Int J Pharm Bio Sci 2014 oct; 5(4): (B) 490 – 498

Research Article **Biotechnology**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

IN VITRO DEMONSTRATION OF TEXTILE DYE BIOREMEDIATION BY FUNGI

PRAVEEN KUMAR G N AND SUMANGALA BHAT K*

Department of Biotechnology, Acharya Institute of Technology, Acharya P.O. Soldevanahalli, Bangalore-560 107

ABSTRACT

Current study has evaluated the decolorization of textile dye Red 3BN by *Penicilium chrysogenum, Cladosporium* sp. and *Aspergillus niger*, isolated from textile industry effluent at Bangalore. The organisms were maintained in the laboratory in Potato Dextrose Agar medium. Production of the enzyme laccase by all the 3 strains of fungi has been confirmed through guaiacol bioassay followed by decolorization of an aqueous solution of the dye by crude extract of laccase prepared from individual fungi maintained in enrichment medium. Validation of fungal decolorization of the dye in real effluent sample supplemented with glucose and peptone under simulated condition has recorded nearly 100% decolorization of the effluent in 16 days by *P. chrysogenum,* and *Cladosporium* sp., whereas *A. niger* exhibited decolorization upto 98.6%. The study has confirmed the potential of these fungi for bioremediation of textile effluent.

KEY WORDS: Decolorization, *Penicilium chrysogenum, Cladosporium* sp., *Aspergillus niger,* Laccase, Azodye, Bioremediation.

SUMANGALA BHAT K
Department of Biotechnology, Acharya Institute of Technology, Acharya P.O. Soldevanahalli, Bangalore-560 107

INTRODUCTION

Dyes belong to a versatile group of chemicals which are consumed by several industries such as textile, printing, paper, food and cosmetics[1]. Use of dyes increased through the evolution of civilization due to the fascination for colors by mankind[2]. Azo dyes represent the largest and most diverse group of synthetic dyes. The average worldwide production of azo dyes is huge touching one million tons annually[3]. The amount of dye in the effluent of textile industry increased to several folds over the years. The major cause for heavy discharge of dyes in effluents of dyeing industry is less effective fixation of the dyes in fabric dyeing process[4]. Colored industrial effluent is the most obvious indicator of water pollution and creates serious aesthetic problem[5]. Release of residual azo dye deteriorates the water quality and is responsible for a number of toxic effects on contaminated ecosystems. Decolorization of wastewater is one of the major issues related to wastewater treatment of textile industries. Physical, chemical as well as biological processes are being used for decolorizing textile effluents[1]. However, the dyes in effluent are more difficult to treat because of their synthetic origin and complex aromatic structures[5]. Environment friendly processes with non-toxic intermediate products need to be devised for safe handling of these pollutants. Bio treatment offers a cheaper, eco-friendly and sustainable alternative for removal of color from textile and other industrial effluents carrying dyestuffs. A number of microorganisms, including bacteria, fungi and yeasts have been reported to decolorize textile dyes[6-8]. Bioremediation techniques include bacterial and fungal biosorption and biodegradation in aerobic, anaerobic, anoxic or combined anaerobic/aerobic treatment methods[9-10].Wastewater composition, type of dye, dosages, costs of treatment, and environmental fate of waste products are some of the factors determining feasibility of the techniques. Microbial bioremediation processes have been recognized for their cost- effectiveness, minimal sludge and environmental sustainability and safety[11]. Microorganisms play a crucial role in mineralization of biopolymers and xenobiotics compounds, like azo dyes[12]. Major groups of microbes employed in bioremediation include bacteria, algae, yeasts and filamentous fungi, like white rot fungi [13-14]. Bioremediation utilizes the metabolic potential of microorganisms to degrade otherwise stable xenobiotic compounds from the environment[15].

MATERIALS AND METHODS

All chemicals used in this study were of AR grade. Textile dye, Red 3BN and effluent sample were collected from a dying industry located at Peenya, Bangalore (Karnataka). The sample was collected from the effluent disposal site of the industry. Carbon and nitrogen sources used were purchased from Himedia Laboratories (Mumbai, India).

(i) Isolation, Screening and Identification of Dye Decolorizing Fungi

The fungi used in the current study were isolated, screened for dye decolrization and identified as per the method described in our previous publication[16].

