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Evaluation of *Calocybe indica* strains for lignocellulolytic enzymes and mushroom yield potential

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ABSTRACT

Calocybe indica*, a milky mushroom is a tropical mushroom of Indian origin that grows well at 28-35°C. Its strains were evaluated for their mycelial growth, extracellular enzymes producing capability and yield potential. For mycelium growth, seven strains of *Calocybe indica* were grown on Potato Dextrose Broth (PDB), Complete Yeast Extract (CYM), Mushroom Minimal Media (MMM) and their broth at 30±2°C. The maximum biomass was recorded in CYM broth in Ci-06 (16.1g/l) and Ci-07 (15.5 g/l). The linear growth on wheat straw after 10 days was observed maximum in Ci-07 (63mm) and Ci-09 strain (66mm). In wheat straw, the maximum biological efficiency of the harvested yield (kg/q dry straw) was observed in the strain Ci-06 (57.3%). Two strains Ci-07 (48.5%) and Ci-09 (45.7%) were found to give yield at par with each other. Maximum number of fruiting bodies were recorded in the strain Ci-06 (1552 No./q dry straw). In culture filtrate, endoglucanase enzyme activity ranged between 1.91-4.42 U/mg, endoxylanase activity ranged between 2.73-4.76 U/mg and laccase activity ranged between 6.94-10.2 U/mg. During spawn run, endoglucanase and endoxylanase enzyme activity ranged between 0.58-1.03 U/mg and 0.74 -2.11 U/mg, laccase activity ranged between 1.51-4.54 U/mg. During pinhead, endoglucanase and endoxylanase activity ranged between 0.61-1.59 U/mg and 1.57-2.96 U/mg, respectively whereas laccase activity ranged between 6.53-10.6 U/mg. In fruiting bodies, endoglucanase and endoxylanase activity of the strains ranged between 0.94-1.88 U/mg and 0.93-2.36 U/mg, laccase activity ranged between 5.43-9.21 U/mg. A positive correlation of yield with biomass and endoglucanase has been observed in *C. indica* strains Ci-03, Ci-06, Ci-07 and Ci-09.

Keywords: Biomass, endoglucanase, endoxylanase, laccase.

INTRODUCTION

Edible mushroom production has been developed industrially in more than 80 countries and in the last 20 years production has increased rapidly to 25 million tones as per claims of Chinese Association of Edible Fungi (Singh, 13). In Punjab, five different varieties of mushroom are cultivated namely *Agaricus bisporus* (Button mushroom), *Calocybe indica* (Milky mushroom), *Lentinula edodes* (Shiitake mushroom), *Pleurotus spp.* (Oyster mushroom), *Volvariella spp.* (Paddy straw mushroom). *Calocybe indica* also known as milky mushroom grows on the substrate rich in organic material in tropical region. It is preferentially cultivated in India as it is a heat loving mushroom that requires a temperature of about 28-35°C with relative humidity of 80-90%.This edible fungus has effective enzyme system for the lignocellulosic substrate degradation. Therefore, it can be cultivated on wide range of agro wastes and byproducts like paddy, wheat, ragi, maize/cotton stalks and leaves, sugarcane bagasse, jute and cotton wastes, tea coffee waste etc (Tewari, 15). It has been studied that fungus could colonize paddy straw, maize stalks and sorghum stalks with high levels of exo and endo-cellulases as the optimum temperature for the growth of this mushroom is (28-35°C). Moreover, the climate of Punjab provides good scope for its cultivation during (May-September).

This mushroom is recommended as a good diet for people with hyperacidity, cardiac and diabetes problem due to the presence of high fiber content, low concentration of fats and carbohydrates. Lignocellulolytic enzymes contain protein which are extracellular containing hydrolytic enzymes (cellulases, hemicellulases, proteases, chitinases, amylases) and ligninolytic enzymes (oxidases and peroxidases). It has been observed that loss of cellulose, hemicellulose and lignin from the biodegradation of waste showed positive correlation with cellulases such as exo-β-1, 4-glucanase (E.C. 3.2. 1.91), endo-β-1, 4-glucanase (E.C.3.2.1.4) , xylanase (E.C. 3.2.1.8) and laccase (E.C.1.10.3.2) activity of fungus. By evaluating the enzymatic system of these strains, the improved mushroom varieties with effective attributes in term of pathogen resistance, high yield and its adaptation to wide range of temperature would be developed.

