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The Use of Agro-Industrial Wastes in the Production of Ligninolytic Enzyme of Some Wild Macrofungi from Turkey

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Abstract: White rot fungi which have the potential to produce high levels of lignolytic enzymes have wide usage in biotechnology. Ligninolytic enzyme production is influenced by many factors such as species diversity, media composition, C / N ratio, pH and temperature. It is thought that species grown in different habitats can produce different amounts of enzymes. Studies have shown that Coriolus versicolor, Funalia trogii, Phanerochaete chrysosporium, Pleurotus ostreatus, P.sajor-caju and P.eryngii are used mostly in biotechnological applications. The aim of this study is to investigate different new species which can produce lignolytic enzymes. In this study, Coriolus versicolor 1, C. versicolor 2, Agrocybe aegerita 1, A. aegerita 2, Armillariella tabescens 1, A. tabescens 2, Fomes fomentarius 1, F. fomentarius 2, Pleurotus ostreatus which collected from the region of Diyarbakir-Mardin were identified and the extracellular enzymes produced by this species were investigated. Whey (PAS) and saboroud dextrose broth (SDB) were used as culture medium, cotton stalk (P) was also used as an enzyme inducer. Laccase, MnP and LİP activities were observed in all studied wild mushrooms. Consequently, the study shows that the enzyme production was induced by cotton stalk. Also, these species and cotton stalks should be evaluated for biotechnological applications.
Keywords: Cotton Stalk, Ligninolytic Enzymes, Macrofungus, Whey

Introduction

Substantial amounts of lignocellulosic waste are produced annually. These are available for utilization as potential sources of food or as substrate for the production of industrial metabolites by microorganisms[1]. The main constituents of wood, cellulose, hemicellulose and lignin can be dissected by microorganisms such as white, brown, soft rotten fungi and some bacteria. However, the most effective microorganisms which can degradate lignin are *Basidiomycetes* fungi which can produce laccases (EC 1.10.3.2), ligninperoxidase (EC 1.11.10.14) and manganese peroxidase (EC 1.11.1.13) [2].

Lignin-degrading enzymes are enthralling since they can be used in various industrial applications, e.g., biosensors [3] pulp bleaching[4-5], labeling in immunoassays[6], bioremediation [7] and green organic synthesis [8]. The increase in the potential application areas of ligninolytic enzymes in biotechnology has increased the interest in researching new species producing enzymes [9].

The ligninolytic enzyme complexes of ‘white rot fungi’ are significantly different. Some of these species release only one enzyme for lignin degradation, while some secrete more than three enzymes [9]. Lignin peroxidase; A protein with high oxidation potential. This enzyme can oxidize phenolic and non-phenolic substances. Manganese peroxidase is considered to dipolymerize natural and synthetic lignin in non-living conditions, but it is believed that it cannot oxidize non-phenolic substances [10].

Laccase, which is a multi-copper oxidase enzyme group, catalyzes the monoelectronic oxidation of substances by spending the molecular oxygen [11]. Furthermore, many aromatic compounds trigger the production of laccase widely [12-13-14]. According to Master and Field [15], lignolytic enzymes occur as secondary metabolites under the condition of limiting nitrogen. However, the high nitrogen concentration in the environment of Pleurotus ostreatus cytumules the lignin mineralization[16]. As is seen, enzyme production is affected by many factors. Due to the necessity of large amounts of low-cost enzymes in biotechnological processes, the use of lignocellulosic wastes for production and the detection of species capable of producing lignolytic enzymes are considered to be appreciated [9]. Today, Phanerochaete chrysosporium and Coriolus versicolor are used extensively in biotechnological studies [16]. However, it is necessary to investigate the new species and to characterize their enzymes by taking into account the different isoforms of enzymes produced by fungi which are very important in biotechnological studies.

Producing Laccase, MnP and LİP by newly isolated 9 white-rot fungus with the substrates being SDB and whey in natural low-cost medium was the aim of this study. Furthermore, the evaluation of the effect of an extra cotton stalk was carried out.

Materials and methods

Strains were newly isolated from Mardin and Diyarbakir in Turkey. It can be observed where the strains and the species were gathered. From the Gollu village of Mardin the cotton stalk was acquired and from the Peynirciler Bazaar in Diyarbakır the whey was acquired. Sabouraud dextrose broth BioChemika was used for microbiology (SDB) and whey (PAS) for the basic growth medium. On malt extract agar the strains were subcultured and maintained at 4 ºC.

Culture conditions and preculture of makrofungus

The production capacities of ligninolytic enzymes of *basidiomycetes* isolates were screened during the growth under agitated culture conditions. The acquired fungal pellets were homogenized and used as inoculum after the incubation at 25 ± 2 ºC on SDB for 7 days.

The method of Songulasvili et al. [9] was used in the preparation of culture conditions. For the selected fungi the cotton stalk was used in order to stimulate ligninolytic enzyme production. At 60 ºC the cotton stalk was dried and it was milled to powder. In a rotary shaker, submerged fermentation of these growth substrates was conducted at 150 rpm and 27 ± 2 ºC in 250 ml flasks which contain 100 ml of the aforementioned medium with residues concentration at 30 g /L. In order to inoculate the flasks which contain this media, mycelial homogenates (4 ml) were used. The samples (5ml) were taken from flasks after the 7th, 10th, and 15th days of mushroom cultivation and on 7th, 10th, and 15th days of incubation period, the enzyme activities were measured.

