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Research article

ISOLATION AND PHYSICO-CHEMICAL CHARACTERIZATION OF EXTRACELLULAR LIGNO-CELLULOLYTIC ENZYMES OF *PLEUROTUS PULMONARIUS* IN SUBMERGED FERMENTATION

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ABSTRACT: *Pleurotus pulmonarius*, a member of oyster mushroom can produced lignocellulosic enzymes laccase, peroxidise and cellulase in liquid potato-dextrose medium in submerged stationary condition. The lignocellulolytic activities were assayed using the extracellular culture filtrate which was partially purified using 0-80% ammonium sulphate saturation. Different physico-chemical studies were performed using the partially purified culture filtrate. The fungus produced more laccase and peroxidase than the cellulase. The optimum laccase production was found on 17th day whereas cellulase & peroxidase productions were found on 9th& 10th day, respectively. K_m of laccase is 4.1mM against guaiacol and 1.25 mM against *o*-dianisidine whereas K_m of peroxidase was 0.72mM and cellulase was 0.06 mM. Optimum pH of laccase was 6.0 but for peroxidase and cellulase it was 7.0. The temperature optima of cellulase (50^{0}C) was more than laccase (40^{0}C) and peroxidase (30^{0}C). All the lignocellulosic enzymes showed a wide range of temperature and pH stabilities. Laccase and peroxidase were fully inhibited by NaCl but it was not so effective against cellulase. *P. pulmonarius* showed higher ligninolytic (Laccase and peroxidase) activity than cellulolytic (cellulase) activity. The lignocellulosic enzymes isolated from submerged fermentation of *P. pulmonarius* might be industrially significant as they showed a wide range of temperature and pH stabilities.
Key Words: Cellulase, Laccase, Lignocellulase, Oyster mushroom, Peroxidase

INTRODUCTION

The edible oyster mushroom *Pleurotus* is a versatile genus of white-rot basidiomycete comprising a number of species most of which are commercially cultivated. Members of this genus grow on a wide range of lignocellulosic substrates including cereal straws, sugarcane bagassae, different common weed plants, wood logs, saw dust, banana pseudostem etc. (Naraian *et al.,* 2005; Das & Mukherjee, 2007). All the reported species of *Pleurotus* are not only edible but also well recognized for their complexity of the enzymatic system including lignocellulolytic activities.

The relevance of lignocellulolytic enzymes are ever increasing due to their application in different processes in industry and biotechnology (Desai & Nityanand, 2011; Dos Santos Bazanella *et al.,* 2013). Lignocellulolytic enzymes have various use in a large areas including the agriculture, bioconversion, food, pulp and paper, textile, cosmetic, pharmaceutical and chemical industries etc. (Kuhad *et al.,* 2007). The lignocellulolytic enzymes are one of the most important facets for the biodegradation of xenobiotics, organo-pollutants, synthetic dyes and different industrial contaminants (Cohen *et al.,* 2001). Thus conversion of agro industrial wastage to beneficial or at least less harmful level will have environmental importance (Zadrazil, 2000; Peixoto-Nogueira *et al.,* 2009). The white-rot basidiomycetes are strong decomposers of phenolic and non-phenolic pollutants due to their capability to synthesize lignocellulolytic extracellular enzymes (Maganhotto de Souza Silva *et al.,* 2005; Eichlerova *et al.,* 2006; Chowdhury *et al*., 2014).

The genus *Pleurotus* includes species that belong to group of white-rot fungi and have unique ability to produce extracellular lignocellulolytic enzymes including laccase and Mn peroxidase (Das *et al.,* 2000,2011; Stajic *et al.,* 2006), xylanase (Elisashvili *et al.,* 2008), CMCase, β-glucosidase and β-xylosidase (Kuhad *et al.,* 2007). These enzymes have shown enormous biotechnological potential as they can be used widely for lignocellulose degradation and detoxification of agro-industrial residuals with high phenolic contents (Adhikari *et al.,* 2013; Mata *et al.,* 2005). In the present work three lignocellulolytic enzymes (laccase, EC. 1.10.3.2; manganese peroxidase, EC 1.11.1.13; and carboxymethyl cellulase (CMCase), EC 3.2.1.4) have been isolated from *P. pulmonarius* in stationary condition and various physico-chemical properties of these enzymes are studied which may be beneficial for future biotechnological application of these enzymes.

MATERIALS AND METHODS

Mushroom strain: *Pleurotus pulmonarious* (MTCC 1805) was collected from Microbial Type Culture Collections, Institute of Microbial Technology, Chandigarh, India and was maintained on potato-dextrose agar (pH 7.0) containing 20% potato extract with 2% dextrose and 2% agar as reported earlier (Das *et al.,* 1997).

Inoculum source and Liquid submerged fermentation (SMF)

An inoculum was taken from the periphery of colonies growing on PDA for 7days. The production of enzymes was studied in liquid medium containing potato extract (20%) with 2% carbon source (dextrose) at pH-7.0 in stationary condition at 25±2 ºC. The fungus was cultured in stationary condition and aliquots of culture filtrate were collected. The aliquots were taken for one day interval from 6^{th} to 21^{st} day and the volume of the culture medium was adjusted to its original volume with fresh medium after each collection. Extracellular culture filtrates were assayed for enzyme/protein activity after mycelium was removed by filtration/ centrifugation.