Therefore, the present investigation was carried out to evaluate *Calocybe indica* for cellulase, xylanase

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and laccase enzymes producing capability along with a possible correlation of the enzyme production from different strains with their yield potential.

MATERIALS AND METHODS

Seven strains of *Calocybe indica* were maintained on PDA slants at 30±2°C. For biomass study, different broth media (CYM, PDB, MMM) was dispensed in each flask, autoclaved at 15 psi for 20 minutes and each broth medium (50 ml) inoculated with two bits of 5 mm diameter of respective strains for 10 days to observe biomass (g/l).

Wheat straw was wetted overnight, heated at 80°C for 30 minutes, allowed to cool, filled in the test tubes of tube size (20 × 2 cm), inoculated with 7-10 spawn grains of each *C. indica* strains and incubated at 30±2°C for 10 days to observe linear growth (mm/d). Cultivation trials were conducted using seven strains of *C. indica* during the summer season at a temperature of 28-32°C (May-September). Wheat grains were boiled in water for 40 minutes, mixed with 2% $CaCO_3$ and 4% $CaSO_4$, filled in glucose bottles autoclaved at 20 psi for 1.5 hours. Wheat straw was used as substrate for growing *C. indica* strains. For its cultivation, wheat straw was spread on cemented floor and wetted with clean, fresh water for 14-20 hrs to attain 65-70 per cent moisture. Wetted substrate was boiled for 45 to 50 minutes, cooled and the substrate was thorough spawned @ 10 per cent on dry weight basis, filled in polythene bags @ 2.5 kg wet straw/bag. After complete mycelial impregnation (4-5 weeks), the bags were cased with casing mixture consisting of farm yard manure (FYM) and sandy soil in the ratio 4:1. About 2.5-3.0 cm thick layer of casing mixture was used. After casing, the bags were watered twice daily. After 10-15 days of casing, mushrooms started appearing in flushes for about 40 days and biological efficiency (BE) was calculated. Endoglucanase activity was measured by the method of (Mandel *et al*., 6). The reducing sugars were estimated using DNS reagent (Miller, 8). Xylanase activity was determined by the method described by (Bucht and Eriksson, 1). Reducing sugar was measured as xylose equivalents by DNS method. Laccase estimation was carried out according to the method of (Turner, 16) with some modification as described by (Singh *et al*., 14). One ml of enzyme extract and 3 ml of buffered guaiacol were added, mixed and tube was placed in colorimeter immediately. The change in absorbance was recorded for every 15 sec upto 120secs at 495nm. The total protein content of culture supernatant was estimated by the method given by (Lowry *et al.,* 5).

RESULTS AND DISCUSSION

Calocybe indica strains were grown in Potato dextrose broth (PDB), Complete yeast extract (CYM), Mushroom minimal media (MMM) broth. The mycelial biomass (g/l) of *C. indica* strains were observed after 10 days of incubation. The mycelial biomass ranged between 9.62 to 16.12 g/l in PDB, 9.25 to 16.13 g/l in CYM and 8.31 to 12.00 g/l in MMM. The maximum biomass in PDB was that of Ci-06 and Ci-07, while on CYM, it was maximum in Ci-06 and Ci-09. The maximum biomass in MMM was observed in Ci-04, Ci-06, Ci-07 and Ci-09 strains of *C. indica* (Table 1). Growth of *Calocybe indica* in 11 different broth media had been studied with maximum mycelial biomass harvested from wheat seed extract medium followed by malt extract medium and potato dextrose medium (Pani, 9). In *C. indica,* maximum biomass (0.22 g/100 ml) was recorded on PDB (Phutela and Phutela, 11).

The mycelial impregnation from grain spawn to the hot water treated wheat straw was observed for

Table 1. Growth study of biomass in broth and linear growth on wheat straw.