(ii) Screening for Laccase Activity in Fungi

Organisms were screened for laccase activity using guaiacol as indicator compound. Development of intense reddish brown color in the medium around the fungal colonies and was considered indicative of laccase enzyme activity [17-20]. The organisms were cultured separately in enrichment medium (gl^{-1}) containing 3.0 peptone, 10.0 glucose, 0.6 KH_2PO_4, 0.001 $ZnSO_4$, 0.4 K_2HPO_4, 0.0005 FeSO4, 0.05 MnSO4, 0.5$MgSO_4$ and 20.0 agar, supplemented with 0.02% guaiacol at pH-6 for evaluation of laccase production by them[21]. The fungal strains were inoculated in sterile petri plates containing the above medium and were incubated at 30°C for a period of seven days. Cultures showing definite color changes were considered as laccase producing strains and used for subsequent studies.

(iii) Preparation of Crude Laccase Extract

Production of laccase by the fungal strains mentioned above was done in enrichment broth medium containing glucose, 1%;

peptone, 0.3%,; KH_2PO_4, 0.06%; $ZnSO_4$, 0.0001%; K_2HPO_4, 0.04%; $FeSO_4$, 0.0005%; $MnSO_4$, 0.05% and $MgSO_4$, 0.05% with pH 6. The medium was inoculated with seven day old sporulated culture suspension of individual strains separately and incubated at 30ºC for six days. The cultures were centrifuged at 10,000 RPM for 30 min at 4°C. The supernatant was filtered through Whatman No. 1 filter paper and used as a crude laccase extract.

(iv) Decolorization of Red 3BN by Crude Laccase Extract

Aqueous solution of Red 3BN (0.005% w/v) was mixed with crude laccase extract at a concentration of (4% v/v) and pH was maintained at 6. The samples were incubated at 30°C for six days (144h). Disappearance of the color by laccase enzyme was monitored by measuring the absorbance at 600nm. The percentage decolorization was calculated by taking the maximum absorbance of the untreated dye solution as a control (100%). The optical density was measured using Shimadzu UV-1800 spectrophotometer (Tokyo, Japan).

(v) Decolorization of Textile Effluent by Fungi

Sample of textile effluent containing Red 3BN was collected from the discharge of local dying industry. The effluent sample supplemented with glucose (@2g/100ml and peptone, 0.1g/100ml) with pH adjusted to 6 was used for the validation of the above results. After autoclaving, the modified textile effluent was inoculated with seven day old cultures (10% v/v) of *P. chrysogenum, A. niger* and *Cladosporium* sp. separately and incubated under shaking condition at 27°C and 150 RPM for sixteen days. Textile effluent without culture inoculum was used as control and that lacking culture inoculum as well as textile effluent was used as blank. Decolorization was monitored and measured on 8th and 16th day through a UV spectrophotometer by measuring the absorbance at 600nm. Extent of decolorization was calculated according to the following formula of Moorthi *et al.*[22].

$D= [(A_0-A_1) /A_0] \times 100$

Where, D - decolorization in %; A_0 – initial absorbance; A_1 - final absorbance

RESULTS AND DISCUSSION

1. Isolation, Screening and Identification of Dye Degrading Fungi

Colony morphology, microscopic observation and culture characteristics have confirmed the identity of the fungi as *P. chrysogenum, Cladosporium* sp. and *Aspergillus niger*[16].

2. Screening for Laccase Activity and Dye Decolorization by the Fungi

Laccase has been reported as one of the principal enzymes of the Lignin Modifying Enzymes (LMEs) group involved in the degradation of organic pollutants like azo dyes and pesticides and three dominant representatives of this group are laccase, lignin peroxidase and Mn-dependent peroxidase[23-27]. Earlier investigations on the mechanism of dye decolorization by fungi have reported lignin peroxidase [28,29] and laccase[30-32] as mediators of the process. Therefore, laccase production has been considered as the target for understanding the decolorization of the azo dye Red3BN by the fungi in the current study.The investigation has confirmed production of laccase by all the 3 strains of fungi (i. e. *A. niger, P. chrysogenum and Cladosporium* sp.) in laccase production screening assay (Fig.1a - c). Synthesis and secretion of laccase by these fungi confirmed during the current study is in conformation with earlier reports for the same organisms[33]. Above result was further confirmed by treating solution of Red 3BN with crude laccase extract from individual fungi separately. Visible reduction in the color of the dye solution was obvious (Fig. 2a-c) in all treatments. Average percentage decolorization recorded at the end of six days is presented in Fig. 3, with highest activity recorded for *P.chrysogenum* with 80% reduction in color.

