Iranica Journal of Energy & Environment 5 (1): 59-66, 2014
ISSN 2079-2115
IJEE an Official Peer Reviewed Journal of Babol Noshirvani University of Technology
DOI: 10.5829/idosi.ijee.2014.05.01.09

BUT

Xylanase, Laccase and Manganese Peroxidase Production from White Rot Fungi

[1,2]Rabia Saleem, [1]Mohsin Khurshid and [2]Safia Ahmed

[1]Department of Biosciences, University of Wah, Wah Cantt, Pakistan
[2]Department of Microbiology, Faculty of Biological Sciences,
Quaid-i-Azam University, Islamabad, Pakistan

(Received: January 8, 2014; Accepted in Revised Form: March 14, 2014)

Abstract: Xylanase, laccase and manganese peroxidase (MnP) were produced and optimized by three strains of white rot fungi *Phanerochaete sordida* MRL3, *Lentinus pigrinus* MRL6 and *Poliporus caliatus* MRL7. These strains were initially isolated from wood decaying samples and then screened on minimal salt media,xylanase activities for the above strains were 55, 77.4 and 64.8 IU/mL, respectively. In addition, laccase activities were 80.65, 112.91 and 101.61 U/L, respectively. The activities forMnP were 123, 182.6 and 106.6 U/L, respectively. The maximum xylanase activity was observed at pH: 5.0, 30°C after 216 hours of incubation period. The maximum activities were 272.7, 278.52 and 292.8 IU/mL and the total protein was 1.24, 1.2 and 1.16 mg/mL, respectively. The maximum laccase activity was observed after 192-216 hours of incubation period, at pH: 5.0 on 30°C, the activities were 483.9, 516.4 and 459.67 U/L and the total protein 1.03, 1.2 and 1 mg/ml, respectively. The MnP activities were observed after 192-216 hours, at pH: 5.0 on 30°C, the activities were 588.66, 645.16 and 585.27 U/L and the total protein 1, 1.1 and 0.96 mg/mL, respectively.

Key words: Enzyme Production · White Rot Fungi · Hemicelluloses · Xylan · Lignin

INTRODUCTION

Lignocellulosic materials are widespread in nature and xylan is a polysaccharide found in hemicellulosic fraction of lignocellulose. Xylan is a potential significant resource for renewable biomass, which can be utilized as a substrate for the preparation of many products such as fuels, solvents and pharmaceuticals. On the other hand, xylanases are needed for making use of hemicelluloses. For most bioconversion processes, xylan must first be converted to xylose or xylo-oligosaccharides. There are several applications of xylanases in industry [1]. Xylanases are used in the prebleaching of kraft pulp to reduce the use of harsh chemicals in the subsequent chemical bleaching stages. The enzymatic treatments improve the chemical liberation of lignin by hydrolysing residual xylan. This reduces the need for chlorine-based bleaching chemicals, which is beneficial for the environment [2]. Currently, the major applications of xylanases are in pulp and paper, feed and baking industries [3].

Lignin, a complex and heterogeneous aromatic biopolymer in woody and herbaceous plants, is one of the most abundant naturalpolymers on earth. White rot fungi are primarily responsible forinitiating the depolymerization of lignin in wood [4]. The extracellular lignolytic enzyme system of white rotfungi has been studied extensively in recent years. Lignin peroxidase,manganese peroxidase (MnP) and laccase are associated with thedegradation of lignin. Several attempts to bleach hardwood kraftpulp by means of enzyme treatment have been reported. Arbeloa *et al*. [5] have showed that treatment of unbleached kraft pulp withlignin peroxidase facilitated subsequent chemical bleaching.

Many efforts have been made to utilize enzymes for the degradation of lignin in the pulp and paper industry. One enzyme known to play a major role in natural delignification is laccase (benzenediol: oxygen oxidoreductase) [6]. The enzyme was first identified in the sap of the Japanese lacquer tree *Rhusvernicifera* [7] Among the large blue copper containing enzymes, laccase is the most widely distributed [8] and

Corresponding Author: Mohsin Khurshid, Department of Biosciences, University of Wah, WahCantt, Pakistan.
Tel: +92-3334301513, E-mail: mohsin.mic@gmail.com.

occurs in various plants and fungi [9]. In the fungi, *Deutromycetes, Ascomycetes* and a wide range of *Basidiomycetes* are known producers of laccases, which are particularly abundant in many lignin-degrading white-rot fungi.

MATERIALS AND METHODS

Microorganism: Five strains of white rot fungi were used initially in this research project supplied byMicobiology laboratory Quaid-i-Azam University, Islamabad; known as *Phanerochaete chrysosporium* MRL1, *Phanerochaete sordida* MRL3, *Sterium hirsutum* MRL4, *Lentinus pigrinus* MRL 6 and *Poliporus caliatus* MRL7.