Strain	Mycelial biomass (g/l)			Linear growth (mm)		
	Media			Days		
	PDB	CYM	MMM	3d	7d	10 d
Ci-01	11.14	10.32	8.31	8.01	24.02	53.61
Ci-03	9.62	10.21	9.30	10.03	36.30	59.03
Ci-04	10.40	9.25	11.21	9.32	31.31	55.32
Ci-06	14.73	16.13	10.44	10.61	31.00	58.04
Ci-07	16.12	13.70	12.00	13.00	35.02	63.03
Ci-08	12.32	13.80	9.33	8.03	30.03	60.02
Ci-09	13.93	15.51	11.75	10.01	33.60	66.01
CD (p=0.05)	1.42	1.31	1.63	2.43	4.21	4.74

C. indica strains upto 10 days at 30±2°C. The linear growth after three days of incubation in *C. indica* strains ranged between 8.01 to 13.00 mm, 24.02-36.30 mm on 7th day and 53.61-66.01 mm on 10th day of incubation. Maximum linear growth after 3 days was observed in Ci-06 and Ci-07. After 7 days, maximum linear growth was observed in Ci-03, Ci-07, Ci-09 strains and after 10 days maximum growth was found in Ci-07 and Ci-09 (Table 1). It was observed that the highest linear growth (87.00 mm) in response to the tissue culture obtained from the mushroom which consisted of stipe with well differentiate pileus (Pani, 10). The maximum linear growth (9.35 cm) was observed in YPDA (yeast potato dextrose agar media) (Uddin *et al*., 17).

For cultivation trials of *C. indica* strains, spawn run period ranged between 15.0-19.0 days. The number of fruit bodies harvested were maximum for Ci-06 (1552.0) followed by Ci-07 (1515.0) and Ci-09 (1030.0). The biological efficiency estimated from the harvested yield (kg/q dry straw) was found to show maximum yield potential for the strain Ci-06 (57.3%). Two strains Ci-07 and Ci-09 were found to give yield at par with each other whereas Ci-03 showed low biological efficiency (20.2%). The average weight of fruiting body of all seven *C. indica* strains ranged between 31.5-51.4 g with maximum weight for Ci-09, Ci-08 and Ci-06 strains and lowest for Ci-03 strain. (Table 2; Fig. 1). During the entire cropping cycle, no disease and pest infestation was recorded. Considering the yield potential of *C. indica* strains, Ci-06 indicated maximum biological efficiency (% BE; Table 2).

Maximum biological efficiency had been reported from the *C. indica* strain Ci-06 (62.0%) followed by Ci-04 (58.1%) (Kumar *et al*., 4). It has been observed that maximum biological efficiency ranged from 51.8% -146.3%. The highest biological efficiency of *C. indica*

Table 2. Yield performance of *Calocybe indica* strains on wheat straw.

Strain	Spawn run (days)	NFB (no./q dry straw)	Yield (kg/q dry straw, % B. E.)	Av. Wt. of fruit body (g)
Ci-1	18.0	753.0	25.5	40.3
Ci-3	17.0	452.0	20.2	31.5
Ci-4	19.0	1015.0	30.4	35.6
Ci-6	15.0	1552.0	57.3	42.5
Ci-7	17.0	1515.0	48.5	38.5
Ci-8	15.0	890.0	32.3	43.5
Ci-9	18.0	1030.0	45.7	51.4
CD (p=0.05)	7.26	151.1	5.67	9.75

