IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

Isolation And Screening Of Laccase Producing Fungi From Textile Sizing Site

Vaishali S. Nirmalkar[1*], Asfiya M.T Shaikh [2]

[1,2]K. M. E Society's G. M. Momin Women's College, Bhiwandi, Maharashtra

Abstract: Laccases are multicopper oxidases known for their ability to degrade phenolic and non-phenolic lignin-related compounds, making them essential for various biotechnological and environmental applications. This study aimed to isolate and screen efficient laccase-producing fungi from soil samples collected at textile sizing sites in Bhiwandi, Maharashtra. Using serial dilution and SDA media, 120 fungal isolates were obtained, of which 90 were screened for laccase activity via guaiacol-based plate assay. Positive laccase producers were identified through the formation of reddish-brown halos, indicating enzymatic oxidation. Thirty-seven isolates exhibited strong laccase activity and were subjected to microscopic analysis, revealing dominance of the genera *Aspergillus*, *Penicillium*, *Trichoderma*, *Fusarium*, and *Rhizopus*. The study highlights the ecological adaptability and enzymatic potential of indigenous fungi in polluted environments. Given laccase's eco-friendly catalytic properties and broad substrate specificity, these isolates hold promise for industrial applications such as dye decolorization, lignin degradation, and detoxification of textile effluents. This research underscores the significance of exploring local microbial biodiversity as a cost-effective and sustainable approach to address environmental challenges, paving the way for future pilot-scale enzyme production and strategic implementation of fungal laccases in *bioremediation* efforts.

Key Words: Guaiacol assay, *Aspergillus*, *Trichoderma*, Bioremediation, Textile sizing site

INTRODUCTION

Human lifestyles can be enhanced through innovations, research, and scientific advancements; however, these developments often have detrimental effects on the environment. The presence of various toxic chemicals in the environment is a significant contributor to water toxicity, adversely affecting both human livelihoods and natural ecosystems. Microorganisms possess a substantial capacity to mitigate the toxicity of numerous chemicals through enzymatic activity. Laccase, in particular, exhibits diverse substrate flexibility, rendering it highly suitable for various applications, including industrial development, pulp and textile dye bleaching, bioremediation, and the detoxification of industrial effluents, thereby preventing environmental degradation (Amutha and Abhijit, 2015; More et al., 2011; Viswanath et al., 2008 and Thakkar and Bhatt, 2020). Laccase plays a crucial role in reducing a wide range of pollutants, which is a critical area of focus for minimizing environmental pollution (More et al., 2011). Laccases (benzenediol: dioxygen oxidoreductases, EC 1.10.3.2) are enzymes classified as blue oxidases, containing copper at their catalytic sites. These glycoproteins oxidize a broad spectrum of phenolic compounds and aromatic amines, releasing free hydroxyl radicals that facilitate the degradation of complex compounds (More et al., 2011; Viswanath et al., 2008; Brijwani et al., 2010; Alfarra et al., 2013 and Thakkar and Bhatt, 2020).

Laccase is ubiquitously present in nature, predominantly occurring in fungi, bacteria, and plants. This enzyme facilitates the oxidation of substrates through the utilization of molecular oxygen, resulting in the production of water as a byproduct. Due to its extensive substrate specificity and environmentally benign catalytic

properties, laccase has attracted considerable interest for industrial and biotechnological applications. It is particularly valuable in the fields of bioremediation, the textile and paper industries, food processing, pharmaceuticals, and biofuel production. Laccase is frequently employed for the degradation of pollutants, dye decolorization, and lignin modification, rendering it an indispensable enzyme for sustainable solutions (Sachchidanand et al., 2020).

Laccases derived from fungi present several advantages over other sources, notably their stability, broad substrate specificity, and capacity to oxidize a variety of phenolic compounds (Wakil et al., 2019 and Bello et al., 2022). Fungal laccases are particularly suitable for the processing of lignocellulosic biomass. Over 60 fungal strains, encompassing classes such as Ascomycetes, Basidiomycetes, and Deuteromycetes, have been identified as laccase producers. The majority of laccases characterized thus far have been obtained from efficient lignin degraders, such as white-rot fungi (Rico et al., 2014). Laccases of fungal origin are associated with lignin degradation and are well recognized as integral components of fungal enzyme systems for lignin breakdown (Ndochinwa et al., 2020).