Culture Refreshment: Five strains of white rot fungi were refreshed thrice time on Malt-extract agar pH: 5.

Screening of Laccase, Manganese Peroxidase and Xylanase: The initial screening minimal salt medium (Yeast extract: 10g, Citric acid: 0.25g, Ammonium sulphate: 5g, K_2HPO_4: 0.26g, $MgSO_4.7H_2O$: 0.5g, $CaCl_2.2H_2O$: 0.02g and Streptomycin: 0.03g/L) with 2% wheat straw was used for detecting the ability of the white rot fungi strains to produce laccases, Mn-peroxidases and xylanases was used according to method developed in the literature [10] with some modifications.

Measurement of Xylanase, Laccase and Manganese Peroxidase Activities: Activity of xylanase was determined by the method described by Tuncer, *et al*. [11] against birchwoodxylan. Laccase activity was measured by using 5mM 2, 6-dimethoxy phenol (DMP) as substrate in 100mM sodium tartarate buffer pH: 4.5. Laccase activity was measured according the method stated in literature [10]. Manganese peroxidase (MnP) was assayed by using 2,6-Dimethoxy phenol (DMP) as substrate. MnP activity was measured by the method discussed in the literature [12].

Media Used for Xylanase, Laccase and MnP Production: Kim media was used for the production of xylanase against three strains of white rot fungi, (Proteous-peptone: 0.5g, Urea: 0.3g, KH_2PO_4: 0.2g, $CaCl_2$: 0.3g, Tween-80: 0.2, $(NH_4)_2SO_4$: 1.4g, $MgSO_4 7H_2O$: 0.3g, $FeSO_4$: 0.05g, $ZnSO_4 7H_2O$: 0.014g, $CoCl_2$ 0.02g and $MnSO_4$: 0.016g/L) described in the literature [13] whichwas used with some modification for growth and enzyme production according the method stated in literature [14]. For laccase production media (Soy meal: 30g, Maltose and 15g, Mycological Peptone: 15g/L) discussed in the literature [15] was used for growth and enzyme production, against three strains of white rot fungi. Medium described by [15] was used for MnP production with some modification for growth and enzyme production (Soy meal: 15g, Maltose: Mycological Peptone: 6g and Wheat straw: 8g/L).

Inoculum Preparation: 50mL medium was dispensed into 250mL conical flask. Medium was given steam autoclaving for 15 minutes at 121°C and 15 psi. The inoculum was allowed to grow at 30°C on orbital shaker (120rev/min) for 3-5 days.

Growth Conditions: 1 liter flask was filled with 225mL medium of (pH: 5). Inoculum at 10% (v/v) level was transferred to each growth flask and put into incubator on 30°C at speed 120 rev/min for 7-9 days. Initial and final pH was between 5 and 6.8.

pH Optimization for the Maximum Production of Xylanase, Laccase and MnP: pH of the liquid medium in each flask containing 100mL of medium was adjusted to pH values of 3.0, 4.0, 5.0, 6.0 and 7.0. Sample taken after every 24 hours was analyzed for enzyme activities. Final pH of samples was also noted.

Incubation Period for the Maximum Production of Xylanase, Laccase and MnP: Production of extracellular enzymes was carried out at a range of temperatures 25, 30 and 37°C. Production medium was incubated at different temperatures. Samples were collected and assayed for enzyme activities and dry cell weight was also estimated.

Time Optimization for the Maximum Production of Xylanase, Laccase and MnP: Three strains of white-rot fungi *Phanerochaete sordida* MRL3, *Lentinus pigrinus* MRL 6 and *Poliporus caliatus* MRL7 were incubated at optimum pH and temperature for 1½ week. Samples were collected after every 24 h assayed for enzyme activities and dry cell weight was also estimated.

Large scale production of these enzymes: After optimization, these enzymes were produced in 3liters flasks for biobleaching purpose. 110mL of Malt-extract media was prepared in 500mL flasks and with three strains of white rot fungi, the inoculum was allowed to grow at 120 revolutions/minutes on 30°C for 4-5day. 1100mL of production media of these three enzymes were prepared in 3 liters flasks and inoculated with 10% inoculum size and incubated on 30°C in orbital shaker for 7-9 days. Assayed for enzyme activities and protein was also estimated.

RESULTS

Initially 5 strains of white rot fungi were screened for xylanase, laccase and MnP activities on minimal salt media with 2% wheat straw. *Phanerochaete sordida*MRL3, *Lentinus pigrinus*MRL6 and *Poliporus caliatus*MRL7 were screened on the basis of their activities. Xylanase activities shown by these three strains were 55.0, 77.40 and 64.83 IU/mL, respectively. Laccase activities shown by these three strains were 80.65, 112.91 and 101.61 U/L, respectively. Manganese peroxidase activities shown by these three strains were 123.0, 182.62 and 106.65 U/L, respectively.

