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Short communication

Biosorption and biodegradation potential of triphenylmethane dyes by newly discovered *Penicillium simplicissimum* isolated from indoor wastewater sample

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ABSTRACT

Penicillium simplicissimum (isolate 10, KP713758), a contaminant from indoor wastewater was studied for biosorption and biodegradation activities towards triphenylmethane (TPM) dyes. This newly discovered isolate demonstrated strong decolorization activities towards Methyl Violet (MV, 100 mg l^{-1}), Crystal Violet (CV, 100 mg l^{-1}) and Cotton Blue (CB, 50 mg l^{-1}), with 98%, 95% and 82% removed within 13, 14 and 1 day(s). Malachite Green (MG, 100 mg l^{-1}), the most recalcitrant dye, was partially decolorized (54%) by day 14. The biodegradation potential of *P. simplicissimum* was detected by the reduction in dye spectra peaks. Induced lignin peroxidase and NADH-DCIP reductase activities further suggested biodegradation potential. Batch studies revealed that decolorization activities of *P. simplicissimum* were influenced by the biomass used, initial dye concentrations, oxygen availability and cell viability, with optimum decolorization achieved using 2 g biomass, 100 mg l^{-1} dye concentration and in the absence of oxygen (except for CB).

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Introduction

Triphenylmethane (TPM) dyes are aromatic colorants characterized by the presence of a central carbon atom surrounded by three phenyl groups to form a chromogen (Przystaś et al., 2012). This class of dyes, consisting of Crystal Violet (CV), Methyl Violet (MV), Malachite Green (MG) and Cotton Blue (CB) is the most versatile with applications in various industries (Jadhav and Govindwar, 2006; Jasińska et al., 2012). TPM dyes are naturally mutagenic and toxigenic towards living organisms (Saratale et al., 2006). Conventional techniques employed to remove TPM dyes involved physicochemical processes, such as adsorption, membrane filtration, coagulation and ozonation (Parshetti et al., 2011). These approaches were generally effective, but incurred high cost and generate toxic sludge. As an alternative, biosorbents are explored to attain a more environmentally-friendly and sustainable approach (Shedbalkar et al., 2008).

Fungal-based biosorbents are highly-desirable biosorbents as they remove toxic dyes via biosorption and/or biodegradation (Jadhav and Govindwar, 2006). Fungal biomass binds dye molecules effectively onto the surface of their cells (biosorption), which may be followed by secretion of enzymes to breakdown complex dye molecules (biodegradation) when live cells are used (Jadhav and Govindwar, 2006; Jasińska et al., 2012). Several fungal species successfully decolorize TPM dyes, which include *Phanerochaete chrysosporium* (Bumpus and Brock, 1988), *Irpex lacteus* (Novotný et al., 2001), *Pleurotus ostreatus* (Novotný et al., 2001), as well as non-white rot fungi such as *Aspergillus* sp. (Saratale et al., 2006), *Penicillium* sp. (Jasińska et al., 2012) (Shedbalkar et al., 2008) and *Fusarium* (Abedin, 2008). Several key enzymes are noted for their biodegradation potential. They include oxidative laccase (Lac), lignin peroxidase (LiP) and manganese peroxidase (MnP) (Zhuo et al., 2011), and reductase enzymes such as MG reductase, NADH-2,6-dichlorophenolindophenol (DCIP) reductase and aminopyrine N-demethylase (Shedbalkar et al., 2008). These enzymes are produced primarily by white-rot fungi (Bumpus and Brock, 1988; Novotný et al., 2001; Saratale et al., 2006), sourced primarily from dye-polluted soils, marine environments and industrial effluents (Abedin, 2008; Gou et al., 2009; Torres et al., 2011).

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In this study, the potential of a fungal contaminant (isolate 10) from an indoor wastewater sample (wastewater tank of the Atomic Absorption Spectroscopy (AAS)) (Ting et al., 2011) was evaluated for its biosorption and biodegradation potential on TPM dyes. The purpose of using this isolate lies with the fact that the survival of this isolate in nutrient-poor water is intriguing and that it is a non-white rot fungi which has not been fully explored for its potential to remove TPM dyes. The species of this isolate was first validated and the sequence deposited in NCBI. The decolorization and biodegradation potential of isolate 10 on four TPM dyes (CV, MV, MG and CB) were established and the influence of biomass concentrations, initial dye concentrations and oxygen requirement on decolorization activities investigated. The biodegradation potential of isolate 10 was determined by evaluating the changes in absorption spectra and quantification of enzymes.