Figure1
Screening of laccase activity in fungi

1a. A.niger **1b. Cladosporium sp.** **1c. P. chrysogenum**

Figure 2a
Decolorization of Red 3BN by A.niger at intervals of 24 h.

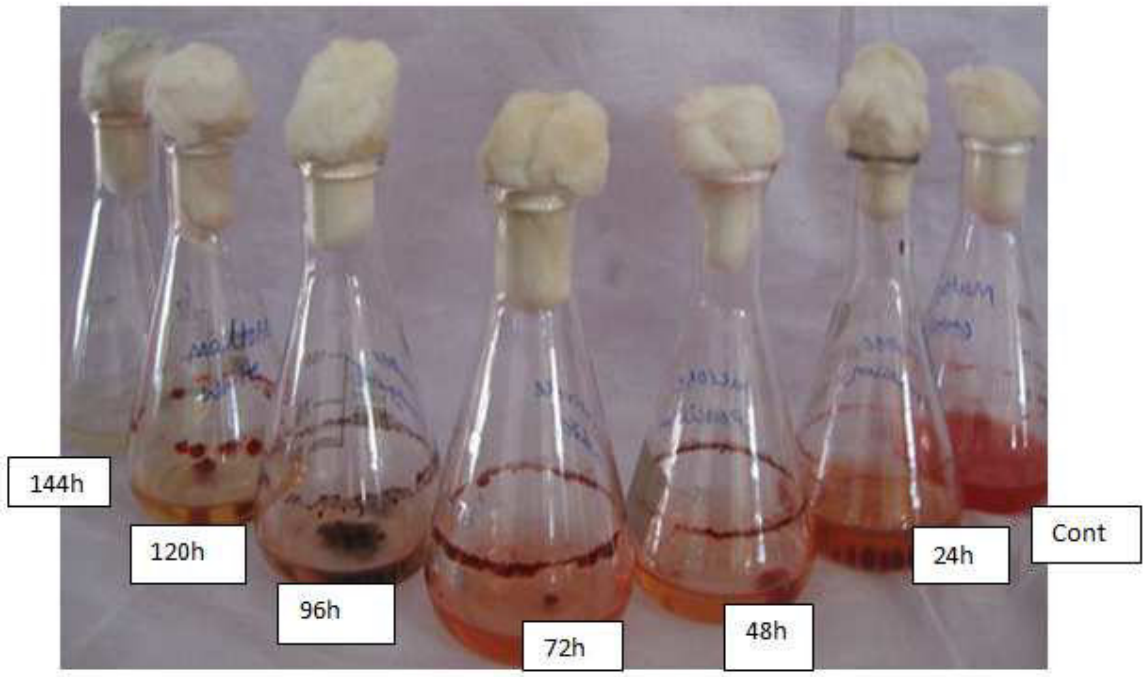


Figure 2b
Decolorization of Red 3BNbyP.chrysogenum at intervals of 24 h.

