

International Research Journal of Biological Sciences _ Vol. 7(11), 18-26, November (2018)

Isolation and screening of fungal strains for bioremediation of textile effluent

Monika Sood, Tanzin Dolma and Pardeep Kaur*

Department of Microbiology, Dolphin (P.G) College of Science and Agriculture, Chunni Kalan-140307, Punjab, India pardeepkaur2108@gmail.com

Available online at: www.isca.in, www.isca.me

Received 28th July 2018, revised 10th October 2018, accepted 7th November 2018

Abstract

Among the various industrial effluents, textile and the dye industry waste play a significant role in water pollution. These dyes are toxic, mutagenic and carcinogen in nature and cause adverse effects on environment as well as on human health. The aim of the study was to isolate potential fungal strains implicated in bioremediation of dye based effluents. From a total of 14 fungal strains isolated from diverse habitats, only 5 strains were found to be positive for Azure B decolorization under solid state condition, with a maximum decolorization index of 2.3mm by fungal strain DW3. Under liquid state conditions, fungal strain CC3 resulted in maximum decolorization of 98.6% at 10th day of incubation. The morphological, cultural and microscopic characterization of potential fungal strain CC3 revealed it as Penicillium sp. The treatment of textile and synthetic effluent (mixture of three dyes Azure B, Congo red and Remazol brilliant blue) by Penicillium sp. CC3 resulted in maximum decolorization to the length observed in the distilled water.

Keywords: Azure B, decolorization, Penicillium sp., CC3, synthetic effluent, textile effluent.

Introduction

Among the most concerned environmental pollutions threatening our biodiversity, water pollution is a major one, where effluents from dye-based industries e.g. textile industries serve as a principal source. The effluent released from textile industries is a complex mixture of many polluting substances such as, heavy metals, pigments and toxic dyes¹. Due to chemical diversity of dyes, they are broadly divided into many categories such as, azo, reactive, anthraquinone, triphenylmethane, heterocyclic, polymeric structures, etc.². Among these types, azo dyes constitute about 70%, while up to 50% of dyes find its ultimate way in the water as $effluent^{3,4}$. Recent studies have shown that azo dyes not only contribute to the mutagenic activity of ground and surface waters⁵ but also lead to aesthetic problems and obstructs light penetration and oxygen transfer into water bodies, hence affecting aquatic life. Therefore, treatment of industrial effluents containing azo dyes before its discharge into wastewater bodies is deemed necessary⁶.

Commonly applied methods for effluent treatment consist of biological, physical and chemical methods⁷. The chemical and physical methods such as oxidative processes, ozonation, photochemical, electrochemical destruction, irradiation and electrokinetic coagulation, are not widely used for the treatment of dye based waste water due to high costs and disposal problem. To recover from these problem green technology is opted for the treatment of the effluent, which includes bacterial and fungal biomass for the adsorption of dye⁸ or low-cost non-conventional adsorbents⁹ e.g. various microorganisms like

bacteria, actinomycetes, fungi and algae have been shown to degrade and biotransform dyes¹⁰. Fungi from the Basidiomycete group, known as white-rot fungi (WRF) and enzymes linked to lignocellulosic decolorization pathways have also been indicated as capable of decolourizing several pollutants of diverse structures including dyes¹¹. These microorganism's secret a various types of enzymes such as laccase, azoreducatase, manganese dependent or independent peroxidase and lignin peroxidase to degrade these dyes¹².

Materials and methods

Effluents: Two types of effluents were used in the study, viz. textile effluent, obtained from Madan dying, Pvt. Ltd., Ludhiana, India, and synthetic effluent, prepared in the laboratory by mixing 3 dyes i.e. Remazol brilliant blue, Congo red, Azure B in equal proportions at a final concentration of 0.01g/L.

Dyes: The structural information of the dyes used i.e. Remazol brilliant blue, Azure B and Congo red is presented in Table-1.

Isolation of fungal strains: The dye decolourizing fungal strains were isolated from diverse habitats such as litter sample (LS), cow dung (CD), cow cud (CC), decomposed wood (DW) and decomposed straw (DS). The isolations were carried out by pour plating technique using Potato dextrose agar (PDA) medium, supplemented with 50μ l/ml of Gentamycin. The inoculated Petri plates were incubated at $28\pm2^{\circ}$ Cin a BOD incubator. The strains with different morphologies were subcultured on fresh PDA medium. The purified fungal strains were stored as slant cultures at 4°C for further experimentation.