Effect of pH on the Production of Xylanase, Laccase and MnP: It is evident from (Figures 1, 2 and 3) that maximum xylanase production was obtained after 216 hours by *P. sordida* MRL3, *L. pigrinus* MRL6 and *P. caliatus* MRL7. Activities were observed 272.74, 278.52 and 292.86 IU/mL, respectively at pH: 5.0. Maximum laccase production was observed after 216 hours at pH: 5.0. Activities were 483.9 U/L after 192 hours, 516.4 U/L after 192 hours and 459.67 U/L. Maximum MnP production was observed at pH: 5.0 after 216 hours, activities were 588.66 U/L after 216 hours, 645.16 U/L after 192 hours and 585.27 U/L. There was decrease in activity with further increase or decrease in pH.

Effect of Temperature on the Production of Xylanase, Laccase and MnP: The optimum temperature for maximum xylanase production was found by growing *Phanerochaete sordida* MRL3, *Lentinus pigrinus* MRL6 and *Poliporus caliatus* MRL7 at different temperatures i.e. 25, 30 and 37°C keeping 1% wheat straw at pH: 5.0 on rotary incubator shaker. It was found that optimum temperature for xylanase production was 30°C; maximum activity was obtained after 216 hours of incubation period. The activities were 272.74, 278.52 and 292.86 IU/mL, respectively.

Maximum laccase production was obtained after 216 hours of incubation period at 30°C; activities were 483.9 U/L after 192 hours, 516.4 U/L after 192 hours and 459.67 U/L after 216 hours, respectively.

It was observed that maximum MnP production was obtained at 30°C after 216 hours of incubation period. The activities of *Phanerochaete sordida* MRL3, *Lentinus pigrinus* MRL6 and *Poliporus caliatus* MRL7 were 588.66 U/L after 216 hours, 645.16 U/L after 192 hours and 585.27 U/L after 216 hours, respectively.

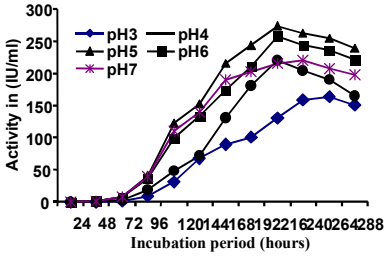

Fig. 1: Effect of pH on the specific activity of Xylanase in shake flask culture with 10% inoculum size on 30°C, in rotary shaker by *P. sordida* MRL3

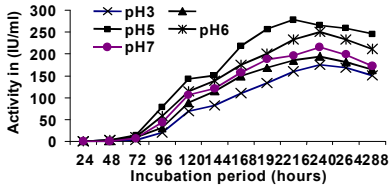

Fig. 2: Effect of pH on the specific activity of Xylanase in shake flask culture with 10% inoculum size at 30°C, in rotary shaker by *L. tigrinus*.

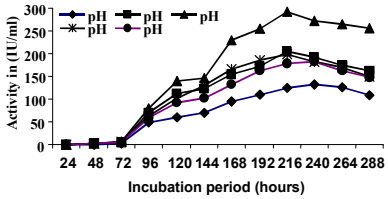

Fig. 3: Effect of pH on the specific activity of Xylanase in shake flask culture with 10% inoculum size at 30°C, in rotary shaker by *P. caliatus* MRL7.

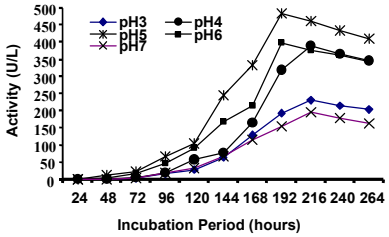

Fig. 4: Effect of pH on the specific activity of laccase in shake flask culture with 10% inoculum size at 30°C, in rotary shaker by *Phanerochaete sordida* MRL3.

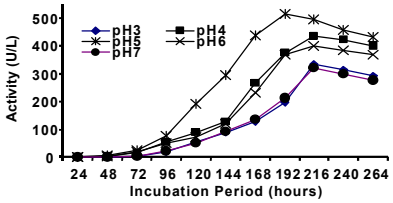

Fig. 5: Effect of pH on the specific activity of laccase in shake flask culture with 10% inoculum size at 30°C, in rotary shaker by *Lentinus pigrinus* MRL6.

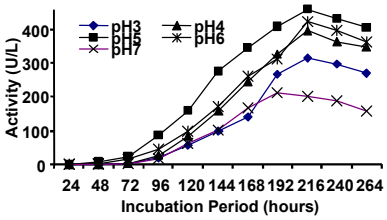

Fig. 6: Effect of pH on the specific activity of laccase in shake flask culture with 10% inoculum size at 30°C, in rotary shaker by *Poliporus caliatus* MRL7.

