ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Production of some bioactive materials by *Pleurotus ostreatus* from pineapple residues and rice straw via solid state fermentation

Mona M. Rashad*, Hala M. Abdou and Abeer E. Mahmoud

Biochemistry Department, Division of Genetic Engineering and Biotechnology, National Research Centre, Dokki 12622, Giza, Egypt

ABSTRACT

The utilization of wastes by bioconversion into value added products may be an innovative solution to the environmental waste problem. The objectives of this work were to study the production of enzymes (amylase, invertase, filter paper cellulase, carboxy methyl cellulase, xylanase and chitosanase) and bioactive phenolic and antioxidant compounds by *Pleurotus ostreatus* NRRL-0366 in solid state fermentation (SSF) using different agricultural residues (rice straw and pineapple waste) at different concentrations. Highest amylase activity (93.75U/gds) was obtained with concentration (triple amount of pineapple waste mixed with rice straw). The maximum activity of invertase enzyme (69.6 U/ gds.) was achieved with double amount of rice straw mixed with pineapple waste. Maximum filter paper cellulase. Activity (18.9U/ gds.) was observed when *P. ostreatus* was grown on rice straw only. The maximum activities of carboxy methyl cellulase, xylanase and chitosanase enzymes (56.55, 55.35 and 46.5 U/ gds), respectively were achieved with concentration (triple amount of rice straw mixed with pineapple waste) respectively. The total polyphenol and antioxidant activities of seven extracts were determined.

Key words: SSF; *Pleurotus ostreatus*; rice straw; pineapple residue; enzymes; antioxidants.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Agro- industrial wastes which are available in large quantities are the most abundant renewable resource on earth. However, the disposal of these wastes presents an increasing environmental problem. Rice straw is an abundant lignocellulosic waste material in many parts of the world. Rice straw production amounts to approximately 731 million tons per year globally, with distribution in Africa (20.9 million tons), Asia (667.6 million tons), and Europe (3.9 million tons)[1]. It contains between 25-45% of cellulose, 20-30% hemicellulose and 10-15% lignin [2].Pineapple residue (crown, peel and central core) accounts for about 50% of the total pineapple fruit weight corresponding to about ten tons of fresh pineapple. This waste contains high amounts of crude fiber and suitable sugars that are necessary for microorganisms growth [3-4].

Enzymes are among the most important industrial products obtained for human needs by microbial sources, in fact, a large number of industrial processes in the areas of industrial, environmental and food biotechnology utilize enzymes at some stage or the other. In solid state fermentations using of agro industrial residues as substrates shows increasing interest for the production of enzymes [5]. *P. ostreatus* is an edible and easy to cultivate[6-8].During the growth of mushroom mycelia and the formation of fruit bodies , different enzymes are secreted extracellular to participate to degrade the insoluble material into simple one [9]. The production of antioxidant phenolic compounds through microbial fermentation processes have been established due to their cost-effectiveness and environmental advantages. During fermentation process, antioxidant phenolics are either produced by microorganisms through secondary metabolic pathway or released from the matrix of the substrate by extracellular enzymatic action. Mushrooms are used as important source of home remedy to protect human body from various disease elicited by oxidative stress [5,7,10]. Mushroom phenolic compound has been found to be an excellent antioxidant and synergist that is not mutagenic [11].

To our knowledge pineapple residue have never been tested to grow mycelial mushroom of *P. ostreatus*. So, the aim of this study was undertaken to study the production of mycelial mushroom by S.S.F. of seven different combination of the two substrates (pineapple residue and rice straw). Moreover, the study aimed to evaluate the effect of substrates type on the enzyme production and antioxidant activities in *Pleurotus ostreatus* culture filtrates.

MATERIALS AND METHODS

Organism:

Pleurotus ostreatus NRRL-0366, kindly provided by agricultural research service, Peoria, Illinois, USA. The stock culture was maintained on potato dextrose agar (PDA) for 1-2 weeks at 28-30°C, then stored at 4°C [12].

Agro-industrial residue (Substrates):

Fresh pineapple residue (crown, peel and central core) was obtained from Egyptian juice processing shops in the local market. Rice straw collected from field and pretreated mechanically by chopping to reduce the particle sizes to 2cm in length. Both were used as substrates for enzymes production and antioxidant activity evaluation. Seven different combinations between pineapple residue and rice straw were used as the basic substrates for solid state cultivation as shown in table (1).