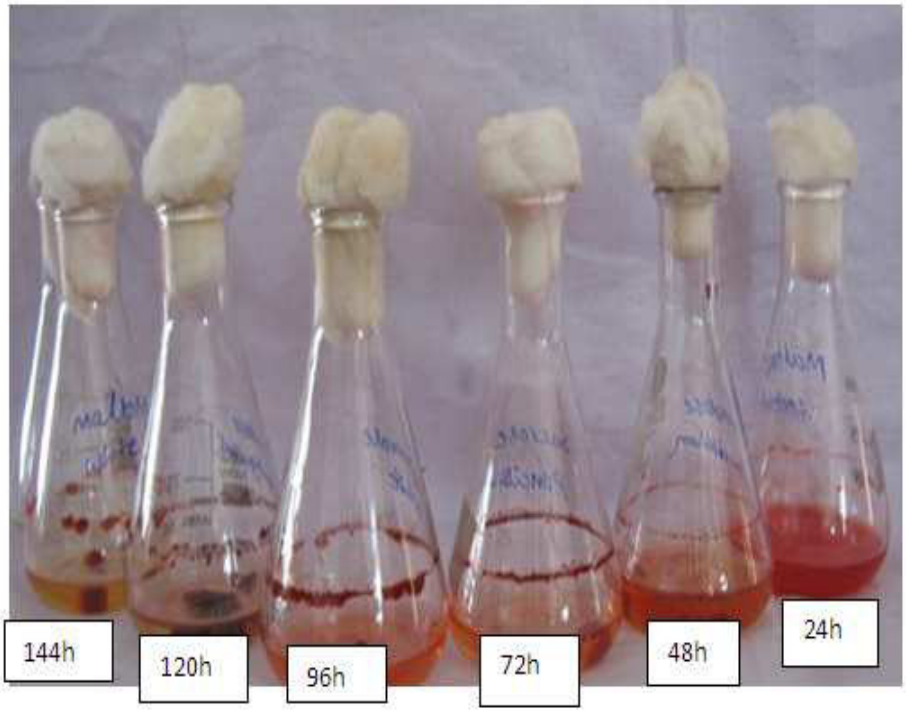


Figure. 2c.
Decolorization of Red 3BN by Cladosporium sp. at intervals of 24 h.

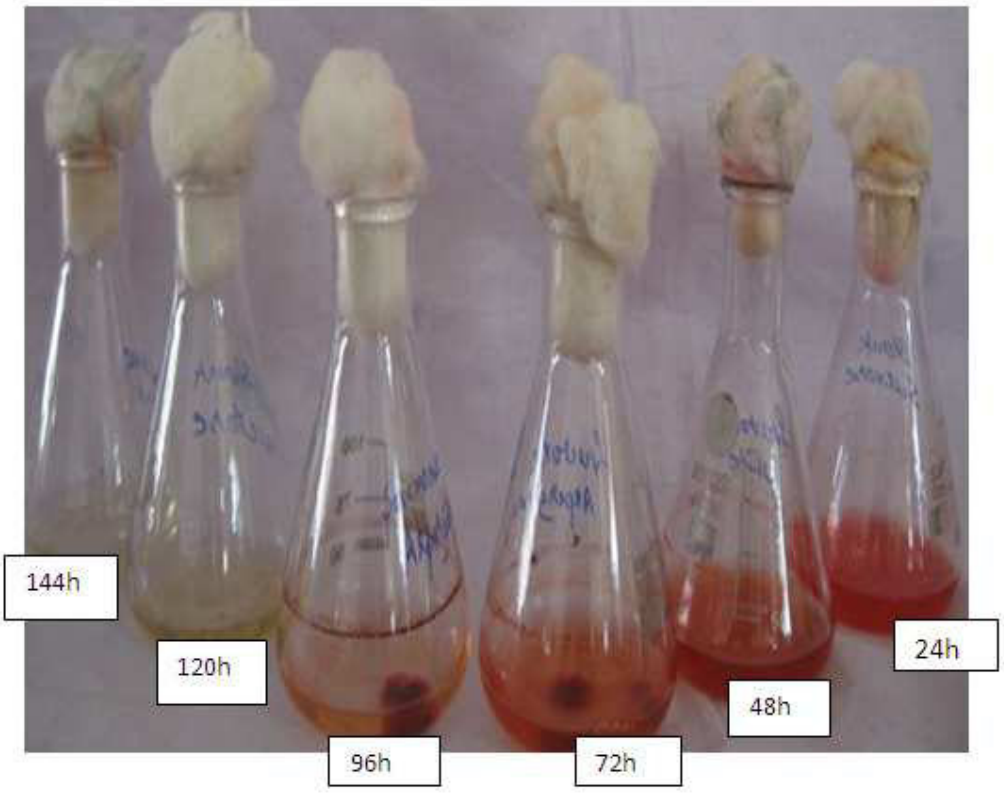
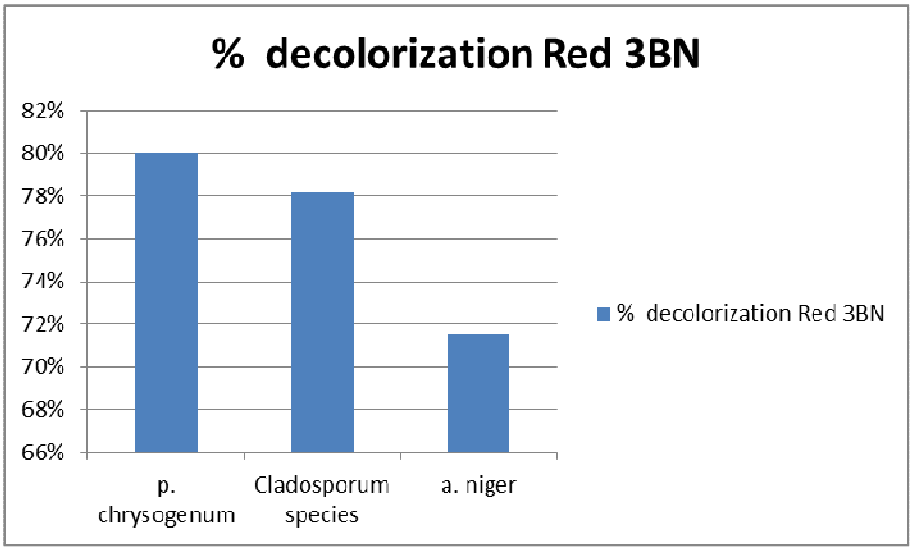