Materials and methods

Taxonomic identification of isolate 10

Isolate 10 was isolated by Ting et al. (2011) as a contaminant from the wastewater of AAS at Monash University Malaysia. The isolate was identified to its species level via DNA sequencing of the 18S rRNA. Isolate 10 was maintained on Potato Dextrose Agar (PDA, Merck) at room temperature (25 ± 2 °C) and subcultured periodically. For DNA sequencing, biomass was first generated by inoculating five mycelial plugs of isolate 10 in 100 ml of Potato Dextrose Broth (PDB, Difco™, France) and incubated for 5 days (25 ± 2 °C). Genomic DNA was extracted from fresh mycelia (30 mg wet weight) using the GF-1 Plant DNA Extraction Kit (Vivantis Technologies, USA). PCR amplification of the internal transcribed spacer (ITS1, 5.8S rRNA, ITS2) gene region was conducted using universal primer pairs of ITS 1 (5′- TCCGTAGGTGAACCTGCGG-3′) and ITS 4 (5′-TCCTCCGCTTATTGATATGC-3′), and purified using the MEGAquick-spin™ Total Fragment DNA Purification Kit (iNtRON Biotechnology, Korea) (Chow and Ting, 2014). DNA sequencing was performed by 1st Base (Malaysia) with the sequence inferred from the NCBI BLAST analysis (http://www.ncbi.nlm.nih.gov/). The sequence was subsequently deposited in GenBank and the accession number (KP713758) obtained. A phylogenetic tree was then constructed using the PhyML and TreeDyn features of Phylogeny.fr platform (http://www.phylogeny.fr/) (Dereeper et al., 2008). The approximate likelihood-ratio test was used to estimate the branch support.

Dye decolorization activity of isolate 10 on various TPM dyes

Each TPM dye (CV, MV, MG and CB) was dissolved in 100 ml of autoclaved MilliQ water (18.2 MQ; Sartorius, Malaysia) to a concentration of 100 mg l^{-1} (except CB at 50 mg l^{-1}). The dyes MV and CB were purchased from Sigma–Aldrich, CV supplied by Merck while MG was acquired from Riendemann Schmidt. Freshly prepared fungal biomass (2.0 ± 0.1 g) was then introduced to the dye solutions. The mixture was incubated (150 rpm, 30 ± 2 °C) (MaxQ 6000, Thermo Scientific, 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 aliquot was withdrawn and centrifuged (10,000 rpm, 10 min) (Microfuge 22R centrifuge, Beckman Coulter, Germany), and the supernatant collected for absorbance analysis. The absorbance of each dye solution was measured at their respective wavelengths (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 of isolate 10 was expressed as decolorization efficiency (DE, %) as follows (Parshetti et al., 2006):

$$\text{Decolorization}\ (\%) = \frac{\text{initial absorbance} - \text{observed absorbance}}{\text{initial absorbance}} \times 100$$

Tolerance of isolate 10 to TPM dyes

Tolerance of live cells to TPM dyes were evaluated by performing viable cell counts on the first and last days of the experiments (Chen and Ting, 2015). Assays were performed using samples from decolorization test and the three optimization experiments (for biomass concentrations, initial dye concentrations and oxygen requirement). The percentage of viable cells (%) was calculated as follows (Chen and Ting, 2015):

$$\text{Viable cells}\ (\%) = \frac{\text{live cell count on the last day of experiment}}{\text{live cell count on the first day of experiment}} \times 100$$

Optimum conditions for dye decolorization by isolate 10

The influences of fungal biomass (g), initial dye concentration (mg l^{-1}) and oxygen requirement on decolorization activities by isolate 10 were investigated. To determine the optimum biomass required, 100 ml of dye solutions were treated with 1.0, 2.0, 4.0, 6.0 and 8.0 (±0.1) g of fungal biomass. At this stage, the initial dye concentrations were pre-determined at 50 mg l^{-1} for CB and 100 mg l^{-1} for CV, MV and MG, and all other experimental conditions kept constant as outlined in Section "Dye decolorization activity of isolate 10 on various TPM dyes". To determine the effect of initial dye concentrations, 50, 100 and 200 mg l^{-1} of each dye solutions were tested while other factors were held constant. For the oxygen requirement test, 100 ml of dye solutions were 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)). The cultures were incubated as standing cultures (static condition for anaerobic set) at room temperature (30 ± 2 °C). A separate set was prepared similarly, excluding paraffin oil overlay, and incubated with agitation (150 rpm, 30 ± 2 °C) to allow aerobic decolorization process to occur.

