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Profiling of morphogenesis related enzymes of milky mushroom *Calocybe indica* (P & C)

Priyadharshini Bhupathi, Akkanna Subbiah Krishnamoorthy and Sivakumar Uthandi

Abstract
Milky mushroom (*Calocybe indica* P&C) is one among the prevalent edible tropical mushrooms cultivated in India. It is well known that secretion of lignocellulolytic enzymes in mushrooms play an important role in degradation of agroresidues but it is understood that certain enzymes are also related to morphogenesis of mushrooms. In this regard, the present study was undertaken to study the function of morphogenesis related enzymes *viz*., *mannitol dehydrogenase (MtDH), xylanase, laccase, tyrosinase and lipoxygenase (LOX)* activities at seven different growth stages viz., pinhead, tiny button, button, I elongation, II elongation, maturation, complete maturation both in pileus and stipe of *Calocybe indica* var. APK2 and CBE-TNAU-1523 wild isolate. The results indicated that activity of *MtDH, xylanase, laccase, tyrosinase and LOX* in the mushroom is found to be maximum at stage five (II elongation) followed by stage four (I elongation) and the rate of increase in activity was higher from stage one to stage five in pileus when compared to stipe in both APK2 variety and CBE-TNAU-1523 wild strain of *Calocybe indica*. Among, all the enzymes assayed, the activity of xylanase was recorded maximum at all the seven stages of mushroom growth followed by lipoxygenase activity. Maximum activity of xylanase was recorded in pileus of APK2 variety and CBE-TNAU-1523 wild strain (3.514µmoles/min/g and 3.551µmoles/min/g, respectively) when compared to stipe.

Keywords: *Calocybe indica* (P&C) var. APK2, CBE-TNAU-1523, morphogenesis related enzymes, mannitol dehydrogenase, laccase, xylanase, tyrosinase and lipoxygenase

Introduction

Huge amount of lignocellulosic agricultural crop residues and agro-industrial by-products generated annually which are rich in organic compounds that are worthy of being recovered and transformed. However, their handling and disposal are often problematic, due to their chemical structure and decomposition properties and there by burnt or incorporated into the soil. But these residues are of particular interest for the agricultural economy of temperate and subtropical countries, since they are produced in large quantities. Philippoussis (2009) accomplished that the cultivation of mushroom is a prominent biotechnological process to enhance the value of agro-industrial production. Previous research work indicates that the lignocellulose degrading mushroom species are used in various solid state fermentation applications for bioremediation and biodegradation of hazardous compounds (Perez *et al*., 2007) [32], biological detoxification of toxic agro-industrial residues (Soccoland Vandenberghe, 2003) [38], biotransformation of agro industrial residues to mushroom food and animal feed (Okano *et al.,* 2006) [30], compost and product developments such as biologically active metabolites, enzymes, and food flavour compounds (Nikitina *et al.,* 2007) [29].

Among applications of solid state fermentation (SSF), mushroom cultivation has proved its economic strength and ecological importance for efficient utilization, value-addition and biotransformation of agro-industrial residues (Zervakis and Philippoussis 2000) [45]. Commercial mushroom productionis yet another efficient and relatively short biological process of food protein recovery from unutilized lignocellulosic materials through enzymatic degrading capabilities of mushroom fungi (Chiu and Moore, 2001) [8].

The use of crop residues depends on the capacity of the fungus to produce a lignocellulolytic enzyme complex (Buswell *et al.,* 1996) [5]. This complex includes the oxidative enzymes laccase and manganese peroxidase (MnP), which are involved in lignin degradation (Shah and Nerud, 2002) [36], and the hydrolytic enzymes xylanase and cellulase (Eira, 2004), which are involved in hemicellulose and cellulose degradation, respectively. In nature, mostly all the basidiomyceteous fungi are able to degrade lignin efficiently by the means of solubilization and mineralization (Kirk and Cullen 1998) [19]. In mushrooms mannitol functions as an osmoregulator by encouraging the influx of water from the environment to develop turgor

