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Biodecolorization and biodegradation potential of recalcitrant triphenylmethane dyes by *Coriolopsis* sp. isolated from compost

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ABSTRACT

Triphenylmethane dyes (TPM) are recalcitrant colorants brought into the environment. In this study, a lesser-known white rot fungus *Coriolopsis* sp. (1c3), isolated from compost of Empty Fruit Bunch (EFB) of oil palm, was explored for its decolorization potential of TPM dyes. The isolate 1c3 demonstrated good decolorization efficiencies in the treatment of Crystal Violet (CV; 100 mg l^{-1}), Methyl Violet (MV; 100 mg l^{-1}) and Cotton Blue (CB; 50 mg^{-1}), with 94%, 97% and 91%, within 7, 7 and 1 day(s), respectively. Malachite Green (MG; 100 mg l^{-1}) was the most recalcitrant dye, with 52% decolorization after 9 days. Dye removal by 1c3 was presumably via biosorption, whereby the process was determined to be influenced by fungal biomass, initial dye concentrations and oxygen requirements. Biodegradation was also a likely mechanism responsible for dye removal by 1c3, occurred as indicated by the reduction of dye spectra peaks. Detection of laccase, lignin peroxidase and NADH-DCIP reductase activities further substantiate the possible occurrence of biodegradation of TPM dyes by 1c3.

1. Introduction

Triphenylmethane (TPM) dyes are synthetic, aromatic colorants used extensively in various textiles, paper, leather, and pharmaceutical industries (Daneshvar et al., 2007). Among the many types of TPM dyes, Crystal Violet (CV), Methyl Violet (MV), Malachite Green (MG) and Cotton Blue (CB) are extensively used resulting in 10–15% of unused dye released into the environment annually (Shedbalkar et al., 2008; Yang et al., 2009). TPM dyes are recalcitrant to degradation due to their synthetic nature and complex aromatic structures (Daneshvar et al., 2007). Their persistence in the environment leads to health and ecological concerns due to their toxic, mutagenic and carcinogenic nature (Jadhav and Govindwar, 2006).

Conventional approaches to remove TPM dyes include application of activated carbon, membrane filters, and chemical coagulation and flocculation (Saratale et al., 2006). These methods are generally effective, but are limited by steep investment costs and generation of toxic sludge (Shedbalkar et al., 2008). Bioremediation is implemented as an alternative as this approach has many benefits to the environment (Shedbalkar et al., 2008). Bioremediation of dyes can be achieved via biosorption or biodegradation. In biosorption, dye molecules bind to the surface of the biomass, while in biodegradation the enzymes are responsible to degrade large toxic dye molecules into simpler and less toxic compounds (Jadhav and Govindwar, 2006).

Historically, dye removal is predominantly performed by typical white rot fungi such as *Phanerochaete chrysosporium*, *Trametes versicolor*, *Pleurotus ostreatus* and *Bjerkandera adusta*; attributed to the role of their extracellular non-specific ligninolytic enzymes (Liu et al., 2004; Salame et al., 2012). These enzymes include laccase (Lac), lignin peroxidase (LiP) and manganese peroxidase (MnP) (Nishiya and Yamamoto, 2007; Zhuo et al., 2011; Anastasi et al., 2011). Fungal species capable of dye removal have been reported to produce at least one of these essential enzymes (Casas et al., 2009). In recent years, other fungal species have emerged with reports in removing TPM dyes. *Aspergillus fumigatus* and *Fusarium solani* (Martius) Sacchardo are two examples where biosorption of Acid Violet 49, CV and MG have been demonstrated (Abedin, 2008; Chaudhry et al., 2013). For biodegradation, only *Aspergillus* sp. has been established to biodegrade MG and MV (Saratale et al., 2006; Kumar et al., 2011, 2012).

