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AKADÉMIAI KIADÓ

White rot fungus *Calocybe indica*: Incredulous factory of lignocellulolytic enzymes and their potential applicability for mushroom cultivation

M. Kaur*, S. Sharma and H.S. Sodhi

Department of Microbiology, Punjab Agricultural University, Ludhiana, 141004, India

ORIGINAL RESEARCH PAPER

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ABSTRACT

Cultivation of specialty mushrooms on lignocellulosic wastes represents one of the most economical organic recycling processes. Compared with other cultivated mushrooms, very little is known about the nature of the lignocellulolytic enzymes produced by the edible fungus *Calocybe indica*, its enzymatic activity profiles during submerged and solid state fermentation. The intracellular activity of laccase (7.67 U mg^{-1}), manganese peroxidase (7.48 U mg^{-1}), cellobiohydrolase (5.46 U mg^{-1}), and endoxylanase (4.21 U mg^{-1}) was best obtained in *C. indica* on 14th and 21st day of incubation. The extracellular activity of laccase (11.57 U mL^{-1}), lignin peroxidase (8.45 U mL^{-1}), and endoxylanases (6.22 U mL^{-1}) were found to be highest on the 14th day. Ligninolytic enzyme activity was substantial during substrate colonisation but quickly dropped during fruiting body development. *C. indica*, on the other hand, showed relatively modest hydrolase activity during substrate colonisation. The activity of hydrolytic enzymes increased dramatically when primordia formed and peaked at the mature fruiting body stage. The yield of the crude enzyme-treated wheat straw utilised for mushroom production was 52.47%. These findings showed that the activities of lignocellulolytic enzymes were regulated in line with developmental phase of growth of *C. indica*.

KEYWORDS

wheat straw, submerged fermentation, solid-state fermentation, lignocellulolytic enzymes, C*alocybe indica*

* Corresponding author. E-mail: manmeet1-mb@pau.edu

1. INTRODUCTION

Calocybe indica (Pur & Chan), commonly known as milky mushroom, belongs to white-rot basidiomycetes, which produce a variety of hydrolytic and oxidative enzymes that allow them to colonise, decompose, and bioconvert a variety of lignocellulosic substrates. Cellulose is a glucose-based linear polymer that can be hydrolysed by endoglucanases, exoglucanases, β-glucosidases, and the most common enzymes in this system are endoxylanases targeting the polysaccharide backbone (Obeng et al., 2017). Lignin peroxidase, manganese peroxidase, and laccase are three major enzymes involved in the breakdown of the complex molecule. The synthesis of enzymes is critical for substrate colonisation as well as fruit body development. Despite the mushroom's economic significance, our understanding of its physiology is incomplete, and further research is needed to fully realise its potential. *C. indica* is considered to release lignocellulolytic enzymes that catalyse the disintegration of the major macromolecular components (cellulose, hemicellulose, and lignin) of its growth substrate in order to gain access to the balanced nutrition required for the development and fruiting (Kumar and Chandra, 2020). The generation of these enzymes by the fungal mycelium is an important part of the colonisation process as well as a factor in mushroom yield. The goal of this study was to determine the profile of lignocellulolytic enzymes during substrate colonisation and growth in the flushes of fruiting bodies formed under solid-state fermentation and evaluation of intracellular and extracellular enzyme activities in submerged fermentation. Furthermore, the putative utilisation of lignocellulolytic enzymes in enhancing mushroom productivity during cultivation was studied.

2. MATERIALS AND METHODS

2.1. Organism and culture conditions

C. indica (CI-03) was obtained from Dr. H.S. Garcha Mushroom laboratory, Department of Microbiology, Punjab Agricultural University, Ludhiana. The pure culture was periodically subcultured on potato dextrose agar (PDA), incubated at 35 ± 2 °C for 7 days to achieve growth continuity and maintained under refrigerated conditions (4 °C).

