World Journal of Pharmaceutical Sciences
ISSN (Print): 2321-3310; ISSN (Online): 2321-3086
Published by Atom and Cell Publishers
Available online at: http://www.wjpsonline.org/
Original Article

Effect of physico-chemical parameters on ligninolytic enzyme production of an indigenous isolate of *Neolentinus kauffmanii* -under submerged culture condition

Johnsy G and Kaviyarasan V

CAS in Botany, University of Madras, Maraimalai campus, Chennai-25, India

Received: 07-03-2014 / Revised: 25-04-2014 / Accepted: 28-04-2014

ABSTRACT

The production of ligninolytic enzymes by an indigenous strain of *Neolentinus kauffmanii* was studied on submerged fermentation. The physical parameters namely, pH, Temperature, the nutritional parameters like suitable carbon and nitrogen sources, metal ions and amino acids were studied for the higher ligninolytic enzyme production. The optimum pH for the both biomass and ligninolytic enzyme production was found to be pH 6.0 in laccase (56.34 U/mL) and 6.5 in peroxidase (47.77 U/mL. Of the different temperature analyzed for the optimal enzyme production in laccase was 25^{o}C (57.8 U/mL) and peroxidase was 30^{o}C showed the maximum activity (47.79 U/mL). Among the different carbon sources tested Xylan supported maximum (63.51 U/mL) Laccase activity, while Peptone supported the maximum activity (64.37 U/mL and 56.97 U/mL) among the different nitrogen sources tested. Among the different metal and amino acids analyzed for ligninolytic enzyme highest enzyme activity was obtained in copper (70.51 U/mL and 53.65 U/mL) and Alanine (57.25 U/mL and 54.98 U/mL). The above result indicates that the *N. kauffmanii* can be used as a biotechnological tool.

Key words: Ligninolytic enzyme, *Neolentinus kauffmanii*, physical parameters, nutritional

INTRODUCTION

White rot Basidiomycetes are unique in their ability to degrade all components of lignocelluloses due to their capability to synthesize the relevant hydrolytic and oxidative extracellular enzymes [1]. These fungi secrete one or more of three extracellular enzymes that are essential for lignin degradation lignin peroxidase (E.C 1.11.1.14) manganese dependent peroxidase (MnP) (E.C 1.11.1.13) and Laccase (E.C 1.10.3.2). The ligninolytic enzymes of Basidiomycetes are of fundamental importance for the efficient bioconversion of plant residues and they are prospective for the various biotechnological applications in pulp, paper, textile, dye industries, bioremediation and many others [2-7]. It is evident that the potential applications of these enzymes in industrial and environmental technologies requires huge amount of these enzymes at low cost. The main issue delaying their implementation at industrial scale is the low yield of ligninolytic enzymes in most white rot fungi [8-10]. Lignin is the most abundant natural aromatic polymer on earth and degradation of this recalcitrant aromatic polymer is caused in nature by white rot fungi through a process that was defined as an enzymatic combustion [11]. The ligninolytic system is an extracellular enzymatic complex that includes peroxidases, laccases and oxidases responsible for the production of extracellular hydrogen peroxidase (H_2O_2) [12, 13]. Those enzyme systems exhibit differential characteristics depending on the species, strains and culture conditions [11]. The potential application of ligninolytic enzymes in biotechnology has stimulated their investigation [14] and the understanding of physiological mechanisms regulating enzyme synthesis in lignocelluloses bioconversion could be useful for improving the technological process of edible and medicinal mushroom production. Laccase belong to multicopper oxidase family. Most laccases were reported from fungal organisms and most biotechnologically useful laccases are also of fungal origin [15]. Lignin peroxidases belong to the family of oxidoreductases [16]. It was first described in the Basidiomycetes *Phanerochaete chrysosporium* [17]. This enzyme has been recorded for several species of white-rot Basidiomycetes [18]. The test fungus *Neolentinus kauffmanii* (AH Smith) VKGJ01 (Accession No. JF808173) is a wood-inhabiting white rot fungus, a

***Corresponding Author Address:** Johnsy G, CAS in Botany, University of Madras, Maraimalai campus, Chennai-25, India; Email: johnsy_moses@yahoo.co.in*

member of family Gleophyllaceae and order Agaricales. These mushrooms have been isolated from the Western Ghats of Kanyakumari district.