Where NFB: Number of fruit bodies; B.E: Biological efficiency

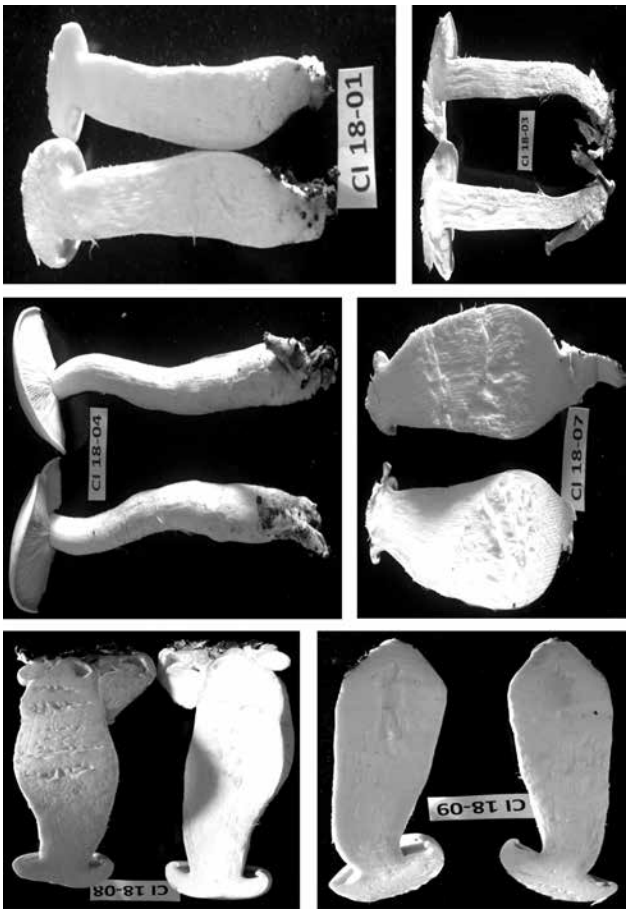

Fig. 1. Cross section of *Calocybe* indica strains.

was observed with wheat straw followed by paddy straw which was 132.4% whereas soybean straw, coconut pith and cotton waste showed 126.1%, 108.7% and 92.07% biological efficiency (Vijaykumar *et al.,* 18). Mangat (7) reported that the boiling of wheat straw for about 45-50 minutes was the best pretreatment for obtaining high yield with the biological efficiency (41.6-62.9%). Kaur (3) observed maximum biological efficiency for the *C. indica* strain Ci-06 (81.82%) and lowest biological efficiency was observed in the strains Ci-01, C-02 and Ci-09 (47.8-51.28%).

For the enzyme activity of endoglucanase, endoxylanase and laccase, the enzymes were extracted from the culture filtrate, at the time of spawn run, pinhead formation and fruiting bodies of *Calocybe indica.* In the culture filtrate, endoglucanase and endoxylanase activity ranged between 1.91-4.42 (U/mg) and 2.73-4.76 (U/mg). Maximum endoxylanase activity was found in Ci-07 followed by Ci-09, Ci-06 & Ci-04. The lowest activity was observed in Ci-03. Laccase activity of the culture filtrate of these strains

ranged between 6.94 - 10.2 (U/mg) with maximum activity in Ci-03 followed by Ci-07, Ci-04, Ci-06. At the time of spawn run, endoglucanase enzyme activity ranged between 0.58-1.03 (U/mg) with maximum activity from the strain Ci-04, Ci-06 and Ci-08. The endoxylanase activity ranged between 0.74 - 2.11 (U/mg) with maximum activity again in the culture Ci-08, Ci-04 and Ci-06. The laccase activity was found to be maximum in the culture Ci-03 and Ci-09. The enzyme extracted from the substrate underneath the mushroom pinheads indicated endoxylanase activity ranged between 0.61-1.59 (U/mg) with maximum activity in Ci-06 and Ci-07 strains followed by Ci-03, Ci-01 and Ci-04. The endoxylanase activity ranged between 1.57-2.96 (U/mg) with maximum activity from Ci-01, Ci-08, Ci-04 followed by Ci-06 and Ci-09. The laccase activity ranged between 6.53-10.6 (U/mg) with maximum activity in Ci-04 and Ci-09 followed by Ci-03, Ci-08 and Ci-06. At the time of fruiting bodies, endoglucanase and endoxylanase activity of the strains ranged between 0.94-1.88 (U/mg) and 0.93-2.36 (U/mg), but statistically the enzyme activity of the harvested mushroom fruit body was at par with each other. The laccase activity ranged between 5.43-9.21 (U/mg) with maximum activity in the fruiting body of Ci-08 & Ci-09 followed by Ci-04, Ci-06 and Ci-07 (Table 3). Cellulase activity in *Calocybe indica* (0.49 U/ml) and *Pleurotus ostreatus* (0.60U/ml) was observed to be maximum at 70°C (Karthikeyan, 2). Maximum activity of endoglucanase and endoxylanase was recorded for *C. indica* strain Ci-6 (2.72, 2.29 U/mg) and Ci-2 (1.70,1.88 U/mg) (Redhu,12).