2.2. Estimation of lignocellulolytic enzyme activities

2.2.1. Submerged fermentation (SmF). For enzyme synthesis, the 7 mm culture discs from the actively developing *C. indica* culture were inoculated into the Mushroom minimal medium (MMM) with the following composition (g L^{-1}): L-asparagine (1.60), D-glucose (20.0), $MgSO_4.7H_2O$ (0.50), KH_2PO_4 (0.46), K_2HPO_4 (1.00), thiamine hydrochloride (0.125), agar (20.0), at pH (6.5) and incubated at 120–160 r.p.m. for respective number of days (7, 14, 21, and 28) (Singh et al., 2020). The biomass was filtered and the mycelial mat was crushed in phosphate buffer (pH-7) and centrifuged (10,000 *g*; 15 min) at 4 °C. The supernatant received after centrifugation was analysed for intracellular activities. The broth left after the separation of mycelium was centrifuged, the clear supernatant obtained was utilised as the crude extract and the extracellular enzyme activities were determined.

2.2.2. Solid state fermentation (SSF). The substrate wheat straw was taken during the mushroom cultivation stages as shown in Fig. 1 (mycelial run, casing, pinhead, first flush, second flush, third flush, and terminated flush), homogenised in phosphate buffer (pH-7) and centrifuged (10,000 *g*; 15 min) at 4 °C to remove residual particles. The clear supernatants obtained were used as crude extract for enzymatic assays.

2.3. Determination of lignocellulolytic enzyme activities

The activity of aryl alcohol oxidase (E.C.1.13.7) was evaluated by observing the production of veratraldehyde after oxidation of veratryl alcohol in 50 mM potassium phosphate buffer at 310 nm (Okamoto and Yanase, 2002). The activity of laccase (E.C.1.10.3.2) was measured spectrophotometrically by analysing the reaction mixture's absorbance change. At 470 nm, the reaction mixture included 0.5 mL distilled water, 1 mL sodium acetate buffer (pH 4.5), 0.5 mL substrate solution (46 mM guaiacol), and 0.5 mL culture supernatant (Singh et al., 1988). The activity of Mn-peroxidase (E.C.1.11.1.13) was assessed using a buffered guaiacol solution, 2 mM $MnSO_4$, and 0.4 M H_2O_2, and the change in light absorbance was measured every 15 s up to 180 s at 465 nm against a blank without hydrogen peroxide (Paszcynski et al., 1988). The enzyme assay for cellobiose dehydrogenase (EC 1.1.99.18) contained 2 mM DCPIP (2,6-dichlorophenolindophenol), 2.5 mM cellobiose, and 4 mM sodium fluoride. The reaction

