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Growth and Lignocellulolytic Enzyme Profile of Different Strains of *Lentinus edode* with Different Carbon Sources

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***Lentinus edodes* (Berk.), the Shiitake mushroom, is highly prized for nutritive and medicinal values. It is traditionally cultivated on the wood logs of Shii tree in Japan. Non availability of the Shii tree in India has necessitated a search for alternative substrates for shiitake cultivation. The investigation focuses on the possibility of using some of the locally available lignocellulosics materials as carbon source in growing media. Three strains of shiitake mushroom (LeC, LeS, OE329) were cultivated on rice husk, wheat bran, corn-cobs and sugarcane bagasse as carbon sources. The maximum linear mycelial growth was observed in LeC strain followed by LeS while a slower mycelial extension was observed in OE329 strain. Among all the three strains endoglucanase activity was found to be highest for LeC (corn-cobs) followed by LeS (corn-cobs) and OE329 (rice husk), while cellobiase activity was maximum with rice husk followed by corn cobs among all the three strains (LeC, LeS, OE329) and maximum exoglucanases was released by LeC followed by LeS and OE329 on their culturing on rice husk as carbon source. Wheat bran as carbon sources resulted in maximum laccases activity and that was on par with rice husk. Thus all the three strains of *L.edodes* showed the preference for rice husk as carbon source. LeC is the only strain that showed maximum growth and enzyme activity on all the C sources tried. There appears to be a direct correlation between the growth and enzyme released by different strains on different carbon sources.**

Key words: Cellulases, Laccases, *Lentinus edodes,* Mycelial growth, Shiitake.

Mushrooms have been recognized as an important health and medicinal food throughout the globe. They are rich sources of proteins, vitamins and minerals; low in fat with high proportion of unsaturated fatty acids and contains no cholesterol (Garcha *et al.,* 1993). World production of mushrooms is estimated around 12 million tons and is growing at the rate of over above 8 % (Rai & Arumuganathan 2008). Shiitake has second position (25.4%) on production After the well-known button mushroom (*Agaricus bisporus*), shiitake is the most cultivated of exotic mushroom in the world (Chang 1999).

Shiitake is one of the best known and characterized mushrooms used for medicinal purposes. Several medicinal properties such as immunological, anti-cancer, antioxidants, antihypertensive, cholesterol- lowering, liver protective, anti-fibrotic, anti-inflammatory, anti-diabetic, anti-viral and anti-microbial activities have been documented in the recent years (Ooi & Liu, 1999; Wasser & Weis, 1999 a,b;).

Sawdust is the most popular basal ingredient used in substrates to produce shiitake (Miller and Jong, 1987; Palomo *et al.,* 1998; Grodzinskaya *et al.,* 2003). Shiitake mushroom is traditionally cultivated on the shii tree

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[*Castanopsis cuspidate* (Thunb.) Schott] or wood logs in Japan. Non availability of the Shii tree in India has necessitated a search for alternative substrates for shiitake cultivation.

Freely available huge amounts of lignocellulosics may offer potential alternative substrates for its cultivation in India. The major constituents of the substrates on which mushrooms grow are cellulose, hemicellulose and lignin. The utilization of the insoluble lignocellulosic substrate by edible mushrooms depends upon the wide array of lignocellulolytic enzymes by fungal mycelium which is a crucial part of the colonization process and is an important determinant of mushroom yield (Thurston 1994). Therefore, the present study is undertaken to evaluate the mycelial growth and lignocellulosic enzyme profile of three different strains of *Lentinus edodes* (shiitake) with view to suggest the best possible alternative substrate for culture of Shiitake in India.

MATERIALS AND METHODS

Culture Maintenance and Inoculum Preparation

Three strains of *Lentinus edodes* used in the present study namely Le-C, Le-S and OE 329 were procured from the Culture Collection Bank of Department of Microbiology, Punjab Agricultural University, Ludhiana and were maintained on Potato Dextrose Agar (PDA) slants at 25±2°C by subculturing fortnightly. The cultures were grown on the same medium as in petriplates and standard mycelial discs (5mm) were cut with the help of a cork borer and used for further growth and enzymatic study.