MATERIALS AND METHODS

Organism and inoculum preparation: Fruiting body of the *N. kauffmanii* (Accession No. JF808173) was isolated from Keeriparai forest of Western Ghats, Tamil Nadu, India, and the culture was maintained on potato dextrose agar medium at 4°C. Inoculum of *N. kauffmanii* was prepared from mycelia grown on the same media incubated at 25°C for 4–6 days. From the plate 7 mm diameter mycelial disc were used as the inoculum.

Selection of medium for laccase production: The white-rot fungus was grown in a chemically defined medium composed of KH_2PO_4 (1 g L^{-1}), $MgSO_4.7H_2O$ (0.5 g L^{-1}), yeast extract (1 g l^{-1}), $CaCl_2.2H_2O$ (0.14 $g1^{-1}$), glucose 10g and thiamine (0.0025 g L^{-1}) [19]. Incubation was carried out statically at 25 ± 1°C in 125 ml Erlenmeyer flask containing 50 ml of the medium inoculated with 7mm agar plug from 6 day old mycelia grown on malt-extract agar. Periodic harvesting of the mycelia was performed using the filter paper. An aliquot of supernatant was collected aseptically and culture filtrates were used as enzyme sources.

Screening of nutritional factors for increasing ligninolytic enzyme activity: After the selection of the best medium for ligninolytic enzyme, seven carbon, nitrogen and metal ion compounds were investigate for their capacity to increase enzyme activity in *N. kauffmanii*. One control treatment was used without the addition of any putative inducer. Various carbon sources such as xylan, glycerol, maltose, sucrose, mannitol, lactose, starch; nitrogen sources such as beef extract, peptone, yeast, NH_4NO_3, ammonium chloride, ammonium nitrate and ammonium tartarate; metal ions such as manganous sulphate, Fe_2SO_4 aluminium oxide, sodium sulphate, Calcium chloride, zinc sulphate and copper sulphate; and amino acids such as Alanine, glycine, asparagine, tryptophan, tyrosine, phenylalanine and Methionine were used. Optimization of physiological parameters such as pH (4.0–8.0) and temperature (20–40°C) were carried out. All chemicals used in this research were of analytical grade and were used without further purification. The compounds were sterilized by filtration using a Millipore membrane (0.45 μm) and added aseptically into the flasks.

Enzyme Activity: The laccase (Lac) activity was determined by measuring the oxidation of 2,2'-azino-bis 3- ethylbenzothiazoline-6-sulphoric acid (ABTS) as increase in absorbance in 3minutes was measured spectrophotometrically at 420nm (ε=36000cm1 M1) [19]. The Manganese peroxidase (MnP) activity by monitoring the oxidation of 20 mM solution of 2,6-dimethoxyphenol (DMP) as the increase in absorbance at 469 nm [20]. One unit of enzyme activity was defined as the amount of enzyme that oxidized 1μM of corresponding substrate per min. The substrate for the Lac and MnP activity assays were both purchased from sigma. One unit of enzyme is the amount of enzyme required for oxidising 1 mmol ABTS and DMP per minute. The experiments were performed at least two times using three replicates. The data presented in the figures correspond to mean values with four replicates.