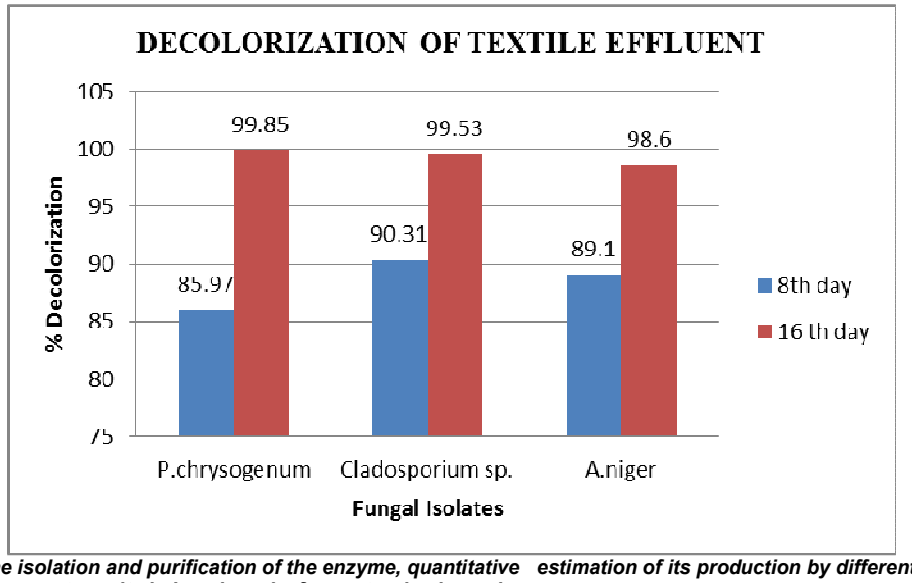
Figure-3
Comparative evaluation of decolorization of Red 3BN by crude laccase extract of fungi



3. Decolorization of textile effluent by fungi

Decolorization of textile effluent by the fungi under simulated conditions was evaluated by inoculating fungal strains to effluent sample supplemented with glucose and peptone under in vitro conditions. Fig.4 depicts percentage decolorization of the effluent observed under laboratory conditions for the 3 strains of fungi. *P.chrysogenum* and *Cladosporium* sp. recorded almost 100% decolorization of the affluent after 16 days of inoculation while *A. niger* showed slightly lesser activity. While comparing the activities of the fungi in dye solution and effluent, relative competence of the strains remained the same with *P.chrysogenum* recording highest activity followed by *Cladosporium* sp. and *A. niger.*Fungi are known for their ability to degrade various complex materials including azo dyes, pesticides and other xenobiotics[15,34,35] . Similar studies on degradation of textile effluent using consortia of microorganisms including bacteria and fungi has reported upto 71% decolorization of the efluent in five days[36]. Another investigation reported 74% decolorization and degradation of azodye into non toxic components by consortia of non- adapted fungal species in the bioremediation of textile effluent[37]. Since current study has assessed the activities of individual fungi separately, potential of theses fungal strains would be much better than those of the earlier report quoted above.

Figure 4
Percentage decolorization of textile effluent by fungal isolates



Further studies on the isolation and purification of the enzyme, quantitative estimation of its production by different fungal strains and validation of the current results in bench scale fermentor is planned.