Table 1: Different Substrate concentrations for solid state cultivation of *P. ostreatus*

Substrates	Pineapple residue : rice straw ratios
1	3:0
2	Ditto 3:1
3	Ditto 2:1
4	Ditto 1:1
5	Ditto 1:2
6	Ditto 1:3
7	Ditto 0:3

Spawn preparation:

The spawn were prepared using wheat grains. The grains were washed thoroughly and 1kg was boiled in 2 liters of water until they became semi-soft. They were then decanted over a sieve to remove excess water and cooled to room temperature. Hundred grams wet weight grains packed in 250ml glass bottles and then they were sterilized at 121°C for 15 minutes. Thereafter each sterilized one was inoculated with *P. ostreatus* NRRL-0366 mycelia and incubated at 25°C in full darkness for two weeks to enable the mycelia to permeate [13].

Solid state fermentation:

Each substrate was sterilized at 121°C for 20 minutes and left to cool. Then inoculated with *P. osteratus* spawn. The fermentation takes place statically in an incubator at 25°C for 10 days.

Extraction method:

The fermented matter was mixed with 10-fold (v/w) distilled water by shaking (180 rpm) at 30ºC for 60 min [14]. The resultant slurry was centrifuged at 13,000xg for 5 min. Finally, the extracts were collected and considered as a source of crude enzyme and used for determination of total phenolic content and total antioxidant activity.

Enzymatic assays:

Amylase assay:

Amylase was assayed by incubating 1 ml of cell free filtrate with 0.1% soluble starch in 0.05M acetate buffer (pH 4.8)[5].After 10 min incubation at 37°C the reducing sugar was determined by Somogyi-Nelson method [15,16] method using glucose as a standard.

Invertase assay:

Invertase activity in the culture filtrate was measured by estimating the liberated reducing sugar released by the hydrolysis of sucrose, as described by Somogyi-Nelson method [15, 16] using fructose as a standard. The reaction mixture contained 1 ml of 0.3 M sucrose, 0.2 ml of the enzyme solution and 0.8 ml of 0.05 M acetate buffer, pH 5.0, and was incubated at 40 °C for 20 min [17].

Filter paper cellulase (FPase) assay:

Whatman No. 1 filter paper (1.0cm x 6.0cm) in 1ml of 0.1M sodium citrate buffer pH 5was used as a substrate and incubated with 1ml of crude extract for 1h at 50°C. [18]. The reducing sugar released was determined by Somogyi-Nelson method [15,16] using glucose as a standard.

Carboxymethyl cellulase (CMCase) assay:

Carboxymethyl cellulose (2% W/V) was used as a substrate prepared in 0.05M sodium citrate buffer (pH 4.8). The reaction mixture contained 0.5ml of culture filtrate and 0.5ml substrate was incubated at 50°C for 30min for enzymatic reaction [18]. After incubation, the reducing sugar was determined by Somogyi-Nelson method [15,16] using glucose as a standard.

Xylanase assay:

Xylanase activity in culture filtrate was measured by estimating the liberated xylose from birch wood xylan as described by Somogyi-Nelson method [15,16] using xylose as a standard. The standard reaction mixture contained 1 % (W /V) of the standard (birch wood xylan) dissolved in 0.05 M acetate buffer pH 5.5 and an appropriate amount of an enzyme in a total volume of 0.3 ml. The reaction mixture was incubated at 50 °C in a water bath for 1h [13].

Chitosanase assay:

An aliquot of 1% chitosan (0.15ml) in 1% acetic acid (pH 4.0) and 0.05 ml of enzyme solution in a total volume of 0.2 ml were incubated at 37°C for 5 min [19]. Finally, the reducing sugar released as a result of the above enzymatic actions were estimated as described by Somogyi-Nelson method [15, 16] using glucosamine as a standard.

Enzymes activities:

One unit of enzyme activity was defined as the amount of enzyme that releases 1 µmol equivalents of reducing sugars (expressed as glucose or fructose or xylose or glucosamine according to each enzyme) in 1 min under the assay conditions. Enzyme activity was expressed as unit per gram of dry substrate.

Determination of total phenolic content:

Phenolic compounds in the samples extracts were estimated by using Folin – Ciocalteu assay [20,21]. One milliliter of sample from aqueous extracts of culture filtrates was mixed with 1ml of Folin-Ciocalteu phenol reagent. After 5min. 1ml of 13% sodium carbonate solution was added to the mixture and adjusted to 10ml with distilled water. The reaction was kept in the dark for 90 min. and its absorbance was read at 725nm. The results were expressed as mg of gallic acid equivalents per 100g dry substrates.