RESULT AND DISCUSSION

In this study the simultaneously optimize the production of two lignin-modifying enzymes (Laccase and Peroxidase) produced by *N. kauffmanii*. For each enzyme, the optimization was performed using the same methodology for both the enzyme activity and its corresponding production rate. In many Basidiomycetes fungus cultivation in the presence of some lignocellulosic residues significantly stimulated ligninolytic enzyme without supplementation of the culture medium with specific inducers [21, 22]. The ligninolytic machinery in most fungus is highly regulated by nutrients such as carbon, nitrogen, copper and manganese. Their production is also affected by fermentation factors, such as medium composition, nature of carbon source, pH of fermentation broth, temperature, amount and nature of nitrogen source as well as the presence of inducers [23-25].

Effect of pH on Ligninolytic enzyme production: The culture condition had significant influence on ligninolytic enzyme production. Mishra et al., [26] reported that a pH in the range of 5.0 to 7.0 and temperature in the range of 30 to 35^{o}C had a significant effect on ligninolytic enzyme production. The most appropriate conditions for the production of LiP and MnP are 4.5 pH and optimum activities of both enzymes are produced under this condition [27]. Wen *et al*., [23] reported the optimum pH of 4.8 for manganese peroxidase production from *P. chrysosporium*. The highest level of enzyme activity was observed pH6.0 in laccase and 6.5 in peroxidase after 17th day of incubation (56.34 U/mL and 47.77 U/mL). The lowest level of enzyme activity was observed in both pH 4.5 and 5.0 when compared to pH6.0 and 6.5. Stajic *et al*. [28] *Pleurotus* sp. which produce maximum peroxidase and laccase activity was

detected at the pH of 6.0 while in *Bjerkandara* sp. the maximum enzyme activity was reported at pH5.2 [29].

Effect of Temperature on ligninolytic enzyme production: Temperature was much significant in the liquid state even through the impact of temperatures was more prominent in the scale up processes, it remains an inevitable factor in all systems due to its impact an microbial growth and enzyme production. The ligninolytic enzyme activity was detected at five different temperatures (20-40^{o}C) in Glucose malt extract medium. The highest level of peroxidase enzyme activity was obtained at 25^{o}C and the laccase activity was obtained in 30^{o}C after the 17th day of harvest (57.8 U/mL and 47.79 U/mL). Similarly, according to Gill *et al*., [30] in *Coriolus vesicolor* and *P. chrysosporium* the optimal activity of the enzyme was expressed at 25- 30^{o}C, where no activity was observed above 30^{o}C. The temperature ranging from 25 to 37^{o}C have to be found to be optimum for ligninolytic enzyme production by different white rot fungus [31]. Higher temperatures denature the metabolic enzymes of microorganisms leading to inhibition of growth and enzyme formation [31].

Effect of Carbon sources on ligninolytic enzyme production: The ligninolytic enzyme production was found to vary with the different carbon sources. Many previous studies have proved that both the nature and concentration of nitrogen sources are powerful nutrition factors regulating ligninolytic enzyme production by wood-rotting Basidiomycetes [32]. Among the different carbon sources that were tested for biomass production and ligninolytic enzyme production, Xylan stimulated the highest (63.51 U/ml) laccase production and sucrose supported highest peroxidase enzyme production (50.3 U/mL). Mansure *et al*., [33] showed that the use of fructose instead of glucose resulted in a 100 fold increase in the specific ligninolytic enzyme activity of basidiomycetes. Increased enzyme activity in media containing these simple sugars can be explained by the high production rate of secondary metabolites when their producing organisms grow in complex media [34]. Leifa *et al*. [35] reported that monosaccharide in general were better than sugar alcohol and complex sugar (cellulose) for biomass production. This was probably due to ease in polymerization and their simple nature. Stajic *et al*., [28] observed the highest laccase activity in the presence of mannitol, glucose and sodium gluconate in two strains of *P. ostreatus*. Off all carbon sources xylan followed by sucrose and starch ensured high laccase activity where as sucrose and starch which provide excellent peroxidase activity. More over the use of these compounds resulted in higher enzyme activity than both lignocellulosic substrates used for submerged fermentation. It was interesting that lactose a poor growth substrate for ligninolytic enzyme production in the test organism (32.65 U/mL).

