Screening and identification of yeast strains possessing synthetic dye-decolorizing and ligninolytic activities

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Introduction

Synthetic dyes — although extensively used in several industrial sectors, due to their aromatic moieties, are often highly recalcitrant. Furthermore dyes can cause considerable environmental pollution, so their removal has received considerable attention. A few microorganisms have been found that can degrade dyes; including filamentous fungi due to their extracellular enzyme systems (Moreira *et al.*, 2001). Yeasts, however, possess the advantage of growing faster than filamentous fungi, and some can easily resist to adverse environments. Nevertheless, **degradation of synthetic dyes by yeasts** has not been extensively studied to date.

Materials and Methods

Four commercial dyes were used — Remazol® Black BA, Levafix® Blue Ca, Levafix® Yellow CA and Levafix® Red CA (Dystar). A standard anthraquinone dye, Remazol Brilliant Blue R (RBBR, Sigma) was also used. Dye stock solutions were added to solid or liquid decolorization media, up to 200 mg.L^{-1} final concentration. **Normal Solid Decolorization Media** (NSDM) and **Normal Liquid Decolorization Media** (NDM) were prepared, as described by Pajot *et al.* (2007), and used for decolorization assays. Yeast strains were isolated from wastewater samples collected at biological treatment and homogenization tanks, from two wastewater stations receiving textile effluents. Newly isolated yeasts, as well as a few ones isolated previously from cheese were evaluated for decolorization ability in in NSDM at 30 ºC, along 36 h with agitation and spectrophotometrically monitorized for colour removal. **Preliminary ligninolytic enzymatic activity** determination for selected yeasts extracellular fluid was performed. Selected yeast strains typing and identification were performed using both classical methods of yeast identification, namely microscopic observations and biochemical standard characterisation, as well as molecular biology methodologies such as 18S rDNA sequencing.

Results

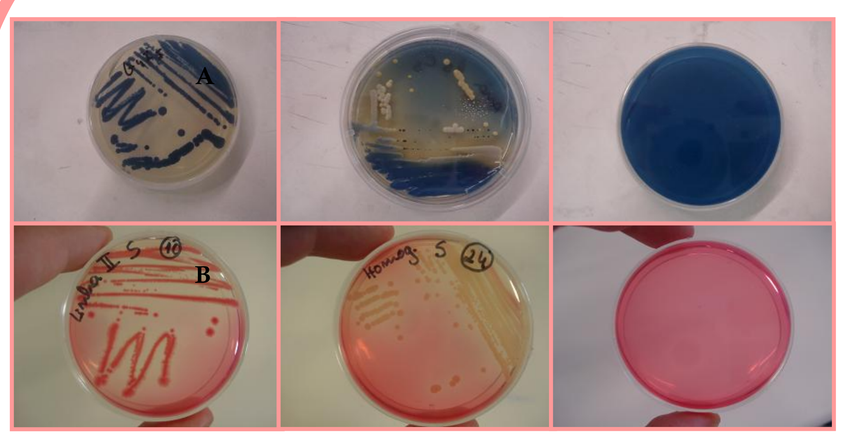


Figure 1. Preliminary observation of decolorization characteristics of strains with performance tested for dye decolorization on NMD medium: (A) Examples of decolorization for Remazol Black BA. (B) Examples of decolorization for Levafix Red CA.

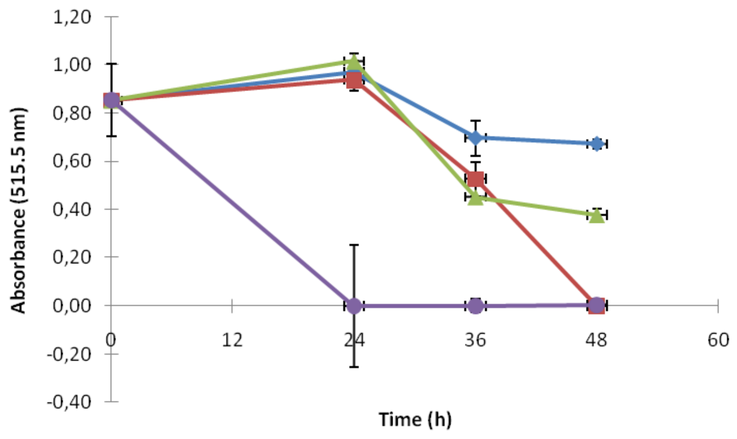
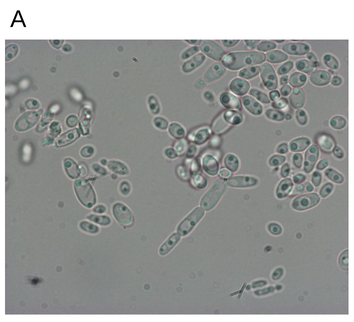
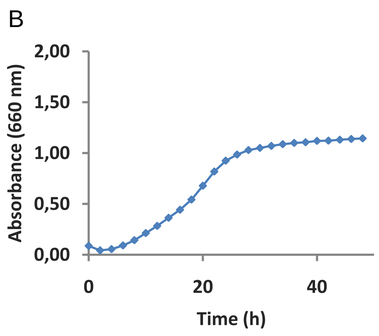


Figure 2. Yeast decolorization of Remazol Brilliant Blue R (A), Levafix Red CA (B) and Levafix Yellow CA (C), in NDM medium after 48 h of cultivation. Abiotic control (♦), isolate HS20 (■), isolate HS24 (▲) and isolate LIIIS36 (●).