Enzyme assays

As for the substrate a total of 5 ml of reaction tubes with a total volume of 5 ml was used for the measurement of laccase activity and as the source 50 mM sodium acetate buffer (pH 4.5) which contains 1 mM Guaiacol and 0.1 ml culture fluid. After 15 minutes of incubation at 37 ° C, absorbance rate was measured at the spectrophotometer (Schimadzu 2550 UV-Visible) at a wavelength of 465 nm [16]. In the study, the absorbance of 37 ° C, 1 minute, 465 nm wavelength was determined as 0.1 Unit increasing activity of 1 unit activity [17].

As described by Tien and Kirk [18] the activity of lignin peroxidase (EC 1.11.1.14) was evaluated by UV spectrometry of the veratrylaldehyde which was produced (e 310= 9300 M−1 cm−1) during veratryl alcohol oxidation. In the reactive mixture there was 2.3 mL sodium tartrate buffer 0.25 M pH 4.5, 0.3 ml veratryl alcohol 200 mM, 0.3 mL hydrogen peroxide 4mM and 0.1 ml Enzyme (denaturing for the blind). The commence of the reaction was observed with hydrogen peroxide and the emergence of veratrilaldehyde was measured at 310 nm.

As also described by Leonowicz et al. [19] the activity of manganese peroxidase (EC 1.11.1.13) was measured based on the oxidative dimerization of 2,6-DMP (2,6-DMP; e 469 = 27 500 M−1 cm−1). An activity unit was defined for all of the evaluated enzymes as the amount of enzyme which provided an increase in the absorbance unit per minute. In this study all of the identified activities in this study were expressed as U / ml.

Results and discussion

Effect of different mediums in laccase activity during the submerged fermentation

Songulashvili et al [9] reports that ligninolytic enzyme complexes of white-rot fungus are significantly different in their composition. These fungi release a single or more of the three extracellular enzymes which are vital for lignin degradation. This diffrence emerges due to different responses to different environments. In this study, 3.89 U / ml at *C. versicolor* 2 is the highest laccase activity, also in *P, ostreatus* (0.61 U / ml) the lowest activity was detected and there was no laccase activity in *A. aegerita* 2 in PAS medium at 7th day (Table 1).

The highest and the lowest levels of laccase activity was detected on the 10th day which was 7.18 U / ml and 2.60 U / ml in *P. Ostreatus* and *A. aegerita* 2, respectively (Table 2). Laccase activity was detected in all studied species at different days of fermentation in PAS medium (Table 1-3). As also described in the literature [20], because of the variations within species, the change of lignolytik enzyme activity in samples of the same species collected from different places is observed. We believe that specificity emerged as a result of different responses to cultivation. Hence, as stated by Kalmıs et al. [21] organisms in nature, incorporating various features, are the largest source for biotechnological applications which is also supported by the findings of this study.

Papinutti, et al. [22] expresses that none of such activities could be increased by the addition of inducers in many species. Since unsupplemented medium (SDB) has a low-cost and provides outcome of high levels of enzyme production, it can be used for increasing the production of laccase. On the 7th, 10th, and 15th day of cultivation in SDB medium the highest laccase activity of different species was detected as, 18.33 U / ml, 11.76 U / ml, 21.86, ve 21.68 U / ml, respectively (Table. 1-3). Plenty of studies were conducted on fungi the studies of Hammel [23], who stated that, lignolysis occur as a result of the lack of nutrients during secondary metabolism,

when the substrate can be obtained, fungus did not make the synthesizing and releasing of ligninolitic agents which are metabolically expensive.

Due to the necessity of low-cost large amounts of enzymes for biotechnological processes, the use of lignocellulosic wastes for production and detection of species that can produce better ligninolytic enzymes is seen as an approach that can be appreciated [9]. On the 7th day, laccase activity was detected as 12.61 U / ml in *A. tabescens* 2 when cotton stalks were used as an inducer and whey as medium (Table 1). During, laccase activity was detected as 2.79 U / ml in PAS medium on the 10th day of fermentation while it was detected 50 times more than in PAS + P (107.83 U / ml). As a result, it was found that cotton stalk encourages a very efficient way of producing the enzyme (Table 2). As it is also stated in the literature that ligninolytic wastes induce the activity of laccase [24-25-26]. When we used cotton stalks, which are available at an advantageous price in our working area, we observed a good result of the laccase activity, thus it is highly possible that the existing material has a good potential for enzyme production. The natural lignocellulosic resources can be used in the production of enzyme both determining the amount of the initial complex, and are thought to be an important factor in the detection of pressure on the enzyme activity [27]. In this study, the highest laccase activity was determined to be 57.24 U / ml, 80.84 U / ml, 150.47 U / ml at *A. tabescens* 1 in SDB + P medium during the fermentation on the 7th, 10th, 15th day respectively (Table 1-3). When laccase activity was only compared to SDB it was determined to be about 50 times more. Samples from different areas of the same species have different enzyme activity. This differences might have caused because of the variation of the species.

Effect of different mediums in manganese peroxidase activity during the submerged fermentation

The results of the effect of the medium on the white - red fungi's MnP activity are shown in table 4.5.6. Manganese peroxidase (MnP) achieved its peak activity during the day of inoculation, not only in control conditions (without cotton stalk) but also in cotton stalk supplemented cultures.

