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Studies for culturing and cultivation of *Lentinus cladopus* Lév.

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Abstract

In this paper the results of the vegetative growth of *Lentinus cladopus* Lév. on twelve solid media and eleven liquid media and reproductive growth on lignocellulosic substrates have been presented. Malt Extract Agar medium (MEA) supported the best mycelial growth from amongst the solid media evaluated while the vegetative growth on Malt Broth (MB) was maximum amongst the liquid media screened for the purpose. *L. cladopus* when grown on different lignocellulosic substrates including wheat straw, paddy straw and their 1:1 mixture gave 33.35 % biological efficiency on 1:1 mixture of wheat straw and paddy straw, which was best as compared to 31.66 % biological efficiency obtained on wheat straw and 12.76 % biological efficiency obtained on paddy straw.

Keywords – *Lentinus cladopus* – lignocellulosic substrates – liquid media – solid media

Introduction

Mushrooms are amongst the most popular food items accepted the world over. The increased consumer demand over the years has resulted in production of mushrooms in large proportions (Thakur 1995) through cultivation which is a highly efficient method for recycling the agricultural residues so as to produce nutritious food (Chang et al. 1998). Many species of genus *Lentinus* Fr. including *L. cladopus* Lév. are reported to be edible which can be cultivated on pasteurized as well as unpasteurized substrates (Morais et al. 2000). Taxonomically *L. cladopus* is quite close to *L. squarrosulus* Mont. in having a similar morphology and hyphal construction but differs from it in having thin pileus, lacking squamules and presence of broader spores. Basidiocarps of this mushroom appear in caespitose clusters. It is characterized by membranous whitish smooth convex depressed to subinfundibuliform pileus and cylindrical concolorous stipe (Peglar 1977). Some preliminary investigations on culturing of this mushroom have been done by Natarajan & Raaman (1981). They are reported to have attempted its cultivation on paddy straw mixed with sawdust (Purkayastha & Chandra 1985). All such species are used by the people because of the presence of significant amount of proteins, lipids, fats, minerals, vitamins in them from dietary point of view (Chang & Miles 2004). This paper presents study on *L. cladopus* which was collected from the wild, brought into pure culture on Potato Dextrose Agar medium and cultured on different solid as well as liquid media for selecting the best medium supporting vegetative growth. Later on it was cultivated on wheat straw, paddy straw and their 1:1 mixture and biological efficiency was also determined.

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Materials and Methods

The Material

The mushroom fruit body of *L. cladopus* was collected from Palampur (Himachal Pradesh) in North-West India from the stem of *Albizia chinensis* (Osbeck) Merrill. It was taxonomically investigated and identified (Pegler 1983). Molecular sequence has been deposited in NCBI Gene Bank (accession number JQ868754). The specimen (PUN-3948) has been deposited in the Herbarium of Botany Department, Punjabi University, Patiala (Punjab), India. The pure culture was raised on the Potato Dextrose Agar medium by tissue culture method (Yadav 2005). The culture has been deposited at Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology (IMTECH) Chandigarh, India under MTCC No. 10948.

Media Used

Both solid as well as liquid media were evaluated for the vegetative growth of *L. cladopus*. The composition of each medium used was based on Tuite (1969). The twelve solid media, used for evaluation are Malt Extract Agar (MEA), Potato Dextrose Agar (PDA), Pea Extract Agar (PEA), Milk Powder Agar (MPA), Potato Malt Agar (PMA), Yeast Extract Agar (YEA), Yeast Potato Dextrose Agar (YPDA), Gram Grain Extract Agar (GGEA), Dimmick Medium (DM), Maize Grain Extract Agar (MGEA), Wheat Grain Extract Agar (WGEA) Czapek Solution Agar (CSA) and eleven liquid media, namely Malt Broth (MB), Potato Dextrose Broth (PDB), Czapek Solution (CS), Glucose Asparagine Medium (GAM), Glucose Peptone Medium (GPM), Richard Solution (RS), Dimmick Medium (DM), Peptone Water (PW), Maltose Peptone Medium (MPM), Bilai Medium (BM) and Koser Citrate Medium (KCM) were used during experimentation for making comparative observation on vegetative growth of *L. cladopus*.

To measure the growth rate of mycelium in various solid media, the diameter of mycelial colonies was measured in cm scale and the average growth rate of mycelium was calculated. The mycelial density was assessed visually and expressed as described by Kadiri (1998) as follows:-

+ Very scanty, 2+ Scanty, 3+ Moderate, 4+ Abundant, 5+ Very Abundant.

