

RESEARCH AND REVIEWS: JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

Production of Laccase by *Auerobasidium Pullulans* and *Cladosporium Werneckii* under Optimized Conditions: Applications in Decolourization of Textile Dyes.

Nelson Adedeji Ademakinwa, and Femi Kayode Agboola*

Department of Biochemistry, Obafemi Awolowo University Ile-Ife, Osun State. Nigeria.

Research Article

Received: 18/11/2013
Revised: 12/03/2014
Accepted: 17/03/2014

*For Correspondence

Department of Biochemistry,
Obafemi Awolowo University Ile-
Ife, Osun State. Nigeria.
Telephone: +2348034738078

Keywords: Laccase, Textile
dyes, *Cladosporium werneckii*,
Auerobasidium pullulans,

ABSTRACT

Textile dye waste-water presents an enormous task in its disposal. This present study aims to identify fungus capable of producing extracellular laccases that under optimized conditions can decolourize textile dyes. *Auerobasidium pullulans* and *Cladosporium werneckii* isolated from soil and decayed wood respectively were identified as laccase producing fungi. Physiological conditions for maximal laccase production were investigated. The fungal biomass was determined to correlate laccase production and its growth. The optimized culture broth was used in decolourization of several textile dyes. *A. pullulans* had maximum laccase activity of 19.18 U/ml on the ninth day of incubation; it utilized glucose, maximum laccase activity was observed at 37 °C and pH 6.0 whereas *C. werneckii* preferred galactose, produced maximum activity of 1.53 U/ml on the twelfth day, had optimum activity at 30 °C and pH 5.0. Both fungal isolates preferred tryptophan as nitrogen source and utilized inoculum size of 24 mm and 0.3 mM of CuSO₄ for optimal laccase production. *A. pullulans* laccase is constitutive whereas *C. werneckii* is inducible. The increase or decrease in laccase activity is in direct proportion to the rate of fungal growth. Optimized culture broth from *A. pullulans* and *C. werneckii* had 1.25 and 2.03 fold increase in laccase activity respectively and they were both able to decolourize malachite green specifically i. e 73% and 35% and had least decolourizing abilities on methylene blue in the absence of inducers and after three hours of incubation. The ability of these newly isolated fungal strains to produce laccase extracellularly and also decolourize dyes would serve as a way to eliminate industrial textile waste water.

INTRODUCTION

Laccases (benzenediol:oxygen oxidoreductases, EC1.10.3.2) which belong to the group of phenol oxidases are copper containing enzymes that catalyse the oxidation of a large variety of organic and inorganic substrate, that includes mono-, di-, and polyphenols, amino phenols, methoxy phenols, aromatic amines and ascorbate in which there is a four electron reduction of oxygen to water [15]. A unique characteristic of laccases aside being a blue copper protein is that its glycosylated. Plant laccases are 20-50 % glycosylated while fungal laccases are 5-20 % glycosylated. This is useful in its classification as plant laccases are involved in polymerization while fungal laccases are involved in depolymerisation reactions [6]. Laccase was first described by Yoshida in 1883 from the exudates of Japanese lacquer tree, *Rhus vernicifera* [36] but it has also been found in mostly fungi [8], bacteria, higher plants [35] and also in insects [34]. Fungal laccases have more roles than bacterial laccases in biotechnological applications mostly because of the high redox potential, +800 mV as noted specifically in delignification [36]

Laccases from fungi are distributed in Ascomycetes, Deuteromycetes and Basidiomycetes and consequently the white rot fungi are thus the most studied for the production of laccase. Laccases have various biological functions in degradation of lignin, morphogenesis and pathogenesis. Their catalytic properties and broad substrate specificity make fungal laccases have great potential in varied industrial and environmental applications including pulp delignification, textile dye transformation, biosensor, xenobiotics degradation, and bioremediation [14]. In contrast to other phenol oxidases, laccase do not require the presence of H₂O₂ for oxidation and this makes

it better suited for more biotechnological applications as found in biodegradation of xenobiotics and decolourization of dyes [22]. About 10,000 dyes produced annually are used basically in the printing and textile industries and these dyes are stable to light, chemicals and even microbial degradation [5]. The release of coloured compounds from these dyes into the environment may affect photosynthesis in aquatic plants and also the concomitant end products are toxic or mutagenic to living organisms [3]. Bioremediation is quite gaining traction as it applies the biological activity of microorganism to degrade toxic chemicals in the environment [20]. The white rot fungi which are the most efficient lignolytic microorganisms which specifically degrade several classes of dyes by concerted actions of three extracellular non-specific and also non stereoselective lignolytic enzymes namely laccases and peroxidases(lignin peroxidase(EC 1.11.10.14) and manganese peroxidase (EC 1.11.1.13) [26]. Laccase had been used to oxidatively detoxify or remove various aromatic xenobiotics and pollutants that are found in industrial waste and contaminated soil or water [2]. Also using laccase for decolourization treatments is a potential procedure quite of advantage to bioremediation technologies solely because there is enzyme production in larger quantities.