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

UV–vis spectral analysis was used to investigate the possible occurrence of dye biodegradation, evident by the change in peaks between control and treated dye samples (Kalpana et al., 2012; Chen and Ting, 2015). The experiment was initiated by inoculating 2.0 ± 0.1 g of fungal biomass to 100 ml of dye solutions, and incubated with agitation (150 rpm, 30 ± 2 °C). Controls were prepared using non-inoculated dye solutions. Two ml of the dye solutions (control and treated) were withdrawn at every 24 h-interval (for the next 14 days), and centrifuged (10,000 rpm, 10 min). Absorption peaks for each TPM dye were detected by means of UV–vis at wavelengths 300–800 nm (Chen and Ting, 2015). Spectra peaks for treated and untreated dye solutions were plotted and compared.

Enzyme assays to detect biodegradation of TPM dyes

This experiment was performed based on optimum conditions established in the previous sections (Sections "Dye decolorization activity of isolate 10 on various TPM dyes" and "Optimum conditions for dye decolorization by isolate 10"). Dye solutions (100 ml at 50 mg l^{-1} concentration) were inoculated with 4.0 ± 0.1 g (for CV, MV and MG) and 8.0 ± 0.1 g (for CB) of fungal biomass. Treated CV, MV and MG solutions were overlaid with paraffin oil and incubated as standing cultures. On the contrary, treated CB was incubated with agitation (150 rpm, 30 ± 2 °C). Control was prepared by inoculating PDB with 5 mycelial plugs of isolate 10 and incubated with agitation (150 rpm, 30 ± 2 °C). Enzymatic activities for both treated and untreated samples 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, for enzymes with increased levels after 6 h of incubation. The three key enzymes assayed in this study were laccase (Lac), lignin peroxidase (LiP) and NADH-dependent 2, 6-dichlorophenolindophenol (NADH-DCIP) reductase.

The Lac assay was performed by detecting oxidation of 2,2′-azinobis(3-ethylbenzthiazoline)-6-sulfonate (ABTS) in control and treated samples via colorimetric change at 420 nm (molar extinction coefficient, ε_{420} = 36,000 M^{-1} cm^{-1}) (Bourbounnais et al., 1995; Chen and Ting, 2015). The reaction mixture contained 5 mM ABTS in 0.1 M sodium acetate buffer (pH 5.0). Lac activities of samples were compared with a standard curve (0.0–0.01 U ml^{-1}) prepared using commercially purified Lac of *Trametes versicolor* (Sigma) (Chen and Ting, 2015). One unit of Lac activity (U) is expressed as the activity of Lac required to catalyze the conversion of 1 μmole of ABTS per minute (Bourbounnais et al., 1995; Chen and Ting, 2015). The LiP activity of isolate 10 was measured by the oxidation of veratryl alcohol to veratryl aldehyde. The reaction mixture contained 8 mM veratryl alcohol in 0.3 M citrate/0.4 M phosphate buffer (pH 4.5). The change in absorbance was read at 310 nm (ε_{310} = 9300 M^{-1} cm^{-1}) (Chen and Ting, 2015). LiP activity was compared against a standard curve (0.00–0.24 U ml^{-1}) of commercially purified LiP (Sigma), with one unit of LiP expressed as the activity of LiP required to catalyze the conversion of 1 μmole of veratryl alcohol per minute (Takamiya et al., 2008; Chen and Ting, 2015). The NADH-DCIP reductase assay was performed with reaction mixture containing 50 μM DCIP and 50 μM NADH in 50 mM potassium phosphate buffer (pH 7.4) (Chen and Ting, 2015). The change in absorbance as a result of DCIP reduction was measured at 590 nm (ε_{590} = 19 mM^{-1} cm^{-1}) (Chen and Ting, 2015). The NADH-DCIP reductase activity (μmol min^{-1} ml^{-1}) was calculated as such (Dionisio-Sese and Tobita, 1998):

$$\frac{\left(\frac{\Delta A}{min_s} - \frac{\Delta A}{min_b}\right) \times V}{\varepsilon \times d \times v}$$

where $\Delta A/min_s$ indicates change in absorbance per min for the sample, $\Delta A/min_b$ is the change in absorbance per min for the blank; V is the total volume of the reaction mixture (ml); ε is the molar extinction coefficient (mM^{-1} cm^{-1}); d is the pathlength (cm); and v is the volume of enzyme solution (ml).

