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Isolation of Ligninolytic Enzymes Producing Microbes from Textile Effluent Contaminated Soil

Omotosho, A. O.,[1*] Oyeleke, S.B[1]., Daniyan, S.Y.,[1] and Egwim, E.C[2].
[1,*]Department of Microbiology, School of Natural and Applied Science, Federal University of Technology, Minna, Nigeria
[2,*]Department of Biochemistry, School of Natural and Applied Science, Federal University of Technology, Minna, Nigeria
Email:omotazeez@yahoo.com

Abstract
Evaluation of some microbial species for their ability to produce ligninolytic enzymes was investigated using streak plate method. Ten (10) microbial strains were isolated from soil contaminated with textile effluents using the spread plate technique. *Aspergillus terreus* and *Aspergillus niger* showed higher expression for laccase with 8.0 mm diameter zones clearance. *Bacillus licheniformis* and *Bacillus subtilis* had the widest clearance zone (12.0 and 8.0 mm) respectively. Only *Aspergillus flavus* however had the potential to produce lignin peroxidase (with 10 mm zones of clearance) of all the fungi isolated in this study. *Streptococcus faecalis, Trichoderma harzianum, Micrococcus luteus,* and *Aspergillus flavus* had the widest clearance zones (6.0 mm) in the Manganese peroxidase screening medium.Some of the microbial species possessed multiple traits for the production of the various ligninolytic enzymes assayed at ≥8 mm diameter zone clearance. Therefore, the research main focus is to identify microorganisms that are potential producers ofligninolytic enzymesgiven that they can be used for industrial waste bioremediation.
Keywords: ligninolytic enzyme, peroxidase, effluents, bioremediation, clearance zones

INTRODUCTION

Ligninolytic enzymes are ubiquitous group of extracellular glycoprotein enzymes that are made up of monomeric protein and a sugar component found in different types of organisms such as plants, bacteria, insects, and fungi (Polak and Jarosz-Wilkolazka, 2012). In plants, laccases are the most documented ligninolytic enzyme and has been documented in different types of plants such as the Japanese lacquer tree, mango, mung bean, peach, tobacco, zea mays (Polak and Jarosz-Wilkolazka, 2012). Whereas, in fungi (especially white-rot fungi), lignin peroxidase, manganese peroxidase and laccases are secreted extracellularly (Hatakka, 1994).

The textile industry is one of the rapidly growing sectors of Africa economy. Textile processes are based on chemical reactions in liquid medium, thereby generating large volume of toxic wastewater. Some of the major processes include sizing and desizing i.e., the saturation of mainly hydrophobic wraps in highly viscous macromolecular solution using starch and strong chemicals such as acids, bases or oxidizing agents (Babu *et al*., 2000).

Main pollution in textile wastewater usually come from dyeing and finishing processes, these processes require the input of a wide range of chemicals and dyestuffs, which generally are organic compounds of complex structure (Babu *et al*., 2000). The role of some bacterial and algal species for the decolourization and degradation of textile dyes has been reported (Baldrian and Snajdr, 2006; Jumarkar *et al*., 2006; Olukanni *et al*., 2006; Togo *et al*., 2008; Cheriaa *et al*., 2009). This is credited to their ability to secret ligninolytic enzymes to remove synthetic dyes. The ligninolytic enzymes enable the microorganisms to oxidize a broad range of substrates, including synthetic dyes (Babu *et al*., 2007; Olukanni *et al*., 2006).This study aimed to isolate ligninolytic enzymes producing microorganisms from textile effluent contaminated soil.

MATERIALS AND METHODS

Sample collection and processing

The nine (9) soil samples, three each per waste dump sites of three textile companies in Sharada industrial area in Kano State, Nigeria. The soil samples were kept in commercially obtained sterile polythene bag and transported to Microbiology laboratory, Federal University of Technology, Minna, for analysis within 6 hours of collection. Samples were preserved by refrigeration at 4°C without any preliminary treatment.