Effect of Nitrogen sources on ligninolytic enzyme production: In this study, all nitrogen sources tested stimulated growth of *N. kauffmanii* increasing the enzyme activity by 2-3 times when compared to the control medium. In general maximal laccase and peroxidase activities were revealed after 17 days of the test fungus cultivation in media with different nitrogen sources beef extract followed by peptone appeared to be best nitrogen source for ligninolytic enzyme accumulation by the test fungus. However, in the test fungus specific laccase and peroxidase activities prove that the positive effect of additional nitrogen on enzyme accumulation may be attributed simply to a higher enzyme activity. The higher level of enzyme production was observed in organic nitrogen sources when compared to inorganic nitrogen. The highest level of ligninolytic enzyme activity was observed in Peptone after 17th day (64.37 U/mL and 56.97 U/mL) which was followed by beef extract and yeast. Wagner *et al*. [36] showed that the presence of glucose during *G. lucidum* cultivation stimulates growth in the medium with peptone and yeast extract as nitrogen sources. Stajic *et al*. [28] showed the best nitrogen sources for peroxidases production were peptone in a concentration of 0.5% and NH_4NO_3 with a nitrogen concentration of 3mM respectively in *P. ostreatus*.

Effect of Minerals on ligninolytic enzyme production: Different metal ions can enhance or inhibit the growth cause morphological and physiological changes and may affect the reproduction of white rot fungus [37]. Different strains and species of Basidiomycetes differ in their sensitivity towards metals during their growth on lignocellulosic substrates [38]. Among the seven metal ions tested copper sulphate showed maximum laccase (70.51 U/mL) and peroxidase (53.65 U/mL) production on 17th day of incubation. The MnP production by the white rot fungus strain L-25 was enhanced by the addition of Mn^{2+} [39]. Galhaup *et al* [40] and Stajic *et al*., [28] reported that the addition of copper sulphate in various concentrations stimulates laccase production in *T. pubuscens, P. eryngii* and *P. ostreatus*. Ligninase production by *Lentinus squarrosulus* and *Psathyrella atroumbonata* was enhanced by 2 to 12 fold addition of Mn^{2+} and Ca^{2+} to the lignocellulosic waste medium [32]. Copper as micronutrient has a key role as a metal activator,

induces both laccase transcription and plays an important role in laccase production [41]. Our results are comparable to those described by other authors, who have reported increase of laccase production with copper ions [32, 42]. Mäkalä *et al.* [43] observed that laccase activity producing *Phlebia radiata* was increased in media with 1.5 mmol/L of Cu^{2+} while Levin *et al.* [44] found that optimal concentration of copper ions for laccase production by *Trametes trogii* is 11 mmol/L.

Effect of Amino acids on ligninolytic enzyme production: Addition of various amino acids in the medium stimulates the ligninolytic enzyme production. The higher laccase and peroxidase activity (57.25 U/mL and 54.98 U/mL) was recorded on 17th day of incubation with alanine at the concentration of 0.01% in the medium (Table 6). Moderate to good level of enzyme activities were obtained with glycine, asparagine, tryptophan and phenylalanine (Table 6). Amino acids such as glycine, tryptophan and methionine increased lignolytic enzyme production by *Ganoderma* sp.kk-02 up to 3.5- fold. Dhawan and kuhad [45] reported maximum lignolytic enzyme production by *Cyathus bulleri* in the presence of methionine. The addition of various amino acids showed stimulating effects on laccase production by *C. buller* 36 and *Ganoderma* sp. kk-0237 [45]. Levin *et al.* [44] found that growth of white-rot fungus *T. trogii, T. villosa* and *C. versicolor F. antarcticus* was considerably inhibited in media with tryptophan as only nitrogen source in comparison with complex nitrogen sources as peptone or yeast extract. Therefore, the addition of aromatic amino acids in cultivation medium on ligninolytic enzyme production was studied. In Table 6 is shown ligninolytic enzyme activities measured during 17 days of cultivation of white-rot fungus *N. kauffmanii*. The addition of aromatic amino acids (Tyrosine and phenylalanine) did not cause the higher laccase and peroxidase production. Collins *et al.* [46] reported a large increase in LiP production when adding tryptophan to the culture of *Trametes versicolor*, *Phanerochaete chrysosporium* and *Chrysosporium lignorum*.

