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ORIGINAL PAPER

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Evaluation of synthetic dye decolorization capacity in *Ischnoderma resinosum*

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Abstract The little studied white rot fungus *Ischnoderma resinosum* was tested for its ability to decolorize seven different synthetic dyes. The strain efficiently decolorized Orange G, Amaranth, Remazol Brilliant Blue R, Cu-phthalocyanin and Poly R-478 on agar plates and in liquid culture at a relatively high concentration of 2–4 and 0.5–1 g l^{-1}, respectively. Malachite Green and Crystal Violet were decolorized to a lower extent up to the concentration of 0.1 g l^{-1}. Decolorization capacity of *I. resinosum* was higher than that in *Phanerochaete chrysosporium*, *Pleurotus ostreatus* or *Trametes versicolor*. In contrast with these thoroughly examined fungi, *I. resinosum* was able to degrade a wide spectrum of chemically and structurally different synthetic dyes. *I. resinosum* also efficiently decolorized dye mixtures. In liquid culture, Orange G and Remazol Brilliant Blue R were decolorized most rapidly; the process was not affected by different nitrogen content in the media. Shaken cultivation strongly inhibited the decolorization of Orange G.

Keywords Decolorization · *Ischnoderma resinosum* · Ligninolytic enzyme · Synthetic dye

Introduction

Synthetic textile dyes belong to the most dangerous pollutants which, as part of the industrial effluents, contaminate steadily higher amounts of waste water. Based on the chemical structure of the chromophoric group, dyes can be classified as azo dyes, anthraquinone dyes, triphenylmethane dyes, phthalocyanine dyes or polyaromatic dyes. Most of them are mutagenic and/or carcinogenic and their removal from the environment is very difficult. Physico-chemical treatment methods (adsorption and precipitation methods, chemical degradation or photodegradation) are financially and often also methodologically demanding and time-consuming and mostly not very effective. Biodegradation by different microorganisms appears to be an alternative. Although many bacteria are able to degrade synthetic dyes under anaerobic conditions, there are usually reduced to even more toxic aromatic amines [1, 8]. Currently, white rot fungi seem to be more prospective organisms.

White-rot fungi are definitely the most intensively studied group of organisms in terms of ligninolytic and bioremediation abilities. Their enzymatic system that involves enzymes participating in lignin modification (laccase, lignin peroxidase, manganese peroxidase, H_2O_2-producing oxidases etc.) is able to transform different xenobiotic compounds such as polycyclic aromatic hydrocarbons, polychlorinated biphenyls, pesticides or synthetic dyes [2, 5, 22]. Recent literature emphasizes the role of hydrogen peroxide and free radicals in these processes [14, 25].

Many white rot fungi (e.g. *Phanerochaete chrysosporium*, *Pleurotus ostreatus*, *Bjerkandera adusta*, *Trametes versicolor* etc.) have been intensively studied in connection with the decolorization processes [3, 6, 13, 17, 18], but no information is available about *I. resinosum*. As we have previously screened more than 200 different basidiomycetes and found a fairly high decolorization capacity in *I. resinosum*, we chose this strain for the further study.

The aim of our present work was to characterize the decolorization and ligninolytic abilities of the little studied white rot fungus *I. resinosum* which seems to be promising for further biotechnological exploitation. We performed a study with several synthetic dyes belonging to different chemical groups to reveal the possible differences in decolorization efficiency, ligninolytic enzyme production, growth properties etc. We tested higher dye concentrations than are usually used in other such studies with the intention to find out where are the limits of the decolorization capacity in our strain.

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Materials and methods

Organisms

Ischnoderma resinosum (Fr.) P. Karst. CCBAS 553, *P. chrysosporium* Burds. CCBAS 571, *P. ostreatus* (Jacq.: Fr.) Kumm. CCBAS 473 and *T. versicolor* (L.: Fr.) Pilat CCBAS 612 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.

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), Crystal Violet and Malachite Green were purchased from Sigma, Orange G, Amaranth, RBBR and Cu-phthalocyanin from Fluka. All chemicals were of analytical grade.