Isolation of bacteria and fungi from soil samples contaminated with textile effluent

Isolation was carried out by inoculating 0.1 mLof 10^{-3}dilution factor of the soil samples onto Nutrient agar (NA)and Sabrouard Dextrose Agar (SDA) plates in triplicates.NA plates were incubated at 37ºC for 48 hours and SDA plates at 25ºC temperature for 3-5 days.The isolates were subcultured by streaking on fresh sterile media to obtain discrete colonies. Pure isolates were subcultured on sterile agar slants and stored at 4ºC for further use.

Identification and characterization of the isolates

Bacterial isolates were identified presumptively on the basis of the following features: colonial morphology, Gram-staining and biochemical test (Chessbrough, 2006). The biochemical test carried out includes glucose utilization, starch hydrolysis, catalase, citric utilization and carbohydrate fermentation. The bacterial isolates were identified by comparing their characteristics with those of known taxa using the Bergy's manual of systemic bacteriology (Chessbrough, 2006).

Different fungal colonies were identified morphologically and by their morphological appearance including the pigmentation, shape, presence of special structure and characteristics of spores as described by Barnett (2003).

Standardization of the bacterial and fungal isolates

The fungal isolates were inoculated in SDA for 5-7 days for maximal growth. Fungal spores were suspended in sterile distilled water by gently rubbing the surface of the flooded agar with a sterile wire loop. The spore suspension was passed through sterile glass wool to remove mycelium and spore concentration was determined spectrophometrically at 650 nm (an absorbance of 1 cm-1 is approximately 5 *10^6 spores) (Acharya *et al.,* 2013).

The turbidity of the 24 hour old cultures were adjusted to 5 *10^8 cfu by diluting the broth culture with sterile peptone water. The bacterial cell concentration was determined spectrophometrically at 600 nm (an absorbane of 0.6 cm-1 is approximately 5 *10^8 cfu).

Screening for lignin peroxidase producers

The standardized test isolates (1mL each) were pipette into sterile petri dishes. Nutrient agar and saubourand dextrose agar supplemented with 0.5 w/v Pyrogallol were poured onto the plate containing 1 mL each of the standardized test bacteria and fungi isolates respectively and the plates were carefully stirred. The agar plates containing bacteria were incubated at 37ºC for 48 hours while fungi cultures were incubated at 25ºC temperature for 72 hours (Savitha *et al.*,2011), thereafter, the formation of reddish hollow zones (due to the oxidation of Pyrogallol) around the test isolates colonies were observed and result recorded(El Monssef *et al.* 2016).

Screening for Laccase producers

Laccase producing organisms were identified on basal medium plate containing following composition (g/L): 3.0 peptone, 10.0 glucose, 0.6 KH_2PO_4, 0.001 $ZnSO_4$, 0.4 K_2HPO_4 0.0005 $FeSO_4$, 0.05 $MnSO_4$, 0.5 $MgSO_4$, 20.0 agar (pH-6) supplemented with 0.02% Guaiacol. The basal medium plate was swab with the test isolates in a circular pattern. The plates incubated at 25°C for 7 days for fungi and 48 hours for bacteria and then screened for the formation of reddish brown zones (due to the oxidation of guaiacol) around the test isolates colonies (Coll *et al.,* 1993, Kalra *et al.,* 2013). The reddish brown zone formed around the test isolates colonies are measured and result recorded.