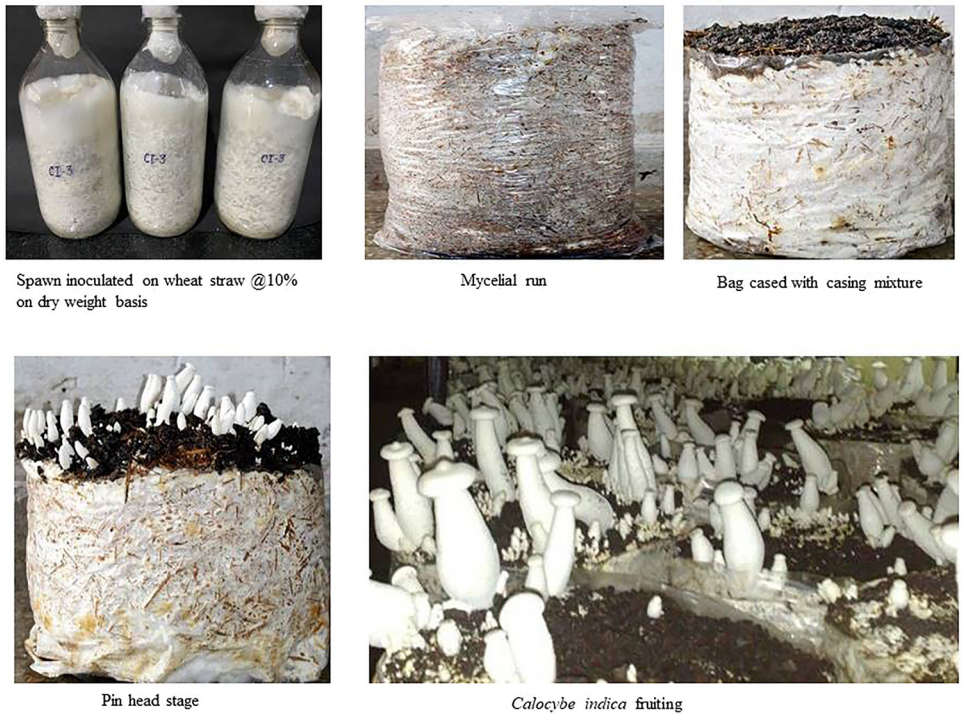

Fig. 1. Stages of mushroom *Calocybe indica*

was initiated by adding the enzyme solution, and the decrease in 600 nm absorbance was observed for the first 5 min at 37 °C (Sadana and Patil, 1985). Using veratryl alcohol as a substrate, the lignin peroxidase (E.C.1.11.1.14) activity assay detects the oxidation of veratryl alcohol to veratryl aldehyde in the presence of H_2O_2 at 310 nm (Tien and Kirk, 1988). The activity of endoxylanase (EC 3.2.1.8) was evaluated by mixing 70 µL of properly diluted sample with 630 µL of birch wood xylan (1% w/v) in 50 mM citrate buffer (pH 5.0) for 10 min at 40 °C (Bailey et al., 1992). β-glucosidase (E.C.3.2.1.21) was determined using pNPG (*p*-nitrophenol-a-D-glucopyranoside) as the substrate. 2 mM pNPG in 50 mM sodium acetate buffer (pH 3.0) and an appropriately diluted enzyme solution were included in the reaction mixture. The reaction was stopped by adding 1N Na_2CO_3 after 10 min of incubation at 45 °C, and the colour generated as a result of pNP release was detected at 410 nm (Saha and Bothast, 1996). The activity of cellobiohydrolase (E.C.3.2.1.91) was measured by incubating the cellobiose solution (0.5 mL) and the enzyme extract (0.5 mL) in a water bath at 50 °C for 10 min. After incubation, 3 mL of DNS was added to each test tube and the tubes were placed in a boiling water bath for 15 min. After allowing the contents to cool to ambient temperature, 1 mL of sodium potassium tartrate solution was added, and the enzyme activity was measured at 510 nm (Mandels and Sternberg, 1976). The activity of endoglucanase (EC.3.2.1.4) was determined by estimating the reducing sugars released after incubation of the substrate with the enzyme extract (Mandels et al., 1976). DNS reagent was used to evaluate the reducing sugars (Miller, 1959). The total cellulase activity (Filter paper activity, FPA) was measured using filter paper as substrate. A reaction mixture containing a string of filter paper (Whatman No.1), 0.8 mL of a 50 mM citrate buffer (pH 5.0), and 0.2 mL of properly diluted supernatant was incubated for 30–120 min at 40 °C (Ghose, 1987).

2.4. Cultivation of *C. indica*

The cultivation trial on *C. indica* was conducted during the months of May-October. The cultivation was carried out under indoor natural conditions with temperature ranging from 25–40 °C and relative humidity (80–90%) was maintained throughout the cropping cycle. For its cultivation, wheat straw was wetted with clean, fresh water for 14–20 h to attain 65–70% moisture. This straw was boiled for 45–50 min, cooled, the substrate was thoroughly spawned at 10% on dry weight basis, and filled into 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 was calculated (Singh et al., 2020).

2.5. Enzyme production and extraction for pretreatment of wheat straw for mushroom cultivation

The enzyme production was carried out in 250 mL Erlenmeyer flask with 5 g of wheat straw, moistened with water at the ratio of 1:4 (w/v). The experimental flasks were autoclaved at 121 °C for 15 min, inoculated with 10% (v/v) of fungal spore suspension (1×10^8 spores/mL) and incubated in a BOD incubator at 28 ± 2 °C. The contents of the experimental flasks were harvested by 0.1 M potassium phosphate buffer (pH 7), added to achieve a solid/liquid ratio of 1: 10 (w/v). The suspension was stirred in an orbital shaking incubator at 120 r.p.m. for 30 min.

The biomass was filtered using Whatman filter paper (No.1) and the filtrates were centrifuged at 6,000 r.p.m. at 4 °C for 10 min. The supernatants obtained were stored in sterilised glass bottles at 4 °C and used as crude enzyme extracts.

The ligninolytic crude enzyme after extraction (8 mL) was dissolved in 0.1 M phosphate buffer (pH-6.0) and was made up to a volume of 30 mL. The enzyme was sprayed on the wheat straw to achieve a moisture ratio of 1:2 (wheat straw: moisture). The days for spawn run, harvesting, yield, the number of fruiting bodies harvested, and an average weight of fruit bodies were assessed.