pressure thereby, helps in fruiting body development (Jennings, 1985) [16]. The extracellular secretion of laccases by basidiomycete fungi are capable of mineralizing lignin that is apparently unique to this group of organisms (Thurston, 1994) [41]. The formation of rhizomorphs (mycelial strands formed from large numbers of tightly adpressed hyphae) is a different developmental change that is associated with laccase synthesis in *Armillaria mellea* (Worrall *et al.,* 1986) [44] and is responsible for making a polyphenolic glue that sticks the hyphae together. Xylanases are similar to cellulases and it can act synergistically to achieve hydrolysis; predominant enzymes within this system are endoxylanases which attack the polysaccharide backbone, and b-xylosidases, which hydrolyze short xylooligosaccharidesto xylose (Montoya *et al.,* 2012) [26]. Tyrosinase is a multifunctional, glycosylated and copper containing oxidase, which catalyzes the first two steps in mammalian melanogenesis and is responsible for enzymatic browning in fresh fruits, vegetables, beverages and mushrooms (Liu *et al.,* 2013) [22]. Lipoxygenase are involved in the biosynthesis of leukotrienes and lipoxins, which are potent mediators of inflammatory responses (Nicolaou *et al.,* 1989) [28]. Also they play role in the production of volatile molecules that can positively or negatively influence the flavor and aroma (Tasaki *et al.,* 2013) [40].

Milky mushroom (*Calocybe indica*) var.APK2 is one of such mushroom varieties, where complete commercial production techniques have been standardized for the first time of Tamil Nadu Agricultural University (Krishnamoorthy, 1998) [20]. The milky mushroom *Calocybe indica* is highly suitable for hot humid climate and can be cultivated almost throughout the year in India except few places (Pani, 2010). Since this mushrooms is morphologically similar to *Agaricus bisporus* (button mushroom), it has been quite popular in southern Indian states and slowly getting popular in other countries (Krishnamoorthy and Balan, 2015). In this regard, the present study focuses the role of the assay of *Calocybe indica* var. APK2 and CBE-TNAU-1523 wild strain morphogenesis related enzymes at different growth stages of milky mushroom.

Materials and methods

Preparation of milky mushroom bed

Beds were prepared following “polybag method” described by Baskaran *et al.,* (1978) [2] using paddy straw.

Preparation of Casing soil

Garden soil (clay loam; pH 8.4) was used for casing throughout the study. The soil moisture content was adjusted to 20 per cent following the method of Devadoss (1971) [9].

Collection of sample

Fresh fruiting bodies of *Calocybe indica* (P&C) var. APK2 and wild strain CBE-TNAU-1523 at seven different growth stages were collected from separately as described by Chakraborthy *et al.,* (2000) and used for morphogenesis related enzyme assay.

Assay of Mannitol dehydrogenase (MtDH)

Mannitol dehydrogenase was assayed as suggested by Chakraborthy *et al.* (2004) with some modifications. One gram of tissue was extracted with 20 mM HEPES (Hydroxyethylpiperazinethanesulfonic acid) KOH buffer (pH 7.5) containing 1 mM EDTA, 2 mM 2- mercaptoethanol and 2 mM PMSF (Phenyl Methyl Sulfonyl Fluoride). Unbroken cells, and cell debri were removed by centrifugation at 32,000 rpm for 60 min at 4°C and the supernatant was used as the cell extract. MtDH activity was determined by incubating the cell extract at 25°C in 20 mM HEPES-KOH (pH 7.5) - 500 mM fructose - 0.25 mM NADPH. The enzyme activity was monitored by recording the change in absorbance at 340 nm. The enzyme assay was repeated three times to avoid experimental errors.

Assay of Laccase

Laccase activity was determined using guaiacol as the substrate according to the method of Sandhu and Arora (1985) [35]. One gram of tissue was extracted with phosphate buffer. Cell debris were removed by centrifugation at 32,000 rpm for 60 min at 4°C and the supernatant was used as the cell extract. Laccase was assayed by adding 0.3 mL enzyme source to 2.5 mL of 30 μM Guaiacol in phosphate buffer (0.1 M) at pH 6.0. Absorbance was read at 470 nm after incubating the reaction mixture for 30 min at 25ºC against zero time control. One unit of laccase activity was calculated as change in absorbance by 0.001 min–1 mL–1 of enzyme source at 25^0C.

Assay of Xylanase

Xylanase activity assay was performed according to Miller (1959) [25]. One gram of tissue was macerate and extracted with sodium citrate buffer (pH 5.3) and centrifugation at 32,000 rpm for 60 min at 4°C and the supernatant was used as the cell extract. The reaction mixture consisting of 1.8 mL of a 1.0% (w/v) suspension of birch-wood xylan in 50mMsodiumcitrate and 0.2 mL of enzyme dilution (in 50 mM sodium citrate at pH 5.3) was incubated at 50ºC for 5 min. Released reducing sugars were determined by dinitrosalicylic acid reagent (DNS) method, by adding 3 mL of DNS solution and then incubating the mixture at 95ºC for5 min. Absorbance was measured at 540 nm. One unit of enzyme is defined as the amount of enzyme catalyzing the release of 1 mmol of xylose equivalent per minute.