DPPH radical- scavenging assay:

The DPPH method was used to determine the radical scavenging activity of culture filtrates [22]. 0.05ml of each of culture filtrate was added to 1.95 ml of methanolic DPPH (0.01mM) and mixed thoroughly. The mixture was left to stand for 45 min in the dark at room temperature and the absorbance was measured at 515nm. A lower absorbance represented a higher DPPH scavenging.
Scavenging activity (%) = [1 - (absorbance sample/absorbance control)] x 100%.

All data were represented as mean of triplicate values

RESULTS AND DISCUSSION

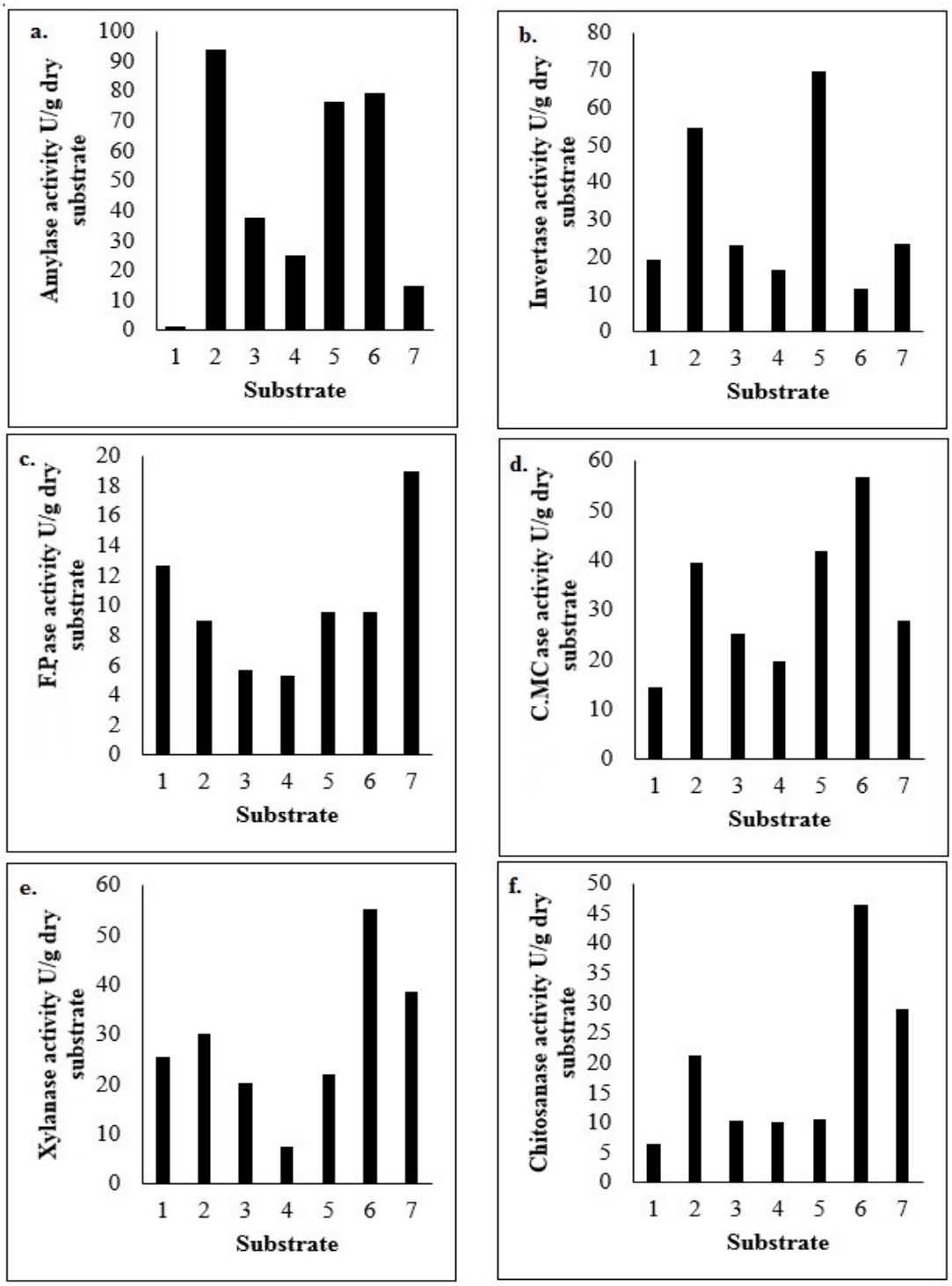
Pineapple residue as it is rich in carbohydrates and other nutrients can serve as an economic substrate for production of useful metabolites through SSF technique [4]. An attempt to supplement this substrate with rice straw (another environmental pollutant) can be a suitable substrate for mushroom *P. ostreatus* NRRL-0366 cultivation. The two substrates (Pineapple residue and rice straw at different ratios) were used for the production of different enzymes (amylase, invertase, cellulases, xylanase and chitosanase) as shown in Figure 1a-f.