CONCLUSION

In the present study stimulation of ligninolytic enzyme production of *Neolentinus kauffmanii* was investigated. By proper selection of the initial pH, temperature, different carbon, nitrogen, metal and amino acids for the test fungus growth and target enzyme synthesis may play an important role in the development of an efficient technology. This study emphasizes the need to explore more organisms to evaluate the real potential of fungi producing ligninolytic enzymes. Target enzyme synthesis may play an important role in the development of an efficient technology. The results of the present study allow us to conclude that wild edible *N. kauffmanii* are good candidates for scale-up ligninolytic enzyme production.

Table 1: Effect of pH on ligninolytic enzyme production

Diff. pH	Laccase (U/ml)	Peroxidase (U/ml)
4.5	17.05±1.22	19.52±0.09
5.0	30.7±0.29	28.28±0.98
5.5	46.7±2.32	33.40±4.19
6.0	56.34±0.88	42.37±3.15
6.5	48.32±4.38	47.77±2.33
7.0	22.01±2.12	32.25±3.14
7.5	21.38±1.28	20.5±1.64
8.0	20.56±2.32	16.5±1.64

Data are expressed as mean ±SD (n=4)

Table 2: Effect of Temperature on Ligninolytic enzyme production

Temperature	Laccase (U/mL)	Peroxidase (U/mL)
20^{o}C	32.1±1.25	29.77±1.61
25^{o}C	48.4±0.32	47.79±0.67
30^{o}C	57.8±1.57	34.24±0.82
35^{o}C	26.5±0.66	28.24±4.12
40^{o}C	19.6±0.23	12.50±0.80

Data are expressed as mean ±SD (n=4)

Table 3: Effect of carbon sources on ligninolytic enzyme production

Carbon sources	Laccase (U/mL)	Peroxidase (U/mL)
Control	27.79±0.66	22.63±3.30
Xylan	63.51±2.93	39.31±2.39
Sucrose	57.31±0.32	50.35±2.24
Mannitol	41.82±2.68	42.10±3.81
Starch	53.21±1.95	49.75±5.04
Glycerol	36.65±1.41	28.43±2.71
Maltose	50.6±1.25	43.12±2.89
Lactose	32.65±1.42	41.32±2.68

Data are expressed as mean ±SD (n=4)

Table 4: Effect of Nitrogen sources on ligninolytic enzyme production

Nitrogen sources	Laccase (U/mL)	Peroxidase (U/mL)
Control	24.06±1.90	23.35±2.18
Beef extract	55.6±0.88	51.14±4.28
Peptone	64.37±0.32	56.97±1.82
NH_4NO_3	52.34±0.95	31.98±2.99
Amm. Chloride	49.62±0.89	27.53±1.51
Amm. Nitrate	35.2±2.99	20.01±2.32
Yeast	43.76±1.60	47.87±1.73
Amm. Tartarate	38.4±1.28	35.27±1.04

Data are expressed as mean ±SD (n=4)

Table 5: Effect of Minerals on ligninolytic enzyme production

Metal Ions	Laccase (U/mL)	Peroxidase (U/mL)
Control	34.56±1.11	30.56±1.16
Manganous sulphate	53.21±0.53	56.73±2.11
Fe_2So_4	34.56±1.85	43.32±2.12
Copper sulphate	70.51±1.27	53.65±2.34
Sodium Sulphate	45.63±2.42	45.53±1.82
Aluminium Oxide	34.86±1.41	27.40±2.11
Zinc Sulphate	24.76±1.38	36.63±1.41
Calcium chloride	27.40±2.11	21.76±1.28