Culture conditions

Static or shaken (reciprocal shaker, 1.9 Hz, amplitude 30 mm) cultivation was carried out in 100-ml Erlenmeyer flasks with 20 ml of N-rich (2 g l^{-1} of ammonium tartrate) or N-limited (0.2 g l^{-1} of ammonium tartrate) Kirk medium [28] or of Malt extract medium (malt extract 1%) at 25°C. All media were supplemented with respective dyes at a final concentration of 0.05, 0.1, 0.5 and 1 g l^{-1} or with a mixture of these dyes. The final concentration of individual dyes in the mixture was 0.5 g l^{-1} (Orange G, RBBR, Amaranth, Cu-phthalocyanin) or 0.05 g l^{-1} (Malachite Green, Crystal Violet). The flasks were inoculated with two wort agar plugs (10 mm diameter), cut from an actively growing part of a colony on a Petri dish and incubated at 27°C for 14 days. Decolorization and enzyme production were followed over a 20-day period and measured on the 3rd, 5th, 7th, 10th, 14th, 18th and 20th day 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 2–4 g l^{-1}. The dishes (four parallels) were inoculated with mycelial plugs (3 mm diameter) cut from actively growing mycelia.

Decolorization assays

Decolorization of the liquid medium was measured in the filtrates (four parallel flasks) after removing the mycelia and monitored spectrophotometrically at the maximum visible wavelength of absorbance (478 nm for Orange G, 595 nm for RBBR, 525 nm for Amaranth, 625 nm for Cu-phthalocyanin, 521 nm for Poly R-478, 616 nm for Malachite Green and 591 nm for Crystal Violet). Systems without fungus served as an abiotic control. The dye sorption effect of mycelia during the decolorization process was determined using the biotic control according to [29]: fungal mycelia inactivated at 55°C for 24 h were harvested by filtration from four parallel flasks and than rinsed with sodium tartrate buffer (pH 4.5) to remove residual extracellular enzymes. After that the dye solution was added to each flask with the mycelial aliquots and the adsorption capacity of mycelia was monitored spectrophotometrically for 1 h. Decolorization activity was tested also on solid media, where the radial growth and the zone of color change on the agar plates were measured daily. All measurements were repeated three times.

Ligninolytic enzyme assays

Enzyme activity was measured in filtrates from four parallel flasks detained after mycelia removal. Activities of extracellular laccase (EC 1.10.3.2) and manganese peroxidase (EC 1.11.1.13, MnP) were determined spectrophotometrically by monitoring the absorbance increase at 425 nm (laccase) or 590 nm (MnP) in the reaction mixture. Laccase activity was assayed according to [4] by monitoring the oxidation of ABTS. Determination of MnP activity using MBTH and DMAB was based on the method of [19] modified according to [10]. MBTH and DMAB were oxidatively coupled by the action of the enzyme in the presence of added H_2O_2 and Mn^{2+} ions to give a purple indamine dye product. The values were corrected for the activities in the test samples (nonspecific peroxidase activity) without manganese where manganese sulfate was substitued by ethylenediaminetetraacetate (EDTA) to chelate Mn^{2+} ions present in the extract. All measurements were repeated three times. One unit of enzyme activity (U) was defined as an amount catalyzing the production of one micromole of green or purple dye per milliliter per minute. Activity of lignin peroxidase (LiP) was determined spectrophotometrically by monitoring the oxidation of veratryl alcohol in the presence of H_2O_2 according to [27].

Determination of growth and biomass production

Radial growth was estimated by measuring the diameter of the colonies grown on the solid agar medium on Petri dishes. Biomass production in liquid media was evaluated by determining the dry mass of mycelia. Mycelia were harvested from the cultivation flasks, washed with distilled water, dried at 105°C for 24 h and weighed.

