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Dyes and Pigments 75 (2007) 38–44
DYES and PIGMENTS
www.elsevier.com/locate/dyepig

Decolorization of high concentrations of synthetic dyes by the white rot fungus *Bjerkandera adusta* strain CCBAS 232

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Received 23 March 2006; received in revised form 5 May 2006; accepted 8 May 2006
Available online 30 June 2006

Abstract

Among thirty different basidiomycetes screened, *Bjerkandera adusta* strain CCBAS 232, *Phanerochaete chrysosporium* strain CCBAS 571 and *Pleurotus ostreatus* strain CCBAS 473 showed the best decolorization properties. *B. adusta* strain CCBAS 232 was able to decolorize a number of chemically different synthetic dyes (Orange G, Amaranth, Remazol Brilliant Blue R, Cu-phthalocyanine and Poly R-478) at relatively high concentrations of 2–4 g L^{-1} both on solid and in liquid medium. This strain also grew easily and produced biomass under these conditions. Unlike *P. chrysosporium* and *P. ostreatus*, *B. adusta* was able to efficiently decolorize all tested dyes. These properties and also a good ligninolytic enzyme production predetermine *B. adusta* strain CCBAS 232 for biotechnological applications.
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Keywords: *Bjerkandera adusta*; Decolorization; Ligninolytic enzymes; *Phanerochaete chrysosporium*; *Pleurotus ostreatus*; Synthetic dyes

1. Introduction

In recent years, synthetic dyes are extensively used in a number of industries, such as textile dyeing or paper printing. Synthetic dyes represent a large group of chemically different compounds, which are classified by their chromophore as azo, anthraquinone, triphenylmethane, heterocyclic or phthalocyanine dyes. Due to their low biodegradability, they cause serious environmental pollution. Most dyes are very stable to light, temperature and microbial attack, making them recalcitrant compounds. Many of them are also toxic or carcinogenic. Conventional wastewater treatment systems are often inefficient and existing physical and chemical technologies are expensive, time-consuming and often methodologically demanding. One of the possible alternatives for treatment of textile effluents is the use of ligninolytic fungi, which produce various isoforms of extracellular oxidases including laccase, Mn peroxidase (MnP) and lignin peroxidase (Lip). These enzymes together with H_2O_2-producing oxidases and secondary metabolites are involved in the degradation of lignin and xenobiotic compounds including synthetic dyes.

One of the strains with high dye-degrading abilities is *Bjerkandera adusta* [1,2]. Although this fungus belongs to the well-known and frequently studied species [3–5], in the field of biodegradation more attention is paid to other species, especially *Phanerochaete chrysosporium* or *Pleurotus ostreatus* [6–10]. Nevertheless, in all these fungi the dyes were tested in the concentrations less than 1 g L^{-1}.

In the current work we studied thirty basidiomycete species for their ability to decolorize two chemically different synthetic dyes: Orange G and Remazol Brilliant Blue R (RBBR). Among them, *B. adusta* together with *P. chrysosporium* and *P. ostreatus* showed the best decolorization properties. The aim of our present work was to characterize the decolorization capacity of the white rot fungus *B. adusta* strain CCBAS 232 with stress on higher dye concentrations than are usually used and to compare its abilities with those of *P. chrysosporium* strain CCBAS 571 and *P. ostreatus* strain CCBAS 473. We used five synthetic dyes belonging to different chemical groups to reveal the differences in decolorization efficiency. As all these species easily

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0143-7208/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.dyepig.2006.05.008

decolorize a number of different synthetic dyes in lower concentrations [4,7,11–13], we searched for the limits of their decolorization capacity. The presented work brings new knowledge about synthetic dye decolorization abilities of *B. adusta*, which seems to be promising for further biotechnological exploitation.

2. Materials and methods

2.1. Organisms

All the strains (listed in Table 1) were obtained from the CCBAS collection (Institute of Microbiology AS CR, Prague, Czech Republic). The strains were maintained by serial transfers and kept on wort agar slants at 4 °C.

