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Decolorization and Degradation of Xanthene Dyes by a White Rot Fungus, *Coriolus Versicolor*

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ABSTRACT

The decolorization of six xanthene dyes (conc. 100 μM) by a white rot fungus, *Coriolus versicolor* (*C. versicolor*), was investigated in liquid culture. The decolorization of Fluorescein, 4-Aminofluorescein, and 5-Aminofluorescein by the fungus was 85.0, 95.0, and 91.9% after 14 days incubation, respectively. However, no decolorization of Rhodamine B, Rhodamine 123 hydrate, and Rhodamine 6G was observed. The first three dyes also were decolorized with cell-free extracts from *C. versicolor*. The decolorization activity was 10.2, 6.7, and 7.2 $\mu M\, min^{-1}\, mg^{-1}$, respectively. Thin layer chromatography (TLC) analyses indicated that degradation of Fluorescein was occurring with the detection of three degradation products.

Key Words: Xanthene dyes; Decolorization; *Coriolus versicolor*.

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INTRODUCTION

Organic synthetic dyes are used extensively in the biomedical, foodstuff, plastic, and textile industries. About 15% of the total world production of dyes is lost in effluent during the synthesis and processing of dyes.[1] When discharged to the environment, the dyes cause both organic pollution and a higher coloration of effluent. The dyes are generally considered as xenobiotic compounds that are very recalcitrant against biodegradable processes. However, during the last several years it has been demonstrated that white rot fungi such as *Phanerochaete chrysosporium* and *C. versicolor* are able to transform dyes (azo, anthraquinone, triphenylmethane, and phthalocyanine) to colorless products or even to completely mineralize them.[2–6]

Xanthene dyes have many uses as colors in textile, paper, food, cosmetics, and ink, due to superior dyeing and coloring properties. The dyes are also poorly biodegradability[7,8] and some of the dyes are toxic compounds.[9] We hypothesized that nonspecific nature of white rot fungi will reasonably be expected to be effective in degradation of the dyes.

In this report, we demonstrated the decolorization and degradation of xanthene dyes by a white rot fungus, *C. versicolor*.

MATERIALS AND METHODS

Chemicals

The xanthene dyes used are shown in Fig. 1. The dyes were purchased from Aldrich Chemical Co. (USA). Fluorescein was purified by preparative TLC eluting with chloroform–methanol (5:1, v/v). The other dyes were used without any purification steps.

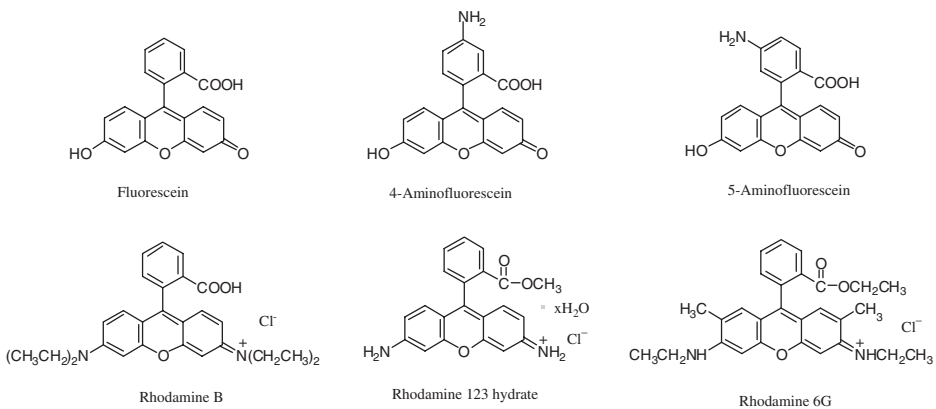

Figure 1. Xanthene dyes used in this study.

Microorganism

Coriolus versicolor IFO 30388 was found as an organic synthetic dyes-decolorizing fungus.[3,10] This fungus was kindly supplied by the Institute for Fermentation, Osaka (IFO), Japan, is optimally grown at 24°C and pH 5.6. It was maintained on potato-dextrose (Difco Laboratories, USA) agar slants at 6°C, transferred to potato-dextrose agar plates, and incubated at 24°C for 10 days before use in this experiment.