Table-1:	Structural	information	of dyes
----------	------------	-------------	---------

Chemical name	Wavelength Λ_{max} (nm)	Туре	Chemical structure, Molecular formula (MF) and Molecular weight (MW)
Remazol brilliant blue	592	Anthraquinone	$(MF:C_{22}H_{16}N_2Na_2O_{11}S_3; MW: 626.53)$
Azure B	650	Azo	$H_{3}C_{N} \xrightarrow{N}_{I} \xrightarrow{N}_{I} \xrightarrow{CH_{3}}_{CH_{3}} CI^{-}$ $(MF: C_{15}H_{16}SN_{3}CI; MW: 305.8)$
Congo red	540	Azo	$(MF:C_{32}H_{22}N_6Na_2O_6S_2; MW: 696.665)$

Screening of fungal strains for dye decolourizing enzymes: Primary screening: The fungal strains were inoculated in a basal medium supplemented with 0.02% (w/v) Azure B. The composition of the medium is as follows (g/L): monopotassium phosphate (1.0), ammonium tartarate (0.5), magnesium sulphateheptahydrate (0.5), calcium chloride (0.01), yeast extract (0.01), copper sulphatepentahydrate (0.001), ferric sulphate (0.001), manganese sulphate monohydrate (0.001). The inoculated Petri plates were incubated at $28\pm2^{\circ}$ C in a BOD incubator under dark conditions and growth was followed for a period of approximately 2 weeks. The formation of a clear zone around the fungal colonies indicate the production of peroxidases such as lignin peroxidase (LiP) and manganese peroxidase (MnP). Decolorization index was calculated as follows:

Decolorization index (mm) = $\frac{\text{Decolorization zone}}{\text{Colony size}}$

Secondary screening: Secondary screening of fungal strains was carried out in liquid basal medium comprising of 0.02% (w/v) of Azure B. Two agar discs (approximately 8-10mm) of actively growing mycelia were inoculated in the medium. The flasks were incubated under dark conditions at $28\pm2^{\circ}$ C in a shaking incubator (100rpm). In addition, control sample consisting of media that contained dye but was not inoculated, were also maintained. Once a day, aliquot was withdrawn from the medium and centrifuged at 5000rpm for 10 minutes. The supernatant was read at absorption maxima of the Azure B i.e. 650nm. The percent decolorization was calculated using the following formula¹¹:

Decolorization (%) =
$$\frac{A_0 - A}{A_0} x \ 100$$

Where: A_0 is the initial absorbance and A is maximum absorbance.

Characterization of fungal strains: The selected fungal strains were initially identified on the basis of their morphological and cultural characteristics. Further identification was done using microscopic observation such as conidia, their shape and arrangements, conidiophores and hyphae, after lactophenol cotton blue staining.

Effluent treatment: Two types of effluents were used for the bioremediation study, textile and synthetic effluent. The effluents were added with basal medium components and autoclaved at 121°C for 15 minutes. The experimental flasks were inoculated with actively growing mycelia discs (approximately 8-10mm diameter) of selected fungal strains and incubated at $28\pm2^{\circ}$ C in a shaking incubator at 100rpm under dark conditions. In addition, control samples were also maintained. Once a day, aliquot was withdrawn from the flasks and centrifuged for 10 minutes at 5000 rpm. For textile effluent, the absorbance of the supernatant was read at a broad visible range i.e. 400-700nm and for synthetic effluent, absorption maxima of dyes used, i.e. 540nm (Congo red), 592nm (Remazol brilliant blue)¹³, 650nm (Azure B)¹⁴ were recorded for percent decolorization.

Seed germination bioassay: The decolorized medium was assessed for the reduction in toxicity on the basis of wheat seed germination bioassay. The wheat seeds were sterilized using 0.01% mercuric chloride (HgCl₂) solution for 50 sec., washed 6-7 times with sterile distilled water to remove traces of HgCl₂ and soaked in water for 3-4h. The bottom of the Petri plates were padded with layers of tissue papers and presoaked seeds were placed in each Petri plate followed by soaking of tissue paper with treated and untreated medium. A control using sterile distilled water was also maintained. The plates were incubated at $28\pm2^{\circ}$ C in a BOD incubator for about 3 days. The tissue was kept moist by intermittently spraying the respective medium and sterile distilled water. The plates were observed daily for the germination and plumule lengths of germinated seeds were recorded.

