ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Selection of intraspecific hybrid fusants of *Lentinus edodes* strains (LeS & LeC) and their yield potential on different substrate combinations

Naresh Kumar Chauhan* and Ravinder Kumar Jaswal
Department of Microbiology, Punjab Agricultural University, Ludhiana-141004.

Manuscript Info

Manuscript History:

Received: 22 November 2014
Final Accepted: 26 December 2014
Published Online: January 2015

Key words:

Lentinus edodes, Protoplast fusion, Substrate combination, Yield potential

Corresponding Author

Naresh Kumar Chauhan

Abstract

Strain improvement of *Lentinus edodes* was done using protoplast fusion technique. Five hybrid fusants viz., L4, L13, L31, L58 and L76 were selected on the basis of faster mycelial run rate (MRR) than the parent strains of *L. edodes* (LeS and LeC). Qualitative endoglucanase activity was highest in hybrid fusant L58 with z/c ratio of 1.27. Similar trend was also observed in qualitative estimation of endoglucanase, xylanase and laccase. All the hybrid fusants showed higher lignocellulolytic activities as compared to the parents. The mushroom minimal media (MMM) revealed higher endoglucanase and laccase activities for all cultures with sawdust supplementation at the rate of 1% while xylanase activities were increased with wheat straw (1%). Out of four substrate combinations, combination 3 (wood chips and sawdust of red marenti) proved best spawn run and yield performance. Among the hybrid fusant L31, L58 and the parent LeS (fruiting cultures), the spawn run was significantly earlier in hybrid fusants compared to the parent LeS at 5% level. The hybrid fusant L58 gave highest biological efficiency of 57.2% with control followed by L31 as compared to the parent LeS (48.2%).

INTRODUCTION

Lentinus edodes (shiitake) is one of the speciality mushrooms, ranked second (25.4%) after *Agaricus bisporus* (31.8%) in the annual world production of mushrooms (Cheung, 2008). It has neutraceutical property due to lentinan, a water-soluble polysaccharide with well documented anti-cancer, anti-tumour properties (Brauer et al., 2002). *L.edodes* has the distinctive advantage of a much longer shelf-life thus more commonly sold dried while most of other mushrooms are sold fresh.

The increase in the yield potential of this mushroom is the need of hour to meet the current demand which can be fulfilled either by strain improvement or by substrate optimization. Non-conventional methods of breeding such as protoplast fusion, genetic recombination by rDNA technology have been increasingly used over the last decades. Among these, protoplast fusion has been successfully used by several workers for strain improvement of different varieties of mushrooms (Ashwini et al., 2014, Chakraborty and Sikdar, 2008 and 2010).

L.edodes is saprophytic white-rot fungi that degrade woody substrates containing recalcitrant, hard to decompose lignin components. It grows naturally in temperate climates on declining or dead hardwoods. A potential lignocellulolytic enzyme system consisting of cellulases, xylanases and laccases enables *L. edodes* to utilize a large number of substrates.

The indoor cultivation of *L.edodes* on hardwood sawdust-based substrates is recently increasing as compared to traditional outdoor cultivation on bed-logs. The sawdust-based cultivation method for *L. edodes* has some advantage, such as a short cultivation period and better-manipulation of flushing (fruit body formation) by control of environmental conditions. This method now accounts for about a half of all production, and is expected to become the major cultivation method. Recently, other lignocellulosic organic substrates such as wheat straw, corn

cobs, hazel nut or their combination have been used for cultivation of *L.edodes* (Sharma et al., 2011, Chang and Miles, 2004).

LeS strain is high yielding strain whereas LeC is low yielding strain. The intraspecific protoplast fusion between LeS and LeC was conducted to get high yielding hybrid strains with improved characters. In the present study, five high yielding intraspecific hybrid fusants of *L. edodes* were selected among the 124 regenerants and optimized for yield potential on different lignocellulosic substrates combinations.

MATERIALS AND METHODS

Two strains of *L.edodes*, LeS and LeC were procured from culture collection bank of the Department of Microbiology, Punjab Agricultural University, Ludhiana were fused through PEG-mediated protoplast fusion technique to obtain 124 regenerants. All the regenerants were maintained on Potato Dextrose Agar (PDA) slants at 25±2^{o}C.