Adding cotton stalk to the cultures led to an increased activity of the fungi's ligninolytic enzymes. After 7 days of inoculation the maximum enzyme activities with cotton stalk were 0,6232, 0,0522 U/ml. in *A. tabescens* 1, and *A. tabescens* 2 in SDB+P, medium, respectively. Also an activity of 0,0444 U/ml MnP in *F. fomentarius* 2 in PAS+P medium was detected. The maximum activity without cotton stalk was 0,1428 U/ml in, *C. versicolor* 2 in PAS medium for MnP.

In this work, *A. tabescens, F. fomentarius* and *P. ostreatus* which were produced in MnP in the 10-day-old cultures were analyzed and presented since it was higher in activity when it is compared to the values at the 7th day and compared to unsupplemented cultures. (Table. 4) The highest activitiy was detected in *A. tabescens* 1 (0,9548 U/ml) in SDB+P medium. Furthermore, MnP activity of *C. versicolor* 2 (0,0053 U/ml) we found a decrease in PAS medium when it is compared to values at the 7th day.

Under submerged culture conditions, the maximum MnP activity was significantly increased (0,9895 U/ml and 0,0470 U/ml) in *A. tabescens* 1 and *P. ostreatus* in SDB+P medium on the 15th day of fermantation (Table 6). MnP activities acquired from this study were found to be much higher [21-24-28-20-30-31] or lower [9-32-33-34-35] than MnP activities of some other species in the literature.

According to the results of this study, the highest activity of MnP was seen in *A. tabescens* 1 in SDB+P medium on the 15th day of fermantation. Contrary to the literature, the aromatic compounds in lignin degradation have been determined to be inducers of peroxidases. P. ostreatus showed no MnP activity in any of the environments on the 7th day of fermentation, however it demmonstrated a high activity on the 15th day of fermentation. We suppose that the reason for this is the change in the C / N ratio in the culture medium. In accordance with the literature, it is considered that the C / N ratio [36-37] has different impacts on lignolytic enzyme production, these different methods can be improved and achieving the stability of high enzyme production can be carried out.

We believe that the inclusion of lignocellulosic materials to the environment fosters MnP activity in A. tabescens-2. The literature shows that the results obtained strongly underline that compounds derived from lignocellulose substrates can act as inducers for the output of lignolytic peroxidases.4,16 However, on the 7th day of fermentation C. versicolor 2 suggests that the procurement of high activity in PAS medium is species specific. As the literature suggests, the inclusion of any inducer to the medium in the species does not promote MnP activity [22] and on day 7 of the PAS medium, we believe that the outcome in C. Versicolor 2 influences the amount and time of maximum possible enzyme activity in the nutrients nitrogen.

In this study, the idea that cotton straw and whey can be used for the development of lignolytic enzymes and other biotechnological enzymes that are affordable and safe is promoted [32].

Effect of different mediums on lignin peroxidase activity during submerged fermentation

LİP was first observed in *Phanerochaete chrysosporium* [38-39]. It is reported that this enzyme is a stronger oxidizing agent than other typical peroxidase and can therefore oxidize not only the general peroxidase

substrates such as phenol and analine but also non-phenolic substances[29]. LİP's Ligninolytic activity, which has a fundamental lignolytic role, is acknowledged as a significant issue in many Basidiomycetes. The reason for that is the fact that LİP activity was not detected in many *Basidiomycetes* studied. However, we think that the evaluation of the usage of new species or strains in biotechnological operations is necessary.

In our study, the highest LİP activities on day 7 were 140.98 U / ml in A. tabescens 2 in SDB medium, 86.02 U / ml in C. versicolor 2 in SDB medium and 79.96 U / ml in A. aegerita 2 was acquired in the SDB medium. On day 7 in A. aegerita 1 no LIP activity was observed. Statistically the highest activity in A. tabescens 2 and A. aegerita 2 were detected in other medium, whereas in C. versicolor 2 no difference between SDB, PAS + P and SDB + P medium was found (Table 7).

On day 10 of LIP activity, the highest activities were detected as 125,45 U / ml in A. tabescens 2 's SDB medium, 86,02 U / ml, C. versicolor 2 in PAS + P medium and 78,85 U. In A. aegerita 2 's / ml was detected in the SDB medium. The lowest activity was acquired in A to C, 2.36 U / ml in the PAS medium of A. tabescens 2. It was observed that the medium where the highest activities were detected were numerically different (Table 8).

On day 15, the highest LİP activities were 47.79, 45.40 and 45.40 U / ml, respectively; F. fomentarius 1, F. fomentarius 2 and C. versicolor 2 were detected in SDB + P, SDB + P and SDB medium. In the SDB medium of F. fomentarius 2, the lowest activity was obtained as 2,39 U / ml. In analytical analyzes, it was found that the highest activities in F. fomentarius 1, F. fomentarius 2 strains varied and C. versicolor 2 in SDB and PAS + P medium were not statistically distinguished (Table 9).

The highest LİP activities acquired as a result of the 15th day fermentation period (Table 9.) were detected to be higher in comparison with the literature [32-33-34-40-41]. In this study, it is believed that C / N ratio has different impact on LİP production, that other approaches can be developed and balance for high enzyme production can be established. It is also evident that the SDB medium, which has high activity, is a basic environment and its preparatory use does not result in any increase. The idea that the use of inducers does not result in any increase is also supported by the literature[38]. We believe that for some species no inducer is inducible in the production of LİP. In our study, the use of different species in biotechnological applications has been acknowledged by the difference in LİP activity in different medium and times. On day 7 of fermentation, LİP activity that was detected in A. tabescens 2 of 140,98 U / ml indicates that this type should be assessed.