CONCLUSION

Decolorization of textile dye Red 3BN by *P. chrysogenum, Cladosporium* sp. and *A. niger* isolated from the effluent sample has been demonstrated under in vitro conditions. Production of the enzyme laccase by all the 3 strains of fungi has been confirmed through guaiacol bioassay followed by decolorization of an aqueous solution of the dye by crude extract of laccase. Validation of fungal decolorization of the dye in real effluent samplehas recorded nearly 100% decolorization of the effluent in 16 days by *P. chrysogenum,* and *Cladosporium* sp. Potential of these fungi for bioremediation of textile effluent has been confirmed.

ACKNOWLEDGEMENT

The authors gratefully acknowledge HOD, Biotechnology, and the Principal Acharya Institute of Technology, Bangalore for providing the facilities for the research work and the Management of AIT, Bangalore for their support and encouragement.

REFERENCES

1. Khalid A., Arshad M. and Crowely D. Bioaugmentation of azo dyes: Hdb. Env Chem, 9: 1-37, (2010)
2. Mohan S. V., Roa C. N., Prasad K. K. and Karthikeyan J., Treatment of stimulated reactive yellow 22 (Azo) dye effluents using *Spirogyra* species. Waste manages, 22: 575-582 (2002)
3. Stolz A., Basic and applied aspects in the microbial degradation of azo dyes. Appl. Microbiol Biotechnol, 56: 69-80 (2001)
4. Easton J., Ed. Colour in dye house effluent. Vol 11, Society of Dyers and Colorists,Bradford,UK, (1995)
5. Banat I. M, Nigam P., Singh D. and Marchant R., Microbial decolourization of textile dye containing effluents a review. Bioresource Technol, 58: 217-227 (1996)
6. Olukanni O. D., Osuntoki A. A. and Gbenle G.O., Textile effluent biodegradation potentials of textile effluent - adapted and non -adapted bacteria. Afr J Biotechnol, 5: 1980-1984 (2006)
7. Wesenberg D., Kyriakides I. and Agathos S.N., White- rots fungi and their enzymes for the treatment of industrial dye effluents. Biotechnol Adv, 22: 161-187 (2003)

