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ORIGINAL ARTICLE

Biodecolorization of brilliant green carpet industry dye using three distinct *Pleurotus* spp.

Ram Naraian[1] · Simpal Kumari[1] · Roshan Lal Gautam[1]

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Abstract

Biodecolorization potentials of three distinct white-rot fungi including *Pleurotus florida* (PF), *Pleurotus eryngii* (PE) and *Pleurotus sajor-caju* (PS) were assessed both in liquid and on solid media supplemented with carpet industry dye brilliant green. All three fungi produced laccase and peroxidase enzymes. The decreasing order of laccase production was achieved as 388 > 334 > 301 IU/mL in cultures of PF > PE > PS during 15th, 20th and 17th days, respectively, while, decreasing order of peroxidase production was as 72 > 64 > 55 IU/mL in PF > PE > PS on 15th day of PF, PS cultures and on 20th day of PE, respectively. All tested fungi very efficiently degraded different levels (2, 4, 6 and 8%; w/v) of highly complex synthetic brilliant green dye to colorless. However, PF was found to be best amongst the three species tested on solid and in liquid media. In comparison to higher concentrations, lower concentrations of dye were rapidly biodecolorized by all fungal strains. The order of highest biodecolorization potentialities was recorded as 99 > 91 > 83% by PF > PE > PS, respectively with 2% (w/v) of dye under submerged conditions. While, experimental sets with 4% (w/v) dye were found as the second most rapidly biodecolorized sets, that resulted in 90 > 78 > 70% by PF > PE > PS strains respectively. Based on the findings of biodecolorization potentialities PF was most efficient fungus as compared with other fungi by degrading 99% of the 2% (w/v) dye. Therefore, PF can be subjected in the eco-friendly and cost-effective industrial effluent cleanup processes for the environmental sustainability.

Keywords *Pleurotus* spp. · Carpet dyes · Biodecolorization · Brilliant green · Carpet industry

Introduction

Industrial discharges of carpet dyes through dyeing of fabrics, leathers and by paper printing in surrounding environment not only cause undesirable color of water reservoirs but also tremendously influence the components of aquatic ecosystem including plants, animals and microbes. More than 10,000 different kinds of textile industry dyes with an annual production of several metric tones are commercially used worldwide (McMullan et al. 2001; Yang et al. 2011; Ning et al. 2018). In India almost 1.5 million liters of fabric mill effluent per day is discharged in open surroundings, that creates environmental problems (COINDS 2000; Patil et al. 2010). Approximately 10–20% of dyes used in distinct dyeing processes do not bind with the textile fibers and are carelessly thrown into the open (Murthy et al. 2014; Skariyachan et al. 2016). Synthetic dyes with highly complex aromatic structure make them more stable and difficult to biodegrade (Grassi et al. 2011; Ratanapongleka and Phetsom 2014).

Microbes of different class such as algae, fungi, bacteria, and actinomycetes have been reported for their distinguished ability to decolorize textile dyes (Khehra et al. 2005; Moosvi et al. 2005). Biodegradation of dyes through fungal strains results in breakdown of the dye molecule and consequently detoxification of harmful dyes (Spadaro and Renganathan 1994; Malachova et al. 2006; Svobodova et al. 2007). Under the typical biodiversity of fungal world, the white-rot fungi are most proficient for aerobic degradation (Batal et al. 2015). In particular, the ability of white-rot fungi to biodegrade various types of dyes has established as a highly effective technology governed through oxidoreduction reactions catalyzed by the extracellular enzymes they produce (Toh et al. 2003; Batal et al. 2015). The biological degradation

✉ Ram Naraian
ramnarain_itrc@rediffmail.com

[1] Department of Biotechnology, Faculty of Science, Veer Bahadur Singh Purvanchal University, Jaunpur, Uttar Pradesh 222003, India

of synthetic dyes in nature occurs due to release of enzymes such as; laccase, lignin peroxidase, manganese peroxidase (Afreen and Fatma 2013; Wong and Yu 1999; Lopez et al. 2004). The production of extracellular laccase is constitutively involved in the degradation process, which represents an essential factor in biodegradation (Parenti et al. 2013; Zhuo et al. 2017). Fungi (especially white-rot fungi) have demonstrated a significant capacity to degrade extraordinary variety of recalcitrant organic compound among others (Hatakka and Hammel 2011; Ergun and Urek 2017).