Growth Studies

Growth of three strains of *L. edodes* was carried out in petri plates on Mushroom Mimimal Medium (MMM) (*Jo et al.,* 2006) supplemented with different carbon sources viz. rice-husk, wheat-bran, corn-cobs, and sugarcane-bagasse

A mycelia disc (5mm diameter) was cut from the culture plate and placed in the centre of the plate such that the mycelial end of the disc touches the surface of the media. The entire process of pouring and inoculation was carried out under aseptic conditions. After inoculation the plates were incubated at 25 ± 2ºC in an inverted position. The radial growth of mycelia was measured with the scale at intervals of three days periodically upto 15 days.

Enzyme Production and Extraction

L. edodes strains were grown in MMM containing different Carbon Sources in Erlenmeyer's flasks of 250 ml capacity in solid state . Each flask was inoculated by an agar bit (10 mm diameter) of *L. edodes* fungal mycelium grown on PDA petri-plate. The flasks were incubated at 25 ± 2ºC for upto 21 days. The enzyme was extracted by agitating the content of flask with water and filtered through the muslin cloth. The process was repeated by adding water to give a final volume of 30 ml.

Enzyme Assays

Three cellulolytic enzymes namely exocellobiohydrolase (EC 3.2.1.91, 1,4-β-D-glucan cellobiohydrolase), endo-1,4-β-D-glucanase (EC 3.2.1.4, 1,4-β-D-glucan glucanohydrolase) and β-glucosidase (EC 3.2.1.21, β-D-glucoside glucohydrolase) were estimated in the extracted sample. The endoglucanase activity was measured by the incubation of carboxymethyl cellulose (CMC) with enzyme extract. Exoglucanase and cellobiase activities were measured by using whatman filter paper and cellobiose respectively as substrates. The reducing sugars liberated during the assay reaction were estimated as glucose by using Nelson - Somogyi method (Somogyi 1952) . The activity of oxidative enzyme laccase was determined by using 2, 2'-azino-bis (3-ethylbenzthiazoline-6-sulfonate) (ABTS) as a substrate using the standard routine protocol given by (Buswell *et al.,* 1995). Oxidation of ABTS was monitored at 420 nm (ΔA) with a molar extinction coefficient value (ε= 36, 000 M^{-1} cm^{-1}). Total soluble phenolics in all the samples were determined using Folin-Ciocalteu colorimetric method based on the procedure described by (Singleton and Rossi 1965) with some modifications. The concentration of total phenol was calculated from the standard curve prepared using gallic acid as substrates and total amount of phenol contents was expressed as GAE (mg/g sample).

RESULTS AND DISCUSSION

Growth Studies

Three strains of *L edodes* (Le-C, Le-S and OE-329) were evaluated during the present

investigation to study the effect of different carbon sources on growth and enzyme production. The linear growth of the mycelium in plates was recorded periodically at an interval of 3 days upto a period of 15 days to observe mycelial extension rate (Table 1). The results showed that growth continued to increase with the increase in growth period. The growth rate was however different for different strains and varied with different carbon sources. Among the four different organic carbon sources maximum linear mycelial growth was observed in Le-C strain followed by Le-S while a slower mycelial extension was observed in OE-329 strain.

Le-C showed maximum linear mycelial growth of 84.0 mm on rice husk as a carbon source while mycelial extension was minimum with sugarcane bagasse (78.3mm). Le-S strain showed maximum mycelial extension of 76.5 mm on corn cobs and minimum of 58.3 mm on sugarcane bagasse. The mycelia extension rate was the lowest for OE-329 strains among all the strains which shows maximum linear mycelial growth on corn cobs (67.5 mm) and minimum on sugarcane bagasse

Table 1. Effect of different Carbon sources (supplements) on Mycelial extension (mm) of three strains of *Lentinus edodes*

Lentinus	Growth Period	Carbon Sources			
Edodes	(Days)	Rice Husk	Wheat Bran	Corn Cobs	Sugarcane Bagasse
Le C	3	9.5	10.5	10.3	10.3
	6	21.3	20.8	19.8	22.0
	9	43.0	42.3	40.5	47.5
	12	65.5	62.3	61.5	68.3
	15	84.0	83.0	79.5	78.3
Le S	3	10.8	8.5	15.5	9.3
	6	20.8	12.4	22.3	13.3
	9	34.8	22.8	38.0	23.8
	12	54.0	40.3	53.0	36.3
	15	76.3	65.0	76.5	58.3
OE 329	3	9.8	9.5	10.0	7.5
	6	18.3	19.2	17.0	14.2
	9	30.3	34.0	36.5	29.5
	12	46.0	48.5	50.2	42.0
	15	62.5	64.0	67.5	56.2