Conclusions

This study shows the need to assess more microorganisms and lignocellulose substrates with different materials to evaluate the actual potential of fungi producing ligninolytic enzymes was shown in this study. In the production of valuable technology, the appointment of appropriate plant additives for fungal growth and target enzyme synthesis may play a key role. We can conclude from the results that were acquired here that A. tabescens 1, A. tabescens 2, F. fomentarius1 and F. fomentarius 2 are suitable applicants for estimating ligninolytic enzyme production. However, there is need for further studies in order to clarify why some complex substrates trigger enzyme production.

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References

[1]. Magnelli P, Forchiassin F, Regulation of the cellulase complex production by *Saccobolussaccoboloides*: Induction and repression by carbohydrates. Mycologia 91(1999) 359 -364.

[2]. Gomes E, Aguıar A P, Carvalho C C, Bonfá M R B, Da Sılva R, Boscolo M, Ligninases Production By Basidiomycetes Strains On Lignocellulosic Agricultural Residues And Their Application İn The Decolorization Of Synthetic Dyes. *Brazilian Journal Of Microbiology*, 40 (2009) 31-39.

[3]. Freire R S, Durán N, Kubota L T , Effects of fungal laccase immobilization procedures for the development of a biosensor for phenol compounds. Talanta 54 (2001) 681–686.

[4]. Balakshin M, Chen C L, Gratzl J S, et al. Biobleaching of pulp with dioxygen in laccase-mediator system-effect of variables on the reaction kinetics. J Mol Catal B: Enzym 16 (2001) 205–215.

[5]. Call H P, Mücke I, History, overview and applications of mediated ligninolytic systems, specially laccase-mediator-sy D'Acunzo F, Galli C, Masci B. Oxidation of phenols by laccase and laccase mediator systems. Eur J Biochem 269 (2002) 5330–5335.

[6]. Kuznetsov B A, Shumakovich G P, Koroleva OV, On applicability of laccase as label in the mediated and mediatorless electroimmunoassay: Effect of distance on the direct electron transfer between laccase and electrode. Biosens Bioelectron 16 (2001) 73–84.
[7]. Mayer A M, Staples R C, Laccase: new functions for an old enzyme. Phytochem 60 (2002) 551–565.
[8]. Karamyshev AV, Shleev SV, Koroleva OV, Laccase-catalyzed synthesis of conducting polyaniline. Enzyme Microb Tech 33 (2003) 556–564.
[9]. Songulashvili G, Elisashvili V, Wasser S P, et al, Basidiomycetes laccase and manganese peroxidase activity in submerged fermentation of food industry wastes. Enzyme Microb Tech 4 (2007) 57–61.
[10]. Papinutti V L, Forchiassin F, Lignocellulolytic Enzymes from Fomes sclerodermeus Growing in Solid-State Fermentation, Journal of Food Engineering, 81(2007) 54–59.
[11]. Riva S, *Laccases: Blue Enzymes for Green Chemistry, Trends in Biotechnology*, 24 (2006) 219-226.
[12]. Ardon O, Kerem Z, Hadar Y, *Enhancement of Laccase Activity in Liquid Cultures of The Ligninolytic Fungus Pleurotus Ostreatus by Cotton Stalk Extract, Journal of Biotechnology,* 51 (1996) 201-207.
[13]. Mansur M, Sua´rez T, Ferna´ndez-Larrea J B, Brizuela M A, Gonzalez A E, *Identification of a Laccase Gene Family in The New Lignin-Degrading Basidiomycete CECT 20197, Apply Environment Microbiology,* 63 (1997) 2637–2646.
[14]. Arora D S, Gill P K, *Comparison of Two Assay Procedures for Lignin Peroxidase, Enzyme Microb. Technol.*, 28 (2001) 602–605.
[15]. Master T, Field J A, *Characterization of a Novel Manganese Peroxidase-Lignin Peroxidase Hybrid İsozyme Produced by Bjerkandera Species Strain BOS55 in The Absence of Manganese, J. Biol. Chem.,* 273 (1998) 15412-15417.
[16]. Cing S, *Tekstil Boyalarının Renginin Gideriminde Mikroorganizma Kullanımı*, Yüksek Lisans Tezi, İnönü Üniversitesi, (2001) Malatya,
[17]. Unal TA, Kolankaya N, Dechlorination of bleached kraft pulp by laccase enzyme produced from some white-rot fungi. Turk J Biol 25 (2001) 67-72.
[18]. Tien M, Kirk TK, Lignin-degrading enzyme from the hymenomycete *Phanerochaete chrysosporium*. Burds. Science 221 (1983) 661–663.
[19]. Leonowicz A, Cho NS, Luterek J, Wilkolazka A, Wojtas-Wasilewska M, et al. Reactions of blue and yellow fungal laccases with lignin model compounds. Biochim 64 (1999) 1150- 1156.
[20]. Chi Y, Hatakka A, Maijala P , Can Co-culturing of two white-rot fungi increase lignin degredation and production of lignin-degrading enzymes. Int Biodeter & Biodegre (2007) 32–39.
[21]. Kalmıs E, Yasa I, Kalyoncu F et al., Ligninolytic enzyme activities in mycelium of some wildand commercial mushrooms Afr J Bıotechnol 7: 23 (2008) 4314–4320.
[22]. Papinutti VL, Diorio LA, Forchiassin F, Production of laccase and manganese peroxidase by *Fomes sclerodermeus* grown on wheat bran. J Ind Microbiol Biotechnol 30 (2003) 157–160.
[23]. Hammel KE , Fungal degradation of lignin, plant litter quality and decomposition. CAB International Oxon (1997) 33-45.
[24]. Erden E, Ucar MC, Kaymaz Y et al., New and different lignocellulosic materials from Turkey for laccase and manganese peroxidase production by *Trametes versicolor*. Eng Life Sci 9: 1 (2009) 60–65.
[25]. Neifar M, Jaouani A, Ellouze GR, et al., Effect of culturing processes and copper addition on laccase production by the white-rot fungus *Fomes fomentarius* MUCL 35117. Lett Appl Microbiol. ISSN 0266-8254 (2009) 1–6.
[26]. Adekunle A E, Guo C, & Liu CZ, Lignin-Enhanced Laccase Production from Trametes versicolor. *Waste and Biomass Valorization*, *8*(4) (2017) 1061–1066. https://doi.org/10.1007/s12649-016-9680-4
[27]. Elisashvili V, Penninckx M, Kachlishvili E, et al., *Lentinus edodes* and *Pleurotus* species lignocellulolytic enzymes activity in submerged and solid-state fermentation of lignocellulosic wastes of different composition. Bioresource Technol 99, 3 (2007) 457–462.
[28]. Elisashvili V, Penninckx M, Kachlishvili E, Asatiani MK, Vesitadze G, Use of *Pleurotus* dryinus for Lignocellulolytic Enzymes Production in Submerged Fermentation of Mandarin Peels and Tree Leave,. Enzyme Microb Technol 38 (2006) 998–1004.
[29]. Papinutti VL, Forchiassin F, Lignocellulolytic Enzymes from Fomes sclerodermeus Growing in Solid-State Fermentation, Journal of Food Engineering, 81 (2007) 54–59.
[30]. Levin L, Herrmann C, Papinutti VL, Optimization of Lignocellulolytic Enzyme Production by the White-rot Fungus Trametes trogii in Solid-State Fermantation Using Response Surface Methodology, Biochemical Engineering Journal 39 (2008) 207-214.
[31]. Sasidhara R and Thirunalasundari T, Lignolytic and lignocellulosic enzymes of *Ganoderma lucidum* in liquid medium European Journal of Experimental Biology 4(2) (2014) 375-379.