Highest amylase activity (93.75 and 79.28 U/gds) were achieved with the culture filtrate of *P. ostreatus* using triple amount of pineapple residue mixed with rice straw (substrate no. 2) and substrate no.6 (triple amount of rice straw to that pineapple residue), respectively (Figure 1a). While, its lower activity was obtained (11.1 U/gds) when *P. ostreatus* was grown on fresh unsupplement pineapple residue (substrate no. 1). Rajoriya [23], stated that the enzymatic screening in the mycelium of two *Pleurotus* species (*P.florida* and *P.sajor-caju*) showed good activities of amylase and lipase.

Supplementation of pineapple residue with double amount of rice straw (substrate no.5) enhanced the activity of *P. ostreatus* invertase enzyme 3.68 times compared to the unspplemented one. (Figure1b)

Rice straw (substrate no.7) is a good substrate for production of *P. ostreatus* FPase (18.90 U/gds) while using pineapple residue supplemented with equal amount of rice straw (substrate no. 4) gave 5.25 U/gds. On the other hand, Pineapple residue is considered as a good substrate for cultivation of *P. ostreatus* to produce CMCase enzyme as shown in data of Figure1d. The activities increased 3.89 times when supplemented with rice straw (substrate no.6).

ISSN: 0975-8585



1. Pineapple waste only.
2. Mixture of pineapple waste and rice straw at ratio P3:R1.
3. Ditto P2:R1.
4. Ditto P1:R1.
5. Ditto P1:R2.
6. Ditto P1:R3.
7. Rice straw only.

Figure 1: Effect of media composition on enzyme production by P. ostreatus NRRL-0366 using SSF (a) Amylase, (b) Invertase, (c) FPase, (d) CMCase, (e) xylanase and (f) Chitosanase.

ISSN: 0975-8585

The data in Figure1e,f showed that the maximum activities of *P. ostreatus* xylanase and chitosanase enzymes (55.35 and 46.50 U/gds ,respectively) were achieved with substrate no. 6 ,while their lowest activities recorded 6.5 U/gds when substrate no.4 and substrate no.1 were used ,respectively. This data disagreed with that reported by Rajoriya et al. [23] who obtained negative results of xylanase in the mycelium of *P. florida* and *P. sajor-caju*.

Phenolics are secondary metabolites commonly found in plants and fungi which exert multiple biological effects including antioxidant activity [24,25].The evaluation of the amount of total phenolic compounds as well as the identification of the main phenolic in mushrooms have both great importance in their nutritional and functional characterization. Kalyoncu et al.[26] found that extraction of 21 wild mushroom species mycelia with water was significantly higher in their antioxidant activities than those of ethanol and chloroform extracts. The total polyphenol content in the seven extracts ranged from 17.96 – 65.42 mg gallic acid equivalent/ 100 gds. (Table 2). The data in table 2 showed that in general the supplementation of the pineapple residue with rice straw improved its phenolic content. The highest phenolic content (65. mg gallic acid equivalent/ 100 gds) was obtained by using double amount of pineapple residue to that of rice straw (substrate no. 3) followed by the substrate no. 4 and 5 which recorded 41.70 and 41.25 mg gallic acid equivalent/ 100 gds.,respectively. The fresh unsupplemented pineapple residue (substrate no.1) showed the lowest value (17.96 mg gallic acid equivalent/ 100 gds).The values obtained from the seven extracts were rich in phenolic compounds and its contents are similar or higher than those found in other edible and medicinal mushrooms [26-31].

Table 2: Total phenolic content in *P. osteratus* culture filtrates of different cultivation media composition

*Substrates	Total phenolic (mg gallic acid equivalent/ 100 gds)
1	17.96
2	38.25
3	65.42
4	41.70
5	41.25
6	36.48
7	32.30

*Substrates 1-7 as mentioned previously in table 1.

Table (3) showed the antioxidant activity of the seven extracts by by DPPH free radical scavenging method. Their activities were ranged from 4.79% - 26.24% which are higher than the value of *P. florida* (9.95%) and less than that of *P. sajor-caju* (45.53%)[23].The highest antioxidant activity (26.24%) were obtained by using the third substrate (pineapple residue and rice straw at ratio 2:1),while the lowest one (4.79%) were achieved by using substrate no.7 (rice straw alone).

