JOURNAL OF PURE AND APPLIED MICROBIOLOGY, June 2014. Vol. **8**(3), p. 2453-2458

Growth and Lignocellulolytic Enzyme Profile of Three Strains of *Lentinus edodes* on Saw Dust of Different Indian Timber Plants

Mohan Jairath, P.K. Khanna[1], Neeta Raj Sharma and Giridhar Soni*

Domain of Biochemistry, School of Biotechnology and Biosciences,
Lovely Professional University, Phagwara, Punjab, India.
[1]Department of Microbiology Punjab Agricultural University, Ludhiana, Punjab, India.

(Received: 16 November 2013; accepted: 23 January 2014)

***Lentinus edodes*, the shiitake mushroom, is highly prized for nutritive and medicinal values. Sawdust of different timber plants have been tried as carbon sources for the growth of three different strains of this mushroom . The maximum linear mycelial growth was observed for Le-C strain followed by Le-S while a slower mycelial extension was observed for OE-329 strain. No direct correlation between growth and enzyme activities could be established. The present study indicated the preference for saw dust of Safeda and Shisham by all the three strains. Le C is the only strain with maximum growth and enzyme activity.**

Key words: *Lentinus edodes*, Shiitake, Saw-dust, Mycelial growth, Cellulolytic enzymes, Laccase activity.

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 (Chang 2007). World production of mushrooms is estimated around 12 million tones and is still growing at the rate of over above 8 % (Rai and Arumuganathan 2008). Shiitake has second position (25.4%) on production (Chang 1999b). After the well-known button mushroom (*Agaricus bisporus*), shiitake is the most cultivated of exotic mushroom in the world.

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 (Ooio & Liu, 1999; Wasser & Weis, 1999a, b; Hobbs, 2003).

Shiitake mushroom is traditionally cultivated on the shii tree [*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 sawdusts of different timber plants offer potential as alternative substrate sources 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 (Boominathan and Reddy 1992, Thurston 1994). Sawdust is the most popular basal ingredient used

* To whom all correspondence should be addressed.
E-mail: giridhar.soni@lpu.co.in

in substrates to produce shiitake (Miller and Jong, 1987; Palomo *et al*., 1998; Grodzinskaya *et al.*, 2003).

Therefore, the present study was 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

The present experiment was carried out at Department of Biotechnology, Lovely Professional University, during February to July 2011. 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 petriplates on Mushroom Mimimal Medium (MMM) (Jo *et al.,* 2006) supplemented with different carbon sources (Table 1). 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 recorde at intervals of three days periodically up to 15 days

Enzyme Production and Extraction

The mycelial biomass of *L. edodes* strains was produced in MMM containing different saw dust of timbers as Carbon Sources (Table 1) in Erlenmeyer's flasks of 250 ml capacity. 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-ß-D-glucan cellobiohydrolase), endo-1,4-ß-D-glucanase (EC 3.2.1.4, 1,4-ß-D-glucan glucanohydrolase) and ß-glucosidase (EC 3.2.1.21, ß-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 (1944) and Somogyi (1952) method. 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, Cai and Chang 1995). Oxidation of ABTS was monitored at 420 nm (”A) with a molar extinction coefficient value (µ = 36, 000 M^{-1} $cmÉ^{1}$). 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 saw dust of different timber plants as 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 2*).

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 six 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. Kapoor *et al.*, (2009) reported maximum growth for the strain Le-S with 10% rice bran supplementation and with 20% wheat bran supplementation.

Le-C showed maximum linear mycelial growth of 87.5 mm on saw dust of safeda as a carbon source while mycelial extension was minimum with Kail (56.3mm). Le-S strain showed maximum mycelial extension of 73.5 mm on shisham and 39.5 mm on Himalayan spruce (partal) saw dust. The mycelia extension rate was the lowest for OE-329 strains among all the strains which shows maximum linear mycelial growth on safeda (71.4 mm) and minimum on kail (25.3 mm). The linear growth rates ranged between 2.70 mm/day to 6.46 mm/day for Le C, 1.88 mm/day to 4.90 mm/day for Le S and 1.67 mm/day to 4.76 mm/day for OE-329 (Figure 1).

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. Similarly, Permana *et al.*, (2000) obtained improvement in yield of *L. edodes* on supplementation of wheat bran upto 15% on dry weight basis of the wheat straw substrate. Agarwal (2007) and Lalitesh (2009) also reported improved mycelial extension rates and higher biological efficiencies of *L. edodes* strain Le-S on supplementation of wheat straw with wheat bran.