The potential of genus *Pleurotus* to degrade organopollutants such as highly toxic industrial dyes, polyaromatic hydrocarbons and several others has been well documented (Cerniglia and Sutherland 2001; Knapp et al. 2001; Buchicchio et al. 2016). *Pleurotus* can degrade a wide variety of environmental pollutants, such as recalcitrant synthetic dyes, industrial effluents, and toxic pesticides (Ottoni et al. 2013; Rivera-Hoyos et al. 2013; Wang et al. 2018). Hence, microbial decolorization offers an efficient cleanup of pollutants through a natural agent to reduce the major coloring groups into carbon dioxide, ammonia and water in consequence to cleavage of distinct bonds in the dyes (Rajendran et al. 2012). Therefore, such biological processes for the decolorization dyes have received great curiosity owing to their cheaper, highly efficient, and eco-friendly practices.

The aim of the current work was to biodecolorize brilliant green dye by employing three distinct white-rot fungi (genus *Pleurotus*) including *P. florida*, *P. sajor-caju*, and *P. eryngii*.

Materials and methods

Fungal strains

The present study was performed by using three distinct basidiomycete fungi viz., *P. florida* (PF), *P. sajor-caju* (PS), and *P. eryngii* (PE). All these fungal strains were procured from the laboratory of Mushroom Training & Research Centre (MTRC), Department of Biotechnology, Faculty of Science, Veer Bahadur Singh Purvanchal University, Jaunpur (UP), India.

Maintenance of fungal culture

Pure culture of each fungal strain was grown on potato dextrose agar (potato, 200 g; dextrose, 20 g; agar, 20 g L^{-1}) slants at 22 ± 2 °C and maintained by regular subculturings every fortnightly and stored in refrigerators at 4 °C.

Carpet industry dye

The most widely used synthetic 'brilliant green' carpet industry dye was used in the form of liquid preparations. The powder of dye was purchased from the local dye market, 'Carpet City' Bhadohi (Latitude: 25°25′ 12.00″N; Longitude: 82°34′ 12.00″E.), U.P., India. The absorption maxima (λ_{max}) of the dye was determined through spectrophotometric wavelength scanning (Elico® SL191UV VIS, USA).

Inoculum preparation

To prepare fungal inoculums, all cultures were separately grown on potato dextrose agar at 22 ± 2 °C. Mycelial plugs of 9 mm diameter from the edge of growing colony were removed using sterile cork borer and used as inoculums.

Biomass determination

Total mycelial biomass was determined during different time intervals (5, 10, 15, and 20 days) of incubations. All experiments were performed in triplicates (n = 3). The mycelia biomass was sampled and separated from appropriate set of culture on alternate day by filtering with the help of Whatman No. 1 paper. After the filtration of mycelium it was oven dried at 60 °C and the total biomass was determined by weighing, which was then expressed in mg/mL.

Laccase activity assay

The preliminary laccase producing characteristic of fungi was determined by laccase plate assay by using 1% (w/v) α-Naphthol (Sigma) solution freshly prepared in 96% (v/v) ethanol. For plate assay, 10 days old fully grown fungal plate was used and 4 mm sized three wells within plate were made in triangular positions. After that two distinct fractions with 50 and 100 µL of pre-prepared α-Naphthol solution were poured into two separate wells, and the third well was filled with distilled water and treated as control. After a period of two hours purple coloration developed which indicated extracellular laccase activity of fungal strain (Stalpers 1978).