Results and discussion

Decolorization of synthetic dyes on agar plates

Our previous findings concerning decolorization ability of different fungi (data not shown) indicated that *I. resinosum* can easily decolorize different synthetic dyes on Petri dishes up to the concentration of 1 g l^{-1}. In the present work, we focused on higher amounts of these dyes (2–4 g l^{-1}). The majority of the dyes tested (except for Malachite Green, Crystal Violet and Orange G) were decolorized on solid media even at a concentration of 4 g l^{-1} (Table 1). Malachite Green and Crystal Violet were decolorized only at a concentration of 0.1 g l^{-1}, Orange G at a concentration of 3 g l^{-1}. Out of all studied dyes, Amaranth was decolorized most rapidly in all cases. Although in the case of several other dyes (RBBR, Malachite Green, Crystal Violet) the decolorization process started earlier (sometimes already on the 3rd day of incubation) and the decolorization degree (measured on the 10th day of incubation) was higher, a total decolorization of the whole Petri dish took a longer time than in the case of Amaranth.

The presence of synthetic dyes reduced the mycelial growth rate in *I. resinosum* to a different extent (see Table 1). Our results showed a positive correlation between the growth rate and the decolorization ability measured as the diameter of the decolorized zone on the Petri dish. Malachite Green at a concentration of 0.5 g l^{-1} inhibited the growth of *I. resinosum* completely. Strong inhibition of growth was found also in the case of Malachite Green (at a concentration of 0.1 g l^{-1}), Crystal Violet (concentrations 0.1–1 g l^{-1}) and RBBR (concentrations 3 and 4 g l^{-1}). On the other hand, no or only slight growth reduction was found in case of Cu-phthalocyanine, Poly R−478 and Amaranth even at a concentration of 4 g l^{-1}. The presence of individual dyes influenced also the character of the hyphae and mycelial colonies on Petri dishes. Higher dye concentrations brought about not only a higher growth reduction, but also the formation of thicker hyphae, irregular shape of the mycelial colony and increased amount of aerial hyphae in comparison with the control (data not shown).

As literature data on *I. resinosum* decolorization abilities are missing, it is difficult to discuss our results. When we tried to compare our data with those for the other white rot fungi, we did not find any paper reporting on such high concentrations of synthetic dyes used in agar plates studies. The majority of authors tested synthetic dyes at concentrations 0.1–0.3 g l^{-1} [12, 13, 20] or

Table 1 Decolorization of synthetic dyes by *I. resinosum* growing on agar plates

Dye	Concentration (g l^{-1})	Growth and decolorization characteristics			
		Colony diameter (mm, %)[a]	Growth (days)[b]	Zone diameter (mm)[c]	Decolorization rate (days)[d]
Orange G (azo dye)	2	65.0 (72.2)	30	33.5	5 (35)
	3	47.5 (52.8)	35	12.5	6 (38)
	4	39.0 (43.3)	36	0	0
Amaranth (azo dye)	2	90.0 (100)	10	90.0	4 (10)
	3	82.4 (91.6)	14	72.5	8 (12)
	4	80.0 (88.9)	18	35.6	10 (18)
RBBR (anthraquinone dye)	2	23.3 (25.9)	46	34.4	3 (35)
	3	17.0 (18.9)	52	26.0	3 (40)
	4	3.0 (3.3)	60	15.2	10 (50)
Cu phthalocyanin (phthalocyanine dye)	2	90.0 (100)	8	76.6	5 (18)
	3	90.0 (100)	8	75.0	6 (24)
	4	90.0 (100)	10	62.9	6 (28)
Poly R (polyaromatic dye)	2	90.0 (100)	10	75.3	4 (24)
	3	90.0 (100)	10	60.1	6 (24)
	4	90.0 (100)	10	50.2	8 (28)
Malachite Green (triphenylmethane dye)	0.05	45.6 (50.7)	24	37.7	3 (22)
	0.1	10.2 (11.3)	28	16.3	3 (24)
	0.5	0	0	0	0
	1	0	0	0	0
Crystal Violet (triphenylmethane dye)	0.05	43.4 (48.2)	28	37.0	3 (28)
	0.1	15.8 (17.6)	30	17.9	6 (30)
	0.5	8.5 (9.6)	0	0	0
	1	3.2 (3.6)	0	0	0