[32]. Fujian X, Hongzhang C, Zuohu L, Solid-State Production of Lignin Peroxidase (LİP) and Manganese Peroxidase (MnP) by *Phanerochaete chrysosporium* Using Steam-Exploded Straw as Substrate, Bioresource Technology 80 (2001) 149-151.

[33]. Arora DS, Chander M, Gill PK, Involvement of Lignin Peroxidase, Manganese Peroxidase and Laccase in Degradation and Selective Ligninolysis of Wheat Straw. Int. Biodeter. Biodegradation 50 (2002) 115-120.

[34]. Kapich AN, Prior BA, Botha A, Galkin S, Lundell T, Hatakka A, Effect of Lignocellulose-Containing Substrates on Production of Ligninolytic Peroxidases in Submerged Cultures of Phanerochaete chrysosporium ME-446, Enzyme and Microbial Technology 34 (2004) 187–195.

[35]. Stajic M, Persky L, Friesem D, Hadar Y, Wasser SP, Nevo E, Vukojevic J, Effect of Different Carbon and Nitrogen Sources on Laccase and Peroxidases Production by Selected Pleurotus Species, Enzyme and Microbial Technology 38 (2006) 65–73.

[36]. Yu G, Wen X, Qian Y, Production of the Ligninolytic Enzymes by Immobilized *Phanerochaete chrysosporium* in an Air Atmosphere, World Journal of Microbiology & Biotechnology 21 (2005) 323–327.

[37]. Membrillo I, Sánchez C, Meneses M, Favela E, Loera O, Effect of Substrate Particle Size and Additional Nitrogen Source on Production of Lignocellulolytic Enzymes by Pleurotus ostreatus Strains, Bioresource Technology*,* 99 (2008) 16, 7842-7847.

[38]. Lankinen P, Lignolitic Enzymes of The Basidiomycetous Fungi *Agaricus bisporus* and Phlebia radiata on Lignocellulose-Containing Media, Academic Dissertation in Microbiology, (2004) Helsinki,

[39]. Hammel K E, Cullen D, Role of Fungal Peroxidases in Biological Ligninolysi, Current Openion On Biotechnology 19 (2008) 166–172.

[40]. Reddy G V, Babu P R, Komaraiah P, Roy K R R M, Kothari I L, Utilization of Banana Waste for The Production of Lignolyitc and Celluloytic Enzymes by Solid Substrate Fermentation Using Two Pleuratus Species (P. ostreatus and P. sajor-caju), Process Biochem 38(2003) 1457–1462.

[41]. Shah M P, Reddy G V, Banerjee R, Babu P R, Kothari I L, Microbial Degradation of Banana Waste Under Solid State Bioprocessing Using Two Lignocellulolytic Fungi *(Phylosticta spp. MPS-001 and Aspergillus spp. MPS-002),* Process Biochemistry 40 (2005) 445-451.