Assay of Tyrosinase

The tyrosinase activity assay was performed according to Boiret *et al.,* (1985) [3] with slight modifications. The one gram of tissue was macerated with phosphate buffer (pH 6.8) at 1:1 (w/v) ratio. The extract was collected and centrifuged at 15,000 rpm for 30 min at 4°C. The supernatant was used as enzyme source. Assay was conducted with the reaction mixture, total volume of 3.0 ml: 3 mM-L-tyrosine, 47 mM-sodium phosphate buffer (pH 6.8) and 25 μl of enzyme source. The rate of formation of dopachrome was measured at 475 nm. Control was maintained without adding enzyme mixture. The enzyme activity was expressed as changes in absorbance at 470 nm

Assay of Lipoxygenase

Lipoxygenase was assayed according to Axelrod *et al.,* (1981) [1]. The cells were homogenized in an ice bath with 0.1 M TRIS-HCl buffer (pH 8.5) containing 1% PVP (w/v), 1 mM CaCl2, 5 mM DTT, and 10% (v/v) glycerol. The homogenate was centrifuged at 11 000 rpm for 20 min at 4 °C, and the supernatant was used as the enzyme extract. 50 mg of linoleic acid was added to 50 mg Tween 20 and mixed with 10 ml of Na_2HPO_4 buffer (0.1 M, pH 8.7) by stirring. The solution was cleared by addition of 250 ml of 1 M NaOH and diluted to 25 ml with the buffer. One ml of the enzyme reaction mixture contained 50 ml enzyme extract, 0.95 mlNa_2HPO_4 buffer and 5 ml of substrate solution. The increase in absorbance was monitored at 234 nm.

Result and Discussion

The enzymes activities were spectrophotometrically assayed in all seven growth stages of the milky mushrooms *viz., Calocybe indica* (P&C) APK2 variety and CBE-TNAU-1523 wild strain at 24 hours interval. The results revealed that the morphogenesis related enzymes *viz.,* mannitol dehydrogenase, xylanase, laccase, tyrosinase and lipoxygenase were secreted in higher levels in pileus when compared to stipe. Among all the enzymes tested, xylanase showed maximum activity followed by lipoxygenase in both the mushrooms. Tyrosinase enzyme recorded lesser activity in all the stages of APK2 variety and CBE-TNAU-1523 wild strain.

Morphological stages of milky mushroom

The different growth stages of *Calocybe indica* is refers to pinhead (stage 1), tiny button (stage 2), button (stage 3), I elongation (stage 4), II elongation (stage 5), maturation (stage 6), complete maturation (stage 7) (Plate 1 and 2).

Mannitol dehydrogenase activity (Mtdh)

In the present study, mannitol dehydrogenase activity was maximum in stage five (0.635 and 0.711 µmoles/min/g in APK2 and CBE-TNAU-1523, respectively) followed by stage four (0.617 and 0.623 µmoles/min/g in both mushrooms). Among the parts of mushroom, pileus recorded maximum (0.635 and 0.711 µmoles/min/g) activity of the enzyme followed by stipe (0.221 and 0.133 µmoles/min/g) in both APK2 and CBE-TNAU-1523 milky mushrooms (Tables 1 and 2). The rate of increase in activity was higher from stage one to stage five when compared to stage six and stage seven. During the mannitol synthesis NADPH is obtained from pentose phosphate pathway (Dutsch and Rast, 1972) [10]. Mannitol, a six carbon polyol that accumulates in growing sporophores *i.e.* in both pileus and stipe during and between the flushes of fruiting body development (Hammond & Nichols, 1976) [13]. The increase in MtDH. activity in developmental stages had been attributed to the increase in Hexose monophosphate (HMP) activity (Hammond, 1981) [14]. In contrast, mannitol dehydrogenase activity was reported to be high during the developmental stage 1 of *A. bisporus* and decreased progressively in subsequent stages (Morton *et al.,* 1985) [27].