Laccase Activity Assay

Laccase activity was performed spectrophotometrically at 25˚C with guaiacol as the substrate unless otherwise mentioned (Das *et al.,* 1997; Ray *et al*., 2012). The reaction mixture generally contained 0.1 ml 1M sodium acetate buffer pH 5.0. 0.2 ml culture filtrate 0.5 ml 20 mM guaiacol (in 20% acetone). The volume was adjusted to 1 ml. The absorbance was taken in 470 nm for guaiacol (ε=6740 $M^{-1}cm^{-1}$). In some cases laccase activity was determined with *o*- dianisidine (Das *et al.,* 1997; Ray *et al*., 2012). Enzyme activity was expressed in International Unit.

Peroxidase Activity (POA) Assay

Peroxidase activity was estimated spectrophotometrically at 25˚C where guaiacol and H_2O_2 was used as substrates. In presence of H_2O_2, guaiacol formed into oxidized guaiacol. The rate of formation of guaiacol dehydrogenated product is a measure of the peroxidase activity and can be assayed spectrophotometrically at 436 nm. The reaction mixture generally contained 1.4 ml 0.1M sodium phosphate buffer (pH-7.0), 0.03 ml 20 mM guaiacol (in 20% acetone), 0.02 ml H_2O_2, and 0.5 ml culture filtrate. The absorbance was taken at 436 nm (Putter, 1974). Enzyme activity was expressed in International Unit.

Cellulase Activity Assay

The production of reducing sugar (glucose) was measured by dinitrosalicylic acid (DNSA) method due to cellulolytic activity. For carboxymethyl cellulase activity, assay mixture was prepared by adding 0.5 ml culture filtrate and 0.5 ml of 1% (w/v) carboxymethyl cellulose (Merck) solution to 1 ml of 100 mM sodium phosphate buffer pH- 7.0 and followed by 3.0 ml 3,5- dinitrosalycylic acid reagent (Ghosh, 1987). The mixture was incubated at 22 ± 1^0 C (unless otherwise mentioned) for 30 min and 1 ml of 40% (w/v) Rochelle salt (Sodium- potassium tartarate) solution was added to stop reaction and kept in boiling water bath for 15 min to develop color. The intensity of red color was measured spectrophotometrically at 540 nm (Miller, 1959). Enzyme activity was expressed in International Unit.

Protein estimation

Protein concentration was determined according to the method of Lowry (1951) using bovine serum albumin as standard.

Partial purification of Enzymes

The enzymes present in the culture filtrate were partially purified after 0 - 80% ammonium sulphate precipitation, followed by extensive dialysis against 0.01mM acetate buffer (pH-5.0) (Das *et al.,* 1997).

Physico-chemical studies of laccase, peroxidase & cellulase enzymes

The physico-chemical studies of laccase, peroxidase & cellulase enzymes were done using their specific substrate (guaiacol/*o*-dianisidine) for laccase, dehydrogenated guaiacol (guaiacol and H_2O_2) for peroxidase, carboxymethyl cellulose for cellulase, unless otherwise mentioned.

Determination of pH optima

The pH optima of laccase, peroxidase and cellulases were determined in the pH range of 3.0 to 8.0 with an interval of 1.0 unit. 20 U of enzyme was incubated with respective substrate at 30^0C in different pH conditions. The buffer systems used were : 0.1 M Na-citrate buffer (pH 3.0-4.0), 0.1 M Na-acetate buffer (pH 4.0-5.0), 0.1M Na-phosphate buffer (pH 6.0-8.0).

Determination of pH stability

pH stability was estimated by incubating 20U of each laccase, peroxidase & cellulase at different pH values (3.0-8.0). The incubation was carried out at 30^0 C up to 4h. The buffer systems were used as the same as mentioned during determination of pH optima. The highest enzyme activity obtained, was taken to be 100% and other values were expressed as percentage relative to the highest value.

Determination of optimum temperature

For determination of optimum temperature for laccase, peroxidase & cellulase enzymes, assay mixture were incubated at different temperature in the range of 30^0 – 70^0 C in a temperature regulated spectrophotometer (Systronics). Prior to enzyme-substrate reaction, the substrate and buffer were also incubated separately so that at the time of reactions all the components could reach the specific temperature. 20 U enzyme was used to start the reaction.

Determination of temperature stability

Temperature stability of the laccase, peroxidase & cellulase were studied by incubating 20 U of each enzymes as well as other reaction mixtures at different temperatures in the range of 30-70^0C for up to 4h. The samples were taken out at different time intervals and assayed at 30^0 C with specific substrates.

Study of some effector molecules

Effects of some metal ions and effector molecules were determined by co-incubating the respective compounds at 100 mM concentration with the respective substrate and enzyme (20U) solution followed by measurement of laccase, peroxidase & cellulase activities by their specific substrate.

RESULTS AND DISCUSSION

The plant biomass is mainly composed of cellulose, hemicellulose and lignin (generally known as lignocelluloses) that are strongly bonded with each other by non-covalent linkages, covered about half of the photosynthetic matter. *Pleurotus* spp. like other white rot fungi produced a number of oxidative and hydrolytic enzymes to degrade the lignocellulosic substances within which cellulase, laccase and manganese peroxidases are important **(**Kuhad et al, 1993).