[a]The first number represents the diameter of the mycelial colony in millimeters (measured on the 10th day of cultivation), the number in parentheses shows the colony diameter in percentage of the control (control = colony diameter of *I. resinosum* growing on the Kirk N-limited agar medium without dyes; the colony diameter of the control was 90.0 mm on the 10th day of cultivation)
[b]Growth: the number represents the day on which the Petri dish (diameter 90 mm) was completely colonized by the mycelia of the fungus
[c]Diameter of the decolorized zone in mm (measured on the 10th day of cultivation)
[d]Decolorization rate: the first number represents the day of cultivation on which the decolorization started; the number in parentheses indicates the day of cultivation on which the Petri dish was completely decolorized

at a concentration lower than 0.1 g l^{-1} [7]. Therefore we performed the experiments with three well-described fungi (*P. chrysosporium*, *P. ostreatus* and *T. versicolor*) using the high concentrations (2, 3 and 4 g l^{-1}) of the dyes to compare the results with *I. resinosum* (Table 2). None of these strains was able to grow on media containing Malachite Green and we did not find any decolorization of this dye. *P. chrysosporium* exhibited fast growth on media in the presence of majority of the dyes, a very high capacity to decolorize both tested azo dyes and a good ability to decolorize Poly R. However, it was not able to decolorize RBBR, Malachite Green and Crystal violet. Also Cu-phthalocyanine was decolorized by this fungus only at the concentration of 2 g l^{-1}. The growth rate of *P. ostreatus* and *T. versicolor* on media with the dyes was in most of the cases comparable with *I. resinosum*. Nevertheless, *P. ostreatus* showed a substantially lower decolorization capacity—it decolorized only Amaranth up to the concentration of 3 g l^{-1} and Crystal violet in the lowest (0.05 g l^{-1}) concentration tested. *T. versicolor* decolorized the majority of the dyes to a certain extent, however, RBBR, Cu-phthalocyanine and Crystal violet only at the lowest concentration. *T. versicolor* decolorized also Orange G only up to the concentration of 3 g l^{-1}, but more rapidly than *I. resinosum*. It is evident that dyes belonging to chemically different groups were not decolorized to the same extent and that structural differences in the dye molecule strongly affect the decolorization process. Comparing decolorization capacity of our tested strains, we can conclude that only *I. resinosum* was able to decolorize all the studied dyes efficiently, including Malachite Green, which is used as a fungicide and also possesses a high toxicity to bacteria and mammalian cells [21]. Although *P. chrysosporium* showed in some cases (Orange G and Amaranth) the highest decolorization capacity among the strains studied, its ability to decolorize other dyes was very limited. Our results indicated that *I. resinosum* possesses a high decolorization ability, which was even higher than that in some well-described fungi. In contrast with these fungi, *I. resinosum* was able to degrade a wide spectrum of chemically and structurally different synthetic dyes. The current results revealed that even the concentration of 4 g l^{-1} of some azo, phthalocyanine, or polyaromatic dyes does not limit the *I. resinosum* decolorization capacity.

Decolorization in liquid media

Our strain *I. resinosum* was more sensitive to the tested dyes in liquid culture than in agar plates (data not

Table 2 Decolorization of synthetic dyes by *P. chrysosporium*, *P. ostreatus* and *T. versicolor* growing on agar plates