2.6. Statistical analysis

All enzyme activities were performed in triplicates and presented in tables and figures. The data were analysed statistically using One-way ANOVA IBM SPSS Statistics version 16.0.

3. RESULTS AND DISCUSSION

3.1. Intra-extracellular distribution of ligninolytic enzyme activities during incubation period

Intra- and extracellular activities of ligninolytic enzymes based on fungal mycelium and the liquid culture filtrate are shown in Figs 2 and 3. *C. indica* produced the highest intracellular laccase (7.67 U mg^{-1}) and manganese peroxidase (7.48 U mg^{-1}) and extracellular laccase (11.57 U mL^{-1}) and manganese peroxidase (3.93 U mL^{-1}) on the 14th day, and the activity started to decline as the incubation period lengthened. Indeed, there is evidence from the literature that laccases of white-rot fungi may be divided into extra- and intracellular forms, with respect to induction of enzyme synthesis, patterns of extra- and intracellular isoforms, and the mechanism of secretion. Although within the present study the majority of the laccase activity was detected within the respective culture filtrates, there was also considerable intracellular laccase activity

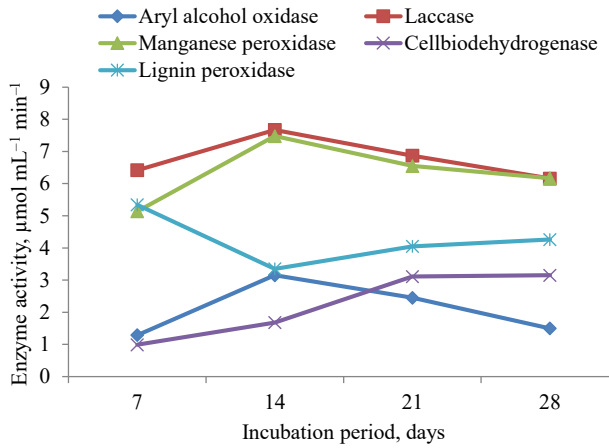

Fig. 2. Intracellular ligninolytic enzyme activities under submerged fermentation

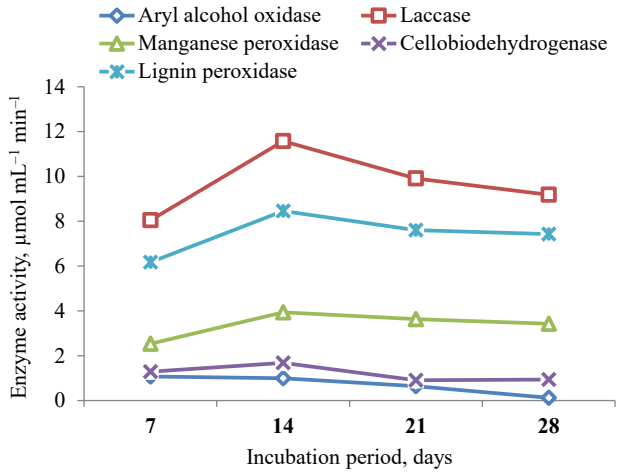

Fig. 3. Extracellular ligninolytic enzyme activities under submerged fermentation

observed. The intracellular activity of the cellobiose dehydrogenase (3.15 U mg^{-1}) peaked on the 28th day. The activities of intracellular aryl alcohol oxidase (3.15 U mg^{-1}) and extracellular cellobiose dehydrogenase (1.68 U mL^{-1}) and lignin peroxidase (8.45 U mL^{-1}) were found to be maximum on the 14th day. However, the intracellular lignin peroxidase (5.34 U mg^{-1}) and extracellular aryl alcohol oxidase (1.07 U mL^{-1}) activities were peaked on the 7th day. The ligninolytic enzymes produced by *C. indica* as secondary metabolites might be induced by nutrient deprivation, thus the enzymatic activity decreased as the incubation period increased. The high laccase activity might be regulated in association with increase in morphogenesis during the mycelial growth and the enzyme level declined rapidly (Sousa et al., 2019). The extracellular activity of enzymes was related to the fungal culture entering the log phase, which is metabolically the most active phase. Similarly, Shantaveera Swamy and Ramalingappa (2015) reported that *Gleophyllum trabeum* produced maximum laccase (18 U L^{-1}), manganese peroxidase (24.43 U L^{-1}), and lignin peroxidase (3.53 U L^{-1}) for 14 days. Sou et al. (2017) reported that mycelium of *Sparassis latifolia* resulted in maximum activity of laccase, manganese peroxidase, and lignin peroxidase on the 14th day of incubation on Kirk's medium. Cesur et al. (2022) observed that laccase activity of *Flammulina velutipes* increased on the 4th day of incubation.