P. pulmonarius produced laccase, peroxidase and cellulase in simple potato dextrose media in stationary condition (Fig.1). The composition of culture media and the culture condition (shaking/stationary have immense effect on extracellular enzyme production in filamentous fungi (De Souza et al 2004). The optimum laccase was produced in stationary condition in 17^{th} day whereas peroxidase in 10^{th} day but cellulase enzyme showed the least time i.e 9^{th} day (Fig.1). Specific activity of laccase is 270 U/mg proteins whereas those of peroxidase and cellulase are 0.35 U/mg protein and 13.51 U/mg proteins, respectively. De Souza *et al*. (2004) reported that most of the laccase was produced after exhaustion of carbon and nitrogen sources. Stajic *et al.* (2007) reported that lignocellulosic enzymes were better produced in solid state fermentation than the submerged fermentation. Dos Santos Bazanella *et al.* (2013) reported 2.86 U/ml laccase in solid state fermentation using wheat bran as substrate and 2.2 U/ml peroxidase activity using pineapple peel as substrate. In present condition peroxidase enzyme was produced in highest amount (19.9U/ml) whereas cellulase was produced in least amount (0.77 U/ml). 15.2 U/ml laccase was produced in submerged fermentation in simple potato-dextrose medium. Snajdr and Baldrian (2007) showed highest laccase activity (72$\pm$ 13 UL^{-1}) in 35 days. Specific activity of laccase is highest (270 U) followed by the specific activity of cellulase (13.51). Specific activity of peroxidase is least i.e. 0.35 U/mg protein. Rana and Rana (2011) reported that *Pleurotus* spp. showed more ligninase activities than cellulase activity. De Souza *et al.* (2002) reported that laccase is the best lignocellulosic enzyme in *P. pulmonarius*.

The physico-chemical studies of any enzyme are very much important to characterize it. Laccase, peroxidase & cellulases are tested for their substrates. Fig 2 showed the K_m values. K_m value of laccase is 4.1 mM against guaiacol and V_{max} is 7.46 µM min^{-1} and 1.25 mM against *o*-dianisidine and V_{max} is 20 µM min^{-1}. K_m value of peroxidase is 0.72 mM and V_{max} is 44 µM min^{-1}. K_m of cellulase is least among the three tested enzymes. The value is 0.06 mM and V_{max} is 0.062 µM min^{-1}.

The pH optima and temperature optima of laccase is pH-6 and 40^0C. De Souza *et al.* (2002) reported the optimum temperature and pH of *P. pulmonarius* is 50^0C and 6.5, respectively. *P. pulmonarius* laccase showed wide range of pH and temperature stability (Fig. 3 & 4).

Like other *Pleurotus* sp. *P. pulmonarius* also produces heat stable laccase enzyme which show optimum activity in acidic pH. Here laccase is full stable in pH 7.0 and comparatively less stable in alkaline pH than acidic pH (Fig. 3a). De Souza *et al.* (2002) reported that laccase of *P. pulmonarius* was more stable in alkaline pH than acidic pH. Possibly the laccase isolated in solid state fermentation is different than that was isolated in submerged fermentation. The K_m and optimum temperature of this laccase was different from *P. pulmonarious* laccase isolated from solid state fermentation (De Souza and Peralta, 2003). Mn- peroxidase of *P. pulmonarius* is comparatively more stable in pH 6.0 but stability looses as it approaches to higher acidic or alkaline pH (Fig.3b). Cellulase also showed better stabilities in pH 6.0 (Fig. 3c).