Laccase from *Trametes* genus have been investigated in recent years for its ability to degrade dyes [7]. Foorontafal *et al* [14] carried out dye decolourization using three fungal laccases of the *Trametes* and *Aspergillus* genus. Maleej-Kammoun *et al* [23] also carried out the dye decolourization of malachite green using laccase from a novel strain of the *Trametes* sp. Due to strict environmental laws, the textile industry in order to save cost is seeking ways to develop cost effective waste water remediation. The previous methods of precipitation, filtration, adsorption and TiO₂ oxidation are costly and not eco-friendly compared to fungi and their enzymes in textile dye decolourization which are well accepted globally in being cost effective and eco-friendly[12]

The aim of this research is to isolate, screen, and produce laccase from two different fungi *Auerobasidium pullulans* and *Cladosporium werneckii* that had been separately isolated from soil with decayed plant materials and decayed wood respectively. The physiological conditions that would favour their growth for maximum laccase production would be determined through various optimization processes. The laccase from optimized culture broth produced by both fungal isolates would be used to decolourize several local textile dyes. This would provide new insights that would assist in reducing environmental hazards caused by textile dye waste water.

MATERIALS AND METHODS

Chemicals and Media

Guaiacol, Catechol and Bradford reagent were obtained from Sigma, USA. For the purpose of this study, all other chemicals used were of analytical grade and of high purity. Local dyes were purchased in textile industries in Lagos, Nigeria.

Microorganism and Maintenance

The fungi used for the purpose of laccase production were isolated from decayed wood and soil containing decayed forest litters separately from unfarmed sites of the Obafemi Awolowo University, OAU Ile-Ife Osun State, Nigeria. Decayed wood and soil containing forest litters were collected in several sterile cellophane bags. Serial dilution was carried out with subsequent plating on Malt Extract Agar (MEA) amended with 0.01% chloramphenicol to inhibit bacterial growth. Several sub culturing was carried out till pure colonies were obtained. The pure fungal isolates were maintained on agar slants at 4 °C until needed for further use

Screening for Laccase Producing Fungi

For the purpose of isolating a laccase producing, the pure fungal isolates were subjected to screening medium for laccase production accord. The screening medium contained (Peptone - 0.3, Glucose- 1.0, KH₂PO₄ - 0.06, ZnSO₄ - 0.0001, K₂HPO₄ - 0.04, FeSO₄ - 0.00005, MnSO₄ - 0.005, MgSO₄ - 0.05, Guaiacol 10 mM, Agar - 2.0). The fungal cultures were inoculated in agar plates and the plates were incubated for 7 days in dark condition. The substrate utilized reddish brown colour in screening medium indicates the positive strain for lignin degradation. The fungi isolates with laccase producing ability from both decayed wood and soil containing decayed plant materials were selected for further studies [19].

Identification of Laccase Producing Fungi

Identification of several fungi isolates would be carried out using the growth characteristics i.e. texture, pigmentation, form, spore formation) and morphology through staining techniques using Lactophenol in cotton blue(for moulds) and gram- staining(for yeasts). The prepared slides were examined under light microscope. Sugar fermentation test was carried out on the yeasts for biochemical characterization [4]

Laccase Production

The well grown fungal agar plugs were separately inoculated into laccase submerged fermentation production mediums. The laccase production medium comprised (g/100 ml) Peptone - 0.3, Glucose - 1.0, KH₂PO₄ - 0.06, ZnSO₄ - 0.0001, K₂HPO₄ - 0.04, FeSO₄ - 0.00005, MnSO₄ - 0.005, MgSO₄ - 0.05 and 10 mM guaiacol. The medium were incubated at 28 °C for 15 days. The amount of enzymes produced was assayed at regular time intervals. The production medium was withdrawn and filtered using Whatman Filter paper No. 1. The filtrate was used as enzyme source [19].

Optimization of Cultural Conditions for Laccase Production

Submerged fermentations were carried out for the identified pure isolates to extracellularly produce laccase in optimal conditions. Various carbon sources(maltose, sucrose and galactose), Nitrogen sources(Urea, NH₄SO₄, arginine, tryptophan, alanine) pH (acetate buffer adjusted with 1 N HCl and 1 N NaOH) Temperature(23- 60 °C), Inoculum sizes(1-5 , 6 mm agar plugs), effect of inducers(Guaiacol, Copper Sulphate) and the effect of incubation time (days) were varied for optimal laccase production. Shaking conditions for each described parameters for optimization was carried out in a rotary orbital shaker while the temperature effect on laccase production by both fungi was carried out under static conditions in temperature controlled incubators.

Laccase Assay

Laccase activity was measured spectrophotometrically with guaiacol as substrate. The reaction mixture contained 0.1 ml of crude filtrate of culture broth, guaiacol in final concentration of 1.0 mM and 0.8 ml of 10 mM acetate buffer pH 5.0 in a final reaction volume of 1.0 ml. The reaction mixture was incubated for 15 minutes at room temperature. The reaction was followed spectrophotometrically at 450 nm; the extinction coefficient of guaiacol is 12,100 M⁻¹ cm⁻¹. One unit of laccase activity is defined as the amount of laccase that catalysed the oxidation of one micromole of guaiacol per minute. The reaction was carried out in room temperature. [29]

Protein Determination

Protein concentration was determined using bovine serum albumin (BSA) as standard. [9]

Determination of Fungal Biomass

The biomass, **B**, obtained from the crude enzyme was determined by estimating the dry weight after collection of the filtrate. The filter paper (Whatman Filter Paper No. 1) containing the already dried fungus was weighed, **F_A**. The weight of unused filter paper, **F_B**, was estimated and was subsequently subtracted from the filter paper containing the dried fungal growth.

$$B = F_A - F_B$$

Dye Decolourization Potential of Optimized Culture Broth

0.5 ml of the optimized culture broth was added to 2 ml (in final concentration of 0.096 mg/L) of each dye solution followed by incubation in a static conditions at room temperature for 3 h. Samples were monitored and decrease in the maximum absorbance was recorded every 30 min. [12]

Percent of dye decolourization was calculated as the formula:

$$\text{Decolourization (\%)} = [(A_i - A_t) / A_i] \times 100, \text{ where,}$$

A_i: initial absorbance of the dye, **A_t**: absorbance of the dye at any time interval. Negative controls (reaction mixtures without enzyme) were designed as a reference to compare decolourization percentage of treated samples.