Furthermore, laccase activity in fungal culture was determined by the oxidation of 2,2′-azino-bis (3-ethylthiazoline-6-sulfonate (ABTS) at 37 °C. The culture extract was prepared by crushing and filtering mycelia in tris-buffer (pH 5.0). The reaction mixture contained 600 mL enzyme extract, 300 mL (0.1 M) sodium acetate buffer pH 5.0 and 100 mL standard solution of ABTS (1 mM). The reaction mixture was incubated at 37 °C and the oxidation of substrate was assessed by measuring absorbance spectrophotometrically at 420 nm (Niku et al. 1990). One unit of enzyme activity was expressed as the amount of enzyme oxidizing 1 mM of ABTS per minute per mL of enzyme extract.

Peroxidase activity assay

Peroxidase activity was determined by the oxidation of veratryl alcohol to veratraldehyde. The standard reaction mixture consisted of 1 mL of 125 mM sodium tartarate buffer (pH 3.0), 500 µL of 10 mM veratryl alcohol, 500 µL of 2 mM hydrogen peroxide solution and 500 µL of the enzyme extract. The reaction was initiated at 37 °C by adding hydrogen peroxide and change in absorbance was recorded spectrophotometrically at 310 nm (Arora and Gill 2001). One unit of enzyme activity was expressed as the amount of enzyme oxidizing 1 µM of veratraldehyde produced per minute per mL of the enzyme extract. Whereas, control contained all components except the enzyme extract. All the assays were conducted in triplicate (n = 3).

Biodecolorization of dye on solid media

For the assessment of dye decoloring efficiency of three fungi on solid medium, each fungi was cultivated on PDA media supplemented with brilliant green dye preparations. The prepared media plates were then inoculated with 9 mm disc of inoculums, which were placed at the center of each plate supplemented with dye, while, uninoculated plate was treated as control. There after the sets of experimental Petri plates were incubated at 28 ± 2 °C and observed for dye decolorization. Each experimental set including control was managed in triplicates (n = 3).

Biodecolorization of dye in submerged culture

The dye decolorization efficiency of fungi in submerged culture was assessed by growing them in 100 mL Erlenmeyer flask containing potato dextrose broth (PDB) supplemented with different concentrations (2, 4, 6 and 8%; w/v) of brilliant green dye in different sets. These preparations were sterilized and inoculated with 6 day old, two 9 mm sized mycelial plugs of each fungus separately, which were then incubated at 28 ± 2 °C for 30 days in a rotatory shaker incubator (150 rpm). Furthermore, samples from each set were filtered to make them mycelium free. Thus, obtained supernatant was used for evaluation in color reduction. Each experimental set was run in triplicates (n = 3). Percent dye decolorization was recorded spectrophotometrically (λ_{max} = 450 nm) by measuring absorbance.

The percent (%) biodecolorization was calculated as following: Biodecolorization (%) $= \frac{100(Abs_{t0} - Abs_{tf})}{Abs_{t0}}$ where Abs_{t0} is the absorbance at initial of day of culture, Abs_{tf} is the absorbance at final day of culture.

Results

Production of mycelial biomass in different fungal cultures

Production of total mycelial biomass of three different white-rot fungi, PF, PS and PE was evaluated on the dry weight basis. The experimental set of PF culture represented higher biomass yields in comparison to the rest of the fungi tested (PS and PE), while, PE showed second most highest biomass yield during different incubation time periods (5, 10, 15, and 20 days). As observed, the production of mycelial biomass in all fungi tested gradually increased with increasing fermentation time up to 20 days. The highest yield of biomass recorded in the cultures of PF, PE, and PS were 18.01, 16.04, 14.01 mg/mL, respectively on the 20th day. Therefore, reducing order of the mycelial biomass yield can be represented as: PF > PE > PS during each day of observation (Fig. 1).

Laccase profile of different *Pleurotus* spp.