In this study, the dye removal and biodegradation potential of a lesser-known white rot fungus, *Coriolopsis* sp. (1c3) was explored. Several species of this genus; *C. rigida*, *Coriolopsis gallica*, *Coriolopsis polyzona* and *C. byrsina*, have been reported as laccase producers able to decolorize anthraquinone, azo, heterocyclic and metal

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textile dyes (Sanchez-Lopez et al., 2008; Gomes et al., 2009; Chairin et al., 2013; Daâssi et al., 2014). Nevertheless, their potential to decolorize TPM dyes remains to be explored. Isolate 1c3 was recovered from the EFB compost of oil palm and was the isolate of interest as it had successfully decolorized the azo dye Remazol Brilliant Blue R (RBBR) with 70% decolorization efficiency (Peh et al., 2011). The isolate 1c3 was tested on four different TPM dyes (CV, MV, MG, CB). The optimum conditions for dye decolorization (presumably via biosorption) under the influence of fungal biomass, initial dye concentration and oxygen requirement were established. The biodegradation potential of 1c3 was detected from evaluations on the change of the absorption spectra, and the quantification of enzymatic activities (laccase, lignin peroxidase and NADH-DCIP reductase activities). This paper reports the results from this study with aims to provide information on the use of 1c3 for the removal of TPM dyes in wastewater.

2. Methods

2.1. Culture establishment and biomass generation

Isolate 1c3 was isolated by Peh et al. (2011) from Empty Fruit Bunch (EFB) compost of oil palm (*Elaeis guineensis*). The fungal isolate was maintained on Potato Dextrose Agar (PDA, Merck) at room temperature (25 ± 2 °C) and sub-cultured periodically. To generate biomass, five mycelial plugs of 1c3 were inoculated into 100 ml of Potato Dextrose Broth (PDB, Difco™, Le Pont de Claix, France) and incubated for 7 days (25 ± 2 °C). The fungal biomass was homogenized with a hand-held homogenizer (LabGEN 125, Cole-Palmer, USA), filtered through Whatman filter paper No. 1, washed with sterile distilled water and weighed to 2.0 ± 0.1 g (fresh weight). This freshly-prepared biomass is used for dye decolorization experiments.

2.2. Dye decolorization tests

Each TPM dye (CV, MV, MG and CB) was dissolved in 100 ml autoclaved MilliQ water (18.2 MQ; Sartorius, Malaysia) to a concentration of 100 mg l^{-1} (except CB to a concentration of 50 mg l^{-1}). To the dye solutions, 2.0 ± 0.1 g of the freshly prepared fungal biomass was introduced and the mixture incubated with agitation (150 rpm, 30 ± 2 °C) (MaxQ 6000, Thermo Scientific, Iowa, USA) for 14 days. Untreated (non-inoculated) dye solutions were designated as negative controls. At every 24 h-interval for the next 14 days, a 2 ml of aliquot was withdrawn, centrifuged (10,000 rpm, 10 min) (Microfuge 22R centrifuge, Beckman Coulter, Germany) and the supernatant collected for absorbance analysis. Each dye solution was measured at its respective wavelength (CV at 590 nm, MV at 584 nm, MG at 617 nm, CB at 599 nm) using a Tecan Infinite M200 plate reader. The dye removal potential by isolate 1c3 is expressed as decolorization efficiency (DE, %) (Parshetti et al., 2006) as follows:

$$\mathrm{DE} = \frac{A_i - A_o}{A_o} \times 100$$

where A_i and A_o represent the initial and observed absorbance post-treatment with 1c3, respectively.

2.3. Optimization of conditions for dye decolorization

Dye decolorization by isolate 1c3 under the influence of fungal biomass, initial dye concentration and oxygen requirement were investigated. The optimum biomass was determined by treating 100 ml of dye solutions with fungal biomass at various weights of 1.0, 2.0, 4.0, 6.0 and 8.0 ± 0.1 g. The initial dye concentrations were kept constantly at 50 mg l^{-1} for CB and 100 mg l^{-1} for CV, MV and MG, as with other experimental conditions outlined in Section 2.2. The influence of initial dye concentrations was tested using 50, 100 and 200 mg l^{-1} of each dye while all other factors remained constant. For the oxygen requirement test, 100 ml of dye solutions were first inoculated with 2.0 ± 0.1 g (fresh weight) of fungal biomass, and overlaid with 5 ml of paraffin oil (filter-sterilized with 0.45 μm mixed cellulose ester syringe filter (Jet Biofil)) prior to incubation as standing static cultures (anaerobic set). A separate flask was prepared with the exclusion of paraffin overlay, and incubated with agitation to allow dye decolorization under aerobic conditions.