Selection of hybrid fusants

Putative hybrid fusants were selected on the basis of MRR. Mycelial bit (4 mm) from each putative hybrid fusant and the parent strains were transferred on the PDA plates (90 mm) in triplicates and incubated at 25±2^{o}C for 9 days. The diameter of each culture was measured after 9 days.

Enzyme activity of hybrid fusants and their parents

The selected hybrid fusants were tested for their ability to produce extracellular enzymes (endoglucanase, xylanase and laccase) on mushroom minimal medium (MMM) having composition (g/l) of L-asparagine 1.6, D-glucose 20, $MgSO_4.7H_2O$ 0.5, KH_2PO_4 0.46, K_2HPO_4 1.0, Thiamine hydrochloride 0.125, pH 6.5. Mushroom minimal media broth (50 ml) was dispensed in flasks and autoclaved at 15 psi for 20 minutes, inoculated with 8mm mycelial bit and incubated at 25±2^{o}C. After 10 days, the mycelia cake was filtered to obtain supernatant which was used to measure enzyme activities.

Qualitative Endoglucanase estimation: In plate clearing assay, mycelia bit (4 mm size) of each strain was inoculated on respective modified MMM medium plate (glucose replaced by 0.5% carboxymethylcellulose, CMC) and incubated at 25±2°C. After 6 days, petri plates were flooded with 4% Congo red solution in alcohol, destained with 1M NaCl for 15minutes each. Clear zone formation indicated enzyme activity level. Colony diameter (c) and zone diameter (z) were at two perpendicular planes to calculate z/c ratio.

Quantitative Endoglucanase estimation: The test tubes containing a mixture of 0.5 ml CMC solution and 0.5 ml of enzyme extract were incubated at 50°C for 30 minutes in water bath (Mandels et al., 1976). The reducing sugars liberated were estimated using DNS reagent (Miller, 1959). A standard curve was prepared using standard glucose (10-100 µg/ml).

Quantitative estimation of Xylanase activity

Xylanase activity was assayed according to the method given by Bucht and Eriksson (1968) with slight modification. 0.5 ml of culture filtrate was taken in test tube containing 0.5 ml of 1% xylan solution and incubated at 50°C for 30 mintues. Reducing sugar was measured as xylose equivalents by DNS method. A standard curve was prepared using standard xylose (10-100 µg/ml). Enzyme activity was expressed as µg sugar liberated per min per ml of culture filtrate.

Quantitative estimation of Laccase activity

Laccase was estimated according to the method given by Turner (1974). One ml of enzyme extract and 3mL of buffered guaiacol solution were added to cuvette, mixed and placed immediately in colorimeter. The change in absorbance was recorded for every 15 second upto 120 seconds at 495nm.An absorbance in O.D by 0.01 in 60 second was taken as one unit. Enzyme activity was expressed as units/ml of culture filtrate.

Cultivation of hybrid fusants and the parents strains of *L.edodes*

Cultivation of the parents strains (LeS and LeC) of *L. edodes* and their five hybrid fusants (L4, L13, L31, L58 and L76) were conducted during October-March under natural indoor climatic conditions. The cultivation trials were conducted at the Mushroom Research Complex, PAU, Ludhiana using modified cultivation methodology (Anonymous, 2003)

Master spawn was prepared in Spawn Laboratory, Department of Microbiology, Punjab Agricultural University, Ludhiana on wheat grains in empty glucose bottles using the standard methodology (Phutela et al., 1986).

Sawdust and wood chips of broad leaves trees viz., mango (*Mangifera indica*), teak (*Tectona grandis*), and red meranti (*Shorea robusta*) supplemented with wheat bran and $CaCO_3$were used as substrate. The wood chips, sawdust, wheat bran and $CaCO_3$ were used in the proportion of 45:45:9:1. Wheat straw (90 part) supplemented with

wheat bran (10 parts) and $CaCO_3$ (1 part) was taken as control.

The substrates were spread and mixed on a cemented floor and soaked with water overnight so as to have a final moisture content of 65-70 percent and were mixed well manually and then filled in polypropylene bags (30 × 10 cm) at the rate of 750g substrate/bag on wet weight basis. A hole was made in the centre of the bag for inoculation and bags were sterilized by autoclaving at 20 psi for 90 minutes, then inoculated @ 2-3 percent on wet weight basis. The bags were then incubated at 25 ± 2ºC in 12 hours light/day and 80 % relative humidity. After about 45-65 days, a thick mycelial sheet was developed on the surface of the substrate.