Lignin is among the most resilient elements found in lignocellulosic biomass, effectively encasing cellulose and hemicellulose. To liberate fermentable sugars, a pretreatment of this biomass is essential, which can be performed using laccase (Shrestha et al., 2016). Laccase plays a vital role in plant disease processes, pigment synthesis, and the degradation of lignocellulose derived from agricultural waste (More et al., 2011).
Laccases obtained from wood-decaying basidiomycetes are especially significant due to their capacity to utilize a variety of substances, including aromatic compounds, phenols, and the fundamental components of lignin, as sources of carbon. Furthermore, laccase is capable of breaking down lignin-like substances such as reactive, polymeric, and heterocyclic dyes (Shrestha et al., 2016). Chemical pretreatment techniques aimed at releasing fermentable sugars are expensive, intricate, and detrimental to the environment, and the by-products resulting from lignin degradation during chemical delignification can obstruct the fermentation process. Employing laccases for lignin depolymerization represents a biological strategy that provides a safer and more cost-effective option for extracting fermentable sugars from lignocellulosic biomass (Shrestha et al., 2016 and Thakkar and Bhatt, 2020).

Laccase was first isolated from the Japanese lacquer tree, *Rhus vernicifera*. The catalytic characteristics inherent to this enzyme have endowed it with significant potential for applications in both environmental and industrial contexts, including the delignification of pulp and paper, the production of valuable chemicals derived from lignin, the decolorization of textile dyes, the detoxification and bioremediation of environmental pollutants such as olive mill wastewater through decolorization and dephenolization, the development of biosensors, the production of biofuels, and the transformation of steroids and antibiotics within the pharmaceutical and food industries, as well as cosmetic applications (Mukhopadhyay and Banerjee, 2015; Sheikhi et al., 2012; Shrestha et al., 2016; Monssef et al., 2016; Janusz et al., 2020; Pourkhanali et al., 2021).

The scholarly inquiry into laccase is driven by its extensive applicability and the escalating global demand for these enzymes. The primary objective of this investigation was to procure soil specimens, isolate fungal species, scrutinize them microscopically, and evaluate the diversity of indigenous fungi capable of oxidizing a wide array of phenolic compounds and aromatic amines, thereby liberating free hydroxyl radicals that promote the breakdown of intricate substances originating from a textile sizing environment. The aim was to conduct a comprehensive analysis and appraisal of the oxidative potential of fungi for prospective broad applications, inspired by the rising requirement for laccase across various sectors. Given its significance for large-scale utilization, the present research endeavour was focused on locally screening for highly proficient laccase-producing fungi that possess the capability to secrete substantial quantities of the enzyme, thereby minimizing production costs. This approach aims to identify fungal strains that not only produce laccase efficiently but also demonstrate resilience in various industrial conditions, enhancing their potential for commercial use.

MATERIALS AND METHODS

Selection of sample sites

Different textile sizing sites of Bhiwandi city were selected for the sample collection.

Collection of soil samples

The samples were collected from different spots in each site in zip lock polythene bags. Almost 5-10 soil samples were taken from each sizing industries. The soil sample was mixed well and processed next day.

Isolation of fungi

Fungal colonies were isolated from soil samples by serial dilution method where SDA (Sabouraud dextrose agar) media was prepared, autoclaved and poured in sterile petri plates. Soil samples (1 gm) diluted up to 10^{-5} dilution was spread on respective solidified SDA plates with the help of sterile spreader. The inoculated petri plates were incubated at 28°C for 48 h. About 120 different fungal isolates differentiated based on physical characteristics obtained after incubation were selected for the further processes. The isolates were further inoculated on SDA plates by point inoculation and incubated at 28°C for 48 h to obtain pure fungal cultures (Khan and Kumar, 2011).