3.2. Ligninolytic enzyme profile during solid state cultivation of *C. indica* on wheat straw

The ligninolytic enzyme activities from early colonisation till fruit bodies showed varied pattern as shown in Table 1. The laccase (14.11 U mg^{-1}), lignin peroxidase (10.39 U mg^{-1}), manganese peroxidase (9.75 U mg^{-1}), and aryl alcohol oxidase (4.65 U mg^{-1}) activities immediately showed increased response at their pinhead stage and dropped down during the harvesting stages, although highest cellobiose dehydrogenase activity was observed during the fourth flush (3.52 U mg^{-1}). Table 2 shows significant maximum positive correlation ($r = 0.880$) amongst all ligninolytic enzymes recorded for manganese peroxidase and laccase indicating a direct

Table 1. Production of lignocellulolytic enzymes from *Calocybe indica*

Oxidative enzymes	Lignocellulolytic enzyme activity (U mg^{-1})							
	Mycelial run	Casing	Pin head stage	First flush	Second flush	Third flush	Fourth flush	Mean
Aryl alcohol oxidase	$0.98 + 0.02^{c}$	$0.76 + 0.1^{c}$	$4.65 + 0.3^{a}$	$3.39 + 0.01^{b}$	$3.34 + 0.04^{b}$	$3.48 + 0.23^{b}$	$3.16 + 0.14^{b}$	2.82
Laccase	$9.75 + 0.1^{b}$	$8.65 + 0.04^{c}$	$14.11 + 0.4^{a}$	$9.38 + 0.03^{b}$	$6.92 + 0.01^{e}$	$6.37 + 0.21^{f}$	$7.35 + 0.16^{d}$	8.93
Manganese peroxidase	$8.25 + 0.03^{b}$	$7.65 + 0.4^{c}$	$9.75 + 0.24^{a}$	$6.04 + 0.03^{d}$	$5.73 + 0.02^{d}$	$5.92 + 0.1^{d}$	$6.07 + 0.02^{d}$	7.05
Cellobiose dehydrogenase	$1.26 + 0.04^{c}$	$0.98 + 0.2^{c}$	$2.08 + 0.01^{b}$	$3.42 + 0.03^{a}$	$3.46 + 0.32^{a}$	$3.50 + 0.01^{a}$	$3.52 + 0.27^{a}$	2.60
Lignin peroxidase	$6.75 + 0.04^{b}$	$5.25 + 0.05^{e}$	$10.39 + 0.19^{a}$	$6.33 + 0.03^{c}$	$5.61 + 0.01^{d}$	$6.38 + 0.3^{c}$	$6.40 + 0.03^{c}$	6.73
Hydrolytic enzymes								
Endoxylanases	$4.08 + 0.01^{d}$	$3.82 + 0.12^{d}$	$6.45 + 0.04^{b}$	$5.91 + 0.01^{c}$	$6.94 + 0.3^{a}$	$7.01 + 0.01^{a}$	$6.84 + 0.13^{a}$	5.86
β-glucosidase	$1.09 + 0.02^{a}$	$0.98 + 0.01^{a}$	$1.21 + 0.28^{a}$	$1.28 + 0.04^{a}$	$1.35 + 0.04^{a}$	$1.3 + 0.4^{a}$	$1.24 + 0.03^{a}$	1.20
Cellobiohydrolase	$3.98 + 0.01^{b}$	$3.25 + 0.05^{c}$	$5.39 + 0.34^{a}$	$5.43 + 0.02^{a}$	$5.52 + 0.04^{a}$	$5.55 + 0.26^{a}$	$5.60 + 0.02^{a}$	4.96
Endoglucanase	$2.86 + 0.04^{d}$	$1.98 + 0.1^{e}$	$5.22 + 0.31^{b}$	$5.45 + 0.04^{b}$	$6.91 + 0.01^{a}$	$2.88 + 0.12^{d}$	$4.40 + 0.05^{c}$	4.24
Total cellulases (FPA)	$1.01 + 0.01^{b}$	$0.78 + 0.02^{b}$	$1.65 + 0.4^{a}$	$1.69 + 0.03^{a}$	$1.73 + 0.13^{a}$	$1.58 + 0.02^{a}$	$1.51 + 0.32^{a}$	1.42

Data represent mean ± SD ($n = 03$). The alphabetical letters are significantly different within each mushroom cultivation stage at $P \leq 0.05$ according to Tukey's post hoc test.