On completion of spawn run, the bags turned brown and bump formation took place. At this stage, the polypropylene bags were removed and cold shock treatment was given to the blocks of substrate impregnated with mycelium by dipping them in chilled water (4-5ºC) for about 5-10 minutes. The bags were then incubated at 18 ± 2ºC and 12-16 hour light/day with 85±5 % relative humidity.

After 1-2 weeks of opening of bags, pinning started in the bags followed by fruit body formation. Fruit bodies were harvested on maturing when they took the shape of umbrella by gently twisting. Any substrate attached to the harvested fruit body was removed. The number and weight of fruit bodies from each bag was recorded and the percent biological efficiency (B.E.) was calculated by the following formula:

$$\text{B.E. (\%)} = \frac{\text{Fresh weight of mushrooms}}{\text{Dry weight of substrate}} \text{ x } 100$$

RESULTS AND DISCUSSION

Selection of fusants

Out of 124 intraspecific fusants, sixty got contaminated due less competitiveness. Most of the fusants were slow growing as compared to the parents. Some putative hybrid fusants were failed to grow in the subsequent sub-culturing processes, reason might be due to unstable genotype or loss of genotype. Similar finding was observed by Mukherjee and Sengupta (1986) where they found some of the regenerated putative hybrids did not grow on mushroom minimal medium.

The 64 hybrid fusants recovered after successive subculturing were plated on PDA medium for further selection on basis of horizontal radial growth. Only five fusants viz., L4, L13, L31, L58 and L76 showed faster MRR of 9.9, 9.5, 9.3, 9.8, 9.3, 7.9, 8.9 mm/day respectively compared to the parent strains at 5% level of significance.

Fusant L4 showed highest MRR on PDA medium at 25±2°C while least radial growth was showed by parent strain LeS. It is reported that the mycelial extension rate is a reliable technique for the selection of fast growing strains from a given stock of germplasm (Khandakar et al., 2008) Tokimoto et al. (1998) found that 24 hybrid fusants out of 400 colonies obtained by protoplast fusion of monokaryons of *Lentinula edodes*.

Colony morphology of hybrid fusants and the parent strains

Colony morphology of selected hybrid fusants was compared with their parents. All the hybrids fusants shared almost similar colony morphology with differences in their growth rate/patterns. Hybrid fusant L4 and the parent LeC showed thick cottony suppressed growth while other showed fluffy growth. The hybrid L58, L76 and the parent LeS produced some exudates in the broth culture. The exudate produced by L58 changed the color of the broth medium from yellowish orange to red which was never been observed in the parents itself indicating the formation of new gene combination (Fig. 1).

Enzyme assay of hybrid fusants and the parent strains

Enzyme activities of various enzymes like, endoglucanase, xylanase and laccase of hybrid fusants and the parents were analyzed to characterize at biochemical level.

Endoglucanase activity

Endoglucanase enzyme activity of hybrid fusants and the parent strains of *L.edodes* was conducted both qualitatively and quantitatively using plate clearing and broth culture assays respectively.

Qualitative estimation

Qualitative estimation of endoglucanase activity was done through zone/colony diameter (Table 1). All the fusants showed higher z/c ratio than parent strains at 5% level of significance. Hybrid fusants L58 (1.27) showed highest endoglucanase production followed by L4 (1.26) among all the strains in plate clearance assay while minimum endoglucanase activity was observed in the parent LeC. Kaur (2007) reported improved endoglucanase activity of hybrid as compared to parents. Sood (2008) revealed higher endoglucanase activity for five hybrid dikaryons while two cultures showed endoglucanase activity at par with the parent, *P. florida* PAU-5.

Quantitative estimation of endoglucanase activity

Extracellular endoglucanase activity of hybrid fusants and parent strains of *L. edodes* was assayed in broth medium after 14 days of incubation. All hybrid fusants showed improved endoglucanase activity than their parents (Table 1).

Supplementation of MMM with saw dust and wheat straw showed higher endoglucanase activities for hybrid fusant L58 followed by hybrid fusant L4 while lowest for LeC. The result obtained was in consistence with qualitative assay. However, sawdust supplementation in MMM almost doubled the endoglucanase activity as compared to wheat straw.