GAL	X	SOR	X
ACT		XYL	X
SAC	X	RIB	
NAG	X	GLY	X
LAT	X	RHA	
ARA		PLE	X
CEL	X	ERY	
RAF	X	MEL	X
MAL	X	GRT	
TRE	X	MLZ	X
2KG	X	GNT	
MDG	X	LVT	
MAN	X	GLU	X
LAC		SBE	X
INO		GLN	

Figure 3. LIIIS36 strain identification. (A) microphotography of yeast cell grown in YM medium (7d.). (B) Growth curve in NDM, 30 ºC with agitation obtained in a 96 microplate reader (Fluostar Optima, BMG Labtech). (C) API ID32C (bioMerieux) biochemical test results.

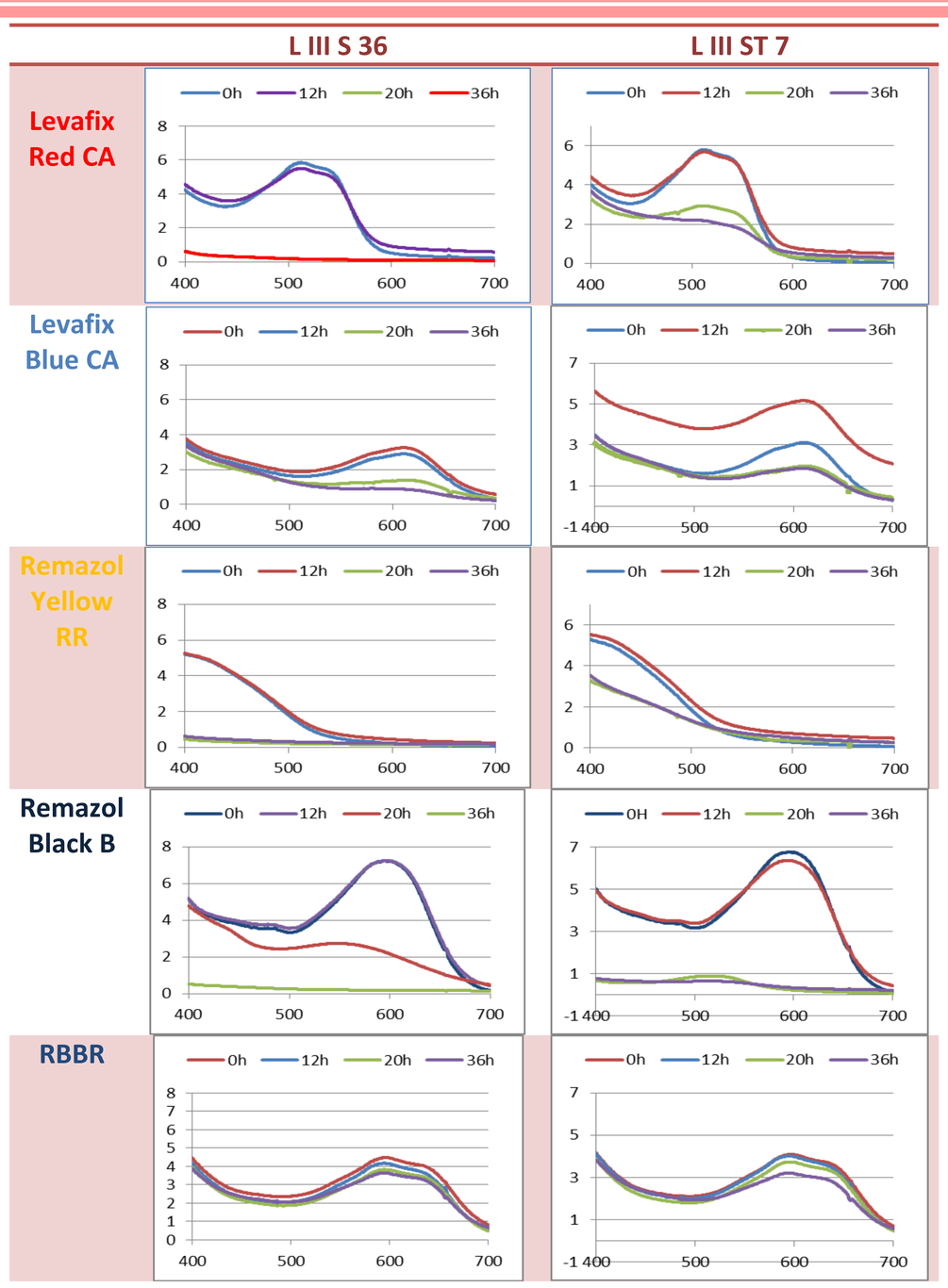


Figure 4. Yeast decolorization (corrected absorbance spectra from 400 to 700 nm) of five selected dyes, in NDM medium, 30 ºC with agitation, for 0, 12, 20 and 36 h samples of strains LIIIS36 and LIIIST7.

Discussion and Conclusions

The preliminary screening from wastewater station samples allowed to isolate 46 yeast isolates. Isolates **HS20**, **HS24** and **LIIIS36** were selected for further evaluation on NDM liquid media, due to their high decolorization ability coupled with low dye adsorption to yeast cells. Strain **LIIIS36** has shown the ability to decolorize more than one of the dyes tested, reaching the maximum decolorization extent in a mere 24-36 h of incubation. Manganese dependent peroxidase activity towards dimethoxyphenol in extracelular fluid was detected for aforementioned strain. Other enzymatic activities are currently under study. Identification with molecular biology is currently underway. Strain LIIIS36 has shown potential for future industrial applications.

References

Moreira, P.R. *et al.* 2001. Decolorization of a Remazol Brilliant Blue R via a novel *Bjerkandera* sp. Journal of Biotechnology, 89: 107-111.

Pajot, H.F. *et al.* 2007. Dye-decolorizing activity in isolated yeasts from the ecoregion of Las yungas (Tucumán, Argentina). Enzyme and Microbial Technology, 40: 1503-1511.

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