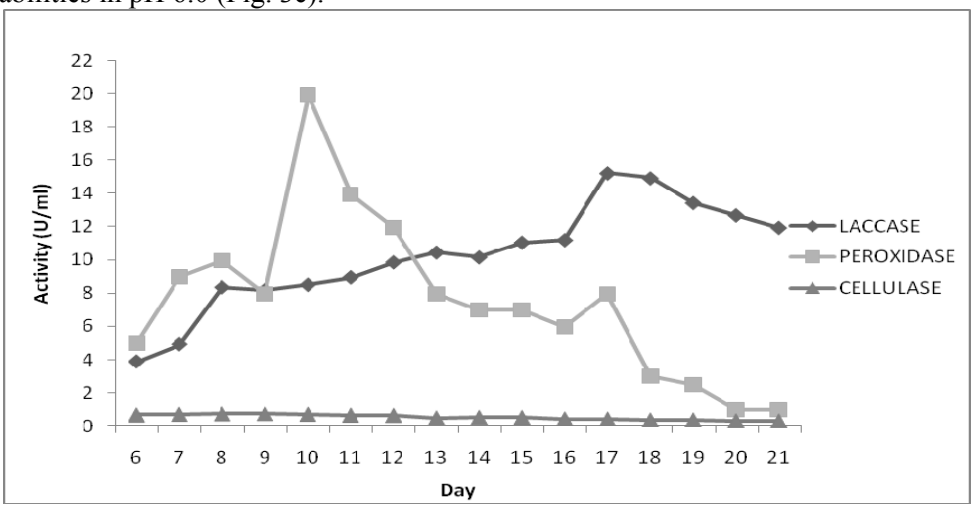
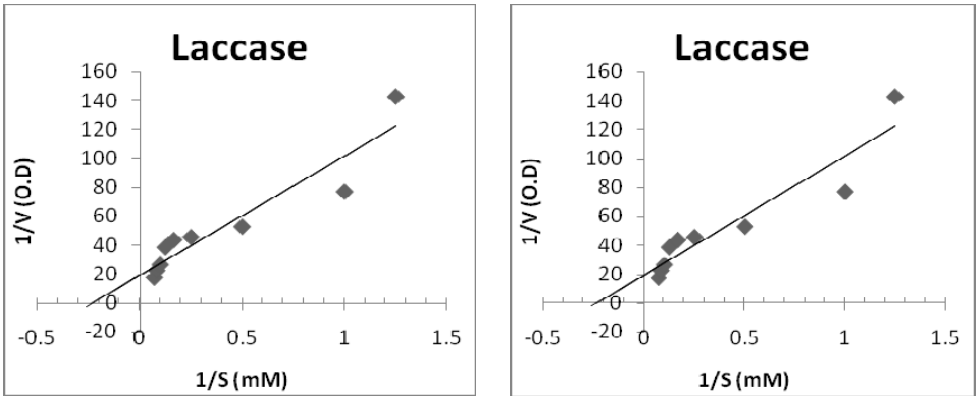
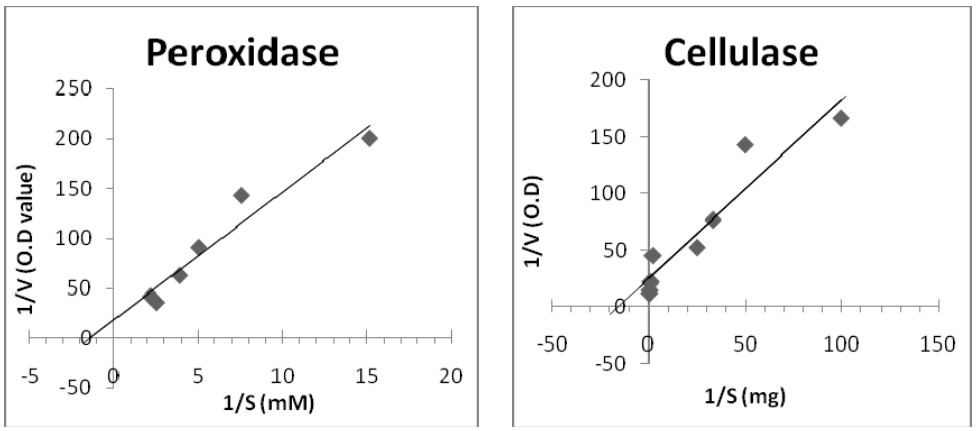


Fig. 1. Production of lignocellulosic enzymes of *P. pulmonarius* in Potato-Dextrose medium in stationary condition.



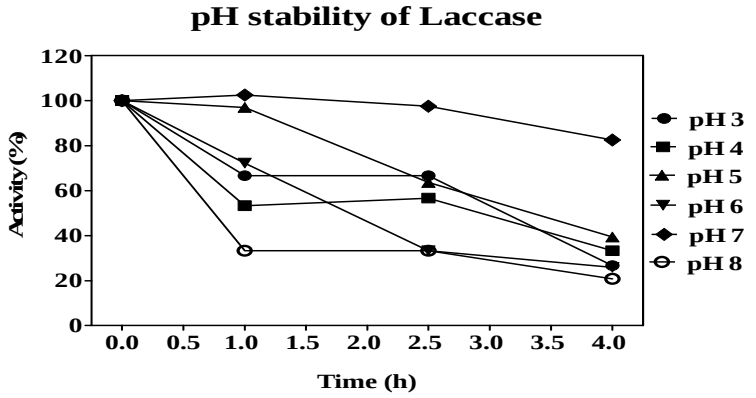
a_1 **a_2**



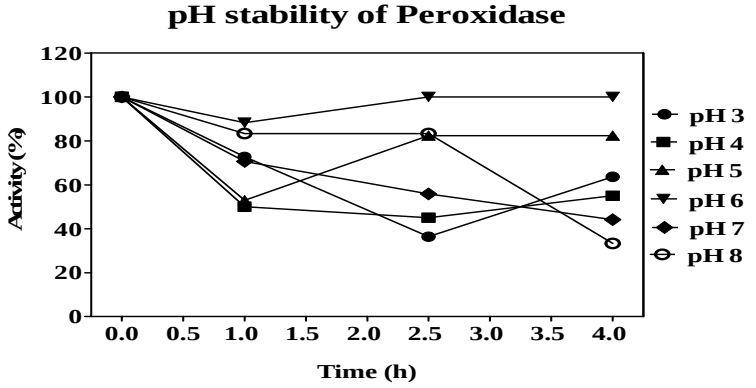
b **c**

Fig. 2. Determination of K_m of different lignocellulosic enzymes of *P. Pulmonarius*. (a_1) K_m of laccase against guaiacol (a_2) K_m of laccase against *o*-dianisidine (b) K_m of peroxidase and (c) K_m of cellulase.