Laccase activity

The laccase activity was found to be significantly higher in stage 5 (1.519 and 1.681µmoles/min/g) in *Calocybeindica* (P&C) var. APK2 and wild strain CBE-TNAU-1523, respectively. Minimum laccase activity was recorded at stage 1 (0.333µmoles/min/g in APK2 variety and 0.411µmoles/min/g CBE-TNAU-1523).Widiastuti *et al.,* (2008) described that the activity of laccase was very high (1.762 U/ml) during the mycelial colonization of *P. ostreatus* and which declined after two weeks and further increased in three weeks, but during fruit body formation the laccase activity reduced sharply. In the cultivated button mushroom *Agaricus bisporus*, laccase activity accumulates during vegetative growth but undergoes rapid inactivation shortly after the onset of fruit body formation (Wood, 1980) [43]. Kalmis and Sargi (2004) [17] reported that there existed an inverted correlation between laccase and manganese peroxidase activities and fruiting body maturation of *Pleurotus ostreatus*, with the minimum of activity occurring when the mushrooms were mature.

Xylanase activity

Among all the enzymes tested xylanase shows maximum activity at all the growth stages of both *Calocybe indica* (P&C) var. APK2 and wild strain CBE-TNAU-1523 but the level of enzyme induction in stage six and seven was decreased when compared to other stages. The results indicated that stage five is having more activity of xylanase3.514µmoles/min/g, followed by stage four (3.443 µmoles/min/g) in APK2variety (Table 1) similar trend was observed in CBE-TNAU-1523 wild strain with higher xylanase activity in stage five (3.551µmoles/min/g) and stage four (3.452 µmoles/min/g) (Table 2). Whereas, stage1 and stage2 recorded less activity of xylanase in both mushrooms. However the activity of xylanase was maximum in pileus when compared to stipe in both mushrooms. Elisashvili *et al.*, (2008) [11] reported that the *P. ostreatus x*ylanase activities gradually increased during second fruiting stage of mushroom and then decreased. Matsumoto (1998) found that the cellulase and xylanase activities increased during the development of the fruiting bodies of *Lentinula edodes*, with highest levels during mushroom maturation which may be due to the fungus need to mobilize large amounts of carbon for mushroom formation.

Tyrosinase activity

Tyrosinase is an enzyme that belongs to Poly phenol oxidase family. The present study indicates that the presence of tyrosinase recorded minimum activity when compared to all the enzymes assayed. Stage five registered significantly high tyrosinase activity (0.043µmoles/min/g)followed by stage four (0.033µmoles/min/g) in APK2 variety and 0.142 and 0.112 µmoles/min/g in CBE-TNAU-1523 (Table 1 and 2). The tyrosinase activity increased with subsequent stages of maturity, which was correlated with senescence of fruiting bodies of *V. volvacea* (Kiran Kumar, 2015) [18] and browning effect in *Agaricus* mushrooms (Singh *et al.,* 2010) [37].

Lipoxygenase

In this present study, lipoxygenase is recorded as a second highest enzyme activity present in all the stages of mushroom next to xylanase. Among the parts of the mushroom high lipoxygenase activity was recorded in pileus (3.435 µmoles/min/g) in stage five followed by stage four (3.159 µmoles/min/g) in variety APK2 (Table 1). In CBE-TNAU-1523, 3.453and 3.132 µmoles/min/g recorded in pileus of stage 5 and stage 4, respectively (Table 2). Hiroi (1988) [15] and Kuribayashi *et al.,* (2002) [21] reportedthat the purified form of lipoxygenase (LOX) showed that free linoleic acid, which is the most abundant fatty acid (72.4% of total fatty acid) present in the pileus of *P. ostreatus* converts linoleic acid to 13-*Z,E*-HPOD (hydroperoxy-(*Z*, *E*)-11, *13*-eicosadienoic acid). In the other hand Mau *et al.,* (1992) [24] reported that 10-oxo-*trans*-8-decenic acid produced concurrently with 1-octen-3-ol might be involved in the development of fruiting bodies of *Agaricus bisporus*. Because it has been assumed that the formation of 1-octen-3-ol requires actions of lipoxygenase and hydroperoxidelyase-like enzyme, involved in the fruiting formation of mushroom.