Table 2. Pearson correlation coefficients between different ligninolytic enzymes of *Calocybe indica*

	Aryl alcohol oxidase	Laccase	Manganese peroxidase	Cellobiose dehydrogenase	Lignin peroxidase
Aryl alcohol oxidase	1	0.283	0.561	–0.444	0.798**
Laccase	0.283	1	0.880**	–0.270	0.487
Manganese peroxidase	0.561	0.880**	1	–0.443	0.645*
Cellobiose dehydrogenase	–0.444	–0.270	–0.443	1	0.731**
Lignin peroxidase	0.798**	0.487	0.645*	0.731**	1

**: Correlation is significant at the 0.01 level (2-tailed); *: Correlation is significant at the 0.05 level (2-tailed).

relationship between the enzymes. Similarly, positive correlation between lignin peroxidase and aryl alcohol oxidase ($r = 0.798$) were recorded. For manganese peroxidase and lignin peroxidase, significant positive correlation at $P < 0.01$ ($r = 0.645$) was observed. Linear positive relationship between cellobiose dehydrogenase and lignin peroxidase ($r = 0.731$) was observed. The positive correlation of the ligninolytic enzyme production was observed with respect to mushroom cultivation stages. The level of enzyme activities seemed to be strongly regulated during the life cycle of *C. indica* in all cultivation stages with distinct variations. Higher levels of oxidase enzyme activity during early stage of the spawn run indicate that ligninolytic enzymes were responsible for degradation of physical barrier of lignin so that more cellulosic and hemicellulosic content was exposed for further degradation to extract the energy for the growth of mushroom fungus. Similar results of ligninolytic enzymes were obtained by Singh et al. (2020), who reported that laccase activity was found to be highest during the pin head stage (6.96 U $mg^{-1)}$ in *C. indica*. According to Elisashvili et al. (2015), during cultivation of *Lentinus edodes* on wheat, laccase and manganese peroxidase activities were higher during substrate colonisation and declined rapidly during primordial appearance and fruit body development.

3.3. Intra-extracellular distribution of cellulolytic enzyme activities during incubation period

As shown in Figs 4 and 5, *C. indica* showed highest intracellular cellobiohydrolase (4.09 U mg^{-1}) and β-glucosidase (0.79 U mg^{-1}) and extracellular cellobiohydrolase (2.82 U mL^{-1}) and β-glucosidase (0.86 U mL^{-1}) activities on the 14th day of incubation period. The intracellular endoglucanase (1.35 U mg^{-1}) enzyme activity was found to be maximum on the 7th day. The enzyme activity of intracellular hydrolytic enzyme, endoxylanase (4.21 U mg^{-1}), peaked on the 28th day. The extracellular endoxylanase (6.22 U mL^{-1}) exhibited maximum activity on the 14th day of incubation related to the culture entering the log phase, which is metabolically the most active phase. However, the extracellular endoglucanase (3.87 U mL^{-1}) showed maximum activity on the 21st day of incubation. The activities of intracellular total cellulases (FPA) (0.81 U mg^{-1}) and extracellular total cellulase (0.84 U mL^{-1}) exhibited notable effects and peaked on the 21st day. Cellulase is an enzyme complex containing mainly endo-β-glucanases, exo-β-glucanases, and cellobiose. Xylanase and cellulase enzymatic systems generally contain multiple enzyme components that have a marked synergism against hemicellulosic residues. The enzymes released from the mycelial hyphae contribute to the mycelium-composite formation by

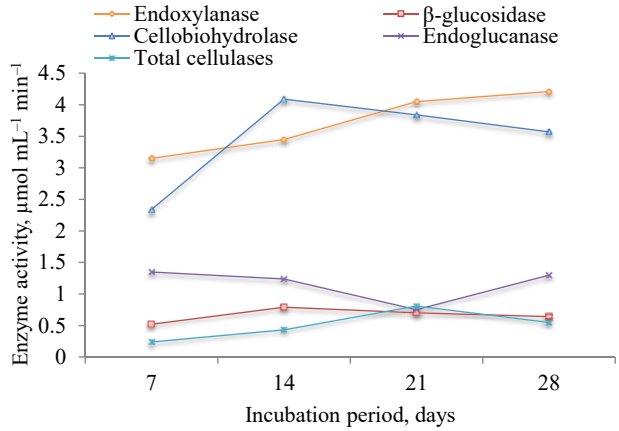

Fig. 4. Intracellular cellulolytic enzyme activities under submerged fermentation