Table 1: Names, classification and maximum absorbance of dyes in 0.1 M citrate buffer pH 4.5)

Dyes	Classification	λ_{max} (nm)	Concentration (mg/L)
Tartrazine	Azo	480	120
Malachite green	Triarylmethane	621	120
Methylene blue	Heterocyclic	610	120
Allura red	Azo	504	120

RESULTS AND DISCUSSION

Isolation and Screening of Laccase Producing Fungi

Serial dilutions up to 10^{-8} for soil with decaying plant materials and 10^{-6} for decayed wood were plated and over sixteen (16) pure fungal isolates were obtained from both sources after repeated sub culturing on Malt Extract Agar (MEA). Only 3 of the sixteen pure isolates were able to produce extracellular laccase. The laccase producing fungi with the most intense halo zone was identified as *A. pullulans* and *C. werneckii* from soil with decaying plant materials and decayed wood respectively.

Effect of Incubation Period

Fig. 1 indicates that, the highest activity was obtained at the ninth day and twelfth day of growth for *A. pullulans* and *C. werneckii* respectively. Fungal laccase formation reached its maximum value with activity of 19.18 U/ml and 1.53 U/ml respectively. A decrease in laccase production was noticed as the incubation period increased. There had been different incubation periods for optimum production of laccase. According to Kaal *et al* [18] the ligninolytic enzyme system of fungi, although may be present in the primary phase of growth, usually is triggered in response to N or C depletion, attaining its maximum in the idiophase when the mycelial dry weight is decreasing. Elisashvili *et al* [13] and Sivakumar *et al* [34] reported that maximum laccase production was obtained at the 7th and 10th day of incubation in case of *Lentinus edodes* and *Ganoderma sp*, respectively. Cavallazzi *et al* [11] found maximum laccase activity with *Lentinula edodes* after 30 days of incubation.

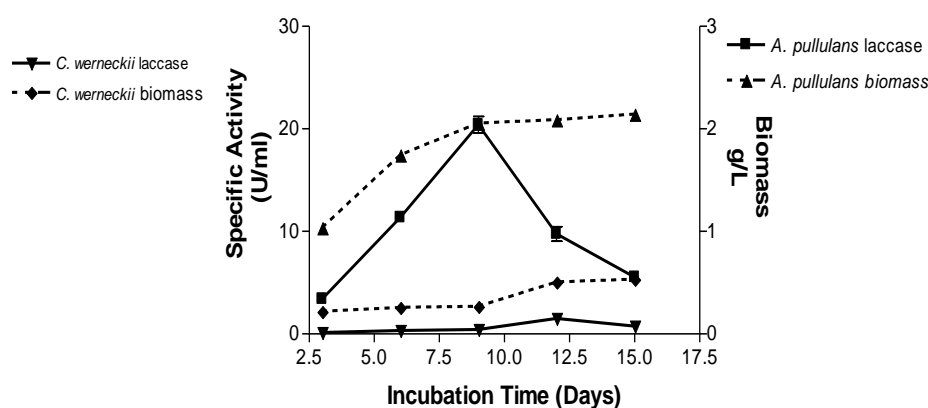


Figure 1: The effect of incubation period on laccase production and fungal growth by *A. pullulans* and *C. werneckii*.

Effect of pH

The pH value ranging from 3.0 to 7.0 was used in this study. As obtained in Fig. 2. From which it is observable that maximal formation of *Cladosporium werneckii* and *Auerobasidium pullulan* laccase took at pH 5.0 and pH 6.0 respectively. Fungal growth was relatively diminished as slight changes in pH affected the growth and hence the laccase production. The fungal laccase formation occurred at a narrow range of pH values, whereas low levels of enzyme were obtained at pH values below and above this value. This may be attributed to the fact that change in pH value may alter the three-dimensional structure of the enzymes [33]. Haltrich *et al* [17] stated that most of fungal cultures prefer a slightly acidic pH in the medium for growth and enzyme biosynthesis, in agreement with the results obtained. In addition, Adejaye and Fasidi [4] reported the *Schizophyllum commune* had its pH optimum at 5.5 while and Sivakumar *et al* [34] observed that *Ganoderma sp* had optimum laccase production at 6.0

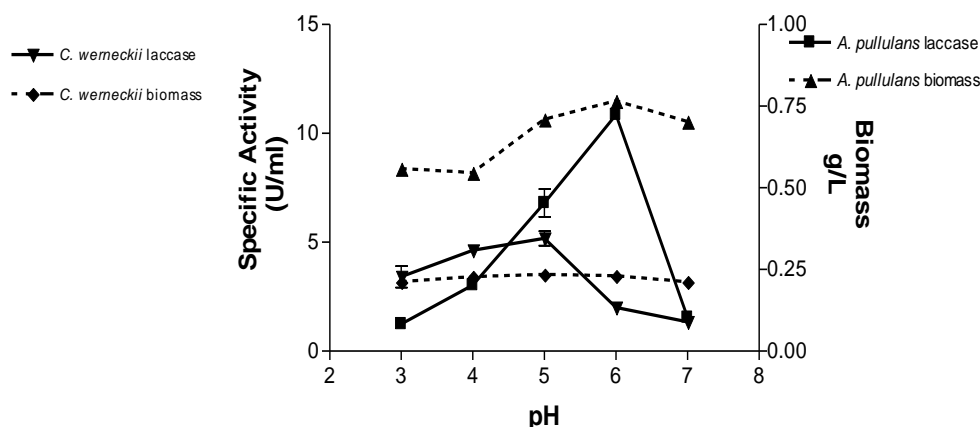


Figure 2: The effect of pH on laccase production by *C. werneckii* and *A. pullulans*.