Table 1. Different Carbon sources used as substrate base

Scientific Name	Common Name	Family
Dalbergia sisoo	Shisham (Tahli)	Fabaceae
Tectona grandis	Sagwan (Teak)	Lamiaceae
Eucalyptus globosus	Safeda	Myrtaceae
Picea smithiana	Himalayan Spruce (Partal)	Pinaceae
Michelia champaca	Aura Wood (Champ)	Magnoliaceae
Pinus wallichiana	Blue Pine (Kail)	Pinaceae

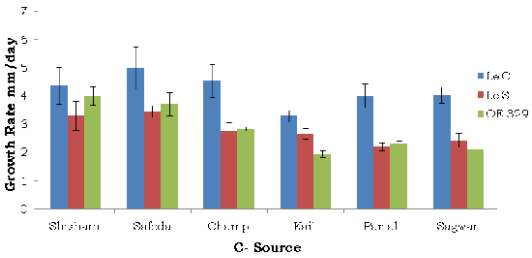

Fig. 1. Effect of different saw dust (C-Source) on growth rate of Shiitake

Table 2. Effect of different Carbon sources (supplements) on mycelial extension (mm) of three strains of *Lentinus edodes* (shiitake mushroom)

Lentinus edodes	Growth Period (Days)	Saw Dusts as Carbon Source					
		Shisham	Safeda	Champ	Kail	Partal	Sagwan
Le C	3	8.3	9.8	9.0	8.8	9.0	10.5
	6	16.8	18.5	19.0	16.2	18.5	20.5
	9	47.3	57.5	48.3	31.5	38.8	35.0
	12	68.8	77.5	69.0	44.0	59.0	56.5
	15	79.8	87.5	81.0	56.3	72.0	70.0
Le S	3	8.8	10.0	7.3	7.5	7.0	6.5
	6	13.8	18.0	12.3	12.5	11.3	11.3
	9	21.0	30.8	26.0	23.8	17.0	19.0
	12	49.0	40.5	34.0	37.8	28.3	35.3
	15	73.5	62.8	55.8	45.5	39.5	46.8
OE 329	3	9.0	8.0	8.8	7.0	7.8	6.5
	6	21.0	17.0	15.6	12.5	13.3	11.0
	9	39.2	35.5	26.2	16.8	19.2	18.5
	12	56.3	52.0	32.8	20.8	27.0	26.5
	15	68.5	71.4	45.3	25.3	36.3	34.0

Enzyme Studies

The 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 and Chang,1993). 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 to assess their capability of producing lignocellulolytic enzymes namely endoglucanase, exoglucanase and cellobiase and lignin modifying extracellular oxidoreductase- laccases.

Saw dusts of various timber plants generally supported the production of endoglucanases (CMCase) of all the three strains through varying degree (Table 3).

OE-329 and Le-C showed maximum activity of 8.23 U/g and 6.06 U/g, with Shisham and Kail sawdust respectively, followed by Le-S (6.03 U/g Partal), whereas the endoglucanases activity of all strains were on par with each other on the remaining substrates.

Exoglucanase (Fpase) activity was maximum in OE-329 (1.65 U/g) and Le-C (0.91 U/g) with shisham (tali) saw dust as substrate followed by Le-S (1.03 U/g) on saw dust of Safeda. Fpase activity on other substrates did not differ significantly (Table 3).

Cellobiase (Cbase) estimation indicated maximum activity in saw dust of safeda as carbon source

Table 3. Lignocellulolytic enzymes profile of different strains of *Lentinus edodes* as a function of Saw Dusts of different timbers as Carbon Sources.

Carbon Sources	*Lentinus edodes* strains		
	Le-C	Le-S	OE-329
	Endoglucanase Activity (U/g)		
Shisham (Tahli)	2.06	1.6	8.23
Sagwan (Teak)	1.06	-	1.57
Safeda	4.21	4.78	1.84
Partal	3.17	6.03	6.11
Champ	1.03	2.64	4.56
Blue Pine (Kail)	6.06	3.81	5.73
	Exoglucanase Activity (IU/g)		
Shisham (Tahli)	0.91	0.81	1.65
Sagwan (Teak)	0.22	0.58	0.49
Safeda	0.89	1.03	1.13
Partal	0.27	0.69	1.11
Champ	-	0.54	-
Blue Pine (Kail)	0.42	0.07	1.01
	Cellobiase Activity (IU/g)		
Shisham (Tahli)	0.82	3.71	17.18
Sagwan (Teak)	-	4.02	0.18
Safeda	28.31	20.62	17.19
Partal	2.13	2.05	-
Champ	8.09	11.48	-
Blue Pine (Kail)	-	9.97	5.52
	Laccase Activity (mIU/g)		
Shisham (Tahli)	34.8	1162	40.4
Sagwan (Teak)	-	2428	15.4
Safeda	249.1	4652	3763
Partal	148.5	1532	-
Champ	-	276.3	-
Blue Pine (Kail)	-	1798	-

Table 4. Total soluble phenolics released on fermentation of *Lentinus edodes* with different Carbon sources

Carbon Sources	Total Soluble Phenolics Released (mg/g)		
	Le-C	Le- S	OE-329
Shisham (Tahli)	1.52	1.35	1.46
Sagwan (Teak)	0.62	1.38	0.64
Safeda	9.25	1.6	1.63
Partal	1.65	1.31	1.57
Champ	1.72	1.59	1.49
Blue Pine (Kail)	1.71	1.41	1.49

in Le-C (28.31 U/g) followed by Le-S (20.62 U/g) and OE-329 (17.19 U/g) that was on par with Shisham (OE-329 followed by Le-S and Le-C) (Table 3).