Table 1 Assay of morphogenesis related enzymes of *Calocybe indica* (P&C) var.APK2 at different growth stages

Enzymes	Different growth stages of mushroom/24 hrs interval (µmoles/min/g)										
	Pinhead (Stage1)	Tiny button (Stage2)	Button (Stage3)	I Elongation (Stage4)		II Elongation (Stage5)		Maturation (Stage6)		Complete maturation (Stage7)	
				Pileus	Stipe	Pileus	Stipe	Pileus	Stipe	Pileus	Stipe
Mannitol dehydrogenase	0.525^{c} (2.81)	0.582^{c} (8.67)	0.604^{c} (1.59)	0.617^{d} (5.31)	0.332^{b} (4.15)	0.635^{c} (3.30)	0.221^{c} (8.94)	0.611^{d} (1.94)	0.131^{c} (8.27)	0.569^{c} (4.37)	0.453^{b} (3.70)
Laccase	0.333^{c} (2.68)	0.417^{c} (8.89)	0.458^{d} (4.45)	1.506^{c} (3.88)	0.312^{c} (10.6)	1.519^{b} (3.74)	0.421^{b} (9.64)	1.453^{c} (4.50)	0.454^{b} (7.04)	0.458^{d} (10.69)	0.324^{c} (3.77)
Xylanase	2.415^{a} (3.30)	2.919^{a} (3.20)	3.427^{a} (6.05)	3.443^{a} (1.92)	1.114^{a} (3.24)	3.514^{a} (4.57)	1.401^{a} (7.07)	3.235^{a} (10.80)	1.291^{a} (4.15)	3.501^{a} (10.68)	0.524^{a} (2.69)
Tyrosinase	0.005^{c} (6.79)	0.019^{c} (2.69)	0.027^{d} (3.67)	0.033^{e} (4.41)	0.004^{d} (6.92)	0.043^{d} (10.3)	0.011^{c} (2.75)	0.011^{e} (10.82)	0.003^{c} (2.07)	0.002^{e} (3.86)	0.003^{d} (6.52)
Lipoxygnase	2.071^{b} (1.99)	2.392^{b} (4.32)	2.807^{b} (3.88)	3.159^{b} (10.7)	0.320^{bc} (1.91)	3.435^{a} (9.97)	0.411^{b} (3.85)	3.117^{b} (3.26)	0.121^{c} (4.15)	3.001^{b} (0.88)	0.012^{d} (0.62)
CD(P=0.05)	0.0916	0.0262	0.0522	0.0618	0.0179	0.0980	0.0408	0.1114	0.0291	0.0421	0.0174

*Mean of three replications
The data in parenthesis are arcsine transformed values. Means in a column followed by the same letter are not significantly different at P = 0.05

Table 2 Assay of morphogenesis related enzymes of CBE-TNAU-1523 (*Calocybe indica* P&C) at different growth stages

Enzymes	Different growth stages of mushroom/24 hrs interval (µmoles/min/g)										
	Pinhead (Stage1)	Tiny button (Stage2)	Button (Stage3)	I Elongation (Stage4)		II Elongation (Stage5)		Maturation (Stage6)		Complete maturation (Stage7)	
				Pileus	Stipe	Pileus	Stipe	Pileus	Stipe	Pileus	Stipe
Mannitol dehydrogenase	0.553^{c} (4.26)	0.572^{c} (4.33)	0.610^{d} (4.47)	0.623^{c} (4.52)	0.231^{c} (2.75)	0.711^{c} (4.83)	0.133^{e} (2.09)	0.614^{d} (4.49)	0.113^{d} (1.92)	0.563^{d} (4.30)	0.113^{d} (1.92)
Laccase	0.411^{d} (3.67)	0.521^{d} (4.13)	1.633^{c} (7.34)	1.673^{b} (7.43)	0.331^{b} (3.29)	1.681^{b} (7.44)	0.441^{b} (3.80)	1.532^{c} (7.11)	0.374^{b} (3.50)	0.731^{c} (4.90)	0.221^{b} (2.69)
Xylanase	2.445^{a} (8.99)	2.815^{a} (9.65)	3.334^{a} (10.5)	3.452^{a} (10.7)	1.002^{a} (5.74)	3.551^{a} (10.8)	1.672^{a} (7.42)	3.422^{a} (10.6)	1.330^{a} (6.62)	3.312^{a} (10.4)	0.246^{a} (2.84)
Tyrosinase	0.045^{e} (3.36)	0.061^{e} (3.69)	0.134^{d} (4.19)	0.112^{c} (4.63)	0.002^{e} (0.62)	0.142^{c} (4.84)	0.011^{d} (2.37)	0.062^{d} (4.29)	0.030^{e} (0.99)	0.020^{a} (1.98)	0.011^{e} (1.00)
Lipoxygnase	2.112^{b} (8.35)	2.351^{b} (8.82)	2.771^{b} (9.58)	3.132^{a} (10.6)	0.221^{d} (2.69)	3.453^{a} (10.7)	0.334^{c} (3.31)	3.121^{b} (10.1)	0.220^{c} (2.68)	3.041^{b} (10.04)	0.124^{c} (2.01)
CD(P=0.05)	0.0518	0.0384	0.0906	0.1433	0.0091	0.1087	0.0249	0.1115	0.0360	0.1549	0.0053