Results and discussion

Isolation and screening of dye decolorizing fungal strains: A total of 14 fungal strains were isolated on PDA medium by pour plating technique and labeled according to their site of isolation such as litter sample (LS1), cow dung (CD1), cow cud (CC1, CC2 and CC3), decomposed wood (DW1, DW2, DW3, DW4, DW5 and DW6), Decomposed straw (DS1, DS2, DS3) . In recent years, microorganisms producing ligninolytic enzymes have widely been reported for dye decolorization and waste water treatment studies¹⁵. Azure B, an azo dye is a substrate for peroxidase enzymes e.g. lignin peroxidase and manganese peroxidase which results in its decolorization from an initial blue color¹⁶. The ability of individual fungal strains to decolorize Azure B was initially investigated under solid state conditions. Five fungal strains (CC3, DW6, DW2, DW3, DS3) were selected on the basis of clear zone production. The strain

DW3 exhibited the highest decolorization index of 2.3mm followed by 1.6mm (DW6), 1.7mm (DS3) and 1. 2mm (CC3 and DW2) (Figure-1).

ISSN 2278-3202

Int. Res. J. Biological Sci.



Figure-1: Azure B decolorization by fungal strain CC3 on solid medium.

The further screening of strains was carried out under liquid state conditions. The rate of decolorization by fungal strains DW6 and CC3 was observed to be very high from the beginning

of the experiment, as these strains resulted in 76.2% and 65.2%, respectively after first day of incubation. Thereafter, a gradual increase in decolorization was observed and finally a maximum decolorization of 95.6% and 98.6% (Figure-2), respectively at 10th day of incubation was achieved. All the other fungal strains showed less than 90% decolorization on 10th day of incubation i.e. 81.9% (DS3), 80.1% (DW2) and 79.9% (DW3). The rate and extent of decolorization by fungal strains in the present study were similar or even better than those by other fungi reported in literature such as 83% decolorization in 11 days by Phanerochaete chrysosporium¹⁷, 65% of decolorization in 20 days by Bjerkandera sp. BOS5517, 70.61% and 85.75% of decolorization in 15 days by *Perreniporia tephropora*¹⁸. As explained by Ghasemi et al.¹⁹ the decolorization of several types of dyes by Phanerochaete chrysosporium were associated with production of extracellular ligninolytic enzymes e.g. MnP and LiP. Another study by Singh et al.²⁰ also reported that enzyme LiP was responsible for 100% decolorization of Direct red dye (30ppm) by P. chrysosporium within 24 h.

Characterization of fungal isolates: On the basis of morphological, cultural and microscopic characterization, the

fungal strains were classified into different genera as follows: DW2 and DW3 as *Rhizopus* sp., DW6 as *Gliocladium* sp., DS3 as *Microsporum* sp. and CC3 as *Penicillium* sp.

Effluent treatment: The fungal strain with greatest decolorization ability i.e. Penicillium sp. CC3 was evaluated using two types of effluents (textile and synthetic). The textile effluent had the following characteristics, neutral pH, pinkish color and presence of suspended particles. The percent decolorization of textile effluent was calculated on the basis of absorption value of decolorized medium, measured at broad visible range (400 - 700nm). A maximum decolorization of 93% was observed at 600nm followed by 92% at 400nm and 91% at 500nm and 700nm each, at 10th day of incubation. (Figure-3 and 4). The synthetic effluent treatment demonstrated percent decolorization of 73.25% for Azure B, 57.14% for Congo red and 55.55% for Remazol brilliant blue (RBB) at 10th day of incubation at 650nm, 540nm and 592nm respectively absorption maximas (Figure-5 and 6). Placido²¹ reported, more than 90% of decolorization of two real effluents from a textile industry by Leptosphaerulina sp.



Figure-2: Azure B decolorization by fungal strain CC3 in liquid medium.



Figure-3: Decolorization of textile effluent by Penicillium sp. CC3



Figure-4:Percent decolorization of textile effluent by Penicillium sp. CC3



Figure-5: Decolorization of synthetic effluent by *Penicillium* sp. CC3.



■560nm ■592nm ■650nm

Figure-6: Percent decolorization of synthetic effluent by Penicillium sp. CC3.

Comparative evaluation of dye decolorization by *Penicillium* **sp. CC3 on different media:** The Figure-7 depicts the comparison of dye decolorization potential of *Penicillium* sp. CC3 on Azure B, textile and synthetic effluent. As observed, *Penicillium* sp. CC3 achieved 73.25%, 93.0% and 98.6% decolorization of synthetic, textile effluents and Azure B respectively at 10th day of incubation. Eichleiova et al.²² stated that difference between decolorization of structurally different dyes is due to the complexity of chromophores present in the mixture resulting in slow decolorization rate of some dyes. Rani et al.⁹ reported biodegradation and detoxification of Malachite green, Nigrosin and Basic fuchsin using two fungal isolates *Aspergillus niger*, and *Phanerochaete chrysosporium*, isolated from dye effluent soil. The maximum decolorization of 81.85% was achieved for Basic fuchsin followed by 77.47%, (Nigrosin), 72.77% (Malachite green) and 33.08% (dye mixture) under shaking

condition, whereas, *P. chrysosporium* recorded maximum decolorization of 90.15% (Nigrosin), 89.8% (Basic fuchsin), 83.25% (Malachite green) and 78.4% (dye mixture).