Dye	Concentration (g l^{-1})	Growth and decolorization characteristics					
		Phanerochaete chrysosporium		*Pleurotus ostreatus*		*Trametes versicolor*	
		Colony diameter (mm, %, days)[a]	Decolorization (mm, days)[b]	Colony diameter (mm, %, days)[a]	Decolorization (mm, days)[b]	Colony diameter (mm, %)[a]	Decolorization (mm, days)[b]
Orange G (azo dye)	2	90.0 (100) 3	90.0 (4)	78.4 (87.1)	0	85.5 (100)	85.9 (6)
	3	90.0 (100) 3	90.0 (5)	75.5 (83.9)	0	85.0 (99.4)	85.4 (8)
	4	90.0 (100) 3	90.0 (6)	75.2 (83.6)	0	82.0 (95.9)	0
Amaranth (azo dye)	2	90.0 (100) 3	90.0 (3)	90.0 (100) 10	35.4 (8)	85.5 (100)	70.2 (6)
	3	90.0 (100) 3	90.0 (4)	85.0 (94.4)	20.2 (10)	85.0 (99.4)	65.5 (8)
	4	90.0 (100) 3	90.0 (6)	80.9 (89.9)	0	80.9 (94.6)	40.8 (10)
RBBR (anthraquinone dye)	2	23.3 (25.8)	0	17.1 (19.0)	0	22.4 (26.2)	20.5 (10)
	3	20.0 (22.2)	0	12.5 (13.9)	0	13.7 (16.0)	0
	4	6.1 (6.7)	0	10.0 (11.1)	0	9.5 (11.1)	0
Cu phthalocyanin (phthalocyanine dye)	2	90.0 (100) 3	85.5 (6)	85.5 (95.0)	0	85.0 (99.4)	55.5 (7)
	3	90.0 (100) 3	0	85.0 (94.4)	0	85.0 (99.4)	50.6 (8)
	4	90.0 (100) 3	0	73.0 (81.1)	0	80.0 (93.6)	20.0 (8)
Poly R (polyaromatic dye)	2	90.0 (100) 3	90.0 (3)	90.0 (100) 10	0	85.5 (100)	65.0 (6)
	3	90.0 (100) 3	85.2 (3)	90.0 (100) 10	0	75.5 (88.3)	0
	4	90.0 (100) 3	60.0 (3)	90.0 (100) 10	0	70.2 (82.1)	0
Malachite Green (triphenylmethane dye)	0.05	0	0	0	0	0	0
	0.1	0	0	0	0	0	0
	0.5	0	0	0	0	0	0
Crystal Violet (triphenylmethane dye)	0.05	17.2 (19.1)	0	30.0 (33.3)	26.5 (6)	29.4 (34.4)	25.5 (6)
	0.1	8.8 (9.8)	0	10.8 (12.0)	0	0	0
	0.5	0	0	0	0	0	0

[a]The first number represents the diameter of the mycelial colony in millimeters (measured on the 10th day of cultivation), the number in parentheses shows the colony diameter in percentage of the control (control = colony diameter of respective strain measured on the 10th day of cultivation on the Kirk N-limited agar medium without dyes, which was 90.0 mm for *P. chrysosporium* and *P. ostreatus* and 85.5 mm for *T. versicolor*), third number (given only in the case of very fast growth rate) indicates the day on which the P.dish (diameter 90 mm) was completely colonized by the mycelia of the fungus

[b]The first number represents the diameter of the decolorized zone in millimeters (measured on the 10th day of cultivation); the number in parentheses indicates the day on which the decolorization started

Table 3 Decolorization ability, biomass production and ligninolytic enzyme production of *Ischnoderma resinosum* cultivated in liquid medium containing the dyes

Dye	Concentration (g l^{-1}l)	Decolorization (%)[a]		Biomass production (%)[b]		Laccase production (%)[c]		MnP production (%)[d]	
		10th day	20th day	10th day	20th day	10th day	20th day	10th day	20th day
Orange G	0.5	92.7	95.4	64.7	88.1	995.2	1,220.7	250.9	27.0
	1	94.4	94.6	38.8	80.7	2,505.4	2,277.1	160.0	56.6
Amaranth	0.5	57.8	99.1	50.5	113.3	155.0	131.5	60.2	88.6
	1	30.3	67.1	85.3	100.0	125.0	100.4	170.6	117.5
RBBR	0.5	94.5	94.8	24.1	45.2	280.1	762.0	510.0	28.9
	1	89.5	93.7	20.7	17.0	235.3	1,169.8	184.2	68.5
Cu phthalocyanin	0.5	70.6	92.2	98.3	180.0	245.8	1,262.2	520.0	12.2
	1	62.2	85.0	86.2	97.8	250.4	246.0	36.5	36.7
Poly R	0.5	23.8	27.2	111.2	157.2	115.5	238.5	260.4	166.5
	1	13.0	17.3	87.9	110.1	125.0	246.4	255.9	124.4
Malachite Green	0.05	49.7	97.0	45.5	9.4	25.5	515.7	230.3	105.9
	0.1	20.0	21.2	40.5	8.7	20.6	38.5	27.7	39.1
Crystal Violet	0.05	34.5	99.6	30.2	11.0	25.0	192.6	180.4	5.1
	0.1	9.4	11.4	12.9	11.6	20.1	31.6	318.9	117.2