*Mean of three replications
The data in parenthesis are arcsine transformed values. Means in a column followed by the same letter are not significantly different at P = 0.05

Plate 1: Different growth stages of *Calocybe indica* var. APK2

S1-Pinhead
S2-Tiny button
S3-Button
S4-I Elongation
S5-II Elongation
S6-Maturation
S7-Complete maturation

Plate 2: Different growth stages of *Calocybe indica* CBE-TNAU-1523wild strain

S1-Pinhead
S2-Tiny button
S3-Button
S4-I Elongation
S5-II Elongation
S6-Maturation
S7-Complete maturation

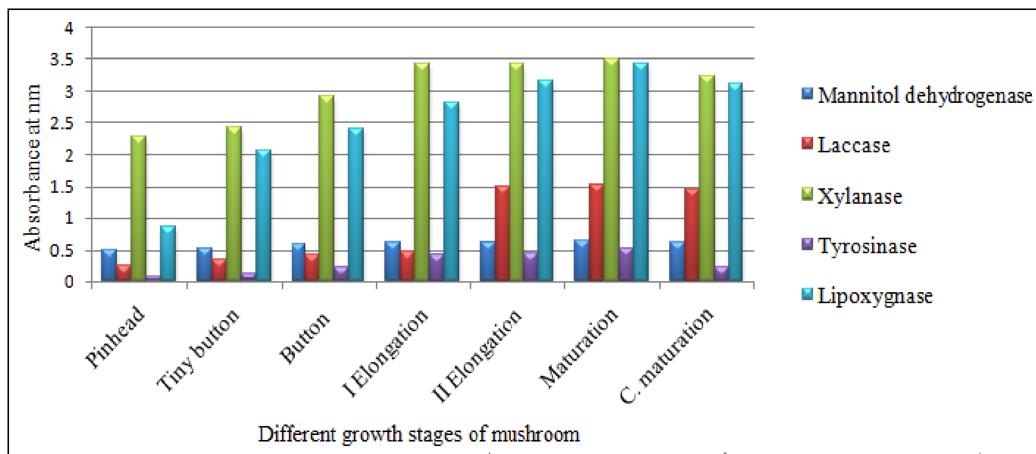

Fig 1: Assay of morhogenesis related enzymes of *Calocybe indica* Var. APK2

Conclusion

The morphogenesis related enzymes *viz.*, mannitol dehydrogenase, laccase, xylanase, tyrosinase and lipoxygenase highly mediate the growth and development of milky mushroom *Calocybe indica* (P&C) var. APK2 and CBE-TNAU-1523 wild strain. The production of morphogenesis related enzymes were higher in vegetative growth phases of *C. indica* and dropped at the harvest of the mature mushrooms. The drastic changes of enzyme activities as observed in milky mushroom *Calocybe indica* from stage one to stage seven was assumed to be due to the influence of internal physiological conditions of the mushrooms which is related to synchronization and oxidation as reported by Bonnen *et al.,* (1994) [4] and Ruhl *et al.,* (2008) [34] in *Agaricus bisporus* and *Pleurotus ostreatus*, respectively. Among all the enzymes assayed xylanase shows maximum activity in all seven growth stages of mushrooms followed by lipoxygenase. Tyrosinase enzyme was observed in all the seven growth stages; however secretion was comparatively less which may be the contributing factor for reduction of browning effect and extension of shelf life in both APK2 variety and CBE-TNAU-1523 wild strain of *Calocybe indica* (P&C). The findings from this study clearly depicts that the lipoxygenase enzyme plays a major role in production of volatile compounds which influence the flavour and fruiting body formation of mushroom. Further research will lead to identification of the gene activity of lipoxygenase enzyme and gene alteration through genome editing techniques to architect the mushroom size and to improve the flavour of milky mushroom.

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References

1. Axelrod B, Cheesbrough TM, Laakso S. Lipoxygenase from soybeans: EC 1.13. 11.12 Linoleate: oxygen oxidoreductase. Methods in enzymology. 1981; 71:441-51.
2. Baskaran TL, Sivaprakasam K, Kandasamy TK. Compact bag method: A new method of increasing the yield of Pleurotus sajor-caju. Indian J. Mushrooms. 1978; 4:10-2.
3. Boiret M, Marty A, Deumie M. Distribution of activity of tyrosinase in the mushroom. Biochemical Education. 1985; 13(2):82-4.
4. Bonnen AM, Anton LH, Orth AB. Lignin-degrading enzymes of the commercial button mushroom, Agaricus bisporus. Applied and Environmental Microbiology.