Seed germination bioassay: The detoxification of the decolorized medium was assessed by wheat seed germination bioassay. The wheat seed were allowed to germinate under three sets of conditions, i.e. in the presence of decolorized/ treated medium, untreated medium and distilled water. The growth

index of seed is measured in terms of plumule length. The wheat seeds grown on untreated medium showed less plumule length due to the toxicity of dye, as compared to the seeds grown in decolorized medium. In fact, the plumule length in presence of decolorized medium was almost equivalent to growth observed in the presence of distilled water. The seeds in decolorized medium showed plumule length of 3.3cm, 3.4cm in distilled water and 1.3cm in untreated medium (Figure-8).



Figure-7: Comparative evaluation of dye decolorization by Penicillium sp. CC3 on different media.



Figure-8: Seed germination bioassay.

In accordance with our study, Li et al.²³ reported phytotoxicity evaluation for both Azure B and its related derivatives by *Bacillus* sp. MZS10, indicating that decolorization metabolites were less toxic then original dye. Barapatre et al.²⁴ grew *Phaseolusmungo* in the presence of textile effluent, distilled water and untreated effluent and reported a higher shoot length of 14.5cm in textile effluent as compared to 10.6cm in untreated effluent.

Conclusion

The bioremediation process for synthetic and textile effluent using fungi is acost-effective process and poses little disturbance to environment as compared to conventional technologies, rendering this technology a very attractive and alternative method of choice. The Penicillium sp. CC3 isolated in the present study resulted in the dramatic decolorization of Azure B (>90%) in an incubation period of 10 days. Furthermore, the toxicity test of the decolorized medium by seeds germination bioassay showed that there was a reduction in the toxicity of the dye after treatment. The toxicity reduction of the dye is attributed to biodegradation of chromophore in dye molecule owing to extracellular peroxidase enzyme production. Thus, the potential isolate, Penicillium sp. CC3 holds a good potential to be used as an dye decolorizing agent in the textile and dying industries, considering its impeccable decolorization potential. In order to ensure the potential application of microorganisms especially for the fungi, for large scale application of dye based waste water treatment, optimization of process parameters and experiments to study the toxic effect of different dves on microbial strains need to be studied. The identification and research of new microbial strains with the aid of molecular techniques will further improve practical applications of microorganism. Thus, it is anticipated that biodegradation will be soon a reliable and competitive dye remediation technology.

Acknowledgment

The authors wish to express their gratitude for the support extended by the authorities of Dolphin (P.G) College of Science and Agriculture, Chunni-Kalan, Punjab, India in carrying out the research work.

References

- 1. Dwivedi P. and Tomar S.R. (2017). Microbial degradation and decolorization of azo and anthraquinonentextile dyes. *Int. J. Pharm. Bio. Sci.*, 8(2), 989-998.
- 2. Cheunbarn T., Cheunbarn S. and Khumjai T. (2008). Prospects of bacterial granule for treatment of real textile industrial wastewater. *Int. J. Agric. Biol.*, 10, 689–692.
- **3.** Hassan M.M., Alam M.Z. and Anwar M.N. (2013). Biodegradation of textile azo dyes by bacterial isolates from dyeing industry effluent. *Int. Res. J. Biological Sci.*, 2(8), 27-31.