[a]Decolorization in percentage of initial amount
[b]Biomass production in percentage of control (control = *I. resinosum* growing in liquid Kirk medium without dyes; biomass production: 1.9 g l^{-1} on the 10th day, 4.4 g l^{-1} on the 20th day)
[c]Laccase production in percentage of control (control = *I. resinosum* growing in liquid Kirk medium without dyes; laccase activity was 20 U l^{-1} on the 10th day, 13 U l^{-1} on the 20th day)
[d]MnP production in percentage of control (control = *I. resinosum* growing in liquid Kirk medium without dyes; MnP activity was 11 U l^{-1} on the 10th day, 41 U l^{-1} on the 20th day)

shown). We therefore used the dyes at concentrations 0.5–1 g l^{-1}. The strain was able to decolorize all the tested dyes to a certain extent (Table 3). An uninoculated control showed no color removal. The physical adsorption of the dyes on the mycelia, monitored during the whole cultivation process (biotic controls), was insignificant in all cases. The dyes most rapidly decolorized were Orange G and RBBR, both in concentrations of 0.5 and 1 g l^{-1}. More than 90% of the dyes was removed within the first 10 days of cultivation. Amaranth and Cu-phthalocyanin at a concentration of 0.5 g l^{-1} were also efficiently decolorized within 20 days. Of the studied dyes, Malachite Green and Crystal Violet were decolorized to the lowest extent. *I. resinosum* was able to decolorize these dyes only at a concentration ten times lower than the other dyes tested. When the initial concentration of Malachite Green or Crystal Violet was only 0.05 g l^{-1}, more than 95% of the dyes was decolorized within 20 days; at 0.1 g l^{-1} the decolorization efficiency strongly decreased.

The results showed that *I. resinosum* is able to decolorize efficiently not only individual synthetic dyes, but also their mixtures (Fig. 1a–c). Three mixtures containing Orange G + RBBR, Cu-phthalocyanin + Malachite Green and Amaranth + Crystal Violet, were tested. The mixtures were decolorized to a high extent already within 14 days of cultivation and after 20 days they were almost completely decolorized.

In the case of the liquid culture, similarly as in agar plates, lower dye concentrations (0.1–0.3 g l^{-1}) were used by the majority of authors [9, 11, 24, 26]. However, Jarosz-Wilkołazka et al. [13] described a successful decolorization of Basic Blue (antraquinone dye) and Acid Red (azo-dye) at a concentration of 1 g l^{-1} by several white rot fungi. Our results showed a high decolorization capacity of *I. resinosum* in liquid culture even at the dye concentrations of 0.5 and 1 g l^{-1}. The good ability of our strain to decolorize a mixture of dyes could be important in the development of further wastewater treatment for textile industries.

Ligninolytic enzyme and biomass production

Our results showed that ligninolytic enzyme and biomass production were affected by the type of the added dye (see Table 3). *I. resinosum* produces laccase and MnP, but no detectable amount of lignin-peroxidase. Orange G had the strongest positive effect on laccase activity during the whole cultivation period (a more than 20-fold increase) in comparison with a culture without any dye added. At the end of the cultivation, a tenfold increase of laccase activity was also found in the medium containing RBBR and Cu-phthalocyanin. On the other hand, Malachite Green and Crystal Violet mostly reduced laccase activity. However, a fivefold (in the case of Malachite Green) or twofold (in the case of Crystal Violet) increase of the laccase activity, found on the 20th day of cultivation in the media with a lower content of these dyes, positively correlated with the efficiency of decolorization. The presence of the dyes influenced also MnP production—mostly a slight increase of activity was found in the early stages of cultivation and a strong decrease in the later ones. The highest increase of MnP production (fivefold) was detected in a medium containing RBBR at a concentration of 0.5 g l^{-1}.