Statistical analysis

All experiments were performed in triplicates. The data were analyzed by One-Way Analysis of Variance (ANOVA) using Statistical Package for the Social Sciences (SPSS) version 20.0. Means were compared using the Tukey–Kramer multiple comparison test (Honestly Significant Difference, HSD, $P < 0.05$), or paired t-test ($P < 0.05$ for paired-comparisons) where relevant.

Results and discussion

Identification of isolate 10

DNA sequencing of the ITS region indicated 99% homology (E value of 0.0; 98–100% query coverage) with those of *Penicillium simplicissimum* strains PBL13 (accession number KM104597.1), P31-62 (KJ439133.1), C62-457 (KJ439103.1), KUC5153 (HM469430.1), PBL3 (KM104578.1) and 99C (KF555227.1) in the NCBI database. Isolate 10 was therefore inferred as *P. simplicissimum* and this indoor isolate was deposited in the database under the accession number KP713758. The phylogenetic tree revealed that isolate 10 was part of a clade comprising *Penicillium* sp., *P. simplicissimum* and *P. wotroi* strains (Fig. 1).

Dye decolorization activities by isolate 10

Isolate 10 have strong decolorization activities (DE, %) towards MV, CV and CB with means of 79%, 76%, and 64%, respectively. The most rapid decolorization occurred for CB with 82% decolorization within 1 day, followed by MV, CV and MG, which required 13 (98%), 14 (95%) and 14 days (54%), respectively (data not shown). Variations in decolorization rate may be attributed to the complex structure of the dye molecules and the presence of inhibitory groups (–CH_3 groups) on CV, MV and MG (Hsueh et al., 2009). This indoor isolate *P. simplicissimum* showed similar traits to environmental isolates in decolorizing TPM dyes (Muthezhilan et al., 2008; Torres et al., 2011). Ability of *P. simplicissimum* (isolate 10) to remove TPM dye is also novel as this species has previously been known to remove azo and phthalocyanine dyes (Bergsten-Torralba et al., 2009). In addition, *P. simplicissimum* (isolate 10) demonstrated potential to decolorize not only CV, CB and MG, but MV as well, which has not been reported previously.

Biodegradation potential of isolate 10

The biodegradation potential of isolate 10 on TPM dyes were concluded from the changes in peak patterns of the absorption spectra (300–800 nm). Upon treatment with isolate 10, peaks for CV, MV and CB were absent (not detected) compared to peak patterns in control (Fig. 2). The reduction or disappearance of absorption peaks suggested the possible occurrence of biodegradation in which chromophoric groups were degraded (absence of peaks) (Kalpana et al., 2012). To date, only a handful of species showed degradation of CV, MV, MG and CB, such as *Phanerochaete chrysosporium* (Bumpus and Brock, 1988), *Aspergillus* sp. (Kumar et al., 2011), *Saccharomyces cerevisiae* MTCC 463 (Jadhav and Govindwar, 2006) and *Penicillium ochrochloron* MTCC 517 (Shedbalkar et al., 2008). This reporting on biodegradation activities of *P. simplicissimum* on TPM dyes is novel as previous studies cited biodegradation on different dyes such as Reactive Red 198, Reactive Blue 214 and Reactive Blue 21 (Bergsten-Torralba et al., 2009). The mechanism is more effective and desirable than biosorption as the latter generates large volumes of dye-treated biomass (sludge) (Zhang et al., 2003). Nevertheless, the biodegradation activities of *P. simplicissimum* (isolate 10) may be influenced by the susceptibility of the viable cells to the toxicity of TPM dyes or the intermediates that formed from the breakdown (as in MV) (Parshetti et al., 2011). Exposure to CV and MG resulted in 0% viable cell recovery, while inoculation to MV and CB allowed the recovery of 0.2% and 188% viable cells, respectively (data not shown). Tolerance to dyes has been linked to higher decolorization