Table 1. Effect of media on laccase activity in submerged fermentation conditions at 7th day

7th day laccase U / ml (means ± SE)*									
Medium[*]	*C. versicolor*1	*C. versicolor* 2	*A. aegerita*1	*A. aegerita* 2	*A. tabescens*1	*A. tabescens* 2	*P. ostreatus*	*F. fomentarius*1	*F. fomentarius* 2
PAS	3.53 ± 0.07[b]	3.66 ± 1.30[b]	3.20 ± 0.12[c]	0.00 ± 0.00[c]	2.80 ± 0.30[b]	0.42 ± 0.01[c]	0.61 ± 0.03[b]	3.21 ± 0.07[b]	2.79 ± 0.03[c]
SDB	4.37 ± 0.90[ab]	20.32 ± 8.34[ab]	3.20 ± 0.01[c]	0.03 ± 0.01[c]	10.53 ± 4.12[b]	1.65 ± 0.28[bc]	2.44 ± 0.08[a]	3.84 ± 0.53[b]	6.61 ± 1.35[b]
PAS+P	5.17 ± 0.81[ab]	17.22 ± 1,82[ab]	4.13 ± 0.10[b]	0.14 ± 0.04[b]	7.25 ± 1.04[b]	12.61 ± 0.26[a]	0.18 ± 0.01[c]	7.36 ± 0.15[a]	12.00 ± 0.11[a]
SDB+P	6.27 ± 0.30[a]	27.23 ± 4.36[a]	4.37 ± 0.03[a]	0.86 ± 0.00[a]	57.24 ± 8.02[a]	2.61 ± 0.42[b]	0.07 ± 0.00[c]	8.96 ± 0.68[a]	11.60 ± 0.54[a]

[*]Means followed by different letters in same line are significantly different ($P < 0.05$) by Duncan's test.
PAS = Whey
SDB = Sabouraud dextrose broth
PAS + P= Whey +cotton stalk
SDB + P= Sabouraud dextrose broth + cotton stalk

Table 2. Effect of media on laccase activity in submerged fermentation conditions at 10th day

10th day laccase U / ml (means ± SE)*

Medium*	*C. versicolor* 1	*C. versicolor* 2	*A. aegerita* 1	*A. aegerita* 2	*A. tabescens* 1	*A. tabescens* 2	*P. ostreatus*	*F. fomentarius*1	*F. fomentarius*2
PAS	3.48 ± 0.25[b]	4.16 ± 1.51[c]	3.02 ± 0.06[b]	2.60 ± 0.01[d]	3.58 ± 0.96[c]	2.79 ± 0.01[c]	7.18 ± 2.18[b]	3.78 ± 0.06[b]	2.66 ± 0.03[c]
SDB	3.76 ± 0.14[b]	5.61 ± 0.55[c]	2.94 ± 0.02[b]	7.36 ± 0.13[b]	2.76 ± 0.02[c]	10.43 ± 1.94[c]	11.16 ± 0.14[ab]	3.03 ± 0.13[b]	11.77 ± 0.19[b]
PAS + P	4.62 ± 0.52[b]	18.15 ± 1.56[b]	7.17 ± 0.95[a]	9.20 ± 0.20[a]	26.84 ± 5.33[b]	107.83 ± 3.48[a]	9.53 ± 0.55[b]	4.45 ± 0.29[b]	88.74 ± 1.36[a]
SDB + P	6.89 ± 0.53[a]	32.45 ± 3.96[a]	4.49 ± 0.10[b]	5.81 ± 0.06[c]	80.84 ± 10.16[a]	39.33 ± 6.79[b]	15.51 ± 1.62[a]	8.44 ± 0.64[a]	12.56 ± 1.49[b]

*Means followed by different letters in same line are significantly different ($P < 0.05$) by Duncan's test.
PAS = Whey
SDB = Sabouraud dextrose broth
PAS + P = Whey + cotton stalk
SDB + P = Sabouraud dextrose broth + cotton stalk

Table 3. Effect of media on laccase activity in submerged fermentation conditions at 15th day

15th day laccase U / ml (means ± SE)*

Medium*	*C. versicolor*1	*C. versicolor* 2	*A. aegerita*1	*A. aegerita* 2	*A. tabescens* 1	*A. tabescens* 2	*P. ostreatus*	*F. fomentarius*1	*F. fomentarius*2
PAS	3.73 ± 0.18[b]	4.26 ± 0.76[b]	6.66 ± 0.78[b]	7.41 ± 0.00[a]	3.91 ± 1.16[c]	17.32 ± 0.00[c]	3.89 ± 0.57[c]	3.46 ± 0.30[b]	3.26 ± 0.03[c]
SDB	3.49 ± 0.13[b]	10.41 ± 2.88[b]	3.68 ± 0.07[c]	3.70 ± 0.08[b]	3.03 ± 0.20[c]	21.86 ± 2.10[c]	12.65 ± 0.09[b]	6.99 ± 0.42[a]	21.68 ± 0.42[b]
PAS + P	2.97 ± 0.03[b]	16.40 ± 1.86[b]	8.87 ± 0.02[a]	6.36 ± 0.65[a]	34.49 ± 13.57[b]	100.24 ± 0.78[a]	11.91 ± 0.98[b]	3.34 ± 0.15[b]	59.04 ± 1.97[a]
SDB + P	5.24 ± 0.26[a]	25.08 ± 0.47[a]	5.91 ± 0.30[b]	4.75 ± 0.14[b]	150.47 ± 1.29[a]	83.85 ± 1.81[b]	20.77 ± 0.30[a]	7.32 ± 0.18[a]	53.62 ± 6.39[a]