Screening of Fungal Isolates for Laccase Production

The fungal isolates were tested for Laccase production potential by plate assay method. This plate assay helps to screen laccase-producing fungi by brown halos formation around colony. From 120 fungal isolates about ninety fungal isolates were screened for Laccase production efficiency. Enzymatic activity was screened based on brown zone formation on composite selective agar medium. The media comprised (per L): 0.3% peptone, 1% glucose, 0.06% KH2PO4, 0.0005% FeSO4, and 0.005% MnSO4, 0.05% MgSO4, 0.0001% ZnSO4, 2% agar and 0.02% guaiacol with pH adjusted to 6. The entire ninety fungal discs were centrally inoculated on sterile solidified composite selective media plates with the help of cork borer. The diameter of disc was 1cm. Plates were incubated at 28°C for 72 h to 120 h. The growth of laccase producing fungi was indicated by the formation of reddish-brown zone surrounding the fungal colony (Sivakumar et al., 2010 and Mathur et al., 2013).

Microscopy of Laccase Producing isolates

Microscopy of all the positive isolate was done by lactophenol cotton blue staining method. In aseptic condition, a loop full of fungal cultures was placed on a clean glass slide, a drop of lactophenol cotton blue stain was mixed with the culture. A clean coverslip was placed over the culture and viewed under the microscope (10X and 45X). Morphological characteristics including colour of the colony and growth pattern studies, as well as their vegetative and reproductive structures were carefully observed under the microscope (Devi and Kumar, 2012).

RESULTS AND DISCUSSION

A diverse array of microorganisms is present in soil, rendering it a crucial ecosystem. Effective isolates are often procured from natural habitats, and the selection of soil samples may influence the efficacy of enzymatic processes. Fungi were isolated employing serial dilution methodologies. The isolated fungi were subsequently analysed for their capacity to produce laccase. A comprehensive screening procedure encompassing 90 fungal isolates was implemented on composite selective agar plates, which incorporated 0.02% guaiacol as a substrate for the laccase enzyme. These plates underwent incubation for a duration ranging from 72 to 120 h. Upon completion of the incubation phase, varying degrees of guaiacol oxidation were noted. The guaiacol oxidizing activities of these isolates were evaluated based on the development of brown halo zones encircling the fungal colonies on composite selective agar plates. The outcomes of the screening indicated that 37 isolates demonstrated enzymatic activity, as evidenced by the formation of brown halo zones surrounding the colonies, suggesting potential for laccase production (Fig. 1). Microscopic examination classified the majority of isolates into the genera *Aspergillus, Trichoderma, Penicillium, Fusarium,* and *Rhizopus.*

Fungal laccases are considered suitable candidates for lignocellulosic biomass processing. Over 60 fungal strains, encompassing various classes such as Ascomycetes, Basidiomycetes, and Deuteromycetes, have been shown to produce laccase (Ndochinwa et al., 2020). Numerous studies have documented laccase production in ascomycetes, including *Gaeumannomyces graminis* (Edens et al., 1999), *Magnaporthe grisea* (Iyer and Chattoo, 2003), *Ophiostoma novo-ulmi* (Binz and Canevascini, 1997), *Mauginella* (Palonen et al., 2003), *Melanocarpus albomyces* (Kiiskinen et al., 2002), and *Monocillium indicum* (Thakker et al., 1992). In addition to plant pathogenic species, laccase production has been reported in certain soil ascomycete species from the genera *Aspergillus, Curvularia,* and *Penicillium,* as well as in some freshwater ascomycetes. Wood-degrading

ascomycetes, such as *Trichoderma* and *Botryosphaeria*, have demonstrated laccase activity. Nearly all species of white-rot fungi have been reported to produce laccase to varying extents (Brijwani et al., 2010).

Fungal laccases are predominantly recognized for their function in the degradation of lignin; however, they also fulfil a myriad of additional roles that encompass various dimensions of multiple processes (An et al., 2018). Given their energy-efficient and eco-friendly nature, laccases present promising applications across a diverse array of industrial and biotechnological domains, including but not limited to wine and juice stabilization, pulp bleaching, advancements in the food industry, enhancement of fibre properties, development of biosensors, detoxification of environmental contaminants, dye decolorization, biosynthesis, polymer fabrication, biofuel cells, pharmaceuticals, the cosmetic sector, and bioremediation of terrestrial and aquatic environments (Bilal et al., 2019; Deska and Kończak, 2019; An et al., 2020a,b; Han et al., 2020). Nonetheless, their integration into biotechnological applications has been hampered by elevated production costs, which culminate in suboptimal enzyme activity and diminished yields. An increasing volume of scholarly attention has been directed towards investigations that elucidate effective strategies for laccase production, which are correlated with enhanced activity and cost reduction (Akpinar and Urek, 2017; Chenthamarakshan et al., 2017; An et al., 2018; Singh and Arya, 2019; Han et al., 2021).