The laccase production potency of each fungi tested through plate assay confirmed that all the tested strains were able to produce this enzyme. As observed the result of laccase plate assay showed dark brown color around the wells, which indicates positive qualitative characteristic of laccase production in the culture of each fungi (Fig. 2). Moreover, the production of laccase enzyme was subsequently analyzed in separate submerged fermentation cultures for the three fungi. All fungi exhibited remarkable production of laccase activity when grown in broth medium under submerged conditions. Laccase activity in the culture of each fungus (PF, PS, and PE) gradually increased with the time of fermentation. The highest laccase production (388 IU/mL) was recorded in the set of *P. florida* on 15th day, which was followed by 334 IU/mL on 20th day by *P. eryngii* culture. However, *P. sajor-caju* exhibited lowest maximum activity of laccase (301 IU/

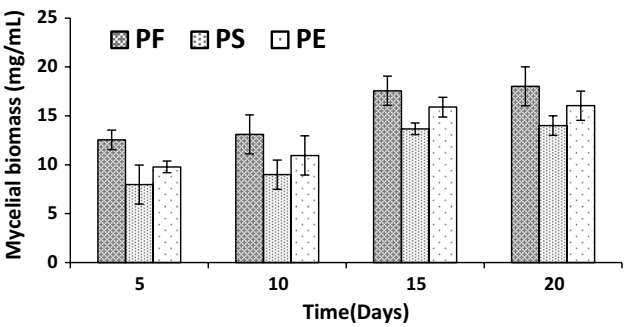

Fig. 1 Showing total mycelial biomass of fungi *P. florida, P. sajor-caju*, and *P. eryngii* after incubation of 20 days

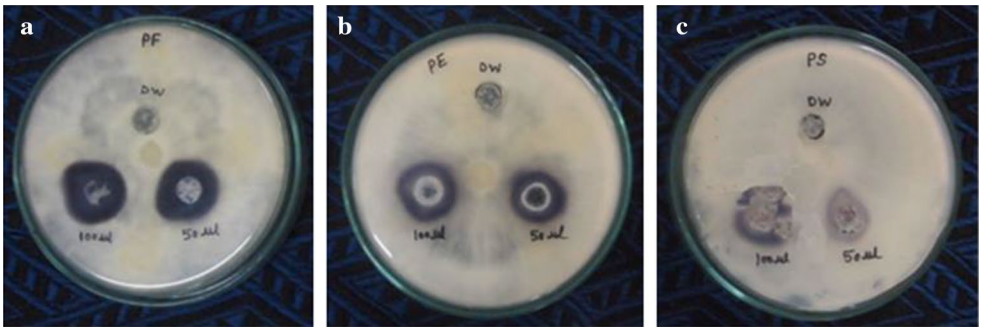

Fig. 2 Plate assay showing qualitative characteristics of laccase production in the cultures of different fungus **a** *P. florida*, **b** *P. eryngii* and **c** *P. sajor-caju* using 50 and 100 µL of test solution

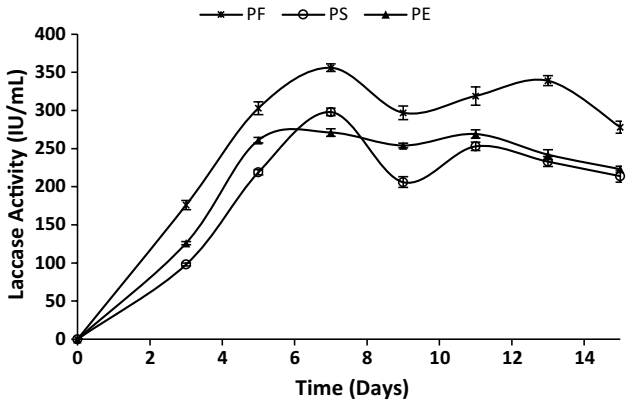

Fig. 3 Comparative laccase enzyme profile of three different white-rot fungi during submerged culture