So as to measure the mycelial growth rate in various liquid media, the mycelial mat from each flask was harvested, washed and dried at 65℃ for 24 hours. The dry weight of mycelium was recorded for two subsequent days and an average of the two was taken as the actual weight.

Spawn Preparation

For spawn preparation, healthy wheat grains were taken and grain spawn was prepared using the standard methodology (FAO 1990). The grains were fully colonized after 10-15 days of inoculation which became ready for use after 17-20 days of incubation.

Substrates and Substrate Preparation for Cultivation

Wheat straw, paddy straw and mixture of two (1:1) were used in the present study for cultivation. The substrates were prepared using standard methodology based on Upadhyay (1990). The colonized bags were incubated for fruiting after complete colonization. The polythene bags were removed on emergence of primordia which took 7-15 days after spawning. Later on each colonized substrate cylinder was immersed in the ice cold water for about 5 minutes for giving chilling treatment. These colonized substrate cylinders were then transferred to cropping room and kept on open racks. During cropping, water was sprayed after every two hours depending upon the requirement to keep the substrate moist. The relative humidity in the cropping room was maintained at 90-95% with the help of humidifier and temperature was maintained between 20-22℃ during fruiting. The fruit bodies were harvested and fresh weight and dry weight of mushrooms harvested in each flush was recorded.

Biological Efficiency

Biological efficiency of the mushroom with individual substrate and mixture was

Table 1 Mean colony diameter (cm) in different solid media ± standard deviation (SD) and mycelial characteristics.

Name of Medium	Mean Colony Diameter (cm) ± Standard Deviation	Mycelium Characteristics & Density
Malt Extract Agar (MEA)	4.88 ± 0.13	Dense thick mycelial growth in concentric rings with white appearance (5+)
Potato Dextrose Agar (PDA)	4.80 ± 0.18	Uniform dense thick mycelial mat with concentric rings and white appearance (4+)
Pea Extract Agar (PEA)	4.58 ± 0.24	Uniform thin mycelial mat, filaments off-white in appearance (3+)
Milk Powder Agar (MPA)	4.41 ± 0.14	Extremely thin filamentous mycelial growth whitish in appearance (3+)
Potato Malt Agar (PMA)	4.35 ± 0.18	Wrinkled, thin uniform mycelial growth, whitish in appearance (3+)
Yeast Extract Agar (YEA)	4.30 ± 0.23	Thin uniform mycelial growth in concentric rings with white appearance (3+)
Yeast Potato Dextrose Agar (YPDA)	4.26 ± 0.17	Thick mycelial growth, mat less thicker than that of Malt Extract Agar and Potato Dextrose Agar, white in appearance (3+)
Gram Grain Extract Agar (GGEA)	4.24 ± 0.12	Thin mat of mycelium, growth in concentric rings with wrinkled margin, off-white in appearance (2+)
Dimmick Medium (DM)	4.21 ± 0.22	Very thin, poorly developed, loosely arranged transparent mycelial filaments off-white in colour (+)
Maize Grain Extract Agar (MGEA)	4.20 ± 0.22	Uniform thin mycelial mat with white appearance (+)
Wheat Grain Extract Agar (WGEA)	4.10 ± 0.46	Thin mycelial growth in concentric rings, light brown coloured patches appear in due course (+)
Czapek Dox Agar (CDA)	4.00 ± 0.48	Extremely thin poor mycelial growth with loosely scattered transparent mycelial filaments, whitish in appearance (+)

calculated on fresh weight basis as per the formula:-

$$\text{Biological Efficiency} = \frac{\text{Fresh weight of mushroom}}{\text{Dry weight of mushroom}} \times 100$$

Results

During evaluation of media, out of the twelve different solid media used for evaluation of vegetative growth, significantly higher vegetative growth was recorded in Malt Extract Agar (4.88 cm) followed by Potato Dextrose Agar (4.80 cm). On an average daily basis the mycelium was found growing at the rate of 0.94 cm in diameter in Malt Extract Agar and mycelium formed a thick dense white mat with concentric rings with maximum mycelial density (5+). Least mycelial growth was recorded in Czapek Dox Agar (4.00 cm) with extremely poor mycelial density (+) in comparison to other media. The mean colony diameter of the fungus with ± standard deviation (SD) and mycelium characteristics in different solid media are presented in table 1.

Out of the eleven different liquid media selected for evaluation of vegetative growth Malt Broth (MB) gave maximum vegetative growth (8.63 mg/ml) followed by Potato Dextrose Broth (7.00 mg/ml). On the other hand, minimum vegetative growth was obtained in Koser Citrate Medium (0.50 mg/ ml). The vegetative growth obtained in eleven different liquid media with ± standard deviation (SD) has been presented in table 2.