Screening for Manganese peroxidase producers

Manganese peroxidase producing microorganisms were identified based on Bavendamm test. Briefly, the test isolates were streaked on a basal medium plates containing glycerol (10g/L), ammonium tartarate (1.84), sodium tartarate (2.3), KH_2PO_4 (2.0), $MgSO_4.7H_2O$ (0.7), $CaCl_2.2H_2O$ (0.14), $FeSO_4.7H_2O$ (0.07), $ZnSO_4.7H_2O$ (0.046), $MnSO_4.7H_2O$ (0.035), $CuSO_4.5H_2O$ (0.007), thiamine (0.0025), yeast extract (1.0), Veratryl alcohol (0.067) and tween80 (0.5) (Dhouib *et al.*, 2005). The oxidation of Veratryl alcohol produces polyphenols which gave brown colouration on the medium. Plates were observed for colour change and result recorded (El Monssef *et al.* 2016).The brownish zone formed around the test isolates colonies are measured and result recorded.

RESULTS

The identity of bacterial isolatesfrom the three dump sites were presented in Table 1. The bacterial isolated were identified as *B. subtilis, B. licheniformis, S. faecalis, M. Luteus* and *B. megatarium*.

Table 1: Biochemical characteristics of the bacterial isolates from textile effluent and contaminated soil

Isolates	G/S	Cat	Coa	S/H	Cit	H_2S	Urea	Oxi	Ind	MR	VP	HAE	Lac	Suc	Glu	Fru	Man	Inference
S1	+R	+	_	+	+	_	_	_	_	_	+	ȧ	_	+	+	+	_	*B. subtilis*
S2	+R	+	_	+	_	_	_	_	_	_	_	_	_	+	+	+	_	*B. licheniformis*
S3	+C	_	_	_	_	_	_	_	_	_	_	ϒ	_	_	_	_	_	*S. faecalis*
SB2	+C	_	_	_	_	_	_	_	_	_	_	_	_	+	_	+	_	*M. Luteus*
SB3	+R	+	_	+	_	_	_	_	_	_	_	_	_	+	_	+	_	*B. megatarium*

Keys:

G/S	= Grams stain	H_2S	= Hydrogen sulphide test	Suc	= Sucrose sugar
Cat	= Catalase test	Oxi	= Oxidase test	Glu	= Glucose sugar
Coa	= Coagulase test	Ind	= Indole test	Fru	= Fructose sugar
S/H	= Starch hydrolysis	MR	= Methyl red test	Man	= Manitol sugar
Cit	= Citrate utilization test	VP	= Voges Proskers test	ȧ	=Alpha haemolysis
Urea	= Urease utilization test	Lac	= Lactose sugar	ϒ	= Gamma haemolysis
+ R	= positive rod	+C	= Positive cocci	HAE	= Haemolysis
+	= Positive	-	= Negative		

*Aspergillus flavus, Aspergillus niger, Candida tropicalis, Trichoderma harzianum*and *Aspergillus terreus*were identified from the textile contaminated soil (Table 2).

Table 2: Characteristics of fungal isolates from effluent and soil contaminated with effluent

Isolates codes	Colour of aerial hyphae	Nature of hyphae	Asexual spore	Character of spores	Inference
S1, S2, S3	Black	Septate	Conidia	Multinucleate vesicle	*A. niger*
S2 , S2	Yellow green	Septate	Conidia	Multinucleate vesicle	*A. flavus*
S3	Dark brown	Septate	Conidia	Multinucleate vesicle	*A. terreus*
S2	Grey yellow	None	None	Single cell	*A. tropicalis*
S3	Green	Septate	Conidia	Single cell	*T. harzianum*

Eight (8) out of 10, including bacteria and fungi isolated from the soil contaminated with textile effluent have reddish brown zone of clearance around their colonies in laccase screening medium as shown in Table3. *A.niger*and *A. terreus*had the widest zone measured 8.0 mm. *B. licheniformis, M. luteus* and *Candida tropicalis* did not produced any noticeable clearance zone. While *Micrococcus luteus, Candida tropicalis* and *Bacillus licheniformis* had no clearance zones.

Six (6) out of 10 including bacteria and fungi isolated from the soil contaminated with textile effluent produce reddish brown clearance zone around their colonies in Lignin peroxidase screening medium as shown in Table 3.*Bacillus licheniformis* and *Bacillus subtilis* had the widest zone measured 12.0 and 8.0 mm respectively. *Bacillus megaterium, Aspergillus terreus* sand *Candida tropicalis* did not produced any noticeable clearance zone.