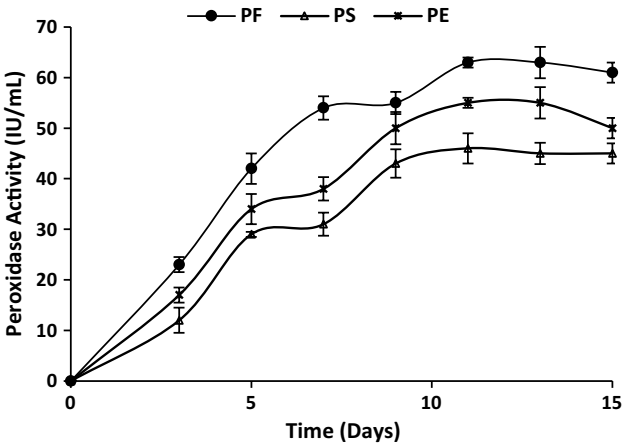

Fig. 4 Comparative peroxidase enzyme profile of three different white-rot fungi during submerged culture

mL) on 17th day in comparison to other fungi (PF and PE) (Fig. 3).

Peroxidase production by different *Pleurotus* spp.

It was observed that all three fungal strains remarkably produced peroxidase enzyme when cultured in broth medium under submerged fermentation conditions. Like laccase activity, peroxidase activity also increased gradually with the incubation time. The highest peroxidase activity (72 IU/mL) was recorded by PF on 15th day, which was followed by 64 IU/mL on 20th day and 55 IU/mL on 15th by PE and PS, respectively (Fig. 4).

Dye decolorization efficiency of different *Pleurotus* spp. on solid media

Decolorization of brilliant green dye was successfully performed on dye supplemented solid media taking three different fungi. The fastest decolorization took place in plates cultured with PF, which was followed in the plates of PE and PS, respectively. Decolorization of brilliant green was observed in separate plates for every particular fungi, where control plate did not show any color reduction (Fig. 5).

Influence of dye concentrations on decolorization in liquid cultures

Different concentrations of brilliant green dye supplemented in separate liquid cultures of PF, PS, and PE were remarkably decolorized during submerged culture conditions. Biodecolorization of lower concentrations of brilliant green dye were found to be faster in comparison to higher concentrations. The influence of dye concentrations on biodecolorization by *P. florida* can be presented in reducing order of 99 > 90 > 80 > and 73%, respectively in 2, 4, 6, and 8% (w/v) of dye. Biodecolorization of dye was variably influenced by each fungal strain. The highest (99%) of dye decolorization took place by the cultures of PF, which was followed to 91% decolorization in the culture of PE, while the PS at 2% (w/v) concentration of dye decolorized only 83% (Fig. 6).

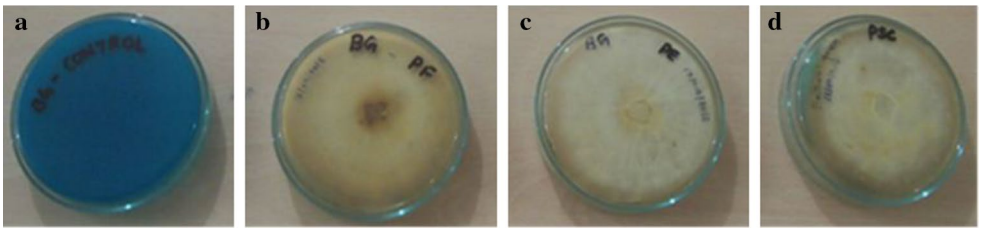

Fig. 5 Showing biodecolorization of brilliant green dye on supplemented media in plates **a** control **b** *P. florida*, **c** *P. eryngii*, and **d** *P. sajor-caju*