- **4.** Lade H., Kadam A., Paul D. and Govindwar S. (2015). Biodegradation and detoxification of textile azo dyes by bacterial consortium under sequential microaerophilic/aerobic processes. *Excli. J.*, 14, 158-174.
- 5. Rajeswari K., Kumar S.R. and Vijagarama, K. (2011). Biodegradation of mixed textile dyes by bacterial strains isolated from dye waste effluent. *Resea. J. Environ. Texicol.*, 5(2), 97-107.
- **6.** Gulzar T., Huma T. and Jalal F et al. (2017). Bioremediation of synthetic and industrial effluents by Aspergillus niger isolated from contaminated soil following a sequential strategy. *MDPI*, doi: 10.3390.
- 7. Robinson T., McMullan G., Marchant R. and Nigam P. (2001). Remediation of dyes in textile effluent: a critical review on current treatment technologies with a proposed alternative. *Biores. Technol.*, 77, 247–255.
- 8. Yang X.Q., Zhao X.X., Liu C.Y., Zheng Y. and Qian S.J. (2009). Decolorizationofazo, triphenylmethane and anthraquinone dyes by a newly isolated *Trametes* sp. SQ01 and its laccase. *Process Biochem.*, 4, 1185-1189.
- **9.** Rani B., Kumar V., Singh J., Bisht S., Teotia P., Sharma S. and Kela, R. (2014). Bioremediation of dyes by fungi isolates from contaminated dye effluent sites for bio-usability. *Braz. J. Microbiol.*, 45(3), 1055–1063.
- Pandu K. and Compala P. (2012). Bioremediation of textile dyes and improvement of plant growth by marine bacteria. M.Sc. Thesis, University of Boras School of Engineering, India.
- **11.** Jayasinghe J., Imtiaj A., Lee W.G. and Im H.K. et al. (2008). Degradation of three aromatic dyes by white rot fungi and the production of lignolytic enzymes. *Microbiology*, 36(2), 114-120.
- 12. Krishnamoorthy R.J., Arul P., Ranjith M., Anandham R., Suganya K. and Prabhakaran J. et al. (2018). Decolourisation and degradation of azo dyes by mixed fungal culture consisted of *Dichotomomycescejpii* MRCH 1-2 and *Phomatropica* MRCH 1-3. *J. Environ. Chem. Eng.*, 6, 588-595.
- **13.** Trivedi N.K., Boricha B.A., Bajaj C.H. and Jasra V.R. (2009). Adsorption of Remazol Brilliant Blue R dye from water by polyaluminium chloride. *Rasayan J. chm.*, 2(2), 379-385.
- 14. Vaishnave P., Kumar A., Ameta R., Punjabi B.P. and Ameta C.S. (2014). Photo oxidative degradation of azure-B by sono-photo-Fenton and photo-Fenton reagents. *Arabian J.Chem.*, 7, 981–985.
- **15.** Bankole O.P., Adekunle A.A., Obidi F.O., Chandanshive V.V. and Govindwar P.S. (2018). Biodegradation and detoxification of Scarlet RR dye by a newly isolated filamentous fungus, *Peyronellaea prosopidis. Sustainable. Environ. Resea.*,doi.org/10.1016/j.serj.2018.03.001.

- Wong S.W.D. (2009). Structure and action mechanism of lignolytic enzyme. *Appl. Biochem. Biotechnol.*, 157, 174-209.
- **17.** Swamy J. and Ramsay J.A. (1999). The evolution of white rot fungi in the decolorization of textile dyes. *Enzyme and microb. Technol.*, 24(3-4), 130-137.
- 18. Mounguengui S., Attéké C., Tchinda S.B.J., Ndikontar M.K., Dumarcay S. and Gérardin P. (2014). Discoloration and biodegradation of two dyes by white-rot fungi Perreniporiatephropora MUCL 47500 isolated in Gabon. *Int.J.Curr.Microbiol.App.Sci.*, 3(6), 731-741.
- **19.** Ghasemi F., Tabandeh F., Bambai S.R., K.R.S. (2010). Decolorization of different azo dyes by Phanerocheate chrysosporium RP78 under optimal condition. *IJEST.*, 7, 457-464.
- Singh H. (2006). Mycoremediation: Fungal bioremediation, John wiley and Son's New jersey USA, ISBN: 9780471755012.

- **21.** Placido J., Chanaga X., Monsalve O.S., Yepes M. and Mora A. (2016). Degradation and detoxification of synthetic dyes and textile industrial effluents by newly isolated *Leptosphaerulina* sp. from Columbia. *Bioresour. Bioprocess.*, 3(6), doi 10.1186/s40643-016-0084-x.
- 22. Eichlerova I., Homolka L. and Nerud F. (2006). Synthetic dye decolorization capacity of white rot fungus *Dichomitussqualens. Bioresour. Technol.*, 97(16), 2153-2159.
- **23.** Li H., Zhang R., Tang L., Zhang J. and Mao Z. (2014). Evaluation of *Bacillus* sp. MZS10 for decolorizing Azure B dye and its decolorization mechanism. *J. Environ. Sci.*, 26, 1125-1134.
- **24.** Barapatre A. and Jha H. (2016). Decolorization and biological treatment of pulp and paer mill effluent by lignin degrading fungus *A. flavus* isolate 10. *Int. J. curr. Microb. Appl. Sci.*, 5(5), 19-32.