Five(5) out of 10 including bacteria and fungi isolated from the soil contaminated with textile effluent were observed to produce hollow brown colouration around their colonies in Manganese peroxidase screening medium as shown in **Table** 3. *Streptococcus faecalis, Trichoderma harzianum, Micrococcus luteus,* and *Asperillus flavus* had the widest zone measured 6.0 mm. *Bacillus megaterium, Aspergillus terreus, Candida tropicalis* and *Aspergillus terreus* did not produced any noticeable clearance zone.

Table 3: Screening of the isolates for their ability to produce Laccase, Lignin peroxidase and Manganese peroxidase

N/S	Isolates (Laccase)	Diameter (mm)	Isolates (LiP)	Diameter (mm)	Isolates (MnP)	Diameter (mm)
1	*Bacillus licheniformis*	Nil	*Bacillus licheniformis*	12	*Bacillus licheniformis*	6
2	*Bacillus subtilis*	2	*Bacillus subtilis*	8	*Bacillus subtilis*	5
3	*Streptococcus faecalis*	2	*Streptococcus faecalis*	4	*Streptococcus faecalis*	Nil
4	*Bacillus megaterium*	2	*Bacillus megaterium*	Nil	*Micrococcus luteus*	Nil
5	*Aspegillus flavus*	2	*Micrococcus luteus*	4	*Bacillus megaterium*	0.5
6	*Asperillus niger*	8	*Asperillus niger*	Nil	*Aspegillus niger*	4
7	*Trichoderma harzianum*	4	*Trichoderma harzianum*	2	*Trichoderma harzianum*	Nil
8	*Micrococcus luteus*	Nil	*Aspegillus flavus*	10	*Aspergillus flavus*	6
9	*Candida tropicalis*	Nil	*Candida tropicalis*	Nil	*Candida tropicalis*	Nil
10	*Aspergillus terreus*	8	*Aspergillus terreus*	Nil	*Aspergillus terreus*	Nil

DISCUSSION

Isolation of new microbial strains of biotechnological applications from various ecological habitats is a prerequisite for industrial growth. The microbial isolates identified were screened for their ability to produce lignin peroxidase, manganese peroxidase and laccase (Table 3). Some of the microbial isolate were able to oxidized the pyrogallol, guaiacol and veratryl alcoholfor lignin peroxidase, manganese peroxidase and laccase in their growth medium. These enzymes may be important enzymes capable of dyes degradation and are required for the survival of microbial committee in a dye contaminated site. These isolates probably have acquired natural adaptation to survive in the presence of the dyes (Khadijah *et al.*, 2009). This correlates the report of Barathi *et al.*, (2015), who isolate microbes capable of producing laccase, lignin peroxidase and manganese peroxidase from sludge samples collected from wastewater treatment sites contaminated with dyes.

Aspergillus terreus and *Aspergillus niger* had the highest clearance zone around its colonies (8.0 mm) as presented in Table 3. This indicates their capability of producing laccase as compared to other microbe isolated from the dump site. Previous reports by Ajani *et al.* (2011); Kantharaj *et al.* (2017) highlights the important of fungi in the production of laccase, hence the results of the present study are comparable with the previous report. The bacteria however, showed low potential of producing laccase and this corroborate the report by Saha and Santra (2014) that bacteria are not suitable source of laccase.Laccase is known to be involved in the depolymerization of lignin, which results in a variety of phenols. In addition, these compounds are utilized as nutrients for microorganisms or repolymerized to humic materials by laccase (Kim*et al.*, 2002).