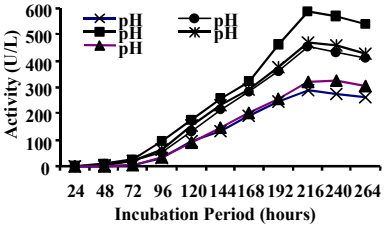

Fig. 7: Effect of pH on the specific activity of MnP in shake flask culture with 10% inoculum size at 30°C, in rotary shaker by *Phanerochaete sordida* MRL3.

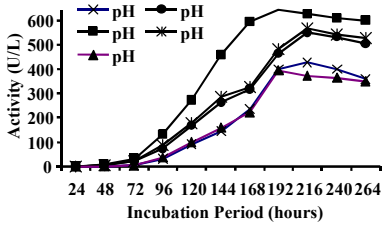

Fig. 8: Effect of pH on the specific activity of MnP in shake flask culture with 10% inoculum size at 30°C, in rotary shaker by *Lentinus pigrinus* MRL6.

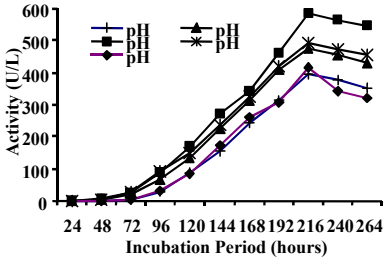

Fig. 9: Effect of pH on the specific activity of MnP in shake flask culture, with 10% inoculum size on 30°C, in rotary shaker by *Poliporus caliatus* MRL7.

Effect of Incubation Period on Production of Xylanase, Laccase and MnP: The maximum specific activity by *Phanerochaete sordida* MRL3 was 272.74 IU/mL and the total protein 1.24 mg/mL was observed after 216 hours of incubation period. The maximum specific activity by

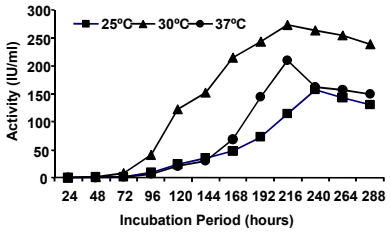

Fig. 10: Effect of Temperature on the specific activity of Xylanase at pH: 5, 10% inoculum size in rotary shaker by *P. sordida* MRL3.

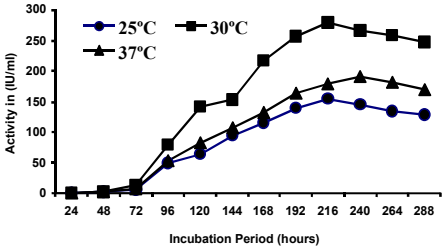

Fig. 11: Effect of Temperature on the specific activity of Xylanase in shake flask culture at pH: 5, 10% inoculum size in rotary shaker by *Lentinus pigrinus* MRL6.

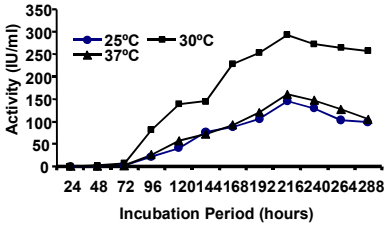

Fig. 12: Effect of Temperature on the specific activity of Xylanase at pH: 5, 10% inoculum size in rotary shaker by *Poliporus caliatus* MRL7.

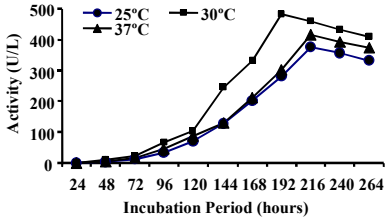

Fig. 13: Effect of Temperature on the specific activity of laccase in shake flask culture at pH: 5, 10% inoculum size in rotary shaker by *Phanerochaete sordida* MRL3.

Lentinus pigrinus MRL6 was observed after 216 hours of incubation period, maximum activity was 278.52 IU/mL and the total protein was 1.2 mg/mL. The maximum specific activity by *Poliporus caliatus* MRL7 was 292.86 IU/mL and the total protein 1.16 mg/mL was observed after 216 hours of incubation period.

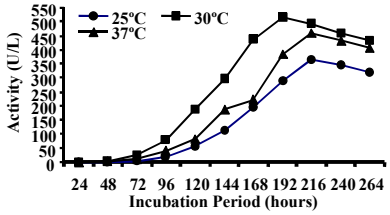

Fig. 14: Effect of Temperature on the specific activity of laccase in shake flask culture at pH: 5, 10% inoculum size in rotary shaker by *Lentinus pigrinus* MRL6.