In CMC medium, specific endoglucanase activity of 200 UI/mg proteins was observed in *Lentinula edodes* (Junior et al., 2003). *L. edodes* IBB 123 had showed endoglucanase activity of 17.90±1.52 U/ml (Elisashvili et al., 2008) whereas *L. edodes* strains exhibited 97±9.8 U/g of endoglucanase activity on wheat straw (Kachlishvili et al., 2006).

Xylanase activity of hybrid fusants and parent strains

All the hybrid fusants showed higher xylanase activity as compared to both the parent strains of *L. edodes* at 5% level of significance (Table 1). Maximum xylanase activity of 23.85 µmol/min/ml was exhibited by hybrid fusant L58 while the parent LeC showed lowest activity of 3.86 µmol/min/ml on supplementation of MMM with wheat straw. In contrast to endaglucanase activity, Wheat straw supplementation of MMM gave more than doubled activity as compared to saw dust.

Xylanase activity was found to be 134±15 (U/g) on wheat straw in *L. edodes* (Kachlishvili et al., 2006), while in *L. edodes* IBB 123 strain, it as 30.15±2.71 U/ml (Elisashvili et al., 2008). Qinghe et al. (2004) revealed maximum xylanase activity of 24.98 µmol/min/ml in 7 days old culture of *Pleurotus ostreatus*.

Laccase activity of hybrid fusants and pwarent strains

Laccase activity of all the hybrid fusants was statistically higher than both the parents at 5% level of significance (Table 1). Hybrid fusant L58 showed highest laccase activity of 8.33 µmol/ml followed by hybrid fusant L4 while lowest laccase activity was observed in parent LeC (0.27 µmol/ml) on supplementation with saw dust. MMM supplemented with saw dust gave higher laccase activity as compared to wheat straw supplementation.

Laccase activity was best exhibited in NPM static condition on 10^{th}, 15^{th}, 20^{th} days with 45, 57.2, 64 Ul^{-1}, respectively in *Lentinus squarrosulus* (Tripathi et al., 2012). *L edodes* culture filtrate progressed abruptly in laccase activity during the period of 14 to 21days, and shown 13Uml^{-1} to 67U ml^{-1} laccase activity, respectively (Cavallazzi et al., 2005).

Cultivation of hybrid fusants and parent strains of *L.edodes*

The wood chips, sawdust of each tree either mango (*Mangifera indica*), teak (*Tectona grandis*), or red meranti (*Shorea robusta*) supplemented with wheat bran and $CaCO_3$ were used in the proportion of 45:45:9:1 to prepare the substrate. Wheat straw (90 part) supplemented with wheat bran (10 parts) and $CaCO_3$ (1 part) was taken as control. All the hybrid fusants and the parent strains of *L. edodes* (L4, L13, L31, L58, L76 LeS and LeC) were evaluated for yield performance on four substrate combinations.

Spawn run was observed in all the substrates but the fruiting bodies appeared only in hybrid fusants L31 and L58 and the parent strain LeS. No fruiting was observed in hybrid fusants L4, L13, L76 and the parent strain LeC. The faster mycelia run was reported in hybrid fusant L31 and L58 as compared to the parent LeS at 5% level of significance. Substrate combination 3 gave the fastest mycelia run which was statistically par with control and combination 2. No significant effect of substrate combinations or varieties was observed on number of fruit bodies at 5% level. The weight of fruit body (WFB) of both the hybrid fusants was significantly higher compared to the parent strain LeS while only combination 3 gave higher WFB compared to control.

Fastest mycelial colonization of *L.edodes* was reported by Ashrafuzzaman et al. (2009) on sawdust of Jackfruit (43 days) followed by sawdust of mango (44 days). Variation in mycelium run rate (MRR) on different substrates might be due to variations in the chemical composition and C: N ratio of substrates. Manjunathan and Kaviyarasan (2010) found that carbon to nitrogen ratio of 1:3 and 1:5 was best for mycelial growth of *Lentinus tuberregium*. Adesina et al. (2011) used waste leaves, barks and logs of some selected fruit trees such as *Anacardium occidentalis*, *Citrus sinensis*, *Mangifera indica*, *Psdium guajava*, *Terminalia cattapa* and *Spondias mombin*, each of which was supplemented with fresh cassava flour, cow-dung, poultry droppings, horse dung, oil palm waste fiber and rice bran. Best mycelial growth was obtained on *S. mombin* followed by *M. indica*, *C. sinensis* and *T. catappa* leaves.