*Means followed by different letters in same line are significantly different ($P < 0.05$) by Duncan’s test.
PAS = Whey
SDB = Sabouraud dextrose broth
PAS + P = Whey + cotton stalk
SDB + P = Sabouraud dextrose broth + cotton stalk

Table 4. Effect of media on MnP ctivity in submerged fermentation conditions at 7 th day

7th day MnP U/ml (means±SE)

Medium*	*C. versicolor* 1	*C. versicolor* 2	*A. aegerita*1	*A. aegerita* 2	*A. tabescens* 1	*A. tabescens* 2	*P. ostreatus*	*F. fomentarius* 1	*F. fomentarius1*
PAS	0,0000±0,0[b]	0,1428±0,1[a]	0,0001±0,0[b]	0,0002±0,0[b]	0,0000±0,00[c]	0,0000±0,00[b]	0,0000±0,0[b]	0,0000±0,0[b]	0,0001±0,0[b]
SDB	0,0003±0,0[a]	0,0007±0,0[b]	0,0004±0,0[a]	0,0006±0,0[a]	0,0009±0,00[c]	0,0001±0,00[b]	0,0001±0,0[b]	0,0001±0,0[b]	0,0032±0,0[b]
PAS+P	0,0000±0,0[b]	0,0000±0,0[b]	0,0000±0,0[c]	0,0001±0,0[b]	0,0364±0,01[b]	0,0348±0,00[ab]	0,0000±0,0[b]	0,0075±0,0[a]	0,0444±0,0[a]
SDB+P	0,0004±0,0[a]	0,0000±0,0[b]	0,0000±0,0[c]	0,0001±0,0[b]	0,6238±0,00[a]	0,0522±0,01[a]	0,0003±0,0[a]	0,0001±0,0[b]	0,0070±0,0[b]

Means followed by different letters in same line are significantly different ($P < 0.05$) by Duncan's test.
PAS = Whey
SDB = Sabouraud dextrose broth
PAS + P = Whey + cotton stalk
SDB + P = Sabouraud dextrose broth + cotton stalk

Table 5. Effect of media on MnP ctivity in submerged fermentation conditions at 10th day

10th day MnP U/ml (means±SE)

Medium[*]	*C. versicolor* 1	*C. versicolor* 2	*A. aegerita*1	*A. aegerita* 2	*A. tabescens* 1	*A. tabescens* 2	*P. ostreatus*	*F. fomentarius* 1	*F. fomentarius1*
PAS	0,0000±0,0[c]	0,0053±0,02[a]	0,0001±0,0[b]	0,0001±0,0[b]	0,0001±0,0[c]	0,0002±0,0[b]	0,0017±0,0[b]	0,0000±0,0[c]	0,0005±0,0[b]
SDB	0,0002±0,0[b]	0,0000±0,0[b]	0,0003±0,0[a]	0,0002±0,0[a]	0,0000±0,0[c]	0,0000±0,0[b]	0,0002±0,0[b]	0,0003±0,0[b]	0,0014±0,0[b]
PAS+P	0,0000±0,0[c]	0,0000±0,0[b]	0,0000±0,0[c]	0,0000±0,0[c]	0,0793±0,01[b]	0,0541±0,01[a]	0,0026±0,0[b]	0,0000±0,0[c]	0,3500±0,0[a]
SDB+P	0,0004±0,0[a]	0,0004±0,0[b]	0,0000±0,0[c]	0,0000±0,0[bc]	0,9548±0,01[a]	0,0521±0,0[a]	0,0078±0,0[a]	0,0024±0,0[a]	0,0103±0,0[b]

[*]Means followed by different letters in same line are significantly different (P < 0.05) by Duncan's test.
PAS = Whey
SDB = Sabouraud dextrose broth
PAS + P = Whey + cotton stalk
SDB + P = Sabouraud dextrose broth + cotton stalk

Table 6. Effect of media on MnP ctivity in submerged fermentation conditions at 15th day

15th day MnP U/ml (means±SE)

Medium*	*C. versicolor* 1	*C. versicolor* 2	*A. aegerita*1	*A. aegerita*2	*A. tabescens* 1	*A. tabescens* 2	*P. ostreatus*	*F. fomentarius* 1	*F. fomentarius1*
PAS	0,0000±0,0[c]	0,0047±0,0[a]	0,0002±0,0[a]	0,0000±0,0[b]	0,0001±0,0[b]	0,0002±0,0[b]	0,0143±0,0[b]	0,0000±0,0[c]	0,0002±0,0[c]
SDB	0,0001±0,0[b]	0,0049±0,0[a]	0,0001±0,0[a]	0,0004±0,0[a]	0,0014±0,0[b]	0,0005±0,0[b]	0,0005±0,0[c]	0,0001±0,0[b]	0,0000±0,0[d]
PAS+P	0,0001±0,0[a]	0,0002±0,0[a]	0,0000±0,0[b]	0,0005±0,0[a]	0,0161±0,0[b]	0,0110±0,0[b]	0,0177±0,0[b]	0,0001±0,0[b]	0,0035±0,0[a]
SDB+P	0,0000±0,0[c]	0,0003±0,0[a]	0,0002±0,0[a]	0,0001±0,0[b]	0,9895±0,07[a]	0,4138±0,01[a]	0,0470±0,0[a]	0,0011±0,0[a]	0,0023±0,0[b]