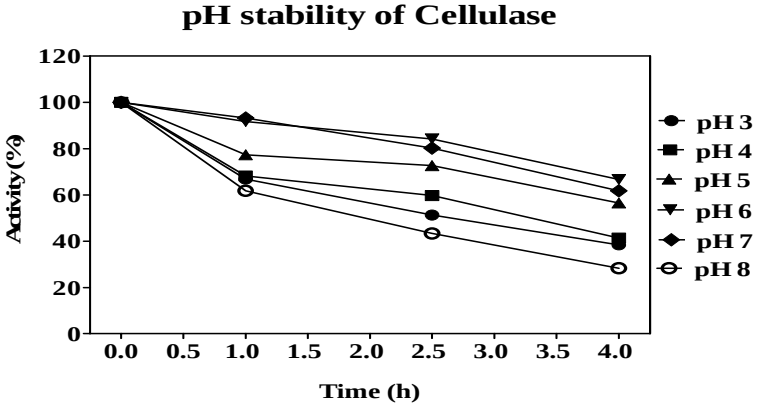
Peroxidase and cellulase of this species show same optimum pH (7.0). The temperature optima of peroxidase is 30^0C and was more or less 80% stable in 30^0C & 40^0C up to 2.5 h. (Fig.4b). Peroxidase of *P. pulmonarius* is not stable in high temperature whereas cellulase shows more than 60% stability in 30^0-50^0C up to 4h (Fig.4c.) again the optimum temperature of cellulase is 50^0C which indicate cellulase of *P. pulmonarius* as a thermostable enzyme. According to most of the researchers lignocellulosic enzymes are generally more stable in a wide range of pH and temperature which make them suitable to fight against the recalcitrant materials of the environment (Kuhad *et al.*, 2007).



3a



3b

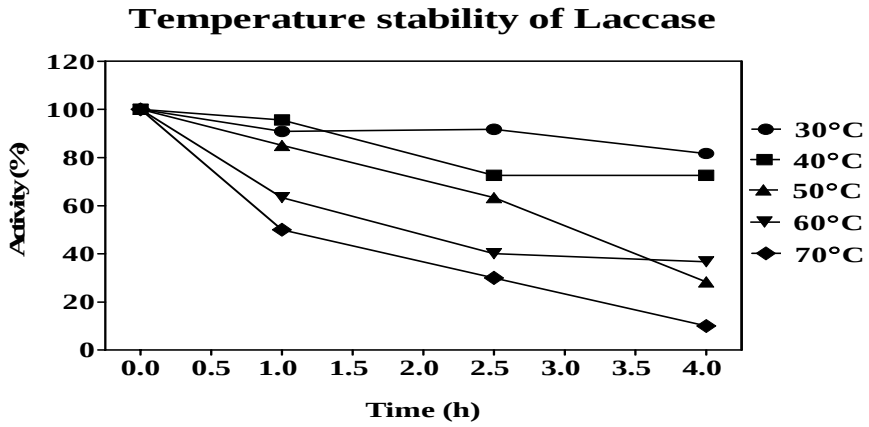


3c

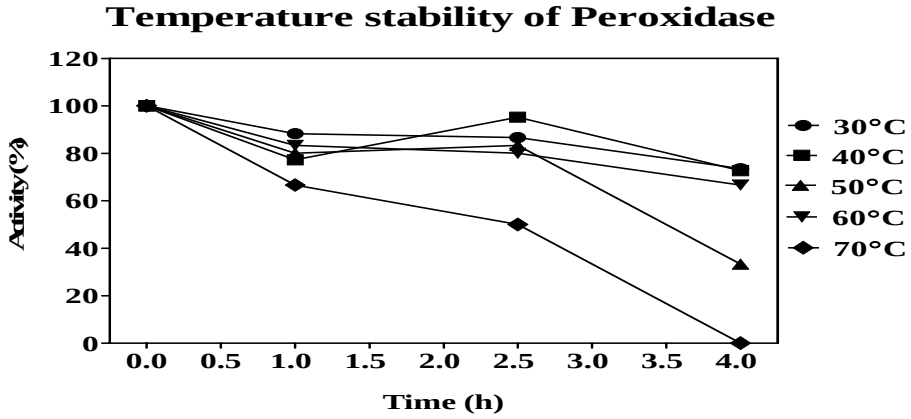
Fig.3a. pH stability of laccase of *P. pulmonarius*. pH stability is estimated by incubating 20U enzyme at different pH value(3.0-8.0). The incubation is carried out at 30^0C up to 4h.

Fig. 3b. pH stability of Peroxidase of *P. pulmonarius*. pH stabilityis estimated by incubating 20U enzyme at different pH value(3.0-8.0). The incubation is carried out at 30^0C up to 4h.

Fig. 3c. pH stability of Cellulase of *P. pulmonarius*. pH stability is estimated by incubating 20U enzyme at different pH value(3.0-8.0). The incubation is carried out at 30^0C up to 4h.