Effect of Temperature

Both fungal isolates were each grown at different degrees of temperature ranging from 23-60 °C. The optimum temperature for *C. werneckii* and *A. pullulans* laccase are 30 °C and 37 °C respectively as observed in figure 3. Increased temperature tends to favour fungal growth though laccase production decreased after reaching its peak at 37 °C and 30 °C for *A. pullulans* and *C. werneckii* respectively. As observed in the results, increasing the temperature above the optimum for both microorganisms, there is decrease in laccase formation and this can be interpreted in terms of the alteration of cell membrane composition and stimulation of protein catabolism. The optimum cultivation temperature depends on the growth kinetics of the microorganism employed rather than on the enzyme produced

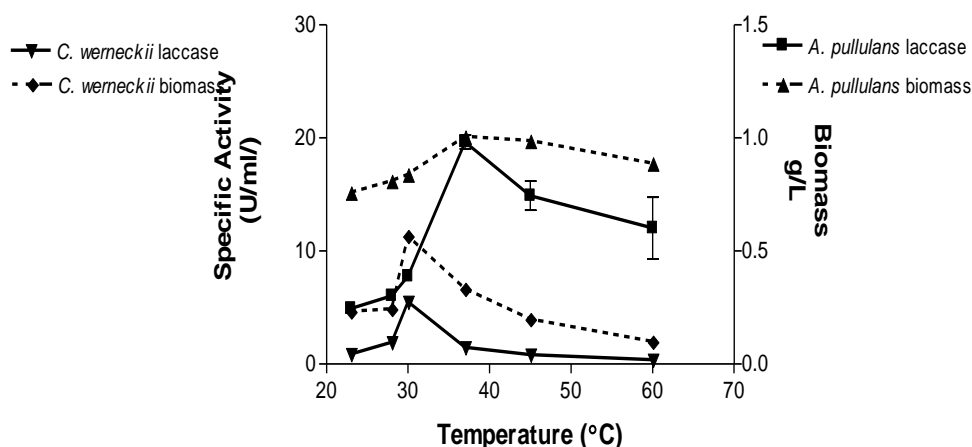


Figure 3 The effect of temperature on laccase production and fungal growth by *A. pullulans* and *C. werneckii*.

Effect of Inoculum Size

For effect of inoculum size on laccase production by both *A. pullulans* and *C. werneckii*, different sizes of inoculum were that ranged from 1-5 agar plugs (6 mm diameter) were cut from actively growing fungal mycelium and was inoculated into production medium. Both *A. pullulans* and *C. werneckii* utilized 4 agar plugs for maximum laccase production. The inoculum size plays an important role in production of enzymes. Increased inoculum sizes further enhanced more production of laccase and fungal growth for both microorganisms. We observed a decline in the dry mycelial weight (biomass) as laccase activity decreased for both organisms. A lower level of inoculum may not be sufficient to initiate growth, whereas a higher level may cause competitive inhibition (Sabu *et al.*, 2005). Sharma *et al.* (1996) reported that inoculum size controls and shortens the initial lag phase, as smaller inoculum size increased the lag phase. Increasing the inoculum size to five had a deleterious effect and its being interpreted as fast depletion of nutrients, resulting in a decrease in metabolic activity.

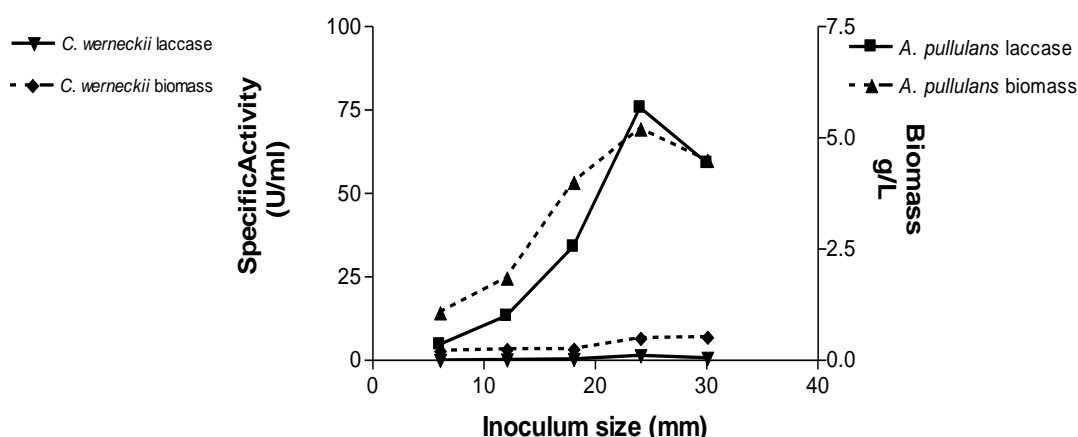


Figure 4: The effect of inoculum size on laccase production by *C. werneckii* and *A. pullulans*.