Yield Performance

The hybrid fusants, L31 and L 58 gave statistically higher yield compared to the parent LeC at 5% level of significance. Maximum biological efficiency (BE) of 57.2% was reported in L58 with control while the parent strain

LeC gave the minimum BE of 40% with substrate combination 1. Among the four substrate combinations, combination 3 gave the highest biological efficiency followed by control while lower yield performance was observed in combination 1 and 2.

Variations in biological efficiencies of shiitake have been reported by different workers with different substrates. Gaitan and Mata (2004) reported biological efficiency on wheat straw substrate ranging from 24.8 to 55.6 percent. Puri et al. (2011) cultivated two different strains of *L. edodes* (L1 and L2) on different saw dusts and agricultural wastes viz., wheat straw, coir pith, poplar saw dust, teak saw dust and Sal sawdust etc. alone and in combinations with one another. They observed 45.9 percent BE for strain L1 with wheat straw. Ten percent supplementation of wheat bran was the best among all the supplements tried. Moreover, wheat straw substrate produced the heaviest and beautiful brown sporocarps with maximum number of fruiting bodies. Kovacsne and Kovacs (2000) and Zervakis et al. (2001) reported wheat straw as the most suitable substrate for production *L. edodes.* Three particle size of wood chips of three trees (oak, maple, and tanoak) were used for cultivation of three strains of *L. edodes* by Donoghue and Denison (1996) to observe highest BE (71%) with CS-287 strain on fine chips of tanoak tree while oak sawdust gave biological efficiency of 44% . According to Fasidi and Kadiri (1991), the increased productivity of *L. subnudus* on a straw based substrate was attributed to the carbohydrates, amino acids and minerals present in the supplements used.

In conclusion, among the five hybrid fusants, the hybrid fusant L58 proved as the potential culture for enhancing yield performance which was well supported with its enzymatic potential followed by L31 compared to the parent strain LeS. Among the four substrate combinations, third substrate combination consisting of sawdust and wood chips of Red meranti was best. The control consisting of wheat straw as substrate showed insignificant difference with combination 3 at 5% level thus can also be used as low cost substrate for the cultivation *L. edodes*.