4a



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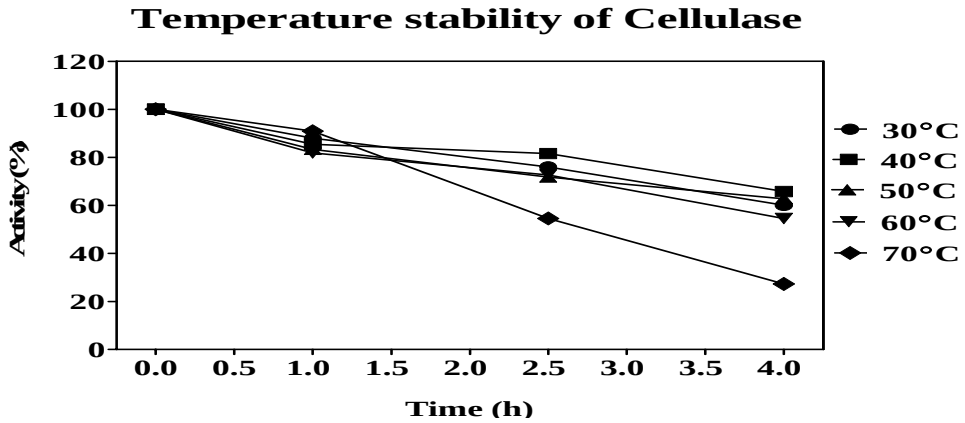
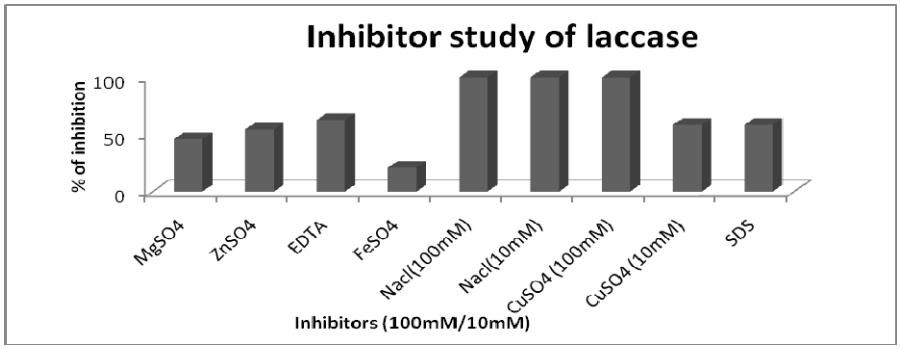


4c

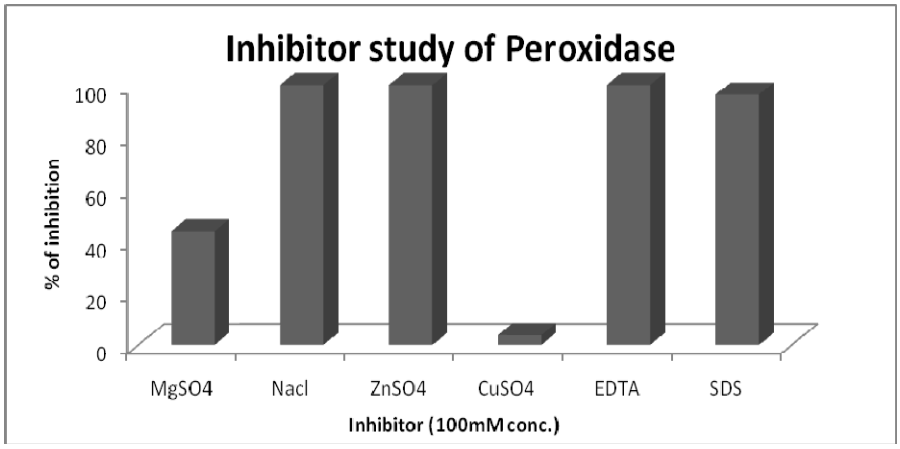
Fig.4a. Temperature stability of laccase of *P. pulmonarius*. Temperature stability is estimated by incubating 20U enzyme at different temperature value (30°C -70°C). The incubation is carried out at pH-5 up to 4h.

Fig. 4b.Temperature stability of peroxidase of *P. pulmonarius*. Temperature stability is estimated by incubating 20U enzyme at different temperature value (30°C -70°C). The incubation is carried out at pH-7 up to 4h.

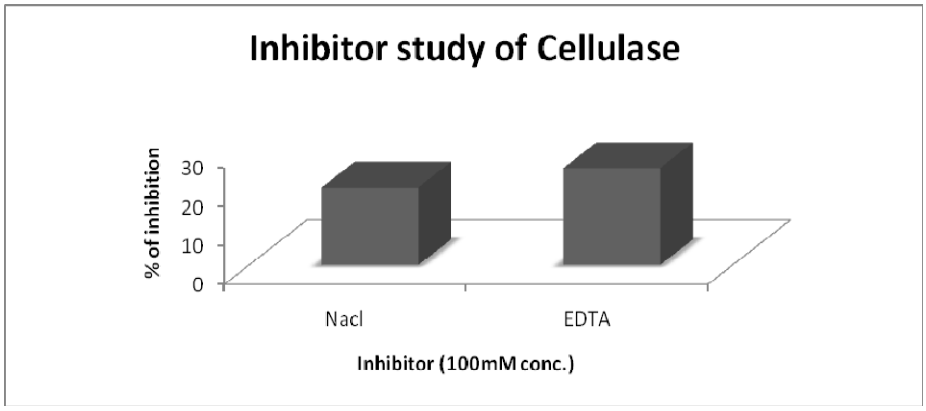
Fig. 4c.Temperature stability of cellulase of *P. pulmonarius*. Temperature stability is estimated by incubating 20U enzyme at different temperature value (30°C -70°C). The incubation is carried out at pH-7 up to 4h.