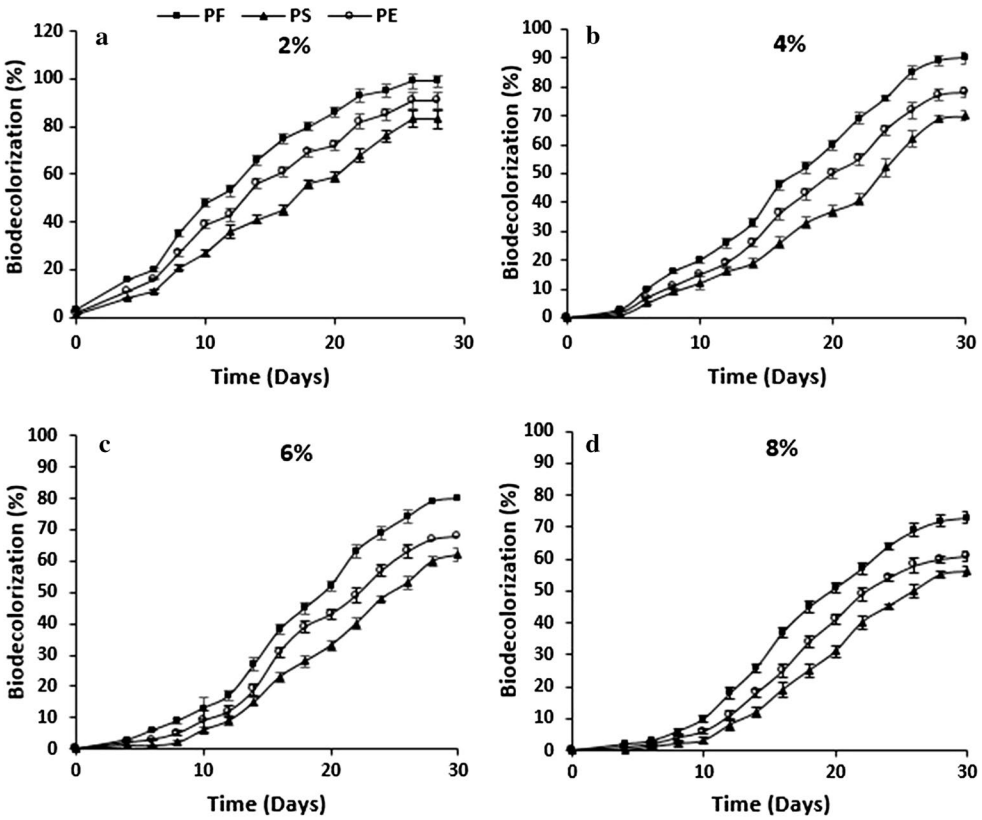

Fig. 6 Effect of different levels **a** 2%, **b** 4%, **c** 6%, and **d** 8% of brilliant green dye on biodecolorization by *P. florida*, *P. eryngii* and *P. sajor-caju*

Discussion

White-rot fungi have been recognized from long ago for their efficient abilities to produce variety of extracellular enzymes capable to degrade carcinogenic carpet dyes. In recent few decades the ability of white-rot fungi to decolorize synthetic dyes is well reported by several researchers (Zhuo et al. 2017; Kumari and Naraian 2016; Hadibarata et al. 2013). Most species of white-rot fungi significantly produce extracellular laccases (Hatakka 1994). Only few microbial enzymes can cause cleavage of associated chromophores and oxidative enzymes; peroxidases and laccases are very much suitable for the degradation/decolorization of textile dyes (Katuri et al. 2009; Duran and Esposito 2000; Duran and Duran 2000). However, in comparison to peroxidases, the laccases work more efficiently and do not depend on co-factors (Goszczynski et al. 1994). White-rot fungi decolorized dye through biodegradation into several smaller compounds via extracellular enzymes like laccase (Hadibarata et al. 2013). Boer et al. (2004) observed a strict relation between production of Mn peroxidase and dye decolorization ability of white-rot fungi. Several reports have advocated that Mn peroxidases from different white-rot fungi are directly involved in dye decolorization (Heinfling et al. 1998; Gold et al. 1988; Jaspers et al. 1994; Michel et al. 1991; Moreira et al. 2000). However, Mn peroxidase from *P. eryngii* strain ATCC 90,787 decolorized four azo and two phthalocyanine dyes directly through enzymatic reactions (Heinfling et al. 1998; Bouacem et al. 2017). The decolorization rate of dyes increases with an increase in incubation time

(Bouacem et al. 2017). The highest percentages of decolorization (89%) were observed for remazol brilliant blue R (RBBR) in 12 h of reaction (Bouacem et al. 2017).