1994; 60(3):960-5.
5. Buswell JA, Cai YJ, Chang ST, Peberdy JF, Fu SY, Yu HS. Lignocellulolytic enzyme profiles of edible mushroom fungi. World Journal of Microbiology and Biotechnology. 1996; 12(5):537-42.
6. Chakraborty TK, Das N, Sengupta S, Mukherjee M. Accumulation of a natural substrate of laccase in gills of Pleurotus florida during sporulation. Current microbiology. 2000; 41(3):167-71.
7. Chakraborty TK, Basu D, Das N, Sengupta S, Mukherjee M. The mannitol cycle in Pleurotus ostreatus (Florida). FEMS microbiology letters. 2004; 236(2):307-11.
8. Chiu SW, Moore DA. Threats to biodiversity caused by traditional mushroom cultivation technology in China. In British Mycological Society Symposium Series, 2001; 22:111-119.
9. Devadoss R. Studies on damping-off of vegetables [dissertation], Tamil Nadu Agricultural University Coimbatore, 1971.
10. Dutsch GA, Rast D. Biochemische beziehung zwischen mannitbildung und hexosemonophosphatzyklus in Agaricus bisporus. Phytochemistry. 1972; 11(9):2677-81.
11. Elisashvili V, Kachlishvili E, Penninckx M. Lignocellulolytic enzymes profile during growth and fruiting of Pleurotus ostreatus on wheat straw and tree leaves. Acta microbiologica et immunologica Hungarica. 2008; 55(2):157-68.
12. Esposito E, Azevedo JD. Fungi: an introduction to biology, biochemistry and biotechnology. Caxias do Sul: Educs. 2004, 11.
13. Hammond JB, Nichols R. Carbohydrate metabolism in Agaricus bisporus (Lange) Sing.: changes in soluble carbohydrates during growth of mycelium and sporophore. Microbiology. 1976; 93(2):309-20.
14. Hammond JB. Variations in enzyme activity during periodic fruiting of Agaricus bisporus. New Phytologist. 1981; 89(3):419-28.
15. Hiroi M. Fatty acid composition of mushroom lipids and chemotaxonomy. Transactions of the Mycological Society of Japan. 1988.
16. Jennings DH. Polyol metabolism in fungi. Advances in microbial physiology. 1985; 25:149-93.
17. Kalmis E, Sargi S. Cultivation of two Pleurotus species on wheat straw substrates containing olive mill waste water. International biodeterioration & biodegradation. 2004; 53(1):43-7.
18. Kiran Kumar N. Studies on molecular basis of morphogenesis, chlamydospores production, shelf life and yield of *Volvariella*spp. [dissertation], Tamil Nadu Agricultural University Coimbatore, 2015.
19. Kirk TK, Cullen D. Enzymology and molecular genetics of wood degradation by white-rot fungi. Environmentally friendly technologies for the pulp and paper industry. Wiley, New York. 1998, 273-307.
20. Krishnamoorthy AS, Muthusamy M, Marimuthu T, Narasimhan V, Muthusankaranarayanan A. APK2 milky mushroom-Extn. Bulletin, RRS, TNAU, Aruppukottai, 1998.
21. Kuribayashi T, Kaise H, Uno C, Hara T, Hayakawa T, Joh T. Purification and characterization of lipoxygenase from Pleurotus ostreatus. Journal of agricultural and food chemistry. 2002; 50(5):1247-53.
22. Liu L, Shi S, Chen X, Peng M. Analysis of tyrosinase binders from Glycyrrhiza uralensis root: evaluation and comparison of tyrosinase immobilized magnetic fishing-HPLC and reverse ultrafiltration-HPLC. Journal of Chromatography B. 2013; 932:19-25.
23. Matsumoto T. Changes in activities of carbohydrases, phosphorylase, proteinases and phenol oxidases during fruiting of Lentinus edodes in sawdust cultures. Reports of the Tottori Mycological Institute (Japan). 1988.
24. Mau JL, Beelman RB, Ziegler GR. Effect of 10-oxo-trans-8-decenoic acid on growth of Agaricus bisporus. Phytochemistry. 1992; 31(12):4059-64.
25. Miller GL, Blum R, Glennom WE, Burton AL. Measurement of methods for assay of xylanase activity. Anal. Biochem. 1959; 2:127-32.