*Means followed by different letters in same line are significantly different ($P < 0.05$) by Duncan's test.
PAS = Whey
SDB = Sabouraud dextrose broth
PAS + P = Whey + cotton stalk
SDB + P = Sabouraud dextrose broth + cotton stalk

Table 7. Effect of media on LİP activity in submerged fermentation conditions at 7th day

7th day LİP U/ml (means±SE)

Medium*	*C. versicolor* 1	*C. versicolor* 2	*A. aegerita*1	*A. aegerita* 2	*A. tabescens* 1	*A. tabescens* 2	*P. ostreatus*	*F. fomentarius* 1	*F. fomentarius 1*
PAS	14,34±0,0[a]	0,00±0,0[b]	0,00	8,36±1,19[b]	2,39±1,19[b]	2,39±1,19[b]	0,00±0,0[b]	11,95±5,97[b]	0,00±0,0[c]
SDB	0,00±5,5[c]	86,02±9,16[a]	0,00	79,96±15,31[a]	3,58±0,0[b]	140,98±9.46[a]	11,95±2,72[a]	0,00±0,0[c]	0,00±0,0[c]
PAS+P	8,36±1,2[b]	53,09±5,21[a]	0,00	0,0000±0,0[b]	0,00±0,0[b]	0,00±0,0[b]	0,00±0,0[b]	22,70±0,59[a]	5,97±0,59[b]
SDB+P	0,00±0,0[c]	66,00±14,22[a]	0,00	7,17±0,0[b]	33,45±7,13[a]	11,95±1,83[b]	7,17±1,52[a]	0,00±0,0[c]	20,31±1,83[a]

*Means followed by different letters in same line are significantly different ($P < 0.05$) by Duncan's test.
PAS = Whey
SDB = Sabouraud dextrose broth
PAS + P = Whey + cotton stalk
SDB + P = Sabouraud dextrose broth + cotton stalk

Table 8. Effect of media on LİP activity in submerged fermentation conditions at 10th day

10th day LİP (means±SE)									
Medium*	*C. versicolor* 1	*C. versicolor* 2	*A. aegerita*1	*A. aegerita*2	*A. tabescens* 1	*A. tabescens* 2	*P. ostreatus*	*F. fomentarius* 1	*F. fomentarius 1*
PAS	23,89±1,19^{b}	0,00±0,0^{c}	0,00±0,0^{b}	0,00±0,0^{b}	3,58±0,0^{b}	2,39±1,19^{c}	0,00±0,0^{b}	32,26±10,94^{b}	0,00±0,0^{c}
SDB	0,00±0,0^{c}	60,93±7,28^{b}	5,97±0,75^{a}	78,85±4,53^{a}	10,75±0,0^{b}	125,45±9,06^{a}	51,37±12,84^{a}	0,00±0,0^{d}	0,00±0,0^{c}
PAS+P	8,36±1,19^{c}	86,02±6,78^{a}	0,00±0,0^{b}	0,00±0,0^{b}	9,56±0,59^{b}	20,31±4,77^{bc}	0,00±0,0^{b}	53,76±0,0^{a}	4,78±0,59^{b}
SDB+P	53,76±4,58^{a}	0,00±0,0^{c}	0,00±0,0^{b}	3,58±0,0^{b}	20,31±2,83^{a}	37,04±8,57^{b}	52,57±5,09^{a}	16,73±0,50^{c}	20,31±1,01^{a}

*Means followed by different letters in same line are significantly different ($P < 0.05$) by Duncan's test.
PAS = Whey
SDB = Sabouraud dextrose broth
PAS + P = Whey + cotton stalk
SDB + P = Sabouraud dextrose broth + cotton stalk

Table 9. Effect of media on LİP activity in submerged fermentation conditions at 15th day

	15th day LİP U/ml (means±SE)								
Medium*	*C. versicolor* 1	*C. versicolor* 2	*A. aegerita*1	*A. aegerita* 2	*A. tabescens* 1	*A. tabescens* 2	*P. ostreatus*	*F. fomentarius* 1	*F. fomentarius* *1*
PAS	8,36±3,16[a]	0,00±0,0[b]	4,78±1,19[b]	0,00±0,0[c]	0,00±0,0[c]	5,97±1,19[bc]	8,36±1,19[c]	9,56±3,16[b]	5,97±1,19[b]
SDB	8,36±1,99[a]	45,40±3,29[a]	15,53±0,75[a]	8,36±0,75[a]	0,00±0,0[c]	0,00±0,0[c]	19,12±0,75[b]	0,00±0,0[b]	2,39±0,75[b]
PAS+P	0,00±0,0[b]	41,82±8,79[a]	0,00±0,0[c]	4,78±0,59[b]	10,75±1,03[b]	38,23±8,79[a]	21,51±0,0[a]	4,78±0,59[b]	5,97±1,19[b]
SDB+P	0,00±0,0[b]	15,53±2,03[b]	0,00±0,0[c]	0,00±0,0[c]	21,51±3,18[a]	21,51±3,18[ab]	0,00±0,0[d]	47,79±4,52[a]	45,40±3,56[a]

*Means followed by different letters in same line are significantly different ($P < 0.05$) by Duncan's test.
PAS = Whey
SDB = Sabouraud dextrose broth
PAS + P = Whey + cotton stalk
SDB + P = Sabouraud dextrose broth + cotton stalk