Laccases are multifunctional oxidases, and their multifunctionality is attributed to the substantial reduction potential that positions them as promising candidates for various biotechnological applications. The prevalent distribution of laccase across numerous fungal genera guarantees their extensive availability, with particular emphasis on wood-decaying basidiomycetes, commonly known as white rot fungi, which are recognized as significant laccase producers (Brijwani et al., 2010).

In conclusion, textile sizing environments provide suitable habitats for microorganisms that synthesize laccase, and they warrant further examination to determine if these isolates can contribute to the bioremediation of waste environments linked to sizing processing. Subsequent research ought to concentrate on utilizing potential laccase-producing isolates for the production and purification of laccase enzymes at a pilot scale.

REFERENCES

1. Akpinar, M., & Urek, R. O. (2017). Induction of fungal laccase production under solid state bioprocessing of new agro-industrial waste and its application on dye decolorization. *3 Biotech*, *7*(2).

2. Alfarra, H. Y., Hasali, N. H. M., & Omar, M. N. (2013). A lignolytic fungi with laccase activity isolated from Malaysian local environment for phytochemical transformation purposes.

3. Amutha, C., & Abhijit, M. (2015). Screening and isolation of laccase producers, determination of optimal condition for growth, laccase production and choose the best strain. *Journal of Bioremediation & Biodegredation*, *6*(4), 1.

4. An, Q., Han, M., Bian, L., Han, Z., Han, N., Xiao, Y., & Zhang, F. (2020a). Enhanced laccase activity of white rot fungi induced by different metal ions under submerged fermentation. *BioResources*, *15*(4), 8369.

5. An, Q., Ma, H. F., Han, M. L., Si, J., & Dai, Y. C. (2018). Effects of different induction media as inducers on laccase activities of *Pleurotus ostreatus* strains in submerged fermentation. *BioResources*, *13*(1), 1143-1156.
6. An, Q., Qiao, J., Bian, L., Han, M., Yan, X., Liu, Z., & Xie, C. (2020b). Comparative study on laccase activity of white rot fungi under submerged fermentation with different lignocellulosic wastes. *BioResources*, *15*(4), 9166.

7. Bello, A., Ameh, J. B., Machido, D. A., & Mohammed-Dabo, A. I. (2022). Screening and Identification of Laccase Producing Fungi from Environmental Samples. *UMYU Journal of Microbiology Research*, *6*(1), 91-98.

8. Bilal, M., Rasheed, T., Nabeel, F., Iqbal, H. M., & Zhao, Y. (2019). Hazardous contaminants in the environment and their laccase-assisted degradation – A review. *Journal of Environmental Management*, *234*, 253–264.

9. Binz, T., & Canevascini, G. (1997). Purification and partial characterization of the extracellular laccase from *Ophiostoma novo-ulmi*. *Current Microbiology*, *35*(5), 278-281.

10. Brijwani, K., Rigdon, A., & Vadlani, P. V. (2010). Fungal laccases: production, function, and applications in food processing. *Enzyme research*, *2010*(1), 149748.

11. Chenthamarakshan, A., Parambayil, N., Miziriya, N., Soumya, P. S., Lakshmi, M. S. K., Ramgopal, A., Dileep, A., & Nambisan, P. (2017). Optimization of laccase production from *Marasmiellus palmivorus* LA1 by Taguchi method of Design of experiments. *BMC Biotechnology*, *17*(1).

12. Deska, M., & Kończak, B. (2019). Immobilized fungal laccase as "green catalyst" for the decolourization process – State of the art. *Process Biochemistry*, *84*, 112–123.