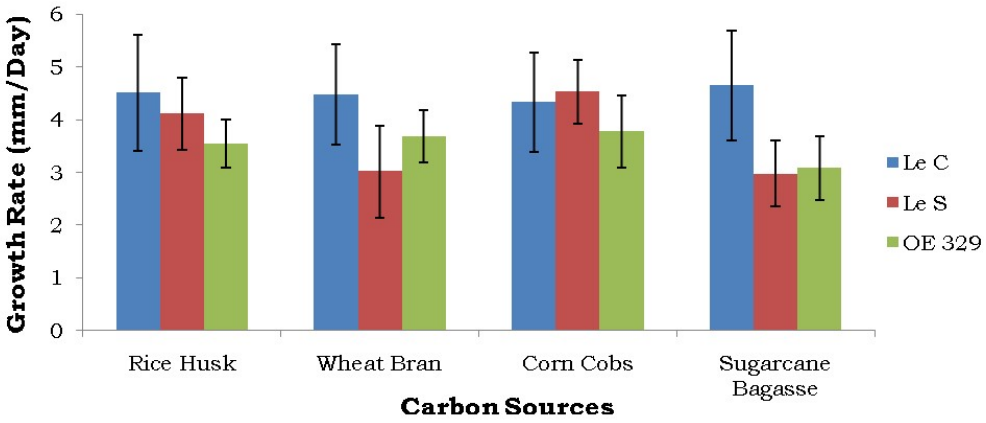

Fig. 1. Effect of different carbon sources on growth rate of shiitake

(56.2 mm). The linear growth rates ranged between 3.17 mm/day to 5.60 mm/day for Le C, 2.05 mm/day to 5.17 mm/day for Le S and 2.37 mm/day to 4.83 mm/day for OE-329 (Fig. 1).

Enzyme Studies

Utilization of insoluble lignocellulosic substrates by mushrooms depends on the production of a pool of hydrolytic (cellulolytic) and oxidative (laccase) enzymes that bring about hydrolysis of the macro molecules of cellulose and lignin components respectively, thereby, librating low molecular weight nutrients (Buswell *et al.,* 1995). In order to assess their capability of producing lignocellulolytic enzymes namely endoglucanase, exoglucanase and cellobiase and lignin modifying extracellular oxidoreductase-laccases.

Release of these enzymes the three strains of *L. edodes* were grown on Mushroom Minimal Media (MMM) broth supplemented with different carbon sources and the amount of water in the medium was adjusted to achieve slummy state. The fermentation conditions were maintained for 21 days at 25 ± 2ºC .

Supplementation of corn cobs and rice husk enhanced the production of endoglucanases (CMCase) of all the three strains (Table 2). Le-C and Le-S showed maximum activity 7.37 and 7.22 U/g, respectively on corn cobs followed by OE-329 (6.62 U/g rice husk), whereas the endoglucanases activity of all strains were on par with each other on the remaining substrates.

Exoglucanase (Fpase) activity was maximum in Le-C (2.18 U/g) and Le-S (1.92 U/g) with rice husk as substrate followed by OE-329 (0.91 U/g) on rice husk. Fpase activity on other substrates did not differ significantly (Table 2).

Cellobiase (Cbase) estimation indicated maximum activity in rice husk as carbon source in OE-329 (49.69 U/g) followed by Le-C (31.10 U/g) and Le-S (25.17 U/g) that was at par with corn cobs (Le-C followed by Le- S and OE-329) (Table 2).

Endoglucanase activity in general, was found to be the highest followed by Cbase and Fpase activity. Kapoor *et al.,* (2009) reported improved mycelial extension rates and higher biological efficiencies of Le-S strain of *L. edodes* on supplementation of wheat straw with wheat bran. They reported the maximum growth for this strain with 10% rice bran and 20% wheat bran supplementation. They also reported that CMCase activity was the highest followed by Cbase and Fpase. CMCase activity increased with increasing incubation period up to the 30th day while the activities of Cbase and FPase did not increase significantly. According to Fasidi and Kadiri (1991) the increased mycelia growth of *L. edodes* on a different substrate was attributed to the carbohydrates, amino acids and minerals present in the supplements used. Leatham (1985) had