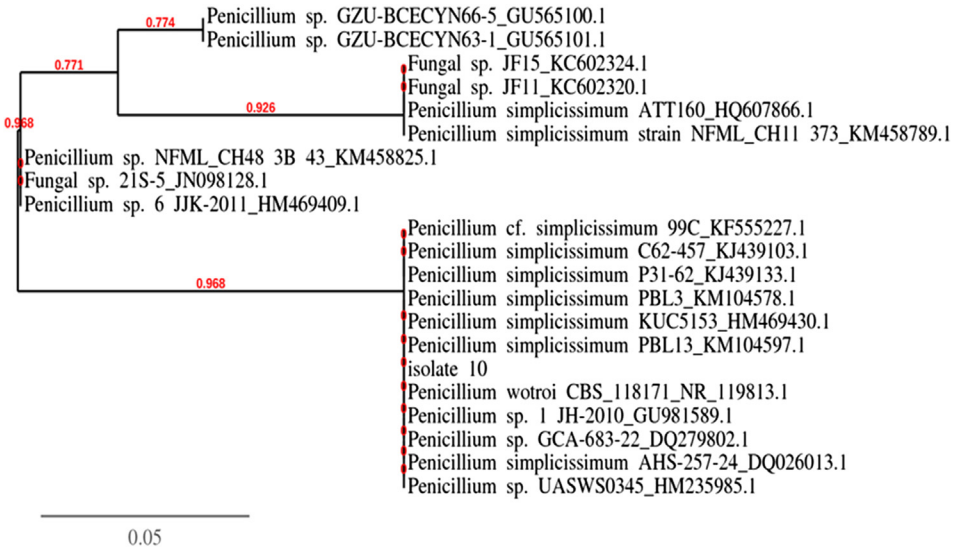

Fig. 1. Maximum likelihood phylogram inferred from the alignment of isolate 10 with nucleotide sequences from NCBI BLAST search. Fungal isolate names are followed with GenBank accession numbers. Values indicated at nodes are the approximate likelihood-ratio test (aLTR) support values.

efficiencies, presumably due to more cells that are available for interaction with the dye as observed for CB (Kumar et al., 2011; Parshetti et al., 2011). Among the four TPM dyes, CB is the least toxic while CV and MG were more toxic and have been used as fungicides in poultry feed and fish cultures, respectively (Jadhav and Govindwar, 2006; Pant et al., 2008).

Optimum decolorization conditions for isolate 10

Application of 4 g of biomass was sufficient to achieve the maximum DE for CV (92%) and MG (87%), while MV and CB required 6 or 8 g to achieve 96 and 93% DE, respectively (Fig. 3). The benefit of using more than 1 g biomass has also been reported in other studies (Jadhav and Govindwar, 2006; Saratale et al., 2006; Shedbalkar et al., 2008; Parshetti et al., 2011; Chen and Ting, 2015), attributed to having more cells to absorb and secrete extracellular enzymes responsible for biodegradation (Abedin, 2008). Decolorization was also achieved more rapidly when higher biomass is used with complete decolorization of CV, MV, CB and MG attained after 1, 1, 1 h 40 min and 18 h with the use of 6, 6, 8

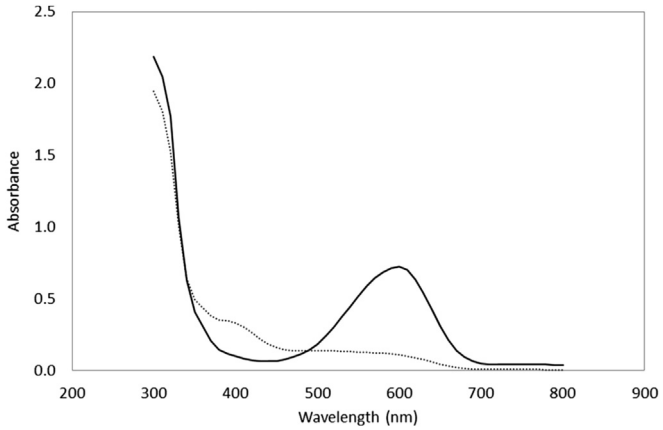

Fig. 2. Exemplary figure of the UV–vis spectrum analysis of Cotton Blue (CB, 50 mg L^{-1}) before (untreated, ——) and after decolorization (treated, – –) by 2 g of isolate 10 at 30 ± 2 °C.