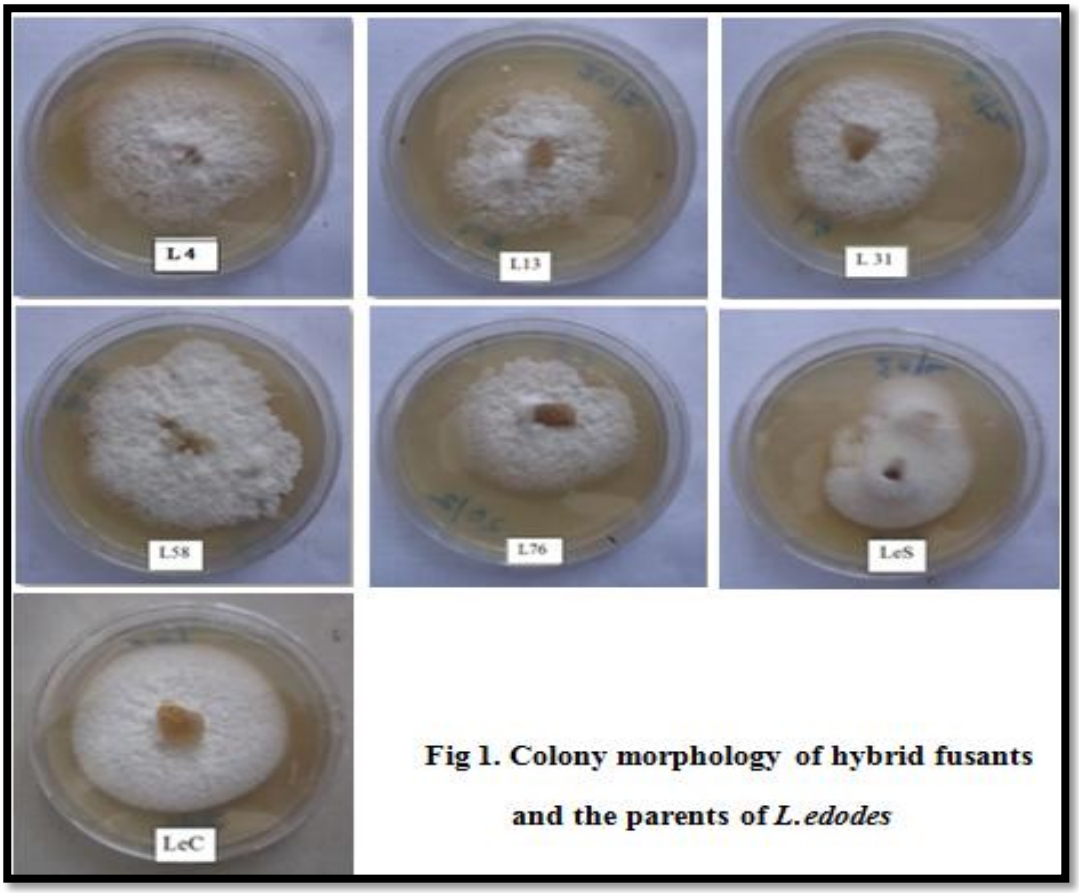

Fig 1. Colony morphology of hybrid fusants and the parents of *L.edodes*

Table 1. Estimation of lignocellulolytic enzymes of hybrid fusants and the parent strains of *L.edodes*

Strains	Zone diameter colony diameter ratio	Endoglucanase activity (µmol/min/ml)		Xylanase activity (µmol/min/ml)		Laccase activity (units/ml)	
	z/c ratio	SD	WS	SD	WS	SD	WS
L4	1.26	7.95	4.05	8.95	22.10	7.42	3.85
L13	1.22	5.42	3.20	7.85	17.90	5.30	3.57
L31	1.20	4.79	2.98	7.65	16.95	4.43	3.60
L58	1.27	8.85	4.85	10.05	23.85	8.33	4.90
L76	1.20	4.79	2.87	7.65	17.87	1.12	3.12
LeS	1.18	3.84	2.06	4.84	16.90	3.44	2.96
LeC	1.15	1.82	0.96	6.72	3.86	0.27	0.07
CD (5%)	**0.025**	**0.0635**	**0.034**	**0.046**	**0.024**	**0.025**	**0.071**

Number of replicates= 3; Mushroom minimal media supplemented with either Sawdust (SD); or Wheat straw (WS) @ 1%; Incubation temperature: 25±2°C; Incubation time: 2 weeks; *Incubation period= 6 days; *MMM without supplementation.

Table 2. Yield potential of hybrid fusants and the parent strain LeS of *L. edodes* on different substrates.

Substrates Composition SD +WC+ WB+ Ca CO_3 45 + 45 + 9 + 1	Complete Mycelial Run (days)	Number of Fruit Bodies (NFB)	Weight of Fruit Bodies (WFB)	% Biological Efficiency (%BE)
L58				
SD(T)+WC(T) + WB + $CaCO_3$	59	10	347	45.8
SD(M)+WC(M)+ WB+ $CaCO_3$	52	13	402	53.6
SD(RM)+WC(RM)+WB +$CaCO_3$	48	12	427	56.9
WS+WB+$CaCO_3$ (control)	49	11	430	57.2
L32				
SD(T)+WC(T) + WB + $CaCO_3$	56	11	341	45.4
SD(M)+WC(M)+ WB+ $CaCO_3$	50	12	410	54.6
SD(RM)+WC(RM)+WB +$CaCO_3$	48	13	422	56.2
WS+WB+$CaCO_3$ (control)	47	13	425	56.6
LeS				
SD(T)+WC(T) + WB + $CaCO_3$	65	9	300	40.0

SD(M)+WC(M)+ WB+ $CaCO_3$	56	13	370	49.3
SD(RM)+WC(RM)+WB +$CaCO_3$	55	13	380	50.6
WS+WB+$CaCO_3$ (control)	58	11	362	48.2
CD(5%) Variety X substrate	2.3	NS	6.7	1.4

WC (T), (M), (RM) = Wood chips of teak, mango and red meranti tree (45 part); SD (T), (M), (RM) = Sawdust of teak, mango and red meranti tree (45 parts); WB= wheat brawn (9 parts); $CaCO_3$ = 1part; WS= wheat straw (90 parts); Dry weight of substrate/bag-750g; Number of replicates-3

REFRENCES

Adesina, F.C., Fasidi, I. O. and Adenipekun, O.C. (2011): Cultivation and fruit body production of *Lentinus squarrosulus* Mont. (Singer) on bark and leaves of fruit trees supplemented with agricultural waste. African. J. Biotechnol. 10 (22): 4608-11.