As per t-values obtained the difference of vegetative growth in Malt Extract Agar (solid medium) and Malt Broth (liquid medium) was significantly higher than in all other media evaluated. Matrix table showing t-values obtained for mycelial growth on solid media and liquid media are given in table 3 and 4, respectively.

From amongst the lignocellulosic substrates used for cultivation wheat straw bags were fully colonized after 7-8 days of inoculation. Bags of mixture substrate took 9-11 days while paddy straw bags took 13-15 days for complete colonization. Small protuberances appeared in each

Table 2 Mean dry weight in different liquid media ± standard deviation (SD).

Name of Medium	Mean Dry Weight (mg/ml) ± Standard Deviation
Malt Broth (MB)	8.63 ± 1.89
Potato Dextrose Broth (PDB)	7.00 ± 0.71
Czapek Dox Solution (CDS)	6.50 ± 3.55
Glucose Asparagine Medium (GAM)	5.70 ± 0.57
Glucose Peptone Medium (GPM)	5.50 ± 1.73
Richard Solution (RS)	2.50 ± 0.79
Dimmick Medium (DM)	2.25 ± 0.35
Peptone Water (PW)	1.38 ± 0.75
Maltose Peptone Medium (MPM)	1.25 ± 0.50
Bilai Medium (BM)	0.83 ± 0.29
Koser Citrate Medium (KCM)	0.50 ± 0.00

in wheat straw substrate and 12.76 % biological efficiency in paddy straw. The observations are summarized in the table 5.

Fig. 1 – Fruiting bodies of *L. cladopus* on 1:1 mixture of Wheat straw and Paddy straw.

Fig. 2 – Fruiting bodies of *L. cladopus* on Wheat straw.

Table 3 Matrix table showing t-values of different solid media used for mycelial growth of *L. cladopus.*

Name of Medium		MEA	PDA	PEA	MPA	PMA	YEA	YPDA	GGEA	DM	MGEA	WGEA	CDA
	Mean Diameter (cm)	4.88	4.80	4.58	4.41	4.35	4.30	4.26	4.24	4.21	4.20	4.10	4.00
Malt Extract Agar (MEA)	4.88	--	0.721	2.206*	4.896**	4.775**	3.919**	5.254**	7.529**	4.718**	5.313**	3.264**	3.534**
Potato Dextrose Agar (PDA)	4.80		--	1.467	3.421**	3.543**	3.125**	4.060**	5.185**	3.782**	4.225**	2.834**	3.125**
Pea Extract Agar (PEA)	4.58			--	1.223	1.533	1.564	2.065*	2.537*	2.114*	2.331*	1.853*	2.164*
Milk Powder Agar (MPA)	4.41				--	0.526	0.733	1.250	1.847*	1.379	1.615	1.292	1.640
Potato Malt Agar (PMA)	4.35					--	0.313	0.677	1.019	0.897	1.056	1.012	1.367
Yeast Extract Agar (YEA)	4.30						--	0.242	0.411	0.889	0.581	0.752	1.095
Yeast Potato Dextrose Agar (YPDA)	4.26							--	0.174	0.311	0.408	0.640	1.004
Gram Grain Extract Agar (GGEA)	4.24								--	0.214	0.321	0.588	0.972
Dimmick Medium (DM)	4.21									--	0.595	0.418	0.775
Maize Grain Extract Agar (MGEA)	4.20										--	0.392	0.758
Wheat Grain Extract Agar (WGEA)	4.10											--	0.301
Czapek Dox Agar (CDA)	4.00												--

* Significant at level 0.05; ** Significant at level 0.01

Table 4 Matrix table showing t-values of different liquid media used for mycelial growth of *L. cladopus.*

Name of Medium		MB	PDB	CDS	GAM	GPM	RS	DM	PW	MPM	BM	KCM
	Mean Dry Wt. (mg/ml)	8.63	7.00	6.50	5.70	5.50	2.50	2.25	1.38	1.25	0.83	0.50
Malt Broth (MB)	8.63	--	1.523	1.153	2.996**	2.563*	6.081**	6.537**	7.136**	7.554**	8.133**	8.612**
Potato Dextrose Broth (PDB)	7.00		--	0.300	2.309*	1.626	7.341**	8.497**	8.978**	10.268**	11.663**	12.974**
Czapek Dox Solution (CDS)	6.50			--	0.498	0.566	2.460*	2.646*	3.139**	3.267**	3.553**	3.781**
Glucose Aspargine Medium (GAM)	5.70				--	0.246	7.356**	9.746**	9.558**	12.606**	16.073**	20.635**
Glucose Peptone Medium (GPM)	5.50					--	3.529**	4.007**	4.796**	5.234**	5.904**	6.468**
Richard Solution (RS)	2.50						--	0.581	2.183*	2.900**	4.293**	5.682**
Dimmick Medium (DM)	2.25							--	1.942*	2.849**	4.765**	7.114**
Peptone Water (PW)	1.38								--	0.289	1.345	2.353*
Maltose Peptone Medium (MPM)	1.25									--	1.400	3.024**
Bilai Medium (BM)	0.83										--	1.976*
Koser Citrate Medium (KCM)	0.50											--