Effect of Carbon Sources

The effect of conventional carbon sources on adaptation of the fungus for the production of laccase is of importance as laccase production is dependent on the microbial taxa employed [27]. For studying the effect of different carbon sources on the formation of laccase, monosaccharide (galactose), disaccharides (maltose) and polysaccharide (soluble starch) were used instead of glucose (control). Each carbon source was added at a concentration of 10.0 g/l to the growing medium as the main carbon source. The obtained results showed that,

galactose and starch was found to be the best inducer for laccase formation from both *C. werneckii* and *A. pullulans* respectively. All other carbon sources repressed the production of laccase albeit to varying degrees (Fig. 5) Bollag and Leonowicz [10] had suggested that easily assimilable components such as glucose, allow for constitutive laccase production but repress its induction in several fungi. In agreement with our results, among the carbon sources, starch supported the maximum laccase production from *Ganoderma sp* [34]. Mansur *et al* [24] showed that the use of fructose instead of glucose resulted in a 100-fold increase in the specific laccase activity of basidiomycetes

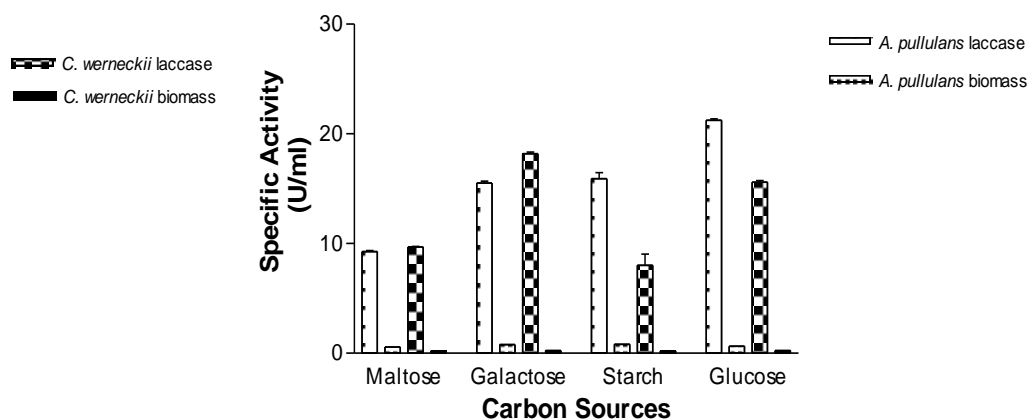


Figure 5: Effect of carbon sources on laccase production and fungal growth (biomass) by *A. pullulans* and *C. werneckii*.

Effect of Nitrogen source

Various nitrogen compounds (urea, L-arginine, L-tryptophan glycine) were added separately to the culture medium in amounts equivalent to the amount of ammonium sulphate (control) in the medium described by. The highest level of enzyme formation expressed in terms of activity was obtained with L-tryptophan for both *A. pullulans* and *C. werneckii*. The rest of nitrogen sources gave also considerable amounts of laccase except L-arginine and glycine which gave the lowest enzyme activity for *C. werneckii* and *A. pullulans* respectively. Different proportions of laccase were obtained in all media containing different nitrogen sources. Gogna *et al* [16] stated that, the most widely used nitrogen sources for fungal ligninolytic enzyme production are ammonium salts. Sivakumar *et al* [34] reported that yeast extract stimulated higher production of laccase by *Ganoderma sp*.

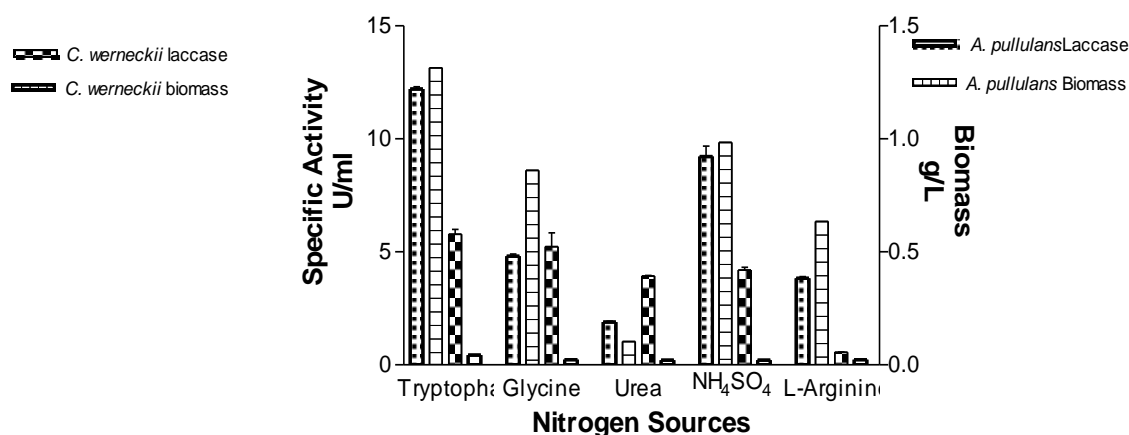


Figure 6: The effect of nitrogen sources on laccase production and fungal growth on both *A. pullulans* and *C. werneckii*.