5a



5b



5c

Fig. 5a.Effect of different inhibitor on laccase activity. Inhibitor study is done by incubating 20U enzyme with different inhibitor molecules in100mM conc. unless otherwise mentioned.

Fig. 5b.Effect of different inhibitor on peroxidise activity. Inhibitor study is done by incubating 20U enzyme with different inhibitor molecule in100mM concentration.

Fig. 5c. Effect of different inhibitor on cellulase activity. Inhibitor study is done by incubating 20U enzyme with different inhibitor molecule in 100mM concentration.

Different effector molecules can act as inducer or inhibitor of enzyme activities (El- Shora et al., 2008). Laccase activities are studied in presence of different effector molecules like $MgSO_4$, $ZnSO_4$, EDTA, $FeSO_4$, NaCl, $CuSO_4$ and SDS. NaCl shows 100% inhibition not only in 100 mM conc. but also in 10 mM conc., $CuSO_4$ shows 100% inhibition in 100 mM conc. $MgSO_4$, $ZnSO_4$, EDTA and SDS show 40-60% inhibition in 100 mM conc. (Fig. 5a.).

NaCl, $ZnSO_4$, EDTA, show 100% inhibition of peroxidase activity. SDS shows 96% inhibition. $CuSO_4$ shows least inhibition (Fig. 5b.). In case of cellulase only 25 and 20% activities are inhibited in presence of EDTA and NaCl, respectively (Fig. 5c). The effect of other molecules is not detected in present experimental conditions.

Though laccase is considered as a Cu- containing enzyme and the production is induced in some cases in presence of lower conc. of $CuSO_4$ as in *Volvariella* (Chen *et al.,* 2003) but here $CuSO_4$ act as an inhibitor, possibly due to its high conc. (100mM) in reaction mixture (Fig. 5). NaCl shows 100% inhibition even in 10 mM conc. Though Das *et al.,* (2000) showed 50 mM NaCl inhibit about 45% laccase activity in *P. florida*. SDS shows 96% inhibition but $CuSO_4$ shows more or less no inhibition in 100 mM conc. Again NaCl showed only 20% inhibition of cellulase activity and EDTA showed 25% inhibition of cellulase activity in *P. pulmonarius.*

Like other *Pleurotus* species, *P. pulmonarius* also produces lignocellulosic enzymes like laccase. peroxidase and cellulase extracellularly in stationary submerged condition. This white rot fungi uses its enzymes in biological pretreatment of lignocellulosics to obtain raw materials and fermentable sources for its growth and development. The lignocellulosic enzymes isolated from submerged fermentation of *P. pulmonarius* might be useful for further possible industrial exploitation as they showed a wide range of temperature and pH stabilities. The more detailed study of lignocellulosic enzymes may open other avenues which can add not only the art of knowledge but also the other physiological/biotechnological applications of these enzymes.

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REFERENCES

Adhikari D, Das N. and Naskar S. (2013). Novel fungal enzymes in environmental remediation. In P. K. Bharati and A. Chauhan(Eds) Environmental biotechnology and application. Discovery Publishing House Pvt. Ltd. New Delhi. pp 71-103.

Chen S, Ma D, Ge W. and Buswell J.A. (2003). Induction of laccase activity in the edible straw mushroom, *Volvariella volvacea*. FEMS Microbiol. Lett.: Vol. 218,145-148.

Chowdhury P, Hari R, Chakraborty B, Mandal B, Naskar S. and N. Das (2014). Isolation, culture optimization and physico-chemical characterization of laccase enzyme from *Pleurotus fossulatus*. Pak. J. Biol.Sci.: Vol.17, 173-181.

Cohen R, Hadar Y. and Yarden O. (2001). Transcript and activity levels of different *Pleurotus ostreatus* peroxidases are differently affected by Mn^{2+}. Environ. Microbiol.:Vol. 3, 312-322.

Das N. and Mukherjee M. (2007). Cultivation of *Pleurotus ostreatus* on weed plants. Bioresource Technol.: Vol. 98, 2723- 2726.

Das N, Naskar S, Chowdhury P, Pasman B. and Adhikari D. (2011). Experimental evidence for presence of a growth regulating extracellular laccase in some *Pleurotus* species. Res. J. Microbiol.: Vol. 6, 496-502.

Das N, Sengupta S. and Mukherjee M. (1997). Importance of laccase in vegetative growth of *Pleurotus florida.* Appl. Environ. Microbiol. Vol. 63, 4120-4122.

Desai, S.S. and Nityanand, C. (2011). Microbial laccases and their applications: A review. Asian J. Biotechnol.: Vol.3, 98-124.

De Souza C.G, Zilly A. and Peralta R.M. (2002). Production of laccase as the sole phenol oxidase by a Brazilian strain of *Pleurotus pulmonarius* in solid state fermentation. J. Basic Microbiol: Vol. 42, 83-90.