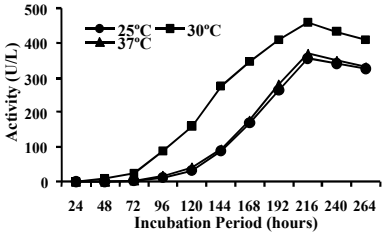

Fig. 15: Effect of Temperature on the specific activity of laccase in shake flask culture at pH: 5, 10% inoculum size in rotary shaker by *Poliporus caliatus* MRL7

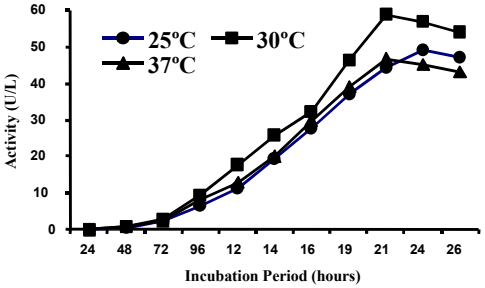

Fig. 16: Effect of Temperature on the specific activity of MnP in shake flask culture at pH: 5, 10% inoculum size in rotary shaker by *Phanerocheate sordida* MRL3.

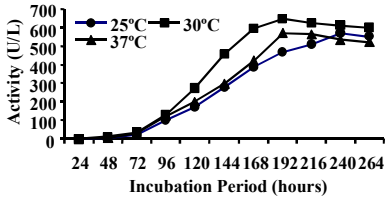

Fig. 17: Effect of Temperature on the specific activity of MnP in shake flask culture at pH: 5, 10% inoculum size in rotary shaker by *Lentinus pigrinus* MRL6.

The maximum specific activity by *Phanerochaete sordida* MRL3 was 483.9 U/L and the total protein 1.03 mg/mL was observed after 192 hours of incubation period. The maximum specific activity by

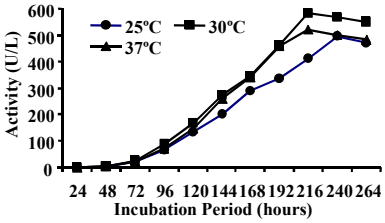

Fig. 18: Effect of Temperature on the specific activity of MnP in shake flask at pH: 5, 10% inoculum size in rotary shaker by *Poliporus caliatus* MRL7.

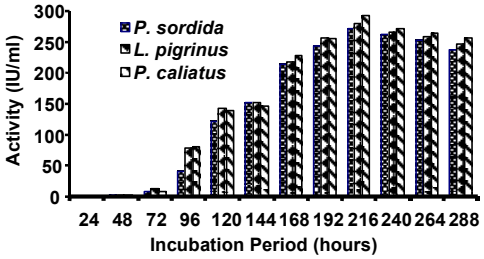

Fig. 19: Effect of Incubation period on the specific activity of Xylanase in shake flask culture at pH: 5, 10% inoculum size in rotary shaker on 30°C by *Phanerochaete sordida* MRL3, *Lentinus pigrinus* MRL6 and *Poliporus caliatus* MRL7.

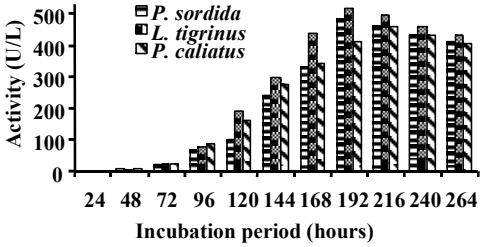

Fig. 20: Effect of Incubation period on the specific activity of laccase in shake flask culture at pH: 5, 10% inoculum size in rotary shaker at 30°C by *Phanerochaete sordida*MRL3, *Lentinus pigrinus* and *Poliporus caliatus* MRL7.

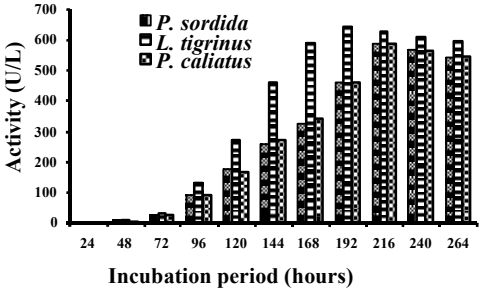

Fig. 21: Effect of Incubation period on the specific activity of MnP in shake flask culture at pH: 5, 10% inoculum size in rotary shaker on 30°C by *P. sordida* MRL3, *L. tigrinus* MRL6 and *P. caliatus* MRL7.