The correlation of the yield with biomass and endoglucanase activity had indicated positive correlation in the *C. indica* strains Ci-03, Ci-06, Ci-07 and Ci-09. These strains with the maximum yield had a positive relation with biomass as well as endoglucanase enzyme production (Fig. 2)

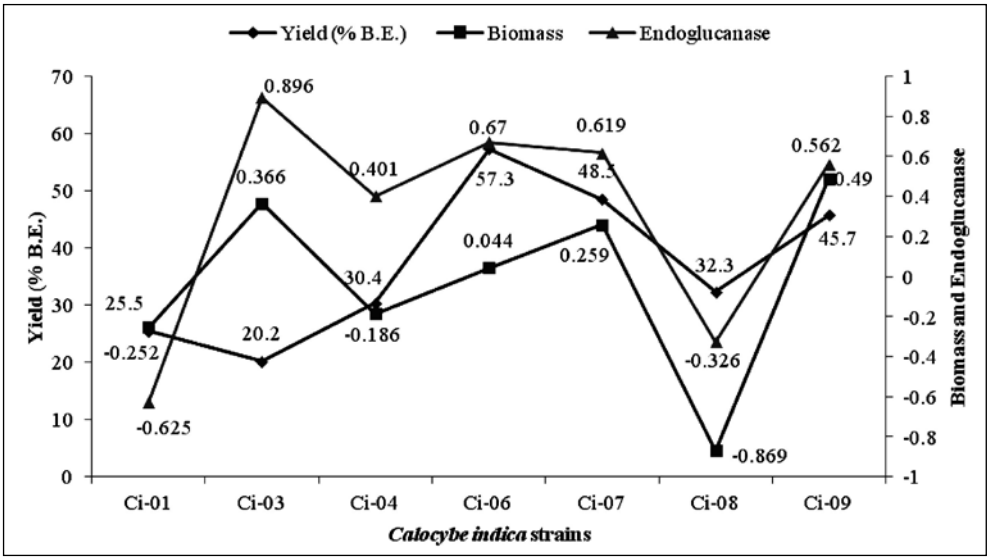

Fig. 2. Correlation of yield with biomass and enzyme (endoglucanase).

Table 3. Enzyme assay of culture filtrate (CF), spawn run (SR), pinhead (PH) and fruiting body (FB) stages.

Strain	Endoglucanase (U/mg)				Endoxylanase (U/mg)				Laccase (U/mg)			
	CF	SR	PH	FB	CF	SR	PH	FB	CF	SR	PH	FB
Ci-01	2.23	0.81	1.25	1.36	3.16	0.74	2.96	2.11	7.23	1.15	6.96	5.43
Ci-03	1.91	0.78	1.29	0.94	2.73	1.13	1.81	2.36	10.2	4.54	8.82	5.98
Ci-04	1.96	1.03	1.12	1.31	3.59	1.81	2.88	2.21	8.22	2.96	10.6	7.21
Ci-06	2.77	0.95	1.59	1.88	3.74	1.70	2.29	1.98	7.97	3.77	7.75	7.03
Ci-07	1.93	0.58	1.43	1.71	4.76	1.13	1.57	1.59	8.61	3.24	6.53	6.33
Ci-08	4.42	0.91	0.61	1.22	2.81	2.11	2.93	1.83	7.13	2.03	8.32	9.21
Ci-09	2.83	0.65	1.09	1.43	3.97	1.42	1.86	0.93	6.94	4.11	10.4	8.24
CD(p=0.05)	0.45	0.19	0.40	NS	0.64	0.43	0.47	NS	1.40	0.65	1.18	1.40

The results concluded that there was a positive correlation with biomass and endoglucanase activity of *C. indica* strains and all these seven strains of *C. indica* are capable of producing lignocelluloytic enzymes.

DECLARATION

The authors declare no conflict of interest.

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