De Souza C.G. and Peralta R.M. (2003). Purification and characterization of the main laccase produced by the white rot fungus *Pleurotus pulmonarius* on wheat bran solid state medium. J. Basic Microbiol.; Vol. 43, 278-286.

De Souza C.G, Tychanowicz G.K, De Souza D.F. and Peralta R.M. (2004). Production of laccase isoforms by *Pleurotus pulmonarius* in response to presence of phenolic and aromatic compounds. J. Basic Microbiol: Vol. 44,129-136.

Dos Santos Bazanella G.C, De Souza D.F. and Castoldi, R. (2013). Production of laccase and manganese peroxidase by *Pleurotus pulmonarius* in solid state cultures and application in dye decolorization. Folia Microbiol: Vol.58, 641-647.

Eichlerova I, Homolka L. and Nerud F. (2006). Ability of industrial dyes decolorization and ligninolytic enzymes production by different *Pleurotus* species with special attention on *Pleurotus calyptratus*, strain CCBAS 461. Process Biochem: Vol.41, 941-946.

Elisashvili V, Kachlishvili E. and Penninckx M. (2008). Effect of growth substrate, method of fermentation and nitrogen source on lignocellulose-degrading enzymes production by white-rot basidiomycetes. J. Ind. Microbiol. Biotechnol: Vol.35**,** 1531-1538.

El-Shora, H.M., Youssef M.M. and Khalaf A.S. (2008). Inducers and inhibitors of laccase from *Penicillium*. Biotechnol.: Vol. 7,35-42.

Ghosh T.K. (1987) Measurement of cellulase activities**.** Pure Appl. Chem**.***:* Vol. 59, 257-268.

Kuhad R.C. and Shing A. (1993). Lignocellulose biotechnology: Current and future prospects. Crit. Rev. Bitotecnol.: Vol. 13,151-172.

Kuhad R.C, Kuhar S, Kapoor M, Sharma K.K. and Singh, A. (2007). Lignocellulolytic microorganisms, their enzymes and possible biotechnologies based on lignocellulolytic microorganisms and their enzymes. pp.1-22, in R.C. Kuhad, A. Singh (Eds) *Lignocellulose biotechnology*. I.K. International, New Delhi.

Lowry O.H. Rosebrough N.J, Farr A.L. and Randall R.J. (1951). Protein measurement with the Folin phenol reagent. J. Biol. Chem.: Vol.193, 265-275.

Maganhotto De Souza Silva C.M, Soares De Melo I. and Roberto De Oliveira P. (2005). Lignolytic enzyme production by *Ganoderma* species. Enz. Microbiol. Technol.: Vol. 37, 324-329.

Mata G, Murrieta Hernandez D.M. and Iglesias Andreu L.G. (2005). Changes in lignocellulolytic enzyme activities in six *Pleurotus* spp. Strains cultivated on coffee pulp in confrontation with *Trichoderma* spp. World J. Microbiol.Biotechnol: Vol. 21,143-150.

Miller G.L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal. Chem**.**Vol.31, 426-428.

Naraian, R, Arora N.K. and Garg S.K. (2009). Improved submerged fermentation of corn cob with mechanically broken oil seed cakes and decolorisation of textile dyes by enzyme extract of *Pleurotus florida* PF05. Res. Environ. Life Sci.: Vol. 2: 83-90.

Peixoto-Nogueira S.C, Michelin M, Betini J.H.A, Jorge J, Aterenzi H.F. and Polizli M.L.T.M. (2009). Production of xylanase by aspergilla using alternative carbon sources: Application of crude extract on cellulose pulp biobleaching**.** J. Ind. Microbiol. Biotechnol: Vol.36, 149-155.

Putter J. (2006). In: Methods of enzymatic Analysis 2 (ED Bergmeyer) Academic press New York. p 685

Rana I.S. and Rana A.S. (2011). Lignocellulolytic enzyme profile of *Agaricus* and *Pleurotus* species cultured on used tea leaves substrate. Advanced Biotech.:Vol.1, 10-14.

Ray S, Das N. and Bishayi B. (2012). Development of a simple method for a new immuno-conjugate utilizing laccase. Res J. Immunol.:Vol. 5, 1-16.

Snajdr J. and Baldrian P. (2007). Temperature affects the production, activity and stability of ligninolytic enzymes in *Pleurotus ostreatus* and *Trametes versicolor***.** Folia Microbiol. Vol.52,498-502.

Stajic M, Persky L, Friesem D, Hadar Y, Wasser Sp, Nevo N, Vukojevic J. (2006). Effect of different carbon and nitrogen sources on laccase and peroxidase production by selected *Pleurotus* species. Enzyme Microbiol. Technol.: Vol. 38, 65-73.

Stajic M, Vukojevic J. and Duletic-Lausevic S. (2007). Influence of the cultivation conditions on ligninolytic enzyme production in *Pleurotus pulmonarius***.** Proc. Nat. Sc.: Vol.113, 303-312.

Zadrazil F. (2000). Is conversion of ligninocellulosics into feed with white-rot fungi realiable? Practical problems of scale-up and technology transfer. Mushroom Sci.: Vol 15, 919-928.

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