Endoglucanase activity in general, was found to be the highest followed by Cbase and Fpase activity except in case of safeda saw dust where cellobiase activity was exceptionally higher in all the three strains of shiitake. 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 saw dust of safeda as carbon source in all three strains (Le-C, 249.1 mU/g; Le-S, 4652.2 mU/g and OE-329, 3762.5 mU/g) that was on par with shisham (Le-C, 34.8 mU/g; Le-S, 1162.0 mU/g and OE-329, 40.4 mU/g) after 21days of incubation. Saw dust of kail and champ 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 3). Similar study of enzyme activity was done for *Pleurotus* and *Volvariella spp*. when grown on varied substrates and slightly different results were obtained. (Manning and Wood,1983; Velázquez-Cedeno *et al.,* 2002).

Total Soluble Phenolic Content

It is evident from the results (Table4) that the maximum total soluble phenolics were released by Le-C (9.25 mg/g) when cultured on Safeda and minimum on Sagwan saw dust (0.62 mg/g).

In case of Le-S maximum phenolic were realized on its inoculation with safeda (1.60 mg/g) and minimum with partal saw dust (1.31 mg/g) as carbon source. While OE-329 released maximum phenolics (1.63 mg/g) on its inoculation in saw dust of safeda as carbon source and minimum concentration observed was 0.64 mg/g on sagwan (teak) as carbon source *(Table 4)*. 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 saw dust of timbers as carbon sources indicates the preference for saw dust of safeda and Shisham. 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 substrates for raising biomass and fruiting bodies of *Lentinus edodes*.

REFERENCES

1. Agarwal, A. "Nutritional, Biochemical and Cultivation Aspects of Shiitake Mushroom (*Lentinula edodes*)." *M.Sc. Thesis*, Punjab Agricultural University, 2007; Ludhiana, India.
2. 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.
3. Bhat, M. K. and Bhat, S. "Cellulose degrading enzyme and there potential industrial applications." *Biotechnol. Adv*., 1997; **15**: 583-620.
4. Boominathan, K. and Reddy, C. A. "Fungal degradation of lignin." *Handbook of applied mycology*, Fungal biotechnology. Marcel Dekker, Inc., New York, 1992; **4**:763–822.
5. Buswell, J. A., Cai, Y. J. and Chang, S. T. "Fungal and substrate-associated factors affecting the ability of individual mushroom species to utilize different lignocellulosic growth substrates." *In: Mushroom Biology and Mushroom Products*, Chinese University Press, Hong Kong, 1993; **1**: 141-150.

6. 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.
7. Chang, S. T. "Development of the world mushroom industry and its role in human health." In: *Souvenir-cum-Abstracts, International Conference on Mushroom Biology and Biotechnology*,2007; NRCM, Solan, India, 3.
8. Chang, S.T. and P.G. Miles, *Mushroom cultivation*: Nutritional value, Medicinal effect and environmental impact. (2nd ed.) CRC Press,2004; New York.
9. 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*, 1999b; 1: 291-300.
10. 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.
11. 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.
12. Hobbs, C.H., "In: Miovic, Michael (Ed.), Medicinal Mushrooms: An Exploration of Tradition, Healing and Culture." *Botanica Press, Williams*.2003.
13. 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.
14. 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.
15. Lalitesh. "Optimizaiton of Conditions for Submerged and Solid State Fermentation for Mycelial Biomass, Exobiopolymers and Sporophore Production of Shiitake Mushroom (*Lentinus edodes*)." *M.Sc. Thesis*, 2009; Punjab Agricultural University, Ludhiana.
16. 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.
17. Manning, K. and Wood, D. A. "Production and regulation of extracellular endo-cellulase by *Agaricus bisporus." J. Gen. Microbiol.*, 1983; **129**: 1839-47.
18. Miles, P.G. and S.T. Chang, "Mushroom biology, concise basics and current developments." *World scientific Publ*., 1997 ; London.
19. Miller, M. W. and Jong, S. C. "Commercial cultivation of shiitake in sawdust filled plastic bags." *Dev-Crop-Sci. Amsterdam: Elsevier Scientific Pub. Co.*1987; **10**: 421-426.
20. Nelson, N. "A photometric adaptation of the Somogyi method for the determination of glucose." *J. Biol. Chem.*, 1944; **153**: 375- 80.
21. Ooio, V.E., Liu, F. "A review of pharmacology activities of mushroom polysaccharides." *Int. J. Med. Mushrooms*,1999; **1**: 195–206.
22. 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.
23. Rai, R. D. and Arumuganathan, T. "Post Harvest Technology of Mushrooms." *Tech Bull*, 2008; NRCM, Solan, 1-84.
24. 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.
25. Somogyi, M. "Notes on sugar determination." *J. Biol. Chem.*, 1952; **195**: 19-23.
26. Thurston, C. F. "The structure and function of fungal laccases." *Microbiology*, 1994; **140**:19 – 21.
27. 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.
28. 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.
29. 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.