Anonymous, (2003): Annual report of All India Coordinated Mushroom Researh Project (2002-2003), pp. 9-10, NRCM, Solan (HP), India,.

Ashrafuzzaman, M., Kamruzzaman, A. K. M., Razi, I. M., Shahidullah, S. M. and Fakir, S. A. (2009): Substrate affects growth and yield of shiitake mushroom. African. J. Biotechnol. 8(13): 2999-3006.

Ashwini, L., Arunagirinathan, N. and Kavitha, M. (2014): Strain Improvement of *Pleurotus* Species by Protoplast Fusion. Intl. J. Adv. Res. Technol. 3(8) ISSN 2278-7763.

Brauer, D., Kimmons, T. and Phillips, M. (2002): Effects of management on the yield and high molecular-weight polysaccharide content of shiitake (*Lentinula edodes*) mushrooms. J. Agri. Food Chem. 50: 5333-37.

Cavallazzi, J. R. P., Kasuya1, C. M. and Soares, M. A. (2005): Screening of inducers for laccase production by *Lentinula edodes* in liquid medium. Brazil. J. Microbiol. 36:383-87.

Chakraborty, U. and Sikdar, S. R. (2008): Production and characterization of somatic hybrids raised through protoplast fusion between edible mushroom strains *Volvariella volvacea* and *Pleurotus florida*. World J. Microbiol. Biotechnol. 24:1481-92.

Chakraborty, U. and Sikdar, S. R. (2010): Intergeneric protoplast fusion between *Calocybe indica* (milky mushroom) and *Pleurotus florida* aids in the qualitative and quantitative improvement of sporophore of the milky mushroom. World J. Microbiol. Biotechnol. 26 (2): 213-225.

Chang, S.T. and Miles, G.P. (2004): Mushrooms: Cultivation, Nutritional Value, Medicinal Effect, and Environmental Impact, Second edition State University of New York at Buffalo MUSHROOMS, CRC Press LLCBoca Raton London, New York, Washington DC.

Cheung, P. C. K. (2008): Nutritional value and health benefits of mushrooms. In: Cheung P C K (ed) Mushrooms as functional foods, pp. 73. John Wiley and Sons, Inc, Hoboken, New Jersey.

Donoghue, J. D. and Denison, C. D. (1996): Commercial production of shiitake (*Lentinula edodes*) using whole-log chips of *Quercus, Lithocarpus*, and *acer*. In: Royse (ed) Mushroom Biology and Mushroom Products. pp. 265-75. © Penn. State Univ. ISBN 1-883956-01-3.

Elisashvili, V., Penninckx, M., Kachlishvili, E., Tsiklauri, N., Metreveli, E., Kharziani, T. and Kvesitadze, G. (2008): *Lentinus edodes* and *Pleurotus* species lignocellulolytic enzymes activity in submerged and solid-state fermentation of lignocellulosic wastes of different composition. Biores. Technol. 99:457-62.

Fasidi, I. O. and Kadiri, M. (1991): Changes in nutrient contents of *Termitomyces robustus* (Beeli) Heim, and *Lentinus subnudus* (Berk.) during sporophores development. Acta. Bot. Hung**.** 36 (1-4): 167-72.

Gaitan, H. and Mata, G. (2004): Cultivation of the edible mushroom *Lentinula edodes* (shiitake) in pasteurized wheat straw-alternative use of geothermal energy in Mexico. Eng. Life Sci**.** 4(4): 363-67.

Junior, J. A. D. S. P., Coreia, M. J. and de Oliveira, N. T. (2003): Cellulase activity of a *Lentinula edodes* (Berk.) Pegler strain grown in media containing carboxymethylcellulose or microcrystalline cellulose. Brazilian Arch. Bio. Technol. 46(3): 333-37.