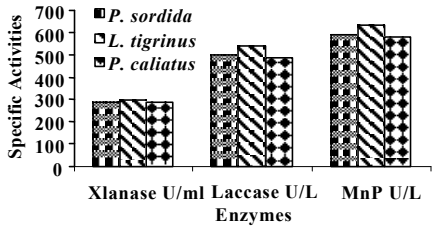

Fig. 22: Production of Xylanase, Laccase and MnP in 1liter flask at pH: 5, 10% inoculum size in rotary shaker on 30°C by *P. sordida* MRL3, *L. tigrinus* MRL6 and *P. caliatus* MRL7.

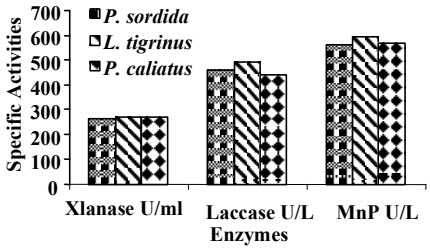

Fig. 23: Production of Xylanase, Laccase and MnP in 3liter flask at pH: 5, 10% inoculum size in rotary shaker on 30°C by *P. sordida* MRL3, *L. tigrinus* MRL6 and *P. caliatus* MRL7.

Lentinus pigrinus MRL6 was 516.4 U/L and the total protein 1.2 mg/mL was observed after 192 hours of incubation period. The maximum specific activity by *Poliporus caliatus* MRL7 was 459.67 U/L and the total protein 1 mg/mL was observed after 216 hours of incubation period.

The maximum specific activity of MnP by *Phanerochaete sordida* MRL3 was observed, 588.66 U/L and the total protein was 1 mg/mL after 216 hours of incubation period. The maximum specific activity by *Lentinus pigrinus* MRL6 was 645.16 U/L and the total protein 1.1 mg/mL was observed after 192 hours of incubation period. The maximum specific activity by *Poliporus caliatus* MRL7 was 585.27 U/L and the total protein 0.96 mg/mL was observed after 216 hours of incubation period.

Large Scale Enzymes Production: After optimization in 250mL flasks, these enzymes were produced in 1liter and 3liters flasks. Xylanase production in 1 liter flask by *P. sordida, L. tigrinus and P. caliatus*, xylanase activities were 287, 295 and 284 IU/mL, respectively. While laccase production in 1 liter flasks by these strains were 496, 540 and 490 U/L, respectively. In case of MnP activities were 586, 632 and 580 U/L, respectively.

In case of 3 liters flask production of these enzymes by *P. sordida, L. tigrinus and P. caliatus*, xylanase activities were 264, 272 and 269 IU/mL, respectively. While laccase activities were 460, 489 and 440 U/L, respectively. TheMnP activities were 560, 592 and 565 U/L, respectively.

DISCUSSION

Xylanase, laccase and manganese peroxidase were produced and optimized by three strains of white rot fungi *P. sordida* MRL3, *L. tigrinus* MRL6 and *P. caliatus* MRL7. Five strains of white rot fungi were screened on minimal salt media with 2% wheat straw for the production of xylanase, laccase and manganese peroxidase activity, these strains were initially isolated from wood decaying samples. These strains were shown high levels of xylanase, laccase and MnP activities. White rot fungi were isolated from woodlands were screened for xylanase, laccase and MnPactivies on minimal salt media, maximum strains showed activities for these enzymes [10]. Three hundred fugal strains were screened for lignin modifying enzymes, some of these strains showed maximum activities of these enzymes [16].

Maximum xylanase production was observed with Kim media, after 216 hours by *P. sordida* MRL3, *L. tigrinus* MRL6 and *P. caliatus* MRL7. A wild strain of *Aspergillus nidulans* isolated from soil produce cellulase-free xylanase activity when developed on submerged cultures using corn cob powder as the main substrate. Maximum xylanase production (220 U/mL) was obtained when the strain was developed in mineral medium supplemented with 3% (w/v) corn cob for 6 days [17].

Xylanase activity was maximally obtained at pH: 5.0 by these three strains. Xylanase was produced by *Thermomyces lanuginosus*, maximum production was observed between pH 5.5 and 9.5. In another study xylanase was produced by *Aspergillus nidulans*, with optimal activity at pH values between 5 and 6 [17].

The optimum temperature for maximum xylanase production was found 30°C. Extracellular xylanase produced by thermophilic fungus *Paecilomyces themophila,* maximum activity was observed at 50 °C [18].

Laccase was produced by *P. sordida* MRL3, *L. pigrinus* MRL6 and *P. caliatus* MRL7, maximum activity was observed with 3% soy meal, 1.5% maltose and 1.5% mycological peptone. Barley bran gave the

Table 1: Xylanase, laccase and Manganese peroxidase activity of different white rot fungi

Strains of white-rot fungi	Xylanase activity in IU/mL	Laccase activity in U/L	Manganese peroxidase activity in U/L
P. chrysosporium MRL1	20.6	11	88.57
P. sordida MRL3	55.0	80.65	123.0
S. hirsutum MRL 4	0.8	14	48.98
L. tigrinus MRL 6	77.4	112.91	182.62
P. caliatus MRL7	64.8	101.61	106.65

highest activities, a maximum value of 639U/L, which was 10 times the value attained in the cultures without lignocellulosics addition [19]. Maltose (2 g L^{-1}) and ammonium tartrate (10 g L^{-1}) were the most suitable carbon and nitrogen source for laccase production. Under optimal culture medium, the maximum laccase activity was determined to be 13.55 U mL^{-1} [20].