Decolorization of Xanthene Dyes by *C. versicolor*

A potato-dextrose agar gel (5 × 5 mm) cutted from the above culture plates was inoculated in sterilized potato-dextrose liquid culture (pH 5.6, 50 mL) containing dye (conc. 100 μM) in a 100 mL Erlenmeyer flask. The culture was incubated at 24°C on a rotary shaker (120 rpm) under aerobic conditions. After an appropriate time, the incubated culture was centrifuged (3000 g × 1 min), and the absorbance of the supernatant was measured at λ_{max} of visible region of each dyes. Decolorization (%) was calculated as follows: Decolorization (%) = [(initial absorbance of λ_{max}) − (observed absorbance of λ_{max})/(initial absorbance of λ_{max})] × 100.

Decolorization Activity of Cell-free Extracts

A potato-dextrose agar gel (10 × 10 mm) cutted from the above culture plates was inoculated in sterilized potato-dextrose liquid culture (pH 5.6, 250 mL) in a 500 mL Erlenmeyer flask. The culture was incubated for 14 days as described above. Cells were harvested by centrifuging (3000 g × 15 min), and washed twice with 70 mM Sørensen buffer, pH 5.6. The cells (25 g wet weight) were redispersed in ice cold Sørensen buffer (50 mL). The cell dispersion was disrupted mechanically with glass beads using a Bead Beater (Biospec Products, USA). The homogenate was centrifuged (20,000 g × 30 min) at 4°C. The supernatant fraction was collected and used as cell-free extracts. The protein content of the cell-free extracts was determined by the method of Bradford.[11]

Decolorization activity of the cell-free extracts was estimated as follows. The reaction mixture consisted of 10 μM dye in 2.5 mL of 70 mM Sørensen buffer (pH 5.6) and 0.5 mL of the cell-free extracts. The reaction was started by the addition of the cell-free extracts, and the decrease in absorbance at λ_{max} of each dye was monitored for 5 min at 25°C. Decolorization activity was defined as the rate of decrease of dye (μM min^{-1}) per mg protein.

Degradation of Fluorescein by *C. versicolor*

Cultures (250 mL × 12; total volume of 3 L) of *C. versicolor* containing 100 μM of Fluorescein were incubated for 14 days as described above. The cultures were adjusted to pH 2.0 with 1 N HCl and extracted twice with an equal volume of

1-butanol. The extracts were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was redissolved in a small amount of methanol and subjected to TLC on Merck Silicagel $60F_{254}$ with chloroform–methanol (5:1, v/v). The bands of the products were detected under UV light, scraped, and extracted with methanol for recording of their UV-visible spectra.

RESULTS AND DISCUSSION

Decolorization of Xanthene Dyes

The results of decolorization (%) of xanthene dyes by growing cell of *C. versicolor* are shown in Table 1. Fluorescein, 4-Aminofluorescein, and 5-Aminofluorescein incubated with *C. versicolor* were slowly decolorized with increasing incubation time. After 14 days of incubation, the decolorization of these dyes reached to 85.0, 95.0, and 91.9%, respectively. However, Rhodamine B, Rhodamine 123 hydrate, and Rhodamine 6G were not decolorized through 14 days of incubation.

Decolorization Activity of Cell-free Extracts

Spectra changes of Fluorescein with the cell-free extracts from *C. versicolor* as an example are shown in Fig. 2. The absorption spectra of Fluorescein can be characterized by λ_{max} of 476 nm. The absorbance at 476 nm decreased with increasing reaction time. The decolorization activity of the cell-free extracts was confirmed from the decrease in absorbance at λ_{max} of each dye is shown in Table 2. The cell-free extracts decolorized Fluorescein, 4-Aminofluorescein, and 5-Aminofluorescein. The decolorization activity was 10.2, 6.2, and 7.9 $\mu M\,min^{-1}\,mg^{-1}$, respectively. Rhodamine B, Rhodamine 123 hydrate, and Rhodamine 6G were not decolorized by the cell-free extracts in these experiment conditions. The ability of the growing cells and the cell-free extracts of *C. versicolor* in decolorizing these dyes would be depend on the substituent groups on the xanthene ring. Fluorescein, 4-Aminofluorescein, and 5-Aminofluorescein all contain a hydroxy substituent on xanthene ring. However, Rhodamine B, Rhodamine 123 hydrate, and Rhodamine 6G contain no hydroxy substituents.