The fungal biomass of any species is generally known as quantitative characteristic of the organism. In the first experiment of the present study total mycelial biomass of three fungi were evaluated. We found *P. florida* produced highest biomass during submerged fermentation. Similar observations were also made in our previous study using *P. florida* (Naraian et al. 2009). In a different study Maziero et al. (1999) worked on various basidiomycete fungus including *P. florida* and *P. sajor caju* and reported similar findings. Moreover, Confortin et al. (2008) also conducted their studies on *P. sajor-caju* but contrary to our findings they reported lesser yield of mycelial biomass.

The lignocellulolytic system of *Pleurotus* spp. has been extensively studied with reference to the production of laccase enzyme. According to the observations highest yield of laccase was achieved by the culture of *P. florida*. The results obtained for laccase were similar to the earlier findings of on several species of *Pleurotus* (Chi et al. 2007; Munari et al. 2008). In a study Eichlerova et al. (2006) reported highest laccase activity by the *P. eryngii.* Synthesis of laccase activity depends on mostly different factors including composition of media and level of nitrogen available (Singh and Chen 2008; Akpinar and Urek 2017).

Moreover, in a subsequent experiment production of peroxidase was also found to be higher by *P. florida*. In next parameter of the study, biodecolorization of brilliant green dye was performed with different concentrations and three different fungi under submerged conditions. It is evident that white-rot fungi of genus *Pleurotus* are strongly capable of decolorizing different groups of synthetic dyes (Chagas and Durrant 2001; Murugesan 2002, 2006). Although fungal strains produce both Mn peroxidase and laccase, the decolorization of synthetic dyes occurs mainly by laccase (Murugesan et al. 2006). As it was recorded in the study, the biodegradation competency varied within species studied. Similar findings were also mentioned by Kunjadia et al. (2016) when working on *P. ostreatus*, *P. sapidus*, *P. florida*, which was 88, 92, 98% of biodecolorization, respectively. Moreover, in another study decolorization of brilliant green dye occurred upto 95% by submerged cultures of *P. ostreatus* (Przystas et al. 2013). In a previous study we found that mono culture of *P. florida* decolorized 86% of dye at the level of 2% (w/v) brilliant green dye (Kumari and Naraian 2016). As it was evident in the study all three species of fungi showed variable efficiency of dye decolorization, which might be due to the extracellular secretion of different enzymes (Lac and MnP) in response to the type of different dyes. It is also obvious that the different species of fungi also respond differentially with the distinct chemical structure of carpet dye. In consequence, the release of extracellular enzymes influences the dye decolorization efficiency of particular fungi.

In recent years, bioremediation of textile dye contaminants from environment is considered as top most priority for the global environmental sustainability (Chen et al. 2013). The volume of effluent discharges of dye processing and manufacturing industries into the open environment sink per sink is rapidly increasing per day (Khandare et al. 2013). Due to such pollutants, our environment is being threatened, reaching a stage of a high risk for sustainable environment and healthy ecosystems. Biological methods are currently considered as environmentally safe for bioconversion of organic pollutants to nontoxic end products (Jin and Ning 2013). The biological degrading system using white-rot fungi for the complex brilliant green carpet industry dye was found to be a potential eco-friendly approach. Therefore, utilization of fungal agents for biodecolorization and biodegradation may play an important role in sustainability of environment by degrading dyes and consequently reducing toxicity of such chemicals negligently thrown in environment.

Conclusion

Biodecolorization of carpet industry dyes using microorganisms such as basidiomycetes and particularly *Pleurotus* species has great potential towards maintaining environment clean and green for sustainability of whole biological system. Based on the findings of the present study a conclusion can be made that genus *Pleurotus* have great ability to degrade highly recalcitrant carpet industry dyes such as brilliant green. Amongst the three fungi tested *P. florida* exhibited greatest potential of dye biodecolorization (99%) by superficially producing higher activities of extracellular laccase and peroxidase enzymes. Although all three fungi successfully biodecolorized the dye, but the remarkable potential of *P. florida* is recommended for the bioremediation of carpet mill dyes to cleanup effluent discharges.

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