Optimum pH for laccase production was at pH: 5.0. When fungi were grown in a medium with pH as optimal for growth (pH: 5.0) the laccase was produced in excess [7]. Laccase produced by *T. modesta* was fully active at pH 4.0 [21].

Maximum laccase activity was observed at 30°C after 216 hrs (9 days) by these three strains. Laccase and manganese peroxidase were detected in liquid medium with ammonium phosphate, yeast extract and ammonium molybdate as nitrogen sources after 3 days of cultivation. Laccase optimal temperature was 45°C [22].

MnP production was optimized for pH, temperature and incubation time, maximum specific activity was observed by *Phanerochaete sordida* MRL3, *Lentinus pigrinus* MRL6 and *Poliporus caliatus* MRL7 at pH: 5.0 at 30 °C after 216 hrs incubation periods. Theproduction of MnP by *Pleurotus ostreatus* in different liquid cultures was investigated. The highest level of activity was observed after 8 days [23].

CONCLUSION

By screening among the white rot fungi; suitable strains were identified for production of xylanase, laccase and manganese peroxidase. Maximum active units enzymes for the desired media compositions, pH and incubation period at optimal temperature were obtained.

REFERENCES

1. Subramaniyan, S. and P. Prema, 2002. Biotechnology of microbial xylanases: enzymology, molecular biology and application. Critical Reviews in Biotechnology, 22(1): 33-64.
2. Viikari, L., M. Tenkanen and A. Suurnäkki, 2001. Biotechnology in the pulp and paper industry. Biotechnology Set, Second Edition, Wiley.
3. Beg, Q., M. Kapoor, L. Mahajan and G. Hoondal, 2001. Microbial xylanases and their industrial applications: a review. Applied Microbiology and Biotechnology, 56(3-4): 326-338.
4. Gold, M.H., H. Wariishi and K. Valli, 1989. Extracellular Peroxidases Involved in Lignin Degradation by the White Rot. Biocatalysis in Agricultural Biotechnology, 389: 127-140.
5. Arbeloa, M., J. D e Leseleuc, G. Goma and J.C. Pommier, 1992. An evaluation of the potential of lignin peroxidases to improve pulps. Tappi Journal, 75(3): 215-221.
6. Call, H. and I. Mücke, 1997. History, overview and applications of mediated lignolytic systems, especially laccase-mediator-systems (Lignozym®-process). Journal of Biotechnology, 53(2): 163-202.
7. Thurston, C.F., 1994. The structure and function of fungal laccases. Microbiology, 140(1): 19-26.
8. Leontievsky, A.A., T. Vares, P. Lankinen, J.K. Shergill, N.N. Pozdnyakova, N.M. Myasoedova, N. Kalkkinen, L.A. Golovleva, R. Cammack and C.F. Thurston, 1997. Blue and yellow laccases of ligninolytic fungi. FEMS Microbiology Letters, 156(1): 9-14.
9. Bourbonnais, R., M. Paice, I. Reid, P. Lanthier and M. Yaguchi, 1995. Lignin oxidation by laccase isozymes from Trametes versicolor and role of the mediator 2, 2'-azinobis (3-ethylbenzthiazoline-6-sulfonate) in kraft lignin depolymerization. Applied and Environmental Microbiology, 61(5): 1876-1880.
10. Muzariri, C.C., J. Mapingire, J. Mazorodze and L. Mandikutse, 2001. Isolation and screening of microorganisms for potential application in remediation of effluent water from the pulp and paper industry. 2nd WARFSA/WaterNet Symposium: Integrated Water Resources Management: Theory. Practice, Cases, pp: 242-250.