Table 2. Lignocellulolytic enzymes profile of different strains of *Lentinus edodes* as a function of different Carbon Sources

Carbon Sources	Endoglucanase Activity (U/g)		
	Le-C	Le-S	OE-329
Rice Husk	3.7	7.07	6.62
Wheat Bran	4.16	0.25	1
Corn Cobs	7.37	7.22	-
Sugarcane Bagasse	2.16	1.89	0.23
Exoglucanase Activity (U/g)			
Rice Husk	2.18	1.92	0.91
Wheat Bran	0.31	-	0.35
Corn Cobs	0.6	1.42	0.22
Sugarcane Bagasse	0.29	0.41	0.15
Cellobiase Activity (U/g)			
Rice Husk	31.1	25.17	49.69
Wheat Bran	-	-	0.49
Corn Cobs	3.12	1.83	1.08
Sugarcane Bagasse	2.82	-	0.98
Laccase Activity (mU/g)			
Rice Husk	564	48.8	64.3
Wheat Bran	1559	1074	1214.5
Corn Cobs	-	445.7	111.7
Sugarcane Bagasse	173.6	26.8	-

- Negligible Activity

Table 3. Total phenols released on fermentation different Carbon sources with *Lentinus edodes*

Carbon Sources	Total Soluble Phenolics Released (mg/g)		
	Le-C	Le-S	OE-329
Rice Husk	3.25	1.45	1.29
Wheat Bran	1.07	1.01	1.27
Corn Cobs	1.65	1.24	0.95
Sugarcane Bagasse	1.77	1.48	1.07

reported that utilization of lignocellulose by *L. edodes* depends on its ability to synthesize hydrolytic and oxidative enzyme. Krishnamoorthy *et al.,* (2002) in a study on the enzyme related biodegradative potiental of *L. edodes* found direct relationship between the productions of cellulases and the yield. Similar observations have been reported for *Pleurotus* and *Volvariella spp*. when grown on varied substrates. (Manning and Wood,1983; Velázquez-Cedeno *et al.,* 2002). Different levels of lignocellulolytic enzymes profile has been reported for *Volvariella volvacea* (Ahlawat *et al.,* 2005) and *Calocybe indica* (Mangat *et al.,* 2008) when grown on bean stalks and wheat straw residues.

The laccases activity was significantly different with growth medium having different carbon sources. The maximum enzyme activity was obtained with wheat bran as carbon source in all three strains (Le-C, 1559.3 mU/g; Le-S, 1074.4 mU/g and OE-329, 1214.5 mU/g) that was on par with rice husk (Le-C, 564.0 mU/g; Le-S, 48.8 mU/g and OE-329, 64.3 mU/g) after 21days of incubation. Corn cobs and sugarcane bagasse does not result in any enzyme activity in case of Le-C and OE-329, however, subsequent enzyme activity was observed in case of Le-S strain (Table 2).

Total Soluble Phenols

The maximum total soluble phenols were released by Le-C (3.25 mg/g) when cultured on rice husk and minimum on wheat bran (1.07 mg/g). In case of Le-S maximum phenolic were realized on its inoculation with sugarcane bagasse (1.48 mg/g) and minimum with wheat bran (1.01 mg/g) as carbon source. While OE-329 released maximum phenolics (1.29 mg/g) on its inoculation in rice husk as carbon source and minimum concentration observed was 0.95 mg/g on corn cobs as carbon source (Table 3). The possible reason behind the variation among the phenolic content was the lignin content present in each carbon sources and the character of each strain of *L. edodes* which releases the pool of hydrolytic and oxidative enzymes for their growth on these substrates.

CONCLUSION

The present investigation on growth and enzyme production by three strains of *L.edodes* on different carbon sources indicates the order of preference rice-husk > corn-cobs > wheat-bran > sugarcane-bagasse. Of all the strains Le-C showed the maximum growth and enzyme activity on either of the carbon sources used. The study can thus help in the formulation of substrate for raising biomass and fruiting bodies of *Lentinus edodes*.