Lignin peroxidase (LiP) plays an important role in the biodegradation of the plant cell wall whose constituent lignin (Piontek *et. al.*, 2001). LiP is able to oxidize aromatic compounds present in dyes with redox potentials higher than 1.4V(NHE) by single-electron abstraction (Piontek*et. al.*, 2001). *Bacillus licheniformis* and *Bacillus subtilis* had the widest zone measured 12.0 and 8.0 mm respectively. This highlights the suitability of these bacterial for the production of LiP relatively to other microbes screened from the dump site (Table 3).Interestingly, *A. flavus* possessed dual traits for synthesizing lignin peroxidase and laccase with ≥8 mm zone clearance. Therefore, *A. flavus* could be used simultaneously in production of these enzymes. Maharani *et al.* (2013) report supported our findings, who reported dump site native bacterial community with multi enzyme production capacity.

Streptococcus faecalis, Trichoderma harzianum, Micrococcus luteus, and *Aspergillus flavus* had the widest clearance zones (6.0 mm) in the Manganese peroxidase screening medium. The production of ligninolytic enzymes from different fungi (*A. niger, A. foetidus, A. fumigatus* and *A. terreus*)has been reported (Sumathi and Manju, 2000; Ali *et al.*, 2007; Jin*et al.*, 2007; And Leeb *et al.*, 2010). Thus, the finding of the present study is in line with the previous reports.

CONCLUSION

The native bacterial and fungal isolated in this study demonstrate varying ligninolytic enzymes production potentials which may be responsible for their survival in the contaminated sites. These enzymes have many industrial applications because of their inherent ability of oxidizing a wide range of phenolic and non-phenolic compounds which have made them to be very useful in many industries including paper, textile and petrochemical industries.

REFERENCES

Acharya, P. B., Shah, A. J., Acharya, D. K., Chabhadiya, S. and Modi, H. A. Manganese Peroxidase Production by Trichoderma pseudokoningii under SSF.*The Microbes: Online International Journal of Microbiology,* 18-29

Ajani A.O., Agarry, S.E., and Agbede, O.O. (2011). A Comparative Kinetic Study of Acidic Hydrolysis of Wastes Cellulose from Agricultural Derived Biomass. *Journal ofApplied Sciences and Environmental Management,* 15 (4) 531 – 537.

Ali, M., Kang, G. H., Yang, H. S., Jeong, J. Y., Hwang, Y. H., Park, G. B. and Joo, S. T. (2007). A comparison of meat characteristics between duck and chicken breast. *Asian-australasian journal of animal sciences*, *20*(6), 1002-1006.

Babu, B. V., Rana, H. T., Ramakrishna, V. and Sharma, M. (2000). COD reduction of reactive dyeing effluent from cotton textile industry. *Journal of the Institution of Public Health Engineers India*, *4*, 5-11.

Baldrian, P. and Šnajdr, J. (2006). Production of ligninolytic enzymes by litter-decomposing fungi and their ability to decolorize synthetic dyes. *Enzyme and Microbial Technology*, *39*(5), 1023-1029.

Barathi, S. and Arulselvi, P. I. (2015). Decolorization, Degradation, And Toxicological Analysis of Textile Dye Effluent by Using Novel Techniques–Review. *International Journal of scientific research and management (IJSRM)*, *3*(2), 2118-2136.

Barnett, J. A., Payne,R. W., Yarrow,D. and Linda,B. (2004). Yeasts: Characteristics and Identification, Amazon.com: Books, 2004.

Cheriaa, J., Bettaieb, F., Denden, I. and Bakhrouf, A. (2009). Characterization of new algae isolated from textile wastewater plant. *Journal of Food, Agriculture and Environment*, *7*(3-4), 700-704.

Chessbrough, M., 2006. District laboratory practice in tropical countries part II. 2nd ed.Cambridge University press, United Kingdom.

Coll, P.M., Abalos, J.M.F., Villanueva, J.R., Santamaria, R. and Perez, P. (1993). Purification and characterization phenoloxidase (Laccase) from the lignin degrading basidiomycete PM1. *Journal of Applied Environmental Microbiol*ogy 59: 2607-2613.

