

When analyzing the SSU rDNA sequences, a lower variability was found. *Trichosporon* sp. HP-2023 showed to differ at 14 base pairs (0.8%) with *T. laibachii* and at 7 base pairs (0.35%) with *T. multisporum*, whereas *T. multisporum* and *T. laibachii* differ at 10 base pairs (0.53%). The even lower variability at the SSU rDNA, when compared to the D1/D2 domain, would be not enough to arrive to a proper discrimination of species.

The high level of similarity found when sequencing and comparing the ITS region of *Trichosporon*

sp. HP-2023 with those of *T. multisporum* and *T. laibachii* was in accordance to previous reports on Trichosporonales (Middelhoven et al. 2004). For Trichosporonales members it is expectable to observe more variability within the D1/D2 domain.

As far as the GenBank reported sequences concerns, *Trichosporon* sp. HP-2023 would be positioned within the Gracile clade, closely related to *T. laibachii* and *T. multisporum*. When the D1/D2 LSU rDNA-based phylogenetic tree was constructed, these three species formed a single cluster with a 94% bootstrap value (Fig. 4). Similar results were obtained with nucleotide partial sequences of SSU rDNA (98% bootstrap value, tree not shown); cyt *b* gene (99% bootstrap value, tree not shown) and ITS1-5.8S-ITS2 region (99% bootstrap value, Fig. 5).

Finally, considering the ITS1-5.8S-ITS2 and the D1/D2 LSU rDNA sequences, *Trichosporon* sp. HP-2023 showed 100% identity with a *Trichosporon* yeast up to date not formally described (*T. akiyoshidainum*) and whose SSU rDNA and cyt *b* gene sequences are not yet available in the public domain. On this context, future information would support ascribing *T. akiyoshidainum* as a valid identification.

Conclusions

The results obtained in this work confirmed the decolorization ability of *Trichosporon* sp. HP-2023 for a wide spectrum of dyes, except in the case of heterocyclics such as fluorescein and methylene blue. Promising results were also obtained concerning dye removal from a simulated effluent containing a mixture of different textile dyes.

The selected yeast *Trichosporon* sp. HP-2023, a strain related to the *T. multisporum–T. laibachii* complex according to molecular fingerprinting and phenotypic characterization, demonstrated an outstanding potential for developing a practical bioprocess for decolorizing textile dye-containing wastewaters.

Further studies focusing on the decolorization mechanism by *Trichosporon* sp. HP-2023 would be particularly valuable for understanding the biological yeast response but also, for evaluating the possibility to implement an alternative eco-friendly treatment and the perspectives for future biotechnological applications.

From a taxonomic point of view, minor physiological differences could be found between *Trichosporon* sp. HP-2023 and its closely related species *T. multisporum* and *T. laibachii*, making molecular genetic analysis the best choice in order to identify members of the *T. laibachii/T. multisporum* complex. Additional analyses such as coenzyme Q or DNA hybridization studies (Middelhoven et al. 2000), procedures not yet available in our laboratory, as well as a formal description concerning *T. akiyoshidainum*, would be expected to confirm the proposed identification and the herein reported findings.

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