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Cellulase from the fruiting bodies and mycelia of edible mushrooms: A review

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ABSTRACT: Cellulose is the most abundant organic polymer constituent of the cell wall of green plants and of various forms of algae. The complexity of lignocellulosic biomass is a major challenge in industrial research. Most mushroom species that naturally grow on soil or wood possess cellulases and the corresponding enzymatic system and, potential candidates for the direct bioconversion of softwood polysaccharides into fermentable sugars. However, there have been fewer studies on mushroom cellulases than on fungi such as *Trichoderma* spp., exploit the full potential of mushroom cellulases. This review will focus on the current status ofmushroom cellulase research and applications and will provide insight into promising future prospects.

KEYWORDS: cellulose, cellulase, fruiting body, mycelia, edible mushroom, sugar

Introduction

As the primary structural component of cell wall of green plants and many forms of algae, cellulose is considered for the most abundant organic polymer on Earth (Klemm et al., 2005). The value of cellulose as a renewable source of energy has made cellulose hydrolysis the subject of intense research and industrial interest (Bhat, 2000). The challenge lies in the complexity of lignocellulosic biomass which is typically comprised of mainly cellulose, lesser hemicelluloses and least of all lignin (Sadhu and Maiti, 2013). At the molecular level, cellulose is a linear polymer composed of repeating D-glucose units linked by β-1,4-glucosidic bonds (Kim et al., 2006). The rigid structure of cellulose provides high resistance to most organic solvents and needs thermo-chemical or physical pretreatment for hydrolysis. Meanwhile, cellulose is used as a food source by a wide variety of organisms including fungi, mushroom, bacteria, plants and protists, as well as a wide range of invertebrate animals, such as insects, crustaceans, annelids, molluscs and nematodes (Tokuda and Watanabe, 2007; Li et al., 2009). These organisms possess cellulases and the complete enzymatic system to facilitate the breakdown of cellulose and subsequent biological conversion to an utilizable energy source glucose (Béguin and Aubert, 1994).

Most of the mushrooms species grow on soil or wood in nature. They have been recognized for their property of degradation of the natural lignocellulosic wastes (Sánchez, 2009). The mushrooms can be divided into two groups based on their carbon preference: “brown-rots” to utilize cellulose and hemicellulose leaving behind a brown residue of lignin, and “white-rots” to degrade strikingly the lignin component besides cellulose and hemicellulose leaving behind a white residue (Stamets, 2005; Schwarze et al., 2013). White-rots are the best lignin degraders which completely metabolize the complex polymer, and have been the most studied (Cohen et al., 2002; Vane et al., 2006). Most of them have the enzymatic capacity to use cellulose, hemicellulose, and other components of lignocellulosic matter as a source of carbon and energy. Brown-rot fungi, which occur predominantly on soft-

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Table 1. Different cellulase assays

Enzyme	Substrate	Assay
Total cellulase	Cotton/Filter paper	3,5-Dinitrosalicylic acid (DNS) assay
Endo-1,4-β-glucanase (Endoglucanase, Endocellulase)	Carboxymethyl cellulose (CMC)/Amorphous cellulose	DNS/HPLC assay
Cellobiohydrolase (Exocellobiohydrolase, Exocellulase)	Avicel/Crystalline cellulose	*p*-Hydroxybenzoic acid hydrazide (PAHBAH) method
β-Glucosidase	*p*-Nitrophenol-β-glucoside (*p*NPG)/Cellobiose	DNS/HPLC assay

woods, have scarcely been studied for biotechnological purposes (Agosin et al., 1989; Stamets, 2000). This makes them potential candidates for the direct bioconversion of softwood polysaccharides into fermentable sugars.

One of the strategies developed to utilize significant quantities of lignocellulosic wastes, generated annually due to photosynthesis, is the culturing of edible mushrooms by solid state fermentation (Chang and Miles, 1991). Top three mushroom species cultivated are the white button mushroom (*Agaricus bisporus*), shiitake (*Lentinula edodes*) and oyster mushroom (*Pleurotus* spp.) (Phan and Sabaratnam, 2012). Cultivation of these mushrooms represents a major industry in the countries of Southeast Asia (Li, 2012).

Taking into account the ecological importance of wood-rotting mushrooms in carbon cycling and wood cellulose degradation, surprisingly few cellulases of mushrooms have been studied compared with other fungi such as *Trichoderma* spp. (Baldrian and Valásková, 2008). This review will introduce some background knowledge of cellulase enzyme and focus on current status in mushroom cellulase research and application, and give insight into some promising prospects for its future.