Effect of Copper sulphate (CuSO₄) Concentration on Laccase Production

Copper as a micronutrient has a key role as a metal activator, induces both laccase transcription, and plays an important role in laccase production [28]. In order to find out the suitable concentration of copper sulphate for induction maximum production of laccase for both *A. pullulans* and *C. werneckii*, a series of concentrations of copper sulphate (0-0.5 mM) was used. *A. pullulans* and *C. werneckii* both showed maximum laccase production at 0.3 mM. It could be deduced that copper sulphate enhanced laccase production but has very little impact on fungal growth for *C. werneckii* but it was able to enhance both laccase production and fungal growth in *A. pullulans*.

However, when the concentration of copper was increased above the optimum it resulted in significant decrease in fungal growth and laccase. This may be attributed to an inhibitory effect of copper at higher concentrations. Baldrian [6] stated that higher copper concentrations may be toxic for fungi, affecting their growth

and enzymatic action. Copper sulphate at a concentration of 30 μ M supported the maximum laccase production by *Ganoderma sp* [34]. Niladevi and Prema [25] obtained maximum laccase activity when copper sulphate was used at a concentration of 1 mM. Galhaup *et al* [15] reported that the addition of copper sulphate in various concentrations ranging between 1 mM and 10 mM stimulated laccase production.

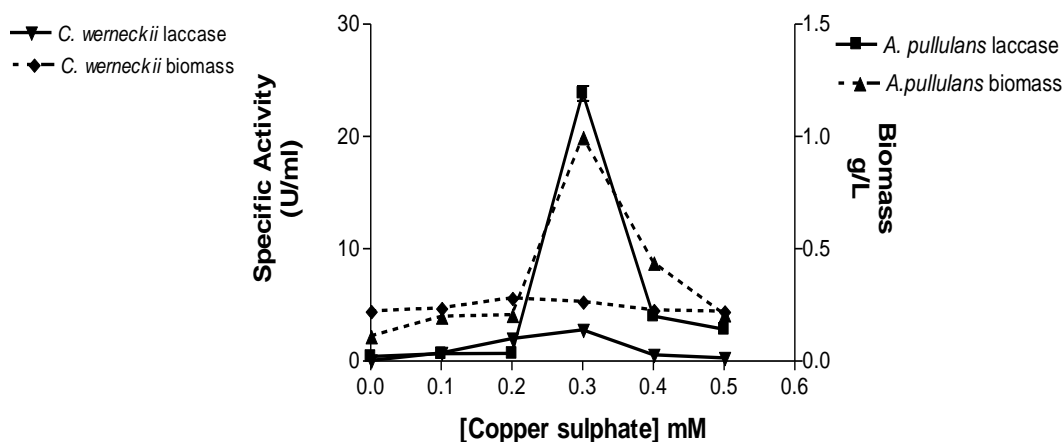


Figure 7: The effect of Copper sulphate concentrations on laccase production by *A. pullulans* and *C. werneckii*.

Dye Decolourization

The ability of laccases to decolourise even dyes of the same class varies and it solely depends on the biological nature of the source of the microorganisms. 60.5% of malachite green (with initial concentration of 60 mg/L) was removed after 15 min incubation of the dye in presence of laccase from *P. variable* while 98% of malachite green decolorization using laccase of *Ganoderma sp*. [37]

In comparison to other classes of dyes the triphenylmethane dyes are resistant to enzymatic treatment and requires time for decolorization [14]

The optimized culture broth from *A. pullulans* had 1.25 fold increase in laccase activity was able to decolourize malachite green (72.456 \pm 0.05%), Allura red (52.23 \pm 0.07%) methylene blue (5.93 \pm 0.08) and Tartrazine (19.82% \pm 0.07) while *C. werneckii* laccase had 2.03 fold increase after optimization and was only able to effectively decolourize malachite green (30.03 \pm 0.97%) after 3 hours of incubation under static conditions without any inducer.

The inability of the optimized laccase broth from both sources to efficiently decolourize methylene blue and tartrazine may be eliminated by inclusion of laccase mediators such as HBT which is a synthetic laccase mediator that assists in laccase oxidation of different substrates by facilitating of electron transfer from O₂ to laccase substrate [37]

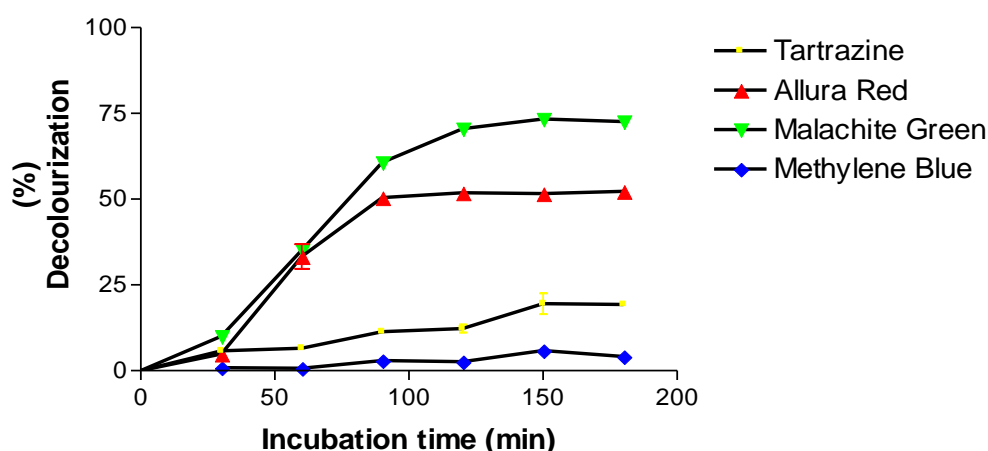


Figure 8: Decolourization of local textile dyes by extracellular laccase by *A. pullulans*