26. Montoya S, Orrego CE, Levin L. Growth, fruiting and lignocellulolytic enzyme production by the edible mushroom Grifola frondosa (maitake). World Journal of Microbiology and Biotechnology. 2012; 28(4):1533-41.
27. Morton N, Hammond JB, Dickerson AG. The Agaricus bisporus mannitol pathway during sporophore growth. Transactions of the British Mycological Society. 1985; 85(4):671-5.
28. Nicolaou KC, Marron BE, Veale CA, Webber SE, Dahlen SE, Samuelsson B *et al*. Identification of a novel 7-cis-11-trans-lipoxin A4 generated by human neutrophils: total synthesis, spasmogenic activities and comparison with other geometric isomers of lipoxins A4 and B4. Biochimica et Biophysica Acta (BBA)-Lipids and Lipid Metabolism. 1989; 1003(1):44-53.
29. Nikitina VE, Tsivileva OM, Pankratov AN, Bychkov NA. Lentinula edodes biotechnology–from lentinan to lectins. Food Technology and Biotechnology. 2007; 45(3):230-7.
30. Okano K, Iida Y, Samsuri M, Prasetya B, Usagawa T, Watanabe T. Comparison of *in vitro* digestibility and chemical composition among sugarcane bagasses treated by four white-rot fungi. Animal Science Journal. 2006; 77(3):308-13.
31. Pani BK. Influence of some growth regulators on biomass production and sporophore yield of milky mushroom (). Asian J Exp Biol Sci. 2011; 2:328-31.
32. Perez SR, Oduardo NG, Savón RC, Boizán MF, Augur C. Decolourisation of mushroom farm wastewater by Pleurotus ostreatus. Biodegradation. 2008; 19(4):519-26.
33. Philippoussis AN. Production of mushrooms using agro-industrial residues as substrates. In Biotechnology for Agro-Industrial Residues Utilisation Springer Netherlands, 2009, 163-196.
34. Ruhl M, Fischer C, Kües U. Ligninolytic enzyme activities alternate with mushroom production during industrial cultivation of Pleurotus ostreatus on wheat straw-based substrate. Curr Trends Biotechnol Pharm. 2008; 2(4):478-92.
35. Sandhu DK, Arora DS. Laccase production byPolyporus sanguineus under different nutritional and environmental conditions. Cellular and Molecular Life Sciences. 1985; 41(3):355-6.
36. Shah V, Nerud F. Lignin degrading system of white-rot fungi and its exploitation for dye decolorization. Canadian journal of microbiology. 2002; 48(10):857-70.
37. Singh P, Langowski HC, Wani AA, Saengerlaub S. Recent advances in extending the shelf life of fresh Agaricus mushrooms: a review. Journal of the Science of Food and Agriculture. 2010; 90(9):1393-402.
38. Soccol CR, Vandenberghe LP. Overview of applied solid-state fermentation in Brazil. Biochemical Engineering Journal. 2003; 13(2):205-18.
39. Subbiah KA, Balan V. A Comprehensive Review of

Tropical Milky White Mushroom (Calocybe indica P&C). Mycobiology. 2015; 43(3):184-94.

40. Tasaki Y, Toyama S, Kuribayashi T, Joh T. Molecular characterization of a lipoxygenase from the basidiomycete mushroom Pleurotus ostreatus. Bioscience, biotechnology, and biochemistry. 2013; 77(1):38-45.
41. Thurston CF. The structure and function of fungal laccases. Microbiology. 1994; 140(1):19-26.
42. Widiastuti H, Wulaningtyas A. Activity of ligninolytic enzymes during growth and fruiting body development of white rot fungi Omphalina sp. and Pleurotus ostreatus. HAYATI Journal of Biosciences. 2008; 15(4):140-4.
43. Wood DA. Production, purification and properties of extracellular laccase of Agaricus bisporus. Microbiology. 1980; 117(2):327-38.
44. Worrall JJ, Chet I, Hüttermann A. Association of rhizomorph formation with laccase activity in Armillaria spp. Microbiology. 1986; 132(9):2527-33.
45. Zervakis G, Philippoussis A. Management of agro-industrial wastes through the cultivation of edible mushrooms. In Proceedings of IV European Waste Forum ‘Innovation in waste management’, CIPA, Milan, 2000.