11. Tuncer, M.r., A.S. Ball, A. Rob and M.T. Wilson, 1999. Optimization of extracellular lignocellulolytic enzyme production by a thermophilic actinomycete *Thermomonospora fusca*BD25. Enzyme and Microbial Technology, 25(1): 38-47.
12. Gold, M.H. and J.K. Glenn, 1988. Manganese peroxidase from *Phanerochaete chrysosporium.* Methods in Enzymology, 161: 258-264.
13. Kim, J.H., M. Hosobuchi, M. Kishimoto, T. Seki, T. Yoshida, H. Taguchi and D.D. Ryu, 1985. Cellulase production by a solid state culture system. Biotechnology and Bioengineering, 27(10): 1445-1450.
14. Rezende, M.I., A.d.M. Barbosa, A.F.D. Vasconcelos and A.S. Endo, 2002. Xylanase production by Trichoderma harzianum rifai by solid state fermentation on sugarcane bagasse. Brazilian Journal of Microbiology, 33(1): 67-72.
15. Heinzkill, M., L. Bech, T. Halkier, P. Schneider and T. Anke, 1998. Characterization of laccases and peroxidases from wood-rotting fungi (family Coprinaceae). Applied and Environmental Microbiology, 64(5): 1601-1606.
16. Dhouib, A., M. Hamza, H. Zouari, T. Mechichi, M. Labat, M. Martínez and S. Sayadi, 2005. Autochthonous fungal strains with high ligninolytic activities from Tunisian biotopes. African Journal of Biotechnology, 4(5): 431-436.
17. Reis, D., M. Costa and R.M. Peralta, 2003. Xylanase production by a wild strain of Aspergillus nidulans. Acta Scientiarum: Biol. Sci., 25: 221-225.
18. Yang, S., Q. Yan, Z. Jiang, L. Li, H. Tian and Y. Wang, 2006. High-level of xylanase production by the thermophilic *Paecilomyces themophila* J18 on wheat straw in solid-state fermentation. Bioresource Technology, 97(15): 1794-1800.
19. Lorenzo, M., D. Moldes, S. Rodrýguez Couto and A. Sanroman, 2002. Improving laccase production by employing different lignocellulosic wastes in submerged cultures of *Trametes versicolor.* Bioresource Technology, 82(2): 109-113.
20. Wang, J., J. Wu, W. Huang and R. Tan, 2006. Laccase production by *Monotospora sp.*, an endophytic fungus in *Cynodon dactylon.* Bioresource Technology, 97(5): 786-789.
21. Nyanhongo, G., J. Gomes, G. Gübitz, R. Zvauya, J. Read and W. Steiner, 2002. Production of laccase by a newly isolated strain of*Trametes modesta.* Bioresource Technology, 84(3): 259-263.
22. Cavallazzi, J.R.P., M.G.D.A. Oliveira and M.C.M. Kasuya, 2004. Laccase production by Lepista sordida. Brazilian Journal of Microbiology, 35(3): 261-263.
23. Kamitsuji, H., Y. Honda, T. Watanabe and M. Kuwahara, 2004. Production and induction of manganese peroxidase isozymes in a white-rot fungus Pleurotus ostreatus. Applied Microbiology and Biotechnology, 65(3): 287-294.

Persian Abstract

DOI: 10.5829/idosi.ijee.2014.05.01.09

چکیده

در این تحقیق، زایلاناز، laccase و پراکسیداز منگنز (MNP) توسط سه گونه از قارچ پوسیدگی سفید *Phanerochaete sordida* MRL3، *Lentinus pigrinus* MRL6 و *Poliporus caliatus* MRL7 تولید و بهینه سازی شد. در ابتدا این گونه از نمونه های چوب پوسیده جدا شد و پس از آن در محیط کشت هایی با حداقل نمک غربالگری شده اند، فعالیت های زایلاناز برای سویه های بالا ۵۵، ۷۷.۴ و ۶۴.۸ IU/mL بود. علاوه بر این، فعالیت های laccase، ۸۰.۶۵، ۱۰۱.۶۱، و ۱۱۲.۹۱ U/L می باشد. میزان فعالیت برای منیزیم اکسید ۱۲۳، ۱۸۲.۶ و ۱۰۶.۶ می باشد. حداکثر فعالیت زایلاناز در pH، ۵، و دمای ۳۰ °C بعد از ۲۱۶ ساعت از گرمخانه گذاری مشاهده شده است. حداکثر فعالیت ۲۷۲.۷، ۲۷۸.۵۲ و ۲۹۲.۸ IU/mL و پروتئین کل ۱.۲۴، ۱.۲ و ۱.۱۶ mg/mL بود. فعالیت حداکثر laccase بعد از ۱۹۲-۲۱۶ ساعت از دوره کمون، مشاهده شد در pH، ۵ و دمای ۳۰ °C ، فعالیت ۴۸۳.۹، ۵۱۶.۴ و ۴۵۹.۶۷ U/L بود و مجموع پروتئین ۱.۰۳، ۱.۲ و ۱ mg/mL بود. فعالیت های MNP بعد از ۱۹۲-۲۱۶ ساعت، در pH، ۵، و دمای ۳۰ °C مشاهده شد، فعالیت های ۵۸۸.۶۶، ۶۴۵.۱۶ و ۵۸۵.۲۷ U/L بود و مجموع پروتئین ۱، ۱.۱ و ۰.۹۶ mg/mL بود.