and 6 g biomass, respectively (data not shown). Higher biomass (6 and 8 g) also retained cell viability and metabolic functions for a longer period of time, allowing decolorization and biodegradation to occur (Bergsten-Torralba et al., 2009). Nevertheless, for some dyes (CV, MG), the use of higher biomass (6–8 g) did not further benefit as saturation of binding sites of the biosorbent by dye molecules may have occurred. It is recommended that 2 or 4 g of *P. simplicissimum* is sufficient to achieve complete decolorization of TPM dyes (DE of 40–90%), as using 6–8 g of biomass will incur additional costs (Fig. 3).

The decolorization of TPM dyes by isolate 10 was also influenced by the initial dye concentrations, except for CB (DE of 50–66% for all concentrations). A higher DE was achieved when lower (50 mg l^{-1}) dye concentrations were used as observed for CV (89%), MV (90%) and MG (64%), compared to when 100 (76%, 79% and 40%, respectively) and 200 mg l^{-1} of dye was used (58%, 67% and 36%) (Fig. 4). Lower initial dye concentrations also allowed decolorization to occur more rapidly. At lower concentrations (50 mg l^{-1}), isolate 10 decolorized CB, CV, MV and MG rapidly, within 1 (82%), 2 (99%), 2 (99%) and 11 (98%) days, respectively (data not shown). An increase to 100 mg l^{-1} took 7, 8, 9 and 9 days to decolorize CB (85%), MG (50%), CV (90%) and MV (96%). At 200 mg l^{-1}, poorer DE was achieved after 7, 8, 9 and 9 days with only 36, 66, 77 and 76% decolorization for MG, CB, MV and CV. From this study, it is evident that the DE of isolate 10 decreased with increasing initial dye concentrations, presumably due to inhibitions of the peptide synthesis by increasing toxicity (Tolba and Saleh, 1963; Saratale et al., 2006). Exposure to 50 mg l^{-1} of CV, MG and MV yielded 0.30%, 0.30% and 0.18% viable cells, while cells were completely dead at 200 mg l^{-1} (0% cell recovery). CB was the least toxic as 634% and 3.8% cells were recovered from 100 to 200 mg l^{-1}, respectively.

Isolate 10 demonstrated better decolorization activities for MG (73%), CV (82%) and MV (82%) in the absence of oxygen (Fig. 5). This suggested decolorization activities occur more efficiently in anaerobic conditions (except for CB) similar to Parshetti et al. (2011). A more rapid decolorization was also achieved in the absence of oxygen; 7 days for CV (94%) and MV (97%) compared to 9 days (90% and 96%) in the presence of oxygen (data not shown). This suggests that decolorization of CV and MV does not require oxygen and most possibly involved reductive reactions by NADH-DCIP reductase (Parshetti et al., 2006). By contrast, decolorization