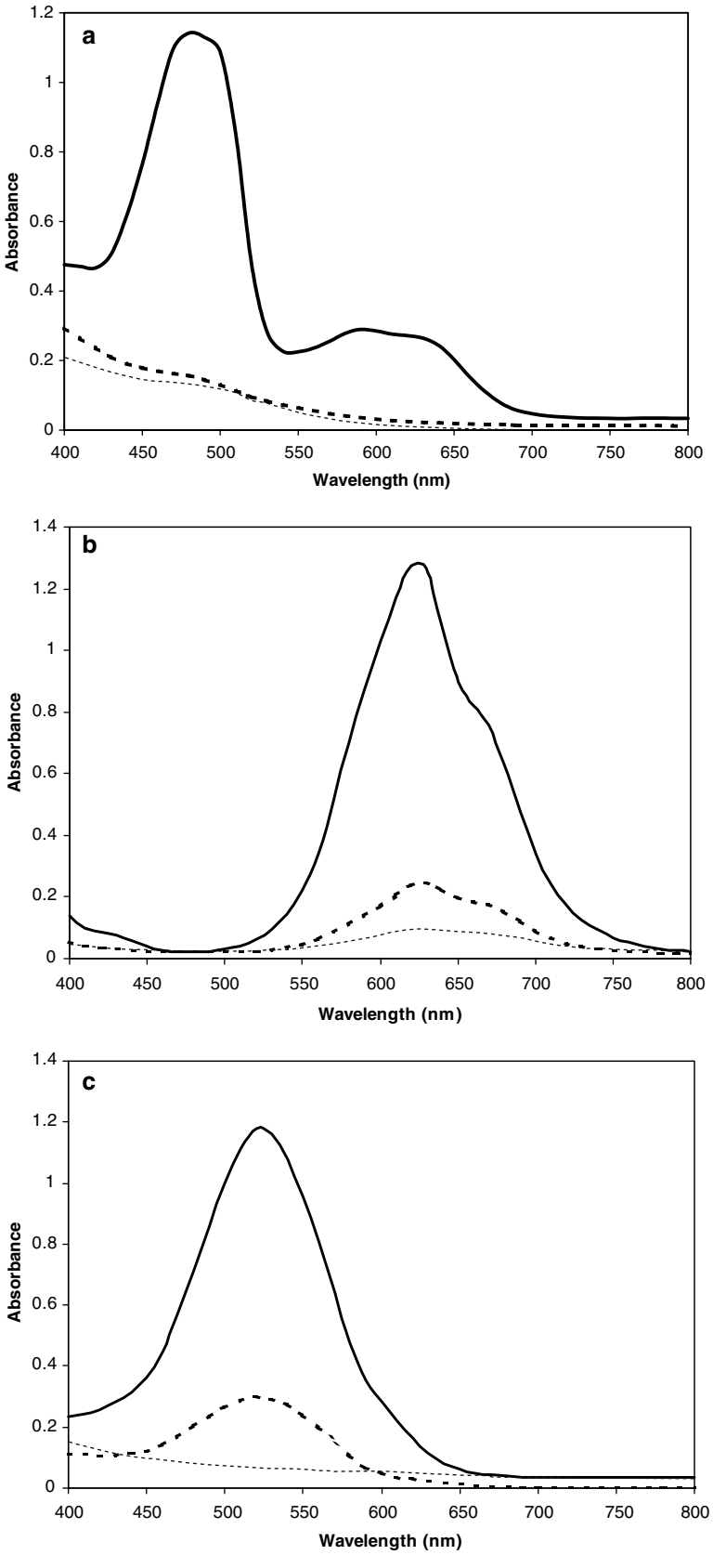

Fig. 1 Decolorization of dye mixtures by *I. resinosum* in liquid medium. The visible spectra of the dye mixtures and absorbance decrease before (*full line*) and during decolorization: after 10 days of cultivation (*dashed line*) and after 20 days of cultivation (*dotted line*) were recorded. **a** Mixture of Orange G (final concentration 0.5 g l^{-1}) and RBBR (0.5 g l^{-1}). **b** Mixture of Cu-phthalocyanine (0.5 g l^{-1}) and Malachite Green (0.05 g l^{-1}). **c** Mixture of Amaranth (0.5 g l^{-1}) and Crystal Violet (0.05 g l^{-1})