13. Devi, M. C., & Kumar, M. S. (2012). Production, optimization and partial purification of cellulase by *Aspergillus niger* fermented with paper and timber sawmill industrial wastes. *Journal of Microbiology and Biotechnology Research*, *2*(1), 120-128.

14. Edens, W. A., Goins, T. Q., Dooley, D., & Henson, J. M. (1999). Purification and characterization of a secreted laccase of *Gaeumannomyces graminis var. tritici*. *Applied and Environmental Microbiology*, *65*(7), 3071-3074.

15. Han, M. L., An, Q., Ma, K. Y., An, W. N., Hao, W. Y., Liu, M. Y., ... & Bian, L. S. (2021). A comparative study on the laccase activity of four basidiomycete fungi with different lignocellulosic residues via solid-state fermentation. *BioResources*, *16*(2),
16. 3017-3031.

17. Han, M., An, Q., He, S., Zhang, X., Zhang, M., Gao, X., ... & Bian, L. (2020). Solid-state fermentation on poplar sawdust and corncob wastes for lignocellulolytic enzymes by different *Pleurotus ostreatus* strains. *BioResources*, *15*(3), 4982.

18. Iyer, G., & Chattoo, B. B. (2003). Purification and characterization of laccase from the rice blast fungus, *Magnaporthe grisea*. *FEMS Microbiology Letters*, *227*(1), 121-126.

19. Janusz, G., Pawlik, A., Świderska-Burek, U., Polak, J., Sulej, J., Jarosz-Wilkołazka, A., & Paszczyński, A. (2020). Laccase properties, physiological functions, and evolution. *International journal of molecular sciences*, *21*(3), 966.

20. Khan, J. A., & Yadav, S. K. (2011). Production of alpha amylases by *Aspergillus niger* using cheaper substrates employing solid state fermentation. *International Journal of Plant, Animal and Environmental Sciences*, *1*(3), 100-108.

21. Kiiskinen, L. L., Viikari, L., & Kruus, K. (2002). Purification and characterisation of a novel laccase from the ascomycete *Melanocarpus albomyces*. *Applied Microbiology and Biotechnology*, *59*, 198-204.

22. Mathur, G., Nigam, R., Jaiswal, A., & Kumar, C. (2013). Bioprocess parameter optimization for laccase production in solid state fermentation. *Int J Biotechnol Bioeng Res*, *4*, 521-530.

23. Monssef, R. a. a. E., Hassan, E. A., & Ramadan, E. M. (2016). Production of laccase enzyme for their potential application to decolorize fungal pigments on aging paper and parchment. *Annals of Agricultural Sciences*, *61*(1), 145–154.

24. More, S. S., PS, R., K, P., Malini, S., & SM, V. (2011). Isolation, purification, and characterization of fungal laccase from *Pleurotus sp*. *Enzyme research*, *2011*(1), 248735.

25. Mukhopadhyay, M., & Banerjee, R. (2015). Purification and biochemical characterization of a newly produced yellow laccase from *Lentinus squarrosulus* MR13. *3 Biotech*, *5*(3), 227-236.

26. Ndochinwa, O., Amadi, O., Nwagu, T., Okpala, G., Nnamchi, C., & Moneke, A. (2020). Screening of Laccase Producing Fungi Using Agro-Wastes under Different Cultural Conditions. *Journal of Advances in Biology & Biotechnology*, 44-57.

27. Palonen, H., Saloheimo, M., Viikari, L., & Kruus, K. (2003). Purification, characterization and sequence analysis of a laccase from the ascomycete *Mauginiella sp*. *Enzyme and Microbial Technology*, *33*(6), 854-862.

28. Pourkhanali, K., Khayati, G., Mizani, F., & Raouf, F. (2021). Isolation, identification and optimization of enhanced production of laccase from *Galactomyces geotrichum* under solid-state fermentation. *Preparative biochemistry & biotechnology*, *51*(7), 659-668.

29. Rico, A., Rencoret, J., Del Río, J. C., Martínez, A. T., & Gutiérrez, A. (2014). Pretreatment with laccase and a phenolic mediator degrades lignin and enhances saccharification of *Eucalyptus* feedstock. *Biotechnology for Biofuels*, *7*, 1-14.