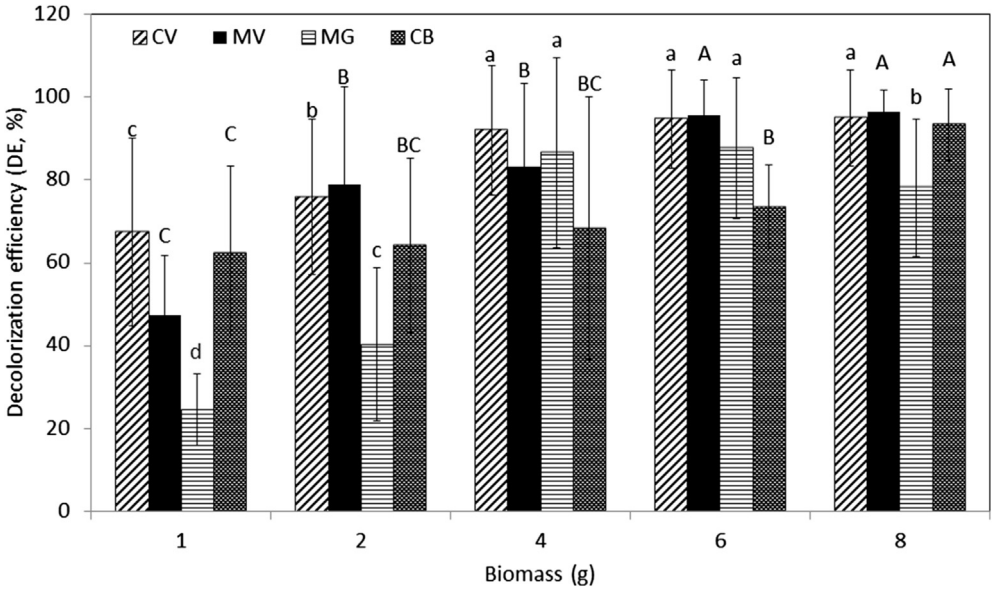

Fig. 3. Influence of biomass (1, 2,4, 6 and 8 g) of isolate 10 at 30 ± 2 °C on the decolorization efficiency (DE, %) of four TPM dyes. Means with the same letters and captions are not significantly different at $HSD_{(0.05)}$. Bars indicate standard error of mean (±SEM).

of CB was more effective with oxygen (64%) compared to without oxygen (57%), suggesting a different group of enzymes (or pathways) may be involved such as the oxidative enzymes Lac and LiP (Zhuo et al., 2011). Nevertheless, the decolorization of CB appeared to be species dependent as *P. ochrochloron* MTCC 517 decolorized CB more efficiently under static conditions with limited oxygen (Shedbalkar et al., 2008). The recovery of (or the lack of) viable cells from this stage reveals that dye removal occurred either via biosorption or biodegradation. Higher recovery of viable cells under aerobic conditions of CV, MV and CB (0.18, 1.8 and 725%, respectively) than in the absence of oxygen (0, 0 and 17%) suggest decolorization occurred in non-viable (CV, MV and MG) and viable (CB) cells, respectively.

Enzymatic activities of isolate 10

Higher levels of LiP and NADH-DCIP reductase were detected in cultures supplemented with TPM dyes compared to control (absence of dyes in PDB) (Table 1), suggesting these enzymes may be specific for biodegradation of TPM dyes via oxidative and reductive processes, respectively (Shedbalkar et al., 2008). These enzymes may be inherent in this species due to their lignin pathway (Yu et al., 2005), but is documented here in relation to TPM dyes for the first time. Among the enzymes, levels of LiP were higher in the presence of MV (3.6×10^{-4} units min^{-1} ml^{-1}) and CB (1.4×10^{-4} units min^{-1} ml^{-1}), whereas NADH-DCIP reductase levels were high in all TPM dyes (Table 1). These enzymes are

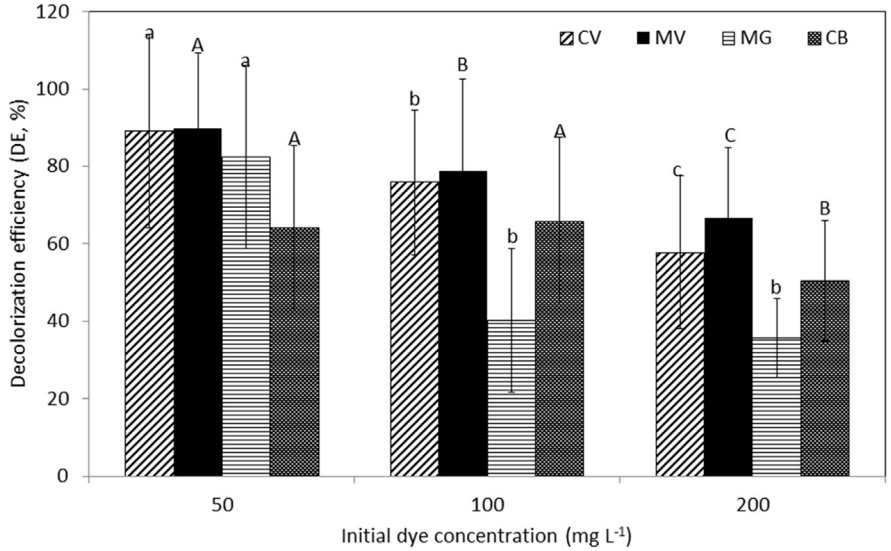

Fig. 4. Influence of initial dye concentrations (50–200 mg l^{-1}) on the decolorization efficiencies of four TPM dyes by isolate 10 at 30 ± 2 °C. Means with the same letters and captions within dye-groups are not significantly different at $HSD_{(0.05)}$. Bars indicate standard error of mean (±SEM).