* Significant at level 0.05; ** Significant at level 0.01

Table 5 Evaluation of lignocellulosic substrates for cultivation of *L. cladopus.*

Name of Substrate	Net Dry Weight of Substrate/Bag (g)	No. of Days for Complete Colonization of Bags after Inoculation	No. of Days taken for appearance of 1st Primordia after Inoculation	Fresh Weight of Mushrooms/Bag (g)			Average Fresh Weight of Mushroom(g)	Biological Efficiency on Fresh Weight Basis (%)
				Bag 1	Bag 2	Bag 3		
Wheat Straw	500	7-8	16	77.134	226.846	170.947	474.927	31.66
Paddy Straw	500	13-15	19	54.240	55.998	81.191	191.429	12.76
Mixture (1:1)	500	9-11	14	148.633	203.226	148.320	500.179	33.35

Fig. 3 – Fruiting bodies of *L. cladopus* on Paddy straw.

incubated substrate bag after few days of complete colonization after which primordia matured into carpophores (Fig. 1, 2, 3). Maximum yield was obtained in 1:1 mixture of wheat and paddy straw followed by wheat straw substrate and paddy straw substrate. Mushroom gave 33.35 % biological efficiency in mixture substrate out of the three substrates followed by 31.66 % biological efficiency

Discussion

L. cladopus is important both nutritionally and nutraceutically (Gulati et al. 2011). This is a maiden attempt to domesticate this mushroom and understand its media requirements for mass culturing. The results obtained in the present study revealed that the best radial mycelial extension (4.88 cm) was supported by Malt Extract Medium among all the solid media and best vegetative growth (8.63 mg) on dry weight basis was supported by Malt Broth medium among all the liquid media evaluated. No work of this nature is available on *L. cladopus*, however for some species including *L. connatus* Berk., *L. squarrosulus* Mont. and *L. subnudus* Berk. there are references

about evaluation of media and substrates for culturing and cultivation. Singh et al. (1990) evaluated Malt Extract Agar and Sabouraud's Agar as the best solid media for vegetative growth of *L. connatus* and Soyabeen Extract Broth as the best liquid medium for vegetative growth of this mushroom. Gbolagade et al. (2006) evaluated Potato Dextrose Agar and Yellow Corn Agar for vegetative growth of *L. subnudus*. Atri et al. (2007) reported maximum radial growth of *L. squarrosulus* mycelium in Yeast Extract Agar. Amongst the liquid media, Yeast Glucose Medium gave the best vegetative yield of *L. squarrosulus* on dry weight basis and Koser Citrate Medium gave the minimum vegetative yield as given by Atri et al. (2007).

Different workers (Farr 1983, Kuo & Kuo 1983, Harris 1986, Singer & Harris 1987) have documented the use of saw dust of various hardwood tree species for the production of shiitake. For successful production of *L. edodes*, Royse (1996) reported a composition of saw dust (80 %), wheat bran (10 %) and millet (10 %). Oghenkaro et al. (2009) while working with *L. squarrosulus* showed highest fruiting in *Brachystegia nigerica* Hoyle & A. P. D. Jones saw dust supplemented with 1 % $CaCO_3$, 1 % sugar and 20 % wheat bran. Upadhyay & Rai (1999) documented the suitability of wheat straw and paddy straw compost for the cultivation of *L. squarrosulus*. Fasidi & Kadiri (1993) reported the stimulation of sporophore emergence by supplementation of agricultural wastes.

The results of the present study are in conformity as is evident from the fact that *L. cladopus* has been successfully cultivated in the laboratory on lignocellulosic substrates. Malt Extract Agar (MEA) proved as the best solid medium and Malt Broth (MB) as the best liquid medium for the vegetative growth of this species. Healthy wheat grains gave good spawn for cultivation and maximum yield was obtained on the mixture of two substrates (1:1- wheat straw: paddy straw) with biological efficiency of 33.35 %.

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