30. Sachchidanand, S. A. K., Kumar, Y., Masih, H., & Singh, K. (2020). Industrial application of fungal laccase produced by solid state fermentation of agro pulse byproducts. *J Pharmacogn Phytochem*, *9*(1), 1977-1985.

31. Sheikhi, F., Roayaei Ardakani, M., Enayatizamir, N., & Rodriguez-Couto, S. (2012). The determination of assay for laccase of *Bacillus subtilis* WPI with two classes of chemical compounds as substrates. *Indian journal of microbiology*, *52*, 701-707.

32. Shrestha, P., Joshi, B., Joshi, J., Malla, R., & Sreerama, L. (2016). Isolation and physicochemical characterization of laccase from *Ganoderma lucidum*-CDBT1 isolated from its native habitat in Nepal. *BioMed Research International*, *2016*(1), 3238909.

33. Singh, G., & Arya, S. K. (2019). Utility of laccase in pulp and paper industry: A progressive step towards the green technology. *International journal of biological macromolecules*, *134*, 1070-1084.

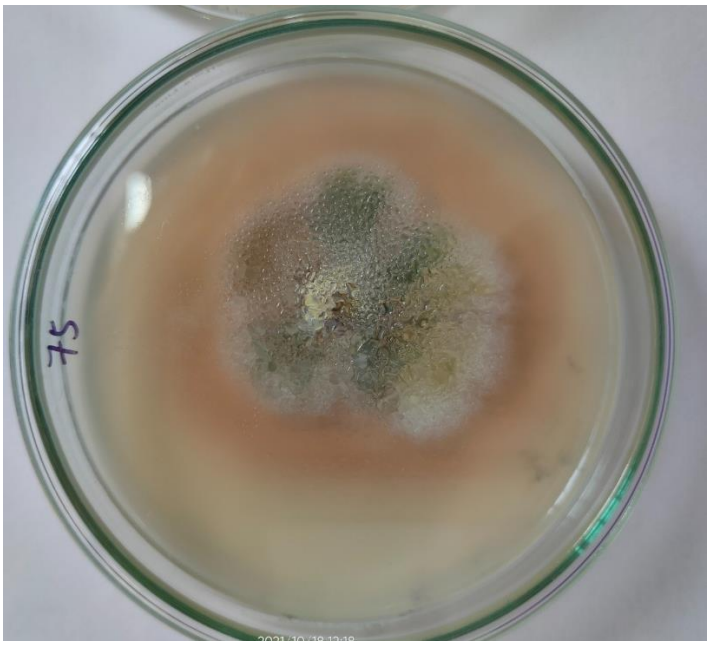
34. Sivakumar, R., Rajendran, R., Balakumar, C., & Tamilvendan, M. (2010). Isolation, screening and optimization of production medium for thermostable laccase production from *Ganoderma sp*. *Int J Eng Sci Technol*, *2*(12), 7133-7141.

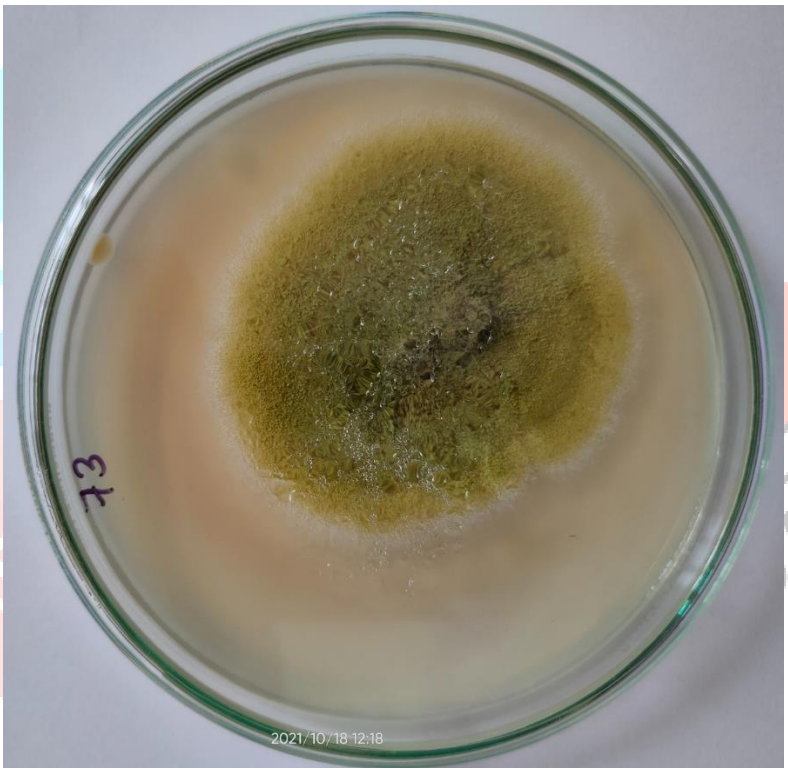
35. Thakker, G. D., Evans, C. S., & Rao, K. K. (1992). Purification and characterization of laccase from *Monocillium indicum* Saxena. *Applied Microbiology and Biotechnology*, *37*, 321-323.