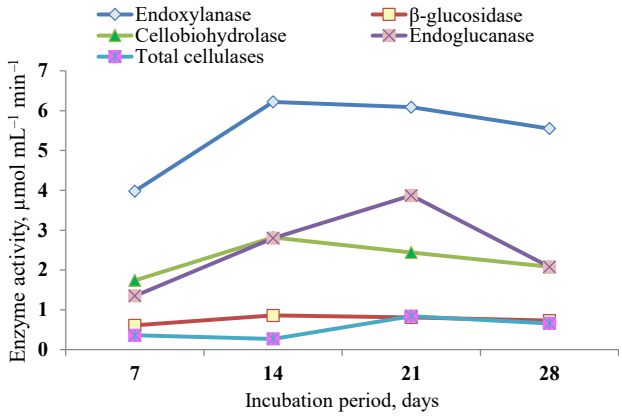

Fig. 5. Extracellular cellulolytic enzyme activities under submerged fermentation

degrading the substrate and increasing the mycelial density (Tacer-Caba et al., 2020). de Lima et al. (2020) reported that a deceleration in mycelium growth rates could occur after the third colonisation week, leading to a decrease in the enzyme activity because of the limitation of utilisable nutrients and carbon sources. Kshirsagar et al. (2020) reported that *Phanerochaete chrysosporium* showed the maximum endoglucanase (166.32 U mL^{-1}) activity on the 7th day of incubation under submerged fermentation. Liu et al. (2020) showed that *P. chrysosporium* presented in the highest cellulase activity (42.2 U mg^{-1}) on the 6th day and the maximum xylanase activity (150.2 U mg^{-1}) on the 8th day.

3.4. Cellulolytic enzyme profile during solid state cultivation of *C. indica* on wheat straw

The endoxylanase enzyme activity was highest during the pin head stage (6.45 U mg^{-1}) and reduced during the terminal stage of *C. indica*. β-glucosidase enzyme revealed maximum activity

in the second flush stage (1.35 U mg^{-1}). The cellobiohydrolase activity increased in all *C. indica* cultivation stages, and the maximum activity was found to be at the terminal stage (5.60 U mg^{-1}). The activities of endoglucanase (6.91 U mg^{-1}) and total cellulases (1.73 U mg^{-1}) increased during the second flush after harvesting as shown in Table 1. The endoglucanase enzyme is endoenzyme, so endoglucanase is produced in higher amount to decrease the polymer chain and consequently provides a faster energy source to meet the demand of the higher biomass production. The observed pattern is characteristic of the white-rot fungi, which have limited cellulolytic activity in the anamorphic stage. They are known for attacking highly lignified substrates and producing enzymes that target lignin polymerisation. Furthermore, there is a link between fungus morphogenesis and its cellulolytic activity. It is well known that cellulolytic enzymes provide energy to the mycelium till the development of the fruit body. The reemergence of cellobiohydrolase and endoxylanases in the latter stages of mushroom development could be attributed to fungal autolysis. The increase in the enzyme activities during fructification may be due to the fungus's need to mobilise large amounts of carbon for mushroom formation. Table 3 presents the highest and positive correlation ($r = 0.852$) amongst all the cellulolytic enzymes recorded for endoglucanase and endoxylanase, indicating a direct relationship between the hydrolytic enzymes. Similarly, significant positive correlation was found between cellobiohydrolase and endoxylanase ($r = 0.836$). The significant positive correlation at $P \leq 0.01$ was observed between β-glucosidase and cellobiohydrolase ($r = 0.682$); endoglucanase and cellobiohydrolase ($r = 0.674$); and endoglucanase and total cellulases ($r = 0.598$). Thus, the positive correlation of the cellulolytic enzyme production was observed with respect to mushroom cultivation stages. The results agreed with the findings of Bhupathi et al. (2017), who showed that the maximum activity of xylanase was recorded in the pileus of APK2 variety and CBE-TNAU-1523 when compared to the stipe in *C. indica*. According to Singh et al. (2020), *C. indica* showed maximum endoxylanase (2.36 IU mg^{-1}) and endoglucanase (1.88 IU mg^{-1}) activities during fruiting body stages.