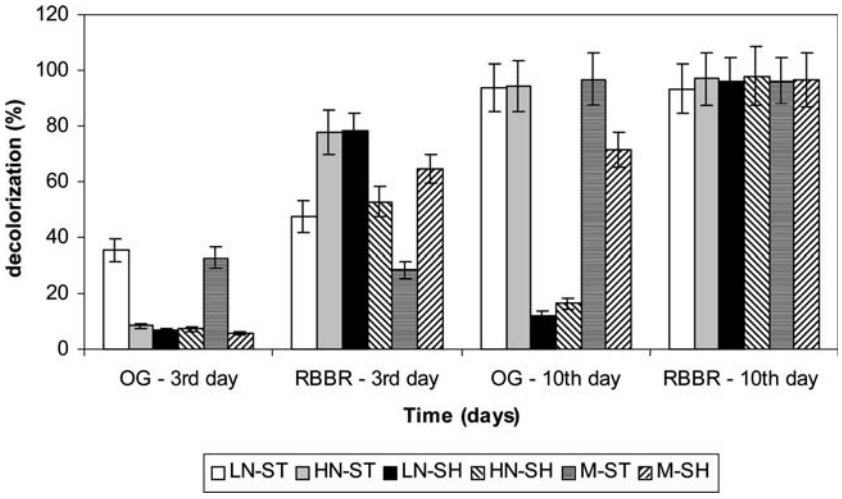

Fig. 2 Decolorization ability of *I. resinosum* cultivated under different conditions. The decolorization of Orange G (*OG*) and Remazol Brilliant Blue R (*RBBR*) was measured in N-limited (*LN*) and N-rich (*HN*) Kirk media and in Malt extract medium (*M*) on the 3rd and 10th day of cultivation. The influence of static (*ST*) and shaken (*SH*) cultivation on decolorization capacity was followed

The growth rate and biomass production in liquid culture was substantially reduced in the media containing RBBR, Malachite Green and Crystal Violet. The strongest inhibition was found especially at the end of cultivation in the media containing Malachite Green and Crystal Violet, when the biomass yield reached only about 10% of that in the media without dyes. Interestingly, the presence of Poly R−478, Cu-phthalocyanine and Amaranth caused in several cases an increase of the growth rate and biomass production of *I. resinosum* (see Table 3).

Our results show that ligninolytic enzymes (especially laccase) are directly involved in the decolorization of synthetic dyes by *I. resinosum*. Nevertheless, this process is obviously more complicated and influenced by many other enzymes and factors, such as different mediators, hydrogen peroxide, etc. [14, 15, 25]. Also the role of biomass production becomes significant if decolorization requires such biomass-associated factors or if certain degradative enzymes are bound to the mycelium [24].

Influence of culture conditions on the decolorization process

The influence of different culture conditions (static vs. shaken cultivation) on the decolorization capacity of *I. resinosum* cultivated in liquid Kirk and Malt extract media containing 0.5 g l^{-1} of Orange G or RBBR is shown in Fig. 2. In the case of RBBR, decolorization capacity was similar under all conditions tested. The decolorization rate was very high and a substantial part of the dye was mostly removed already within first 3 days of incubation. The dye was almost completely decolorized within 10 days. Our findings indicated that the shaken cultivation strongly inhibited the decolorization of Orange G, especially when the fungus grew in Kirk medium. These findings were somewhat unexpected, because many papers report on an increase of decolorization capacity in shaken cultures [13, 23, 24]. On the other hand, Kim et al. [16] also described a decrease in RBBR decolorization in shaken cultures. In our studies, RBBR decolorization capacity of the strain was not negatively influenced in the shaken culture, in contrast with Orange G.

In conclusion, our results show that the white-rot fungus *I. resinosum* is capable of decolorizing a number of synthetic dyes, even at relatively high concentrations. This ability together with the capacity to decolorize a mixture of the dyes predetermines this strain for biotechnological applications.

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