Data are expressed as mean ±SD (n=4)

Table 6: Effect of Amino acids on ligninolytic enzyme production

Amino acids	Laccase (U/mL)	Peroxidase (U/mL)
Control	34.78±3.31	30.36±2.96
Alanine	57.25±1.83	54.98±0.77
Glycine	33.84±2.99	46.85±0.58
Asparagine	40.32±2.32	39.39±1.04
Tryptophan	38.27±3.05	25.29±2.34
Tyrosine	21.49±1.28	47.25±1.83
Phenylalanine	16.32±0.23	24.19±4.47
Methionine	23.04±2.89	16.32±0.23

Data are expressed as mean ±SD (n=4)

REFERENCES

1) Arora DS, Gill PK. Effects of various media and supplements on laccase production by some white rot fungi. Bioresource Technology 2001; 77(1): 89- 91.
2) Osma JF, Saravia V, Toca Herrera J, Rodriguez Couto S. Mandarin peelings: the best carbon source to produce laccase by static cultures of *Trametes pubescens*. Chemosphere 2007; 67: 1677-1680.
3) Rosales E, Rodriguez Couto S, Sanroman MA. Reutilization of food processing wastes for production of relevant matabolites. Application to laccase production by *Trametes hirsute*. Journal of food Engineering 2005; 66: 419-423.
4) Stoilova L, Krastanov A, Stanchev V. Properties of crude Laccase from *Trametes versicolor* produced by solid substrate fermentation. Advance Bioscience and Biotechnology 2010; 1(3): 208-215.
5) Asgher M, Iqbal HMN, Asad MJ. Kinetic characterization of purified laccase produced from *Trametes versicolor* IBI-04 in solid state bioprocessing of corncobs. Bioresources 2012; 7(1): 1171-1188.