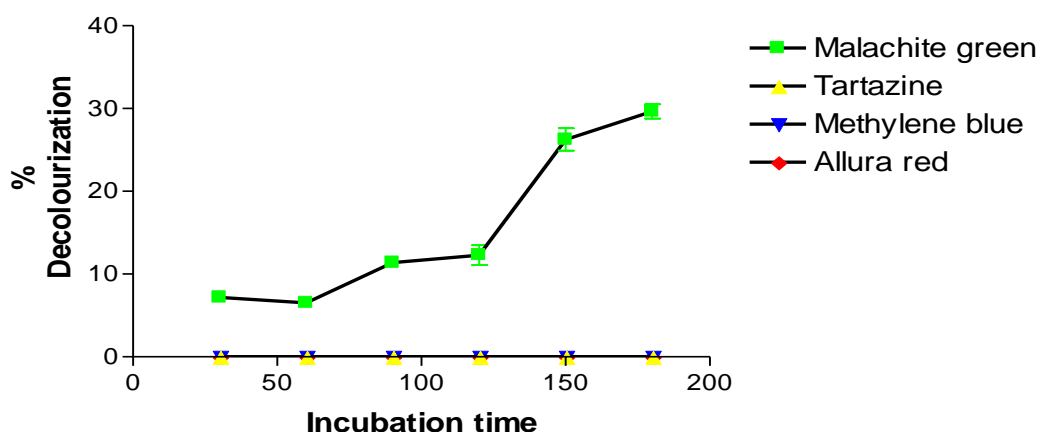


Figure 9: Decolourization of local textile dyes by laccase from *C. werneckii*.

CONCLUSION

The crude extracellular laccases produced by both *A. pullulans* and *C. werneckii* after optimization showed varying potentials in the decolourization of several locally purchased textile dyes. This provides useful insights and a cheap way in utilizing these microorganisms for bioremediation purposes.

ACKNOWLEDGEMENT

The authors wish to thank Mr Tunde Oni of the Department of Microbiology, Obafemi Awolowo University Ile-Ife Osun State, Nigeria for his assistance on the microbial aspect of this study.

REFERENCES

1. Adejaye OD and Fasidi, IO. Effect of Cultural Conditions on Biomass and Laccase Production in Submerged Medium by *Schizophyllum commune* (Fries), a Nigerian Edible Mushroom. *EJEAFF Che.* 2010; 9(3), 600-609
2. Aghaie-Khouzani M, Forootanfar H, Moshfegh M, Khoshayand MR, Faramarzi MA. Decolorization of Synthetic Dyes using Optimized Culture Broth of Laccase producing *Ascomycete Paraconiothyrium Variabile*. *Biochem Eng J.* 2012. 60:9–15
3. Ansari AA and Thakur BD. Biochemical Reactor for Treatment of Concentrated Textile Effluent, *Colourage.* 2006; 2:27-31
4. Aneja KR and Mehrotra RS. Introduction to Mycology. New Age International. 2005
5. Ayed L, Mahdhi A, Cheref A, Bakhrouf A Decolorization and Degradation of Azo Dye Methyl Red by an Isolated *Sphingomonas paucimobilis*: Biototoxicity and Metabolites Characterization. *Desalination.* 2011; 274:272–277.
6. Baldrian, P. Interaction of Heavy Metals with White Rot Fungi. *Enzyme Microb Technol.* 2003; 32, 78–91.
7. Baldrian P. 2004. Purification and Characterization of Laccase from the White Rot Fungus *Daedalea quercina* and Decolorization of Synthetic Dyes by the enzyme. *Appl Microbiol Biotechnol.* 2004; 63:560–563.
8. Baldrian P. Fungal Laccases Occurrence and Properties. *FEMS Microbiol Rev.* 2006; 30: 215–242.
9. Bradford M. A rapid and sensitive method for the quantification of microgram quantities of protein, utilizing the principle of protein dye binding. *Anal Biochem.* 1976; 72: 248-254
10. Bollag JM, Leonowicz, A. Comparative studies of extracellular fungal laccases. *Appl Environ Microbiol.* 1984; 48: 849–854.
11. Cavallazzi JRP, Kasuya CM, Soares MA. Screening of Inducers for Laccase Production by *Lentinula edodes* in liquid medium. *Braz J Microbiol.* 2005; 36:383-387
12. Eichlerova I, Homolka L, Nerud F. Synthetic dye Decolorization Capacity of White Rot Fungus *Dichomitus squalens*. *Bioresour Technol.* 2006; 97:2153–2159
13. Elisashvili V, Penninle M, Kachlishvili E, Tsiklauri N, Metreveli E, Kharziani T, Kvesitadze G. *Lentinus edodes* and *Pleurotus* species Lignocellulolytic Enzymes activity in submerged and solid-state fermentation of Lignocellulosic Wastes of Different Composition. *Bioresour. Technol.* 2008; 99: 457-462.
14. Forootanfar H, Movahednia MM, Yaghmaei S, Tabatabaei-Sameni M, Rastegar, H, Sadighi A, Faramarzi MA. Removal of Chlorophenolic Derivatives by soil isolated *Ascomycete* of *Paraconiothyrium variabile* and Studying the Role of its Extracellular Laccase. *J Hazard Mater.* 2012; 209:199–203
15. Galhaup C, Goller S, Peterbauer C, Strauss J, Haltrich D. Characterization Of The Major Laccase Isoenzyme from *Trametes pubescens* and Regulation of its Synthesis by Metal Ions. *Microbiol.* 2004; 148: 2159–2169