Kaur, J (2007): Selection and breeding for the improvement of oyster mushroom, *Pleurotus florida*. M.Sc. Thesis, Punjab Agricultural University, Ludhiana, India.

Kachlishvili, E., Penninckx, M. J., Tsiklauri, N. and Elisashvili, V. (2006): Effect of nitrogen source on lignocellulolytic enzyme production by white-rot basidiomycetes under solid-state cultivation. World J. Microbiol. Biotechnol. 22(4): 391-97.

Kovacsne, M. and Kovacs, A. (2000): Shiitake growing on straw substrate- A suitable alternative. Cult. Technol. Farm Econ. View Points Champignon. 418: 295-98.

Khandakar, S., Yesmin, N. C. S. and Amin, S. M. R. (2008): Effect of media on mycelial growth of edible mushrooms. Bangladesh J. Mush. 2: 53–56.

Mandels, M., Andreotti, R. and Roche, C. (1976): Measurements of saccharifying cellulase. Biotechnol. Bioengg. Symp. 6: 21-23.

Manjunathan, J. and Kaviyarasan, V. (2010): Studies on the Growth Requirements of *Lentinus tuberregium* (Fr.), An Edible Mushroom. Middle-East J Scientific Res 5 (2): 81-85.

Miller, G. L. (1959): Use of Dinitrosalicylic acid reagent for the determination of reducing sugars. Analytical Chemistry. 31: 426-28.

Mukherjee, M. and Sengupta, S. (1986): Mutagenesis of protoplasts and regeneration of mycelium in the mushroom *Volvariella volvacea*. Appl. Environ. Microbiol. 52:1412-14.

Phutela, R. P., Garcha, H. S., and Sodhi, H. S. (1986): Studies on Spawn preparation of *Agaricus bisporus* (Lange) Sing. Annals. Bio. 2(2): 180-84.

Puri, S., Bhatt, R. and Mishra, K. K. (2011): Cultivation of *Lentinula edodes* (Berk.) Pegler on sawdust substrates and agricultural wastes. Inter. J. Sci. Nat. 2(4): 752-56.

Qinghe, C. A. I., Xiaoyu, Y. U. E. and Tiangui, N. I. U. (2004): The screening of culture condition and properties of xylanase by white-rot fungus *Pleurotus ostreatus*. Process Biochem. 39 (11): 1561-66.

Rajarathnam, S. and Bano, S. (1991): Biological utilization of edible fruiting fungi. In: Arora, D. K. Mukerji and E Math. (eds.). Handbook of applied mycology, Food and Feed. Vol.3. Mercel Dekker, Inc, New York, USA.

Sharma,V. P., Kumar, S. and Sharma, S. R. (2011): Cultivation of Shiitake (*Lentinula edodes*).In: Singh, Manjit., Vijay, B., Kamal, S. and Wakchaure, G. C. (eds.) Mushrooms Cultivation, Marketing and Consumption . pp. 207-14. Directorate of Mushroom Research (ICAR), Solan, (HP), India.

Sood, P. (2008): Enzymatic characterization and yield potential of *Pleurotus florida* dikaryons. M.Sc. Thesis, Punjab Agricultural University, Ludhiana, India.

Tokimoto, K., Fukuda, M., Matsumoto, T. and Fukumasa-Nakai, Y. (1998): Variation of fruiting body productivity in protoplast fusants between compatible monokaryons of *Lentinula edodes*. J. Wood Sci. 44: 469-72.

Tripathi, A., Upadhyay, R. C. and Singh, S. (2012): Extracelular lignolytic enzymes in *Bjerkandera adusta* and *Lentinus squarrosulus*. Indian J. Microbiol. 52(3):381-87.

Turner, E. M. (1974): Phenoloxidase activity in relation to Substrate and development stage in the mushroom, *Agaricus bisporus*. Trans. British. Myco. Soc. 63: 541-47.

Zervakis, G., Ioannidou, S., Philippoussis, A. and Diamantopoulou, P. (2001): Mycelium growth kinetics and optimal temperature conditions for the cultivation of edible mushroom species on lignocellulosic substrates. Folia Microbiol (Czech Republic). 46 (3): 231-34.