6) Qumar Yasmeen, Muhammad Asgher, Munir A Sheikh, Haq Nawaz. Optimization of ligninolytic enzymes production through Response Surface Methodology. Bioresources 2013; 8(1): 944-968.
7) Pandeng Zhou, Chaoying Fu, ShiyuFu, Huaiyu Zhan. Purification and characterization white laccase from the white rot fungus *Panus conchatus*. Bioresources 2014; 9(2): 1964-1976.
8) Weenink XO, Punt PJ, Van den Hondel CAMJJ, Ram AFJ. A new method for screening and isolation of hypersecretion mutants in *Aspergillus niger*. Applied Microbiology and Biotechnology 2006; 69: 711–717.
9) Bailey MJ, Adamitsch B, Rautio J, VonWeymarn N, Saloheimo M. Use of a growth-associated control algorithm for efficient production of a heterologous laccase in *Trichoderma reesei* in fed-batch and continuous cultivation. Enzyme Microbial Technology 2007; 41: 484–491.
10) Cho NS, Wilkolazka AJ, Staszczak M, Cho HY, Ohga S. The role of laccase from white rot fungi to stress conditions. Journal of the Faculty of Agriculture 2009; 54(1): 81-83.
11) Kirk TK, Farrell RL. Enzymatic "combustion": The microbial degradation of lignin. Annual Review of Microbiology 1987; 41(1): 465-501.
12) Yang X, Wang J, Zhao X, Wang Q, Xue R. Increasing manganese peroxidase production and biodecolorization of Triphenyl methane dyes by novel fungal consortium. Bioresource Technology 2011; 102(22): 535-541.
13) Martorell MM, Hipolito PF, De Figueroa LIC. Dye-decolourizing yeasts isolated from Las Yungas rainforests. Dye assimilation and removal used as selection criteria. Intl. Biodeterior and Biodegradation 2012; 66(1): 25-32.
14) Vikineswary S, Abdullah N, Renuvathani M, Sekaran M, Pandey A, Jones GEB. Productivity of laccase in solid substrate fermentation of selected agro-residues by *Pycnoporus sanguineus*. Bioresource Technology 2006; 97(1): 171-177.
15) Alcalde M. Laccase: Biological functions, molecular structure and industrial applications. In: Polaina, J. and Maccabe, A.P. eds. Industrial enzymes: Structure, function and applications 2007; 26: 461-476.
16) Hammel KE, Cullen D. Role of fungal peroxidases in biological ligninolysis. Current Opinion in Plant Biology 2008; 11(3): 349-355.
17) Tien M, Kirk TK. Lignin peroxidase of *Phanerochaete chyrosprium*. Methods Enzyme 1998; 161: 238–248.
18) Pointing SB, Pelling AL, Smith GJD, Hyde KD, Reddy A. Screening of basidiomycetes and xylariaceous fungi for lignin peroxidase and laccase gene-specific sequences. Mycological Research, 2005; 109(1): 115-124.
19) Johannes C, Majcherczyk A, Hüttermann A. Degradation of anthracene by laccase of *Trametes versicolor* in the presence of different mediator compounds. Applied Microbiology and Biotechnology 1996; 46: 313-317.
20) Field JA, Vledder RH, Van Zelst JG, Rulkens WH. The tolerance of lignin peroxidase and manganese-dependent peroxidase to miscible solvents and the *in vitro* oxidation of anthracene in solvent: Water mixtures. Enzyme Microbiol Technology 1996; 18: 300-308.
21) Mikiashvili N, Elisashvili V, Wasser S, Nevo E. Carbon and nitrogen sources influence the ligninolytic enzyme activity of *Trametes versicolor*. Biotechnology Letter 2005; 27: 955–959.
22) Kapich AN, Prior BA, Botha A, Galkin S, Lundell T, Hatakka A. Effect of lignocellulose-containing substrate on production of ligninolytic peroxidase in submerged cultures of *Phanerochaete chrysosporium* ME-446. Enzyme Microbiol Technology 2004; 34: 187-195.
23) Wen X, Jia X, Li J. Enzymatic degradation of tetracycline and Oxytetracyclin by crude manganese peroxidase prepared from *Phanerochaete chrysosporium*. Journal of Hazardous Material 2010; 177 (1-3): 924-928.
24) Stajic M, Kukavica B, Vukojevic J, Simonic J, Veljovic Jovanovic S, Duletic Lausevic S. Wheat straw conversion by enzymatic system of *Ganoderma lucidum*. Bioresources 2010; 5: 2362-2373.
25) Iqbal HMN, Asgher M, Bhatti HN. Optimization of physical and nutritional factors for synthesis of lignin degrading enzymes by novel strain of *Trametes versicolor*. Bioresources 2011; 6(2): 1273-1287.
26) Mishra A, Kumar S, Pandy AK. Laccase production and simultaneous decolorization of synthetic dyes in unique inexpensive medium by new isolates of white rot fungus. Intl. Biodeterior. Biodegradation 2011; 65(3): 487-493.
27) Couto R, Lopez SE, Sanroman MA. Utilization of grape seeds for Laccase production in solid-state fermentors. Journal of Food Engineering 2006; 74(2): 263-267.
28) Stajic M, Persky L, Hadar Y, Friesem D, Duletic Lausevic S, Wasser SP, Nevo E. Effect of copper and manganese ions on activities of laccase and peroxidase in three Pleurotus species grown on agricultural wastes. Applied Biochemical and Biotechnology 2006; 168: 87-96.
29) Mester T, Field JA. Optimization of manganese peroxidase production by the white rot fungus *Bjerkandera* ostreatus strain BOS55. FEMS Microbiology Letter 1997; 155: 161-168.
30) Gill PK, Arora DS. Effect of culture conditions on manganese peroxidase production and activity by some white rot fungi. Journal of Industrial Microbiology and Biotechnology 2003; 30: 28–33.
31) Tripath M, Mishra KAS, Mishra AS, Vaithiyanathan S, Prasad R, Jakhmola RC. Selection of white rot Basidiomycetes for bioconversion of mustard (*Brassica compestris*) straw under SSP into energy substrates for rumen microorganisms. Letter Applied Microbiology 2008; 46(3): 364-370.
32) Galhaup C, Wagner H, Hinterstoisser B, Haltrich D. Increased production of laccase by the wood-degrading Basidiomycetes *Trametes pubescens*. Enzyme and Microbiol Technology 2002; 30: 529-536.
33) Mansure M, Suarez T, Fernandez-Larrea JB, Brizuella MA, Gonzales AE. Identification of laccase gene family in the new lignin-degrading basidiomycetes CECT 20197, Applied and Environmental Microbiology 1997; 63: 2637-2646.
34) Martin JF, Demain AC. Control of antibiotic biosynthesis. Microbial Review 1986; 44: 230-251.
35) Leifa F, Andra TS, Ashok P, Carlos RS. Effect of nutritional and environmental conditions on production of exopolysaccharide of *Agaricus brasiliensis* by sub-merged fermentation and its antitumor activity. LWT Journal 2007; 40: 30-35.
36) Wagner R, Mitchell DA, Sassaki GL, Amazonas MALA. Links between morphology and physiology of *Ganoderma lucidum* in submerged culture for the production of exopolysaccharide. Journal of Biotechnology 2004; 114: 153-164.
37) Wuyep P, Khan A, Nok AJ. Production and regulation of lignin degrading enzymes from *Lentinus squarrosulus* (mant) Singer and *Psathyrella atroumbonata*. African Journal of Biotechnology 2003; 2(11): 444-447.
38) Sathiya-moorthi P, Periyar-selvam S, Sasikalaveni A, Murugesan K, Kalaichelvan. Decolorization of textile dyes and their effluents using white rot fungi. African Journal of Biotechnology 2007; 6(4): 424-429.
39) Fujihara S, Hatashita M, Sakurai A, Sakakibara M. Production of Manganese peroxidase by white rot fungi from potato processing waste water. Role of amino acids on biosynthesis. African Journal of Biotechnology 2010; 9(5): 725-731.
40) Galhaup C, Goller S, Peterbauer K, Clemens Strauss T, Haltrich D. Characterization of the major laccase isoenzyme from *Trametes pubescens* and regulation of its synthesis by metal ions. Microbiology 2003; 148: 2159-2169.