REFERENCES

1. Ahlawat, O. P., Ahlawat, K. and Dhar, B. L. Influence of lignocellulolytic enzymes on substrate colonization and yield in monosporous isolates and parent strains of *Volvariella volvacea* (Bull. Fr.). Sing. *Indian J. Microbiol*., 2005; **45**: 205-210.
2. Buswell, J. A., Cai, Y. J. and Chang, S. T.. Effect of nutrient nitrogen and manganese on manganese peroxidase and laccase production by *Lentinula edodes*. *FEMS Microbiol. Lett.,* 1995; **128**: 81-88.
3. Chang, S.T., World production of cultivated edible and medicinal mushroom in 1997 with emphasis on *Lentinus edodes* (Berk.) Sing. in China. *Int. J. Medicinal Mushroom*, 1999; **1**: 291-300.
4. Fasidi, I. O. and Kadiri, M. Use of agricultural wastes for the cultivation of *Lentinus subnudus* (Polyporaceae) in Nigeria. *Rev. Biol. Trop*. 1991; **41**: 411-15.
5. Garcha H S, Khanna P K and Soni G L Nutritional importance of mushrooms. In Mushroom Biology and Mushroom Products (ST Chang, JA Buswell and SW Chin eds) The Chinese Univ. Press H K. 1993;pp 227-36.
6. Grodzinskaya, A. A., Infante, H. D., Piven, N. M. Cultivation of edible mushrooms using agricultural and industrial wastes. *Agronomia-Tropical-Maracay*, 2003; **52**(4): 427-447.
7. Jo, W. S., Rew, Y. H., Choi, S. G., Seo, G. S., Sung, J. M. and Uhm, J. Y The Culture Conditions for the Mycelial Growth of *Phellinus* spp. *Kor. J. Mycol.,* 2006; **34**: 200-205.
8. Kapoor, S., Khanna, P. K. and Katyal, P. Effect of Supplementation of Wheat Straw on Growth and Lignocellulolytic Enzyme Potential of *Lentinus edodes*. *World Journal of Agricultural Sciences* 2009; **5**(3): 328-331.
9. Leatham, G. F. Extracellular enzymes produced by cultivated mushroom *Lentinus edodes* during degradation of a lignocellulosic medium. *Appl. Environ. Microbiol*., 1985; **50**(4): 859-867.
10. Mangat, M., Khanna, P. K., Kapoor, S. and Sohal, B. S. Biomass and Extracellular Lignocellulolytic Enzyme Production by

Calocybe indica Strains. *Global J. of Biotechnol. & Biochem.,* 2008; **3**: 98-104.

11. Manning, K. and Wood, D. A. Production and regulation of extracellular endo-cellulase by *Agaricus bisporus. J. Gen. Microbiol*., 1983 ; **129**: 1839-47.
12. Miller, M. W. and Jong, S. C. Commercial cultivation of shiitake in sawdust filled plastic bags. *Dev Crop Sci*. 1987; **10**: 421-426.
13. Nelson, N., A photometric adaptation of the Somogyi method for the determination of glucose. *J. Biol. Chem*., 1944; **153** : 375- 80.
14. Ooio, V.E.,and Liu, F. A review of pharmacology activities of mushroom polysaccharides. *Int. J. Med. Mushrooms*, (1999).;1: 195–206.
15. Palomo, A., Door, C., Mattos, L. Comparative study of different substrates for the growth and production of Lentinus edodes Berk ("Shiitake"). *Fitopatologia*, 1998; **33**: 71-75.
16. Rai, R. D. and Arumuganathan, T. Post Harvest Technology of Mushrooms. *Tech Bull*, NRCM, Solan, 2008; 1-84.
17. Singleton, V. L. and Rossi, J. A. Jr. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagent. *Am. J. Enol. Vitic*, 1965; **16**: 144-158.
18. Somogyi, M. Notes on sugar determination. *J. Biol. Chem*. 1952; **195**: 19-23.
19. Thurston, C. F. The structure and function of fungal laccases. *Microbiology*,1994; **140**:19–21.
20. Velázquez-Cedeño, M. A., Farnet, A. M., Ferré, E. Variations of lignocellulosic activities in dual cultures of Pleurotus ostreatus and Trichoderma longibrachiatum on unsterilized wheat straw. *Mycologia*, 2002; **96**(4): 712-719.
21. Wasser, S.P. and Weis, A.L. Medicinal properties of substances occurring in higher Basidiomycetemushroom: Current Perspective. *International Journal of Medicinal mushrooms,* 1999a.; **1**: 31-62.
22. Wasser, S.P. and Weis, A.L. Therapeutic effects of substances occurring in higher Basidiomycete mushroom: A modern perspective. *Critical Review of immunology,* 1999b; **19**: 65-96.