Dhouib, A., Hamza, M., Zouari, H., Mechichi, T., Hmidi, R., Labat, M., Martinez, M.J. and Sayadi, S. (2005). Screening for ligninolytic enzyme production by diverse fungi from Tunisia. *World Journal of Microbiology and Biotechnology*, *21*(8-9), 1415-1423.

El Monssef, R. A. A., Hassan, E. A. and 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.

Hatakka, A. (1994). Lignin-modifying enzymes from selected white-rot fungi: production and role from in lignin degradation. *FEMS microbiology reviews*, *13*(2-3), 125-135.

Jumarkar, N., Murty, D. S., Bhatt, N. S. and Madamwar, D. (2006). 'Decolorization of diazo dye remazol brilliant orange 81 by a novel bacterial consortium'. *World Journal of Microbiology andBiotechnology* 22: 163 – 168.

Kantharaj, P., Boobalan, B., Sooriamuthu, S. and Mani, R. (2017). Lignocellulose degrading enzymes from fungi and their industrial applications. *International Journal of Current Research and Review*, 9(21), 1-13.

Khadijah, O., Lee, K. K. and Mohd Faiz, F. A. (2009). Isolation, screening and development of local bacterial consortia with azo dyes decolourising capability. *Malaysian Journal of Microbiology*, 5(1), 25-32.

Kim, Y. S., Cho, N. S., Eom, T. J. and Shin, W. S. (2002). Purification and characterization of a laccase from Cerrena unicolor and its reactivity in lignin degradation. *Bulletin of the Korean Chemical Society*, *23*(7), 985-989.

Maharani, V., Vijayalakshmi, S. and Balasubramanian, T. (2013). Degradation and detoxification of reactive azo dyes by native bacterial communities. *African Journal of Microbiology Research*, *7*(20), 2274-2282.

Olukanni, O. D., Osuntoki, A. A. and Gbenle, G. O. (2006). Textile effluent biodegradation potentials of textile effluent-adapted and non-adapted bacteria. *African Journal of Biotechnology*, *5*(20):1980 – 1984.

PerinbamKantharaj, B. B., Sooriamuthu, S. and Mani, R. (2017). Lignocellulose degrading enzymes from fungi and their industrial applications. *International Journal of Current Research and Review*, *9*(21), 1.

Piontek, K., Smith, A. T. and Blodig, W. (2001). Lignin peroxidase structure and function. *Biochemical Society Transactions*,2:111-116.

Polak, J.and Jarosz-Wilkolazka, A. (2012). Fungal laccases as green catalysts for dye synthesis. *Process Biochemistry*, *47*(9), 1295-1307.

Saha, A. and Santra, S.C. (2014). Isolation and characterization of bacteria isolated from municipal solid waste for production of industrial enzymes and waste degradation. *Journal Microbiology Experiment*,1(1):12–19.

Savitha, S. D., Tennali, G. B., Channur, N., Anup, A. C., Deshpande, G. and Murtuza, B. A. (2011). Isolation of laccase producing fungi and partial characterization of laccase. *Journal ofBiotechnology, Bioinformatics and Bioengineering*. 1(4), 543-549.

Sumathi, S. and Manju, B. S. (2000). Uptake of reactive textile dyes by *Aspergillus foetidus*. *Enzyme and Microbial Technology*, *27*(6), 347-355.

Togo, C. A., Mutambanengwe, C. C. Z. and Whiteley, C. G. (2008). Decolourisation and degradation of textile dyes using a sulphate reducing bacteria (SRB)–biodigester microflora co-culture. *African journal of Biotechnology*, *7*(2).

World, Bank, (1995). Nigeria Strategic Options for Redressing Industrial Pollution World Bank, Industry and Energy Division. 1st Edition., West Central Africa Department, Annexes, 60-62.