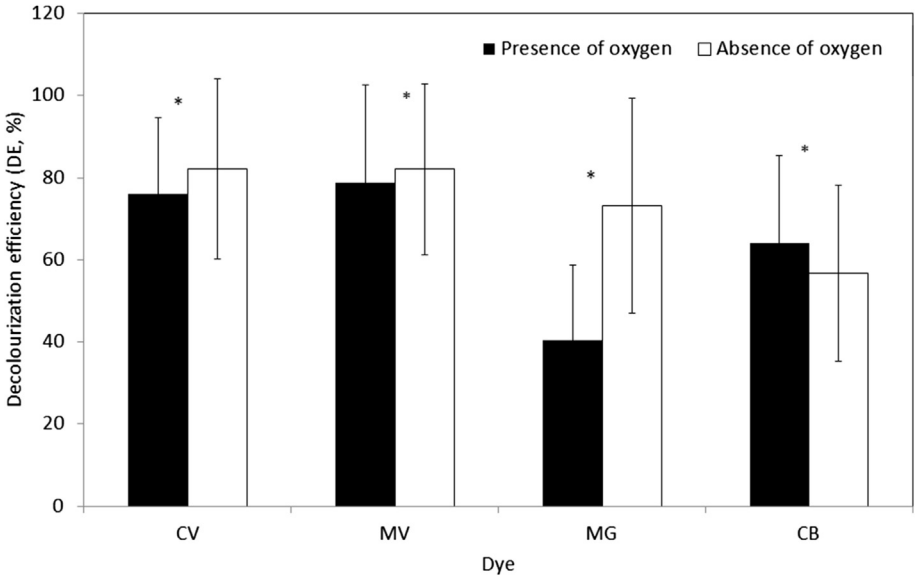

Fig. 5. Influence of oxygen availability on the decolorization of four TPM dyes by isolate 10 at 30 ± 2 °C. "*" indicates significant difference based on paired T-test ($p < 0.05$). Bars indicate standard error of mean (±SEM).

Table 1
Activities of laccase (Lac), lignin peroxidase (LiP) and NADH-DCIP reductase in the supernatants of TPM dye solutions and PDB (control) after treatment with isolate 10. Data is presented in mean ± standard error (SEM) with asterisk indicating significant difference from those in PDB based on t-test ($p < 0.05$).

Enzyme	Enzyme activity				
	PDB	CV	MV	MG	CB
Lac[a]	0.00023 ± 0.000003	0.00014 ± 0.0004	0.00010 ± 0.00002*	0.00005 ± 0.00002*	0.00009 ± 0.00002*
LiP[a]	NA	0.00010 ± 0.00006	0.00036 ± 0.00009*	0.00009 ± 0.00005	0.00014 ± 0.00006*
NADH-DCIP reductase[b]	NA	5103 ± 972*	6637 ± 1704*	2141 ± 367*	5174 ± 1043*

NA: no activity.
[a] Units min^{-1} ml^{-1}.
[b] μmol min^{-1} ml^{-1}.

involved with MG reductase, aminopyrine *N*-demethylase, tyrosinase and manganese peroxidase aid in degrading CV, MV and MG (Bumpus and Brock, 1988; Kumar et al., 2011; Jasińska et al., 2012). This study documents the biodegradation potential of *P. simplicissimum* detected via enzymatic activities.

Conclusions

This study reports the effectiveness of *P. simplicissimum* (isolate 10), isolated as a contaminant from the indoor wastewater, and in decolorizing TPM dyes CV, MV, MG and CB. The decolorization efficiency was influenced by fungal biomass concentration (minimum 2 g), initial dye concentration (tolerable to 100 mg L^{-1}), cell viability and was found to occur more efficiently under anaerobic conditions (except for CB). TPM dye decolorization by isolate 10 involved biodegradation (induction of LiP and NADH-DCIP reductase activities), leading to reduced absorbance peaks of the dyes. This isolate may be a potential cost-effective and easily cultured biosorbent or biodegradation agent for removal of TPM dyes.

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