36. Viswanath, B., Chandra, M. S., Pallavi, H., & Reddy, B. R. (2008). Screening and assessment of laccase producing fungi isolated from different environmental samples. *African Journal of Biotechnology*, *7*(8).

37. Thakkar, A. T., & Bhatt, S. A. (2020). Isolation, identification and optimization of laccase from *Alternaria alternata*. *J Appl Biol Biotechnol*, *8*(3), 64-9.

38. Wakil, S. M., Eyiolawi, S. A., Salawu, K. O., & Onilude, A. A. (2019). Decolourization of synthetic dyes by laccase enzyme produced by *Kluyveromyces dobzhanskii* DW1 and *Pichia manshurica* DW2. *African Journal of Biotechnology*, *18*(1), 1-11.


75


73
2021/10/18 12:18

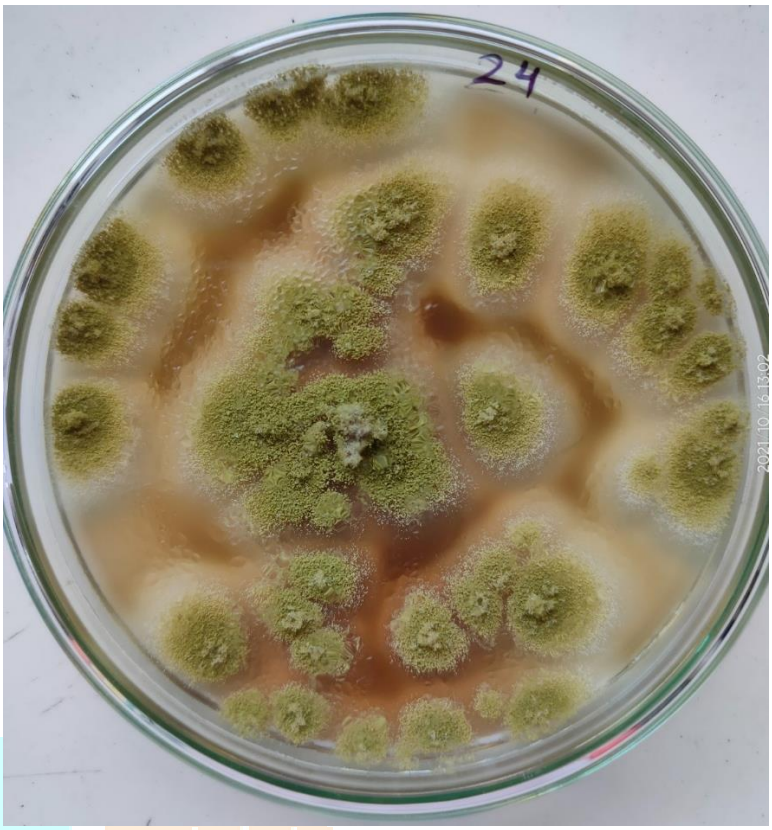


Fig. 1: Fungal isolates showing the laccase positive activity (Brown halo observed surrounding the colonies)