41) Palmieri G, Giardina P, Bianco C, Fontannella B, Sannia G. Copper induction of laccase isoenzymes in lignonolytic fungus *Pleurotus ostreatus*. Applied Environmental Microbiology 2000; 66: 920-924.
42) Fonseca MI, Shimizu E, Zapata PD, Villalba LL. Copper inducing effect on laccase production of white rot fungi native from Misioes (Argentina). Enzyme Microbiol and Technology 2010; 46: 534-539.
43) Mäkalä MR, Lundell T, Hatakka A, Hildén K. Effect of copper, nutrient nitrogen, and wood-supplement on the production of ligninmodifying enzymes by the white-rot fungus *Phlebia radiata*. Fungal Biology 2013; 117(1): 62-70.
44) Levin L, Hermann C, Papinutti VL. Optimization of lignocellulolytic enzyme production by the white-rot fungus *Trametes trogii* in solid-state fermentation using response surface methodology. Biochemical Engineering Journal 2008; 39(1): 207-214.
45) Dhawn S, Kuhad RC. Effect of amino acids and vitamins on laccase production by the bird's nest fungus *Cyathus bulleri*. Bioresource Technology 2002; 84: 35–38.
46) Collins PJ, Field JA, Teunissen P, Dobson AD. Stabilization of lignin peroxidases in white rot fungi by tryptophan. Applied and Environmental Microbiology 1997; 63(7): 2543-2548.