2.2. Chemicals

2,2′-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 3-methyl-2-benzothiazolinone hydrazone (MBTH), 3-dimethylaminobenzoic acid (DMAB), Remazol Brilliant Blue R (RBBR) and Poly R-478 were purchased from Sigma; Orange G, Amaranth and Cu-phthalocyanine from Fluka. All chemicals were of analytical grade.

2.3. Culture conditions

Static cultivation was carried out in 250-mL Erlenmeyer flasks with 50 mL of N-limited (0.2 $g L^{-1}$ of ammonium tartrate) Kirk medium [14] at 25 °C. The medium was supplemented with the respective dye at a final concentration of 2, 3 and 4 $g L^{-1}$. The flasks were inoculated with five wort agar plugs (10 mm diameter) cut from an actively growing part of a colony on a Petri dish. Decolorization and enzyme production were followed over a 28-day period and measured on the 7th, 14th, 21st and 28th days of cultivation.

Cultivation on solid media was carried out at 25 °C in Petri dishes (90 mm diameter) containing N-limited Kirk medium with the respective dye at a final concentration of 2, 3 and 4 $g L^{-1}$. The dishes (four parallels) were inoculated with mycelial plugs (3 mm diameter) cut from actively growing mycelia.

2.4. Decolorization assays

Decolorization of the liquid medium was measured in the filtrates (four parallel flasks) after removing the mycelia and

Table 1
Decolorization of synthetic dyes Orange G and RBBR by different basidiomycetes

Strain	Orange G		RBBR	
	Growth (mm)[a]	Decolorization (mm)[b]	Growth (mm)[a]	Decolorization (mm)[b]
Agaricus bisporus CCBAS 305	26	0	25	32
Agaricus xanthodermus CCBAS 225	12	0	10	33
Agrocybe cylindracea CCBAS 312	75	0	65	65
Armillaria melea CCBAS 330	17	0	17	17
Bjerkandera adusta CCBAS 232	90	90	90	90
Ceriporia metamorphosa CCBAS 269	90	0	90	90
Clitocybe gallinacea CCBAS 342	5	0	8	30
Collybia confluens CCBAS 354	12	0	7	0
Coprimus atramentarius CCBAS 356	28	0	17	17
Ganoderma lipsiense CCBAS 746	50	0	27	0
Hapalopillus rutilans CCBAS 544	11	0	25	25
Hericium clathroides CCBAS 548	35	35	25	23
Inonotus andersonii CCBAS 557	80	0	73	0
Inonotus obliquus CCBAS 559	55	0	45	45
Kuehneromyces mutabilis CCBAS 383	40	40	35	40
Laccaria proxima CCBAS 146	40	40	30	40
Lentinus edodes CCBAS 389	56	30	40	33
Lepista saeva CCBAS 401	34	0	18	25
Phanerochaete chrysosporium CCBAS 571	90	90	90	90
Phellinus pomaceus CCBAS 265	36	28	17	10
Phellinus punctatus CCBAS 262	37	37	32	34
Pholiota adiposa CCBAS 683	50	35	40	27
Pholiota lenta CCBAS 780	60	60	35	35
Pleurotus ostreatus CCBAS 473	90	90	90	90
Polyporus brumalis CCBAS 589	30	0	30	30
Polyporus ciliatus CCBAS 592	90	90	70	70
Pycnoporus sanquineus CCBAS 596	90	75	90	90
Schizophyllum commune CCBAS 752	85	0	68	0
Stropharia semiglobata CCBAS 504	50	50	33	35
Tyromyces lacteus CCBAS 616	90	45	90	90

[a] Radial growth rate measured as a diameter of mycelial colony on the 14th day of cultivation on Kirk N-limited medium containing 0.2 $g L^{-1}$ of the respective dye.
[b] Decolorization measured as a diameter of decolorized zone on a Petri dish on the 14th day of cultivation on Kirk N-limited medium containing 0.2 $g L^{-1}$ of the respective dye.

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