Table 1. Decolorization of xanthene dyes with growing cell of *C. versicolor*.

Dye	λ_{max} (nm)	Decolorization (%) after:				
		1	4	7	10	14 (days)
Fluorescein	476	3.3	14.0	79.3	85.0	85.0
4-Aminofluoresceine	476	1.2	5.3	51.1	88.2	95.0
5-Aminofluoresceine	474	1.2	9.3	52.2	89.9	91.9
Rhodamine B	554	0	0	0	0	0
Rhodamine 123 hydrate	500	0	0	0	0	0
Rhodamine 6G	527	0	0	0	0	0

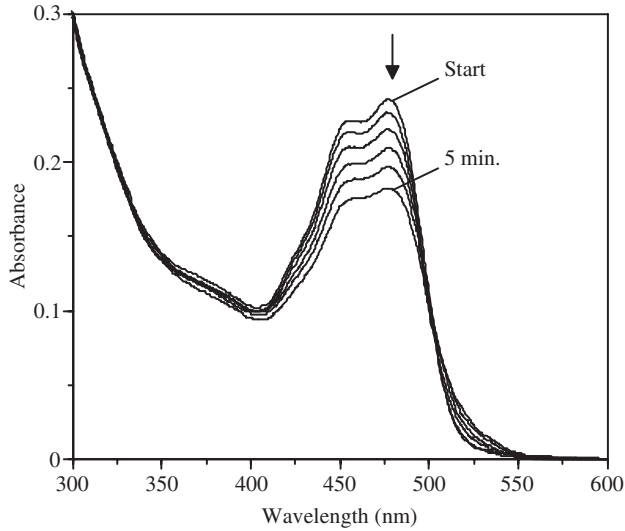

Figure 2. Change of absorption spectra of Fluorescein by cell-free extracts. Spectra were taken at intervals of 1 min (0–5 min) with a Shimadzu model Multispec-1500 UV-visible photodiode array spectrophotometer; Protein contents of cell-free extracts: 0.24 mg/mL.

Table 2. Decolorization activity of cell-free extracts.

Dye	Decolorization activity ($\mu M\ min^{-1}\ mg^{-1}$)
Fluorescein	10.2
4-Aminofluorescein	6.2
5-Aminofluorescein	7.9
Rhodamine B	0
Rhodamine 123 hydrate	0
Rhodamine 6G	0

C. versicolor is capable of producing one or more lignin-type peroxidases and laccase.[12] In attempt to detect activity of these enzymes, we detected mainly laccase activity in the cell-free extracts.[10] Laccase catalyzes the oxidation of various phenolic compounds and the ring cleavage of lignin model compounds. Therefore, we presumed that a hydroxy substituent on the xanthene ring for decolorization of the dyes was beneficial for the attack by laccase from this fungus.

Degradation of Fluorescein by *C. versicolor*

The degradation products of Fluorescein in the incubated culture after 14 days were detected by TLC. The trace amounts of a colored and two colorless products

Table 3. *Rf* values on TLC and λ_{max} of products from fluorescein.

Product	*Rf* value	λ_{max} (nm)[a]
1	0.85	243, 298
2	0.43	452
3	0.02	unclearness (broad)

[a]Solvent; methanol.

were contained in the 1-buthanol extracts of the culture. The *Rf* values on TLC and λ_{max} of the products are shown in Table 3. The appearance of three spots of the products on TLC made sure the degradation of Fluorescein by *C. versicolor*. Further attempts to obtain mass spectra and nuclear magnetic resonance spectra data for these products were unsuccessful.

Our results indicate that the growing cell and the cell-free extracts of *C. versicolor* efficiently decolorized hydroxy substituted xanthene dyes, Fluorescein, 4-Aminofluorescein, and 5-Aminofluorescein. Degradation of Fluorescein by *C. versicolor* was indicated by formation of a colored and two colorless products in culture broth. Further studies are necessary to clear degradation processes of the dyes by white rot fungi for reason of detoxification and/or mineralization of xanthene dyes.

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