Table 3: DPPH radical scavenging activity of *P. osteratus* culture filtrates of different cultivation media composition

*Substrates	DPPH free radical scavenging activity (%)
1	18.77
2	25.19
3	26.24
4	10.58
5	11.05
6	14.62
7	4.79

*Substrates 1-7 as mentioned previously in table 1.

CONCLUSION

Pineapple residue is considered as a good substrate for growing the oyster mushroom especially when supplemented with rice straw using SSF technique for producing some bioactive materials (enzymes, phenolics and antioxidants) which can be used in multi applications.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the financial support by the National Research Centre, Egypt (Project no.10130105).

REFERENCES

[1] Binod P, Sindhu R, Singhania RR, Vikram S, Devi L, Nagalakshmi S, Kurien N, Sukumatan RK, Pandey A. Bioresour. Technol 2010; 83:1-11.
[2] SunY, Cheng J .Bioresour Technol 2002; 83:1-11.
[3] Hemalatha R, Anbuselvis J. Chem Pharm Res 2003; 5:240-242.
[4] Rashad MM, Mahmoud AE, Nooman MU, El-Torky AMM, Keshta AT, Mahmoud HA .Res J Pharm Biol Chem Sci 2015; 6:467-474.
[5] Rashad MM, Abdou HM, Mahmoud AE, Nooman MU. Australian J basic Appl Sci 2009; 3:3352-3360.
[6] Kues U, Y Liu. Appl Microbiol Biotechnol 2000; 54: 141-152.
[7] Rashad MM, Abdou HM. Adv Food Sc 2002; 24:79-84.
[8] Aggarwal P, Pushpa S, Sakshi S, Jyoti A. A review. Intern Res J Pharm 2012; 3: 65-70.
[9] Giardina PR, Cannio L, Materiani L, Marzullo G, Palmiari G, Sannis G. Appl Environ Microbiol 1995; 61: 2408-2413.
[10] Chen H, Ju Y, Li J, Yu M.. Intern J Biol Macromol 2012; 50: 214-218.
[11] Ishikawa NK, Kasuya MCM, Vanetti MD. Brazilian J Microbiol 2001; 32: 206-210.
[12] Jodon MH, Royse D. J Penrsylvania Agric Exp Sta Bull 1979; 258: 4-10.
[13] Rashad MM, Fathy SA, Abdou HM, Mahmoud AE, Jwanny EW. Modell Measure Control 2002; 64:1-18.
[14] Yang S, Yan Q, Jiang Z, Li L, Tian H, Wang Y. Bioresour Technol 2006; 97: 1794-1800.
[15] Nelson N. J Biol Chem 1944; 153: 375-380.
[16] Somogyi M. J Biol Chem 1952; 165: 19-23.
[17] Ghosh K, Dhar A,Samanta TB. Indian J. Biochem. Biophys. 2001;38: 180-185.
[18] Gupta C, Jain P, Kumar D, Dixit AK, Jain RK. Intern J Appl Microbiol Biotech Res 2015; 3: 11-19.
[19] Chiang CL, Chang YM, Chang CT, Sung HY. J Agric Food Chem 2005; 53: 7579-7585.
[20] Singleton VL, Rossi JA. American J Enology and viticult 1965; 16:144-158.
[21] Olajire AA, Azeez L. African J Food Sci Technol 2011; 2: 22-29.
[22] Sanchez – Moreno C, Larrauri JA , Sauracalixto F. REs Int 1999 ; 32: 407-412.
[23] Rajoriya A, Panda A, Gupta N. Tropical plant research 2014; 1: 22-26.
[24] Dimitrios B. Trends food Sc Technol 2006; 17:505-512.
[25] Kim MY, Seguin P, Ahn JK, Kim JJ, Chum S, Kim EH. J Agric Food Chem 2008; 56: 7265-7270.
[26] Kalyoncu F, Oskay M. Kayalar H. Mycology 2010; 1, 195-199.
[27] Mau JL, Lin HC, Chen CC. J Agric Food Chem 2002; 50: 6072-6077.
[28] Asatiani, MD, Elisashvil VL, Wasser SP, Reznick AZ, Nevo E. Biosci Biotechnol Biochem 2007;71:3090-3092.
[29] Barros L, Ferrrera LCFR, Baptista P. Food Sci Technol Intern 2008;141: 47-55.
[30] Jayakumar T, Thomas PA, Ceraldine P. Innovative food Sci Emerging Technol 2009;10: b228-234.
[31] Carvajal AESS, Koehnlein EA, Soates AA, Nakashima ATA, Bracht A, Peralta RM. LWT Food Sci Technol 2012;46: 493-499.