8. Robinson T., Chandran B. and Nigam P., Studies on the production of enzymes by white-rot fungi for the decolourisation of textile dyes. Enzyme Microbial Technol, 29: 575-579 (2001)
9. Pazarlioglu N. K., Urek R.O. and Ergun F., Biodecolourization of direct blue 15 by immobilized *Phanerochaete chrysosporium*. Process Biochem, 40: 1923-1929 (2005)
10. Forgacs E., Cserhati T. and Oros G., Removal of synthetic dyes from waste waters. Env Int, 30: 953-971 (2004)
11. Chen K.C., Wu J.Y., Liou D.J. and Hwang S.C.J., Decolorization of the textile dyes by newly isolated bacterial strains. J Biotechnol, 101: 57–68 (2003)
12. Lie T. J., Leadbetter J. R. and Leabetter E. R., Metabolism of sulfonic acids and other organosulfur compounds by sulfate-reducing bacteria. Geo Microbiol I, 15(2): 135-149 (1998)
13. Mullan M. C. and Meehan C., Microbial decolourization and degradation of textile dyes. Appl Microbial Biotechnol, 56 (1-2): 81-87(2001).
14. Kaushik S., Juwarkar A., Malik A. and Satya S., Biological removal of Cr (VI) by bacterial isolates obtained from metal contaminated sites. J Env Sci and Health, Part A, 43: 419-423 (2008)
15. Watanabe K., Microorganisms relevant to bioremediation. Curr Opin Biotechnol, 12: 237-241 (2001)
16. Kumar Praveen G. N. and Sumangala K Bhat, Fungal degradation of Azo dye-Red 3BN and optimization of physico-chemical parameters. ISCA J. Biol Sci., 1 (2): 17-24 (2012)
17. Coll P. M., Abalos J. M. F., Villanueva J. R., Santamaria R. and Perez P., Purification and characterization phenoloxidase (Laccase) from the lignin degrading basidiomycete PM1. Appl Environ Microbiol. 59 :2607-2613 (1993)
18. Kiiskinen L. L., Ratto M., and Kruus K., Screening for novel laccase producing microbes. J Appl Microbiol, 97:640-646 (2004)
19. Vishwanath B., Chandra M. S., Pallavi H. and Reddy B. R., Screening and assessment of laccase producing fungi isolated from different environmental samples. Afr J Biotechnol,7 (8):1129-1133 (2008)
20. Ang T. N., Ngoh G. C. and Chua A. S. M., A quantitative method for fungal ligninolytic enzyme screening studies. Asia-Pac J Chem Eng, DOI:10.1002/apj.451 (2010).
21. Vinoth Kumar V., Selvaraj D. K., Premkumar P. and Subramanian S., Screening and induction of laccase activity in fungal species and its application in dye decolorization. Afr J Microbiol Res, 5 (11): 1261-1267 (2011)
22. Moorthi S. P., Selvam P. S., Sasikalaveni M. A. K. and Kalaichelvan P.T., Decolorization of textile dyes and their effluents using white rot fungi. Afr J Biotechnol, 6: 424-429 (2007)
23. Nyakundi W., Magoma G., Ochora J. and Nyende A.B. , Biodegradation of diazinon and methomyl pesticides by white rot fungi from selected horticultural farms in rift valley and central provinces, Kenya. J Appl.Technol Envt anitation, 1(2) :107-124 (2011)
24. Cajthaml T. M. Möder P., Kacer V.Š. and Popp P., Study of fungal degradation products of polycyclic aromatic hydrocarbons using gas chromatography with ion trap mass spectrometry detection. J Chromatography A, 974: 213-22 (2002).
25. Mansur M. M. E., Arias J. L., Copa-Patino MF and González AE, The white-rot fungus *Pleurotus ostreatus* secretes laccase isozymes with different substrate specificities. Mycologia, 95(6): 1013-20 (2003)
26. Pointing S. B., Feasibility of bioremediation by white-rot fungi. Appl Microbiol Biotechnol, 57: 20-33(2001)
27. Veignie E. C., Rafin P. W., Cazier F., Preliminary evidence of the role of hydrogen peroxide in the degradation of benzo[a]pyrene by a non-white rot fungus *Fusarium solani*. Environ Pollution, 129: 1-4. (2004)
28. Jadhav U., Dawkar V., Telke A., and Govindwar S. P., Decolorization of Direct Blue GLL with enhanced lignin peroxidase enzyme production in Comamonas sp. UVS J Chem Technol Biotechnol, 84:126-132 (2008a)
29. Jadhav U., Dawkar V., Ghodake G., and Govindwar S.P., Biodegradation of Direct

Red 5B, textiles dye by newly isolated Comamonas sp. UVS J Hazard Mater, 158: 507-516 (2008b)
30. Wang C., Zhao M., Li D., Cui D., Lu L., and Wei X. D., Isolation and characterization of a novel Bacillus subtilis WD23 exhibiting laccase activity from forest soil. Afr J Biotechnol, 9 (34): 5496-5502 (2010)
31. Hao J., Song F., Huang F., Yang C., Zhang Z., Zheng Y., and Tian X., Production of laccase by a newly isolated deuteromycete fungus *Pestalotiopsis* sp. and its decolorization of azo dye. J Ind Microbiol Biotechnol, 34:233-240 (2007)
32. Couto S. R. and Herrera J. L. T., Industrial and biotechnological applications of laccases: A review. Biotechnol. Adv, 24:500-513 (2006).
33. Abdel-Azeem A. M. and Salem F.M., Biodiversity of laccase producing fungi in Egypt. Mycosphere, 3(5): 900–920 (2012)
34. Laxminararayana E., Thirumalachary M., Kumar M. R. and Singaracharya M. A., Decolorization and biodegradation of sulphonated azo dyes by fungi to clean dye contaminated soil environments. J Nat Env Sci, 1(1): 35-42 (2010)
35. Das S. S. M. and Anitha S., Mycoremediation of monocrotophos, Int J Pharma and Biosci, 12(1): 337-342 (2011)
36. Rajendran R., Karthik S. S. and Uma Maheswari K., Aerobic biodecolorization of mixture of azo dye containing textile effluent using adapted microbial strains. J Envt Sci Technol, DOI: 10.3923/jest.2011.
37. Gopi V., Akhilesh U. and Soundararajan N., Bioremediation potential of individual and consortium non-adapted fungal strains on azo-dye containing textile effluent. Adv Appl Sci Res, 3(1): 303-311 (2012).