2.4. Ultraviolet–visible (UV–vis) spectral analysis to detect biodegradation potential

UV–vis spectral analysis was used to detect the possible occurrence of biodegradation, particularly when change in peaks between control and treated dye samples were observed (Kalpana et al., 2012). The experiment was initiated by inoculating 2.0 ± 0.1 g of harvested fungal biomass in 100 ml of respective dye solutions and incubation followed (150 rpm, 30 ± 2 °C). Non-inoculated dye solutions were included as negative controls. For the next 14 days, 2 ml aliquots were withdrawn at every 24-h interval, centrifuged (10,000 rpm, 10 min) and the supernatant was measured by means of UV–vis between the wavelengths of 300–800 nm, using a Tecan Infinite M200 plate reader, to detect absorption peaks of each TPM dye (Parshetti et al., 2011). Spectra peaks for dyes with and without treatment of 1c3 were plotted and compared.

2.5. Enzyme assays to detect biodegradation potential

Dye solutions (50 mg l^{-1} dye concentrations) were treated with 1c3 according to optimum conditions derived from the results. Approximately 2.0 ± 0.1 g of 1c3 were introduced into CV and MV solutions, 4.0 ± 0.1 g in CB and 8.0 ± 0.1 g in MG solutions. Treated CV, MV and CB solutions were incubated with agitation, while MG was overlay with paraffin oil. The control was prepared by inoculating PDB with 5 mycelial plugs and incubated with agitation (150 rpm, 30 ± 2 °C). For both treated and control sets, the enzymatic activities were determined at every 1 h interval for the first 6 h, gradually increasing to 18, 24, 48, 72, 96, 120, 144, 168, 192, 216, 240, 264, 288, 312 and 336 h, when enzyme levels were discovered to increase after 6 h of incubation. The three key enzymes assayed were laccase (Lac), lignin peroxidase (LiP) and NADH-dependent 2, 6-dichlorophenolindophenol (NADH-DCIP) reductase.

To perform the laccase (Lac) assay, 300 μl of supernatant was introduced to 2.3 ml reaction mixture (1400 μl distilled water, 600 μl of 0.1 M pH 5 sodium acetate buffer, 300 μl of 5 mM ABTS) and incubated at 30 ± 2 °C (Wisebath, WB-15; Daihan Scientific, Seoul, Korea) for 2 min. Hydrogen peroxide (300 μl of 1 mM H_2O_2) was added and the change in absorbance was immediately measured at 1 min intervals for 15 min. The calorimetric change due to oxidation of ABTS was detected at 420 nm (molar extinction coefficient, ε_{420} = 36,000 M^{-1} cm^{-1}) using a Tecan Infinite M200 plate reader (Bourbounnais et al., 1995). Lac activity was compared to a standard curve constructed from 0.0 to 0.01 U ml^{-1} concentrations of commercially purified laccase of *Trametes versicolor* (Sigma). One unit of laccase activity (U) is expressed as the activity of laccase required to catalyze the conversion of 1 μmole of ABTS per minute (Bourbounnais et al., 1995).

To quantify lignin peroxidase (LiP), 60 μl of supernatant were added to 2.85 ml of the reaction mixture (1890 μl distilled water, 600 μl of 0.3 M citrate-0.4 M phosphate buffer with pH 4.5, 300 μl of 8 mM veratryl alcohol) and incubated as previously described. Hydrogen peroxide (150 μl of 5 mM H_2O_2) was added and the change in absorbance as a result of the oxidation of veratryl alcohol