3.5. Productivity: spawn run, substrate colonisation, and fruit body development

C. indica produced the maximum yield (52.47 kg/q dry straw) and fruit body weight (28.12 g) in the enzyme treated wheat straw as shown in Table 4. The spawn run was completed in fewer days (19) as compared to non-treated straw (21), and the maximum number of fruiting bodies

Table 3. Pearson correlation coefficients between different cellulolytic enzymes of *Calocybe indica*

	Endoxylanase	β-glucosidase	Cellobiohydrolase	Endoglucanase	Total cellulases (FPA)
Endoxylanase	1	0.537	0.836**	0.852**	0.330
β-glucosidase	0.537	1	0.682*	0.459	0.317
Cellobiohydrolase	0.836**	0.682*	1	0.674*	0.026
Endoglucanase	0.852**	0.459	0.674*	1	0.598*
Total cellulases (FPA)	0.330	0.317	0.026	0.598*	1

**: Correlation is significant at the 0.01 level (2-tailed); *: Correlation is significant at the 0.05 level (2-tailed).

Table 4. Enzymatic cultivation of *Calocybe indica* on wheat straw

	Treatments		CD ($P \leq 0.05$)
	Control	Crude enzyme	
Spawn run (d)	21.00 ± 0.2	19.00 ± 0.3	
First harvest (d)	28.00 ± 0.5	24.00 ± 0.4	
Yield (kg/q dry straw)	38.16 ± 0.12	52.47 ± 0.2	2.27
NFB (no./q dry straw)	1707 ± 0.4	1865 ± 0.54	NS
Av. wt. of a FB (g)	22.35 ± 0.1	28.12 ± 0.2	
Disease/Pest	–	–	

Data given is mean of three replicates; NFB: number of fruiting bodies; FB: fruiting body; d: number of days; Av. Wt.: average weight.

(1865 no./q dry straw) harvested was during the enzymatic treatment. The enzyme treated wheat straw promoted efficient cell wall disintegration, resulting in maximum yield and fruiting body growth. This might be due to the increase in the digestibility of wheat straw due to the decrease in silica content and the breaking of the bonds between cellulose, hemicelluloses, and lignin content. Bioconversion of enzymatic wheat straw into mushroom fruiting bodies is a physiologically efficient method. The efficiency to colonise and utilise wheat straw substrate depends on the fungus's ability to create extracellular enzymes required to break down the main components of the lignocellulosic waste biomass (Suwannarach et al., 2022). The biological treatment processes are safe, eco-friendly, easy to perform, and involves lower operational costs as compared to other treatment methods. The amount of lignocellulolytic enzymes produced by *C. indica* growing on a cheap medium gave us an opportunity to conduct a study at a laboratory scale. Enzymes are highly efficient, it takes a very little amount of enzymes to make a lot of chemistry. Thus, small pilot level equipment is adequate to supply enough catalyst for regular enzyme production.

However, the process model for production of lignocellulolytic enzymes from *C. indica* includes feedstock transport, sterilisation, fungal growth, enzyme production, process optimisation, fermentation residence time, system monitoring (pH and temperature), extraction, and downstream processing. Accessibility of oxygen between heat generation and substrate particles in the available space are the major challenges in solid state fermentation for the production of enzyme at the large scale. Moreover, half of the cost of production is associated with capital investment (depreciation, insurance, maintenance, etc.). Accurate assessment of enzyme activity should be carried out for fresh samples so all these parameters could be optimised, only than can cost effectiveness be described.

4. CONCLUSIONS

The study underlines that the expression of biosynthetic potential of *C. indica* is highly dependent on the method of fungi cultivation. *C. indica* produces enzymes that are necessary for lignocellulose degradation, as well as for developmental regulation. However, *C. indica* successfully colonising wheat straw treated with crude enzyme might be considered for its growth and yield. Therefore, *C. indica* production can be a boon to small scale mushroom growers. The

lignocellulolytic enzymes with high activities and efficiencies are ecofriendly tool of nature that could be used extensively in biotechnological applications and various industries that require high amounts of enzymes.

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