16. Gogna E, Vohra R, Sharma P. Biodegradation of rose bengal by *Phanerochaete chrysosporium*. Lett Appl Microbiol. 1992; 14: 58–60.
17. Haltrich D, Nidetzky B, Kulbe KD, Steiner W, Zupancic S. Production of Fungal Xylanases. Biores. Technol. 1996; 58: 137-161
18. Kaal EEJ, Field JA, Joyce TW. Increasing Ligninolytic Enzyme Activity in Several White-Rot Basidiomycetes by Nitrogen-Sufficient Media. Bioresour Technol. 1995; 53: 133–139.
19. Kiiskinen LL, Viikari L, Kruus K. Purification and characterisation of a novel Laccase from the ascomycete *Melanocarpus albomyces*. Appl Microbiol Biotechnol. 2002; 59: 198–204.
20. King RB, Long GM, Sheldon JK. Practical Environmental Bioremediation, Field Guide, Boca Raton, Lewis Publishers. 1998
21. Krishna C. Production of Bacterial Cellulases by Solid State Bioprocessing of Banana Wastes. Bioresour Technol. 1999; 69: 231- 239.
22. Law WM, Lau WN, Lo KL, Wai LM, Chiu SW. Removal of Biocide Pentachlorophenol in Water system by the Spent Mushroom Compost Of *Pleurotus pulmonarius*. Chemosphere. 2003; 52:1531–1537.
23. Maalej-Kammoun, M., Zouari-Mechichi, H., Belbahri, L., Woodward, S., Mechichi, T. Malachite Green Decolourization and Detoxification by the Laccase from a Newly Isolated Strain Of *Trametes sp.* Int Biodeter Biodegr. 2009; 63:600–606.
24. Mansur M, Suarez T, Fernandez-Larrea JB, Brizuela MA, Gonzalez AE. Identification of a Laccase Gene Family In The New Lignin-Degrading Basidiomycete CECT 20197. Applied Environ Microbiol. 1997 63, 2637-2646.
25. Niladevi, K.N., Prema, P., (2007). Effect of Inducers and Process Parameters on Laccase Production by *Streptomyces psammoticus* and its application in dye decolourization. Bioresour Technol. 2007; 99: 4583-4589.
26. Notvony C, Cajthaml T, Rothschild N, Dorsoretz C and Sasek, V. *Irpex lacteus* a white rot fungus applicable to water and soil bioremediation. Appl. Microbiol Biotech. 2000; 54: 850-853
27. Osma JF, Saravia V, Toca Herrera JL, Rodriguez C. Mandarin peelings: The Best Carbon Source to Produce Laccase by Static Cultures of *Trametes pubescens*. Chemosphere. 2007; 67:1677-1680
28. Palmeiri G, Cennamo G and Sannia G. Remazol brilliant blue decolourization by the fungus *pleurotus ostreatus* and its oxidative enzymatic system. Enzyme Microbial Tech. 2005; 36, 17-24
29. Pointing SB, Pelling AL, Smith GJ, Hyde KD. Screening of Basidiomycetes and xylariaceous fungi for lignin peroxidase and laccase specific gene sequence. Mycol Res. 2005; 109: 115-124
30. Sabu A, Pandey A, Daud MJ, Szakacs G. Tamarind Seed Powder and Palm Kernel Cake: Two Novel Agro Residues for the Production of Tannase under solid state fermentation by *Aspergillus niger* ATCC 16620. Bioresour. Technol. 2005 96(11):1223-1228.
31. Schmidt A, Huber R. The Blue Oxidases, Ascorbate Oxidase, Laccase and Ceruloplasmin Modelling and Structural Relationships. European J Biochem. 1990; 187(2):341-352
32. Sharma DK, Tiwari M, Behere BK. Solid state fermentation of new substrates for Production of Cellulase and other Biopolymer Hydrolyzing Enzymes. Appl Biochem Biotechnol. 1996; 15: 495- 500.
33. Shulter ML, Kargi F. Bioprocess Engineering Basic Concept, Prentice Hall of India Pvt Ltd, New Delhi, In-dia. 2000
34. Sivakumar, R, Rajendran, R, Balakumar C, Tamilvendan C. Isolation, Screening and Optimization of Production Medium for Thermostable Laccase Production From *Ganoderma Sp.* Int J Eng Sci Technol. 2010; 2(12):7133-7141.
35. Telke AA, Kalyani DC, Jadhav UU, Parshetti GK, Govindwar SP. Purification and Characterization of an Extracellular Laccase from a *Pseudomonas sp.* LBC1 and its Application for the Removal of Bisphenol. A J Mol Catal. 2009; 61:252–260.
36. Thurston CF. The Structure and Function of Fungal Laccases. Microbiol. 1994; 140(1): 19–26.
37. Zhuo R, Ma L, Fan F, Gong Y, Wan X, Jiang M, Zhang X, Yang Y. Decolorization of different dyes by a newly isolated white-rot fungi strain *Ganoderma sp.* En3 and cloning and functional analysis of its laccase gene. J Hazard Mater 2011; 192:855–873.