Cellulase assay

Cellulase is any of several enzymes that catalyze cellulose hydrolysis, the decomposition of cellulose and of some related polysaccharides. The cellulases include endoglucanases (Endo-1,4-β-glucanase, EGs), cellobiohydrolases (Exocellobiohydrolase, CBHs) and β-glucosidases (BGs) (Dashtban et al., 2009).

Endoglucanases randomly hydrolyze internal glycosidic bonds in cellulose chains, generating oligosaccharides of various lengths and consequently new chain ends. It is generally active against acid-swollen amorphous cellulose, soluble derivatives of cellulose such as carboxymethyl cellulose (CMC) and cellooligosaccharides (Wood, 1989). Cellobiohydrolases cleave β-1,4-glycosidic bonds from chain ends releasing cellobiose and some glucose molecules. These enzymes are active against crystalline substrate such as Avicel, amorphous celluloses and cellooligosaccharides, while inactive against cellobiose or CMC. β-Glucosidases hydrolyze soluble cellodextrins and cellobiose to glucose from non-reducing end. It is active against analog substrate *p*-nitrophenol-β-glucoside (*p*NPG) and natural substrate (e.g., cellobiose). Cellulase activity is mainly evaluated by a reducing sugar assay using 3,5-Dinitrosalicylic acid (DNS) as colorimetric agent to measure the end products of cellulose hydrolysis activities. Different cellulase assays are summarized in Table 1.

Mushroom cellulases

Commonly, mushrooms have two phases in their life cycle viz., the mycelium (vegetative phase) that lasts longer in the life cycle and the fruiting body (reproductive phase) that bears the spores. The mycelia grow through the substrate, biodegrade the substrate and support the formation of fruiting bodies.

Three important commercially cultured mushrooms *Lentinus edodes*, *Volvariella volvacea* and *Pleurotus sajor-caju* exhibited varying abilities to utilize different lignocellulosics as growth substrates (Buswell et al., 1996).

Mycelia

Different types of lignocellulosic wastes have been used as nutrient sources for mushroom cultivation. The effects of different substrates on mushroom mycelial growth and yield of cellulases have been widely studied. Some of cellulases isolated from mushroom mycelia were presented in Table 2.

The cellulase production (Endo-β-1,4-glucanase, exo-β-1,4-glucanase and β-glucosidase) of *Pleurotus sajor-caju* by solid state fermentation (SSF) using sugarcane pressmud as substrate was optimized for 10 days of

Table 2. Cellulase isolated from mushroom mycelia

Enzyme	Mushroom	Characterization	Main medium ingredients	Reference
Endo-β-1,4-glucanase, Exo-β-1,4-glucanase and β-glucosidase	*Pleurotus sajor-caju*	Optimum pH 5.0 and temperature 25°C	Sugarcane pressmud	Pandit and Maheshwari, 2012
Cellobiohydrolase, carboxymethyl cellulase and β-glucosidase	*Pleurotus sajor-caju*	Maximum activities of 15.6, 83.4 and 56.1 U (mg protein)$^{-1}$ after 20 days	Cotton-waste	Tan and Wahab, 1997
Endo- and exoglucanase	*Pleurotus ostreatus* (Jacq.) P. Kumm. (type NRRL-0366)	Maximum activities of 2.46 and 1.80 U/ml at 27°C and pH 5.5	Avicel PH101	Daba et al., 2011
Cellulase and laccase	*Oudemansiella radicata* (Relhan ex Fr.)	Maximum cellulase activity of 490 units/ml/min	Submerged (SMF) and solid-state (SSF) fermentations	Balaraju et al., 2010
Endo-β-1,4-glucanase, Exo-β-1,4-glucanase and β-glucosidase	*Sparassis crispa* DSMZ 5201	Maximum activities of 0.41, 0.27 and 0.55 unit/mg protein	YMG meidium	Kim et al., 2004
Filter paper assay, carboxymethyl cellulase and β-D-glucosidase	*Stereum ostrea*	Maximum activities of 4.67, 4.15 and 5.51 U/ml at 6th day	Submerged conditions	Praveen et al., 2012
β-Glucosidase, cellulase and avicelase	*Ganoderma neo-japonicum*	5 days incubation at 25°C	Potato dextrose agar	Jo et al., 2011
Endo-1,4-β-glucanase, cellobiohydrolase, and β-glucosidase	*Volvariella volvacea*	-	Microcrystalline cellulose (Avicel) or filter paper	Cai et al., 1999
Endoglucanase EG1	*Volvariella volvacea*	42 kDa, pI 7.7, maximal at pH 7.5 and 55°C	Crystalline cellulose	Ding et al., 2001
β-Glucosidase	*Volvariella volvacea*	95 kDa with *N*-terminal of PPSDFAKANIDEIVEQLTLD	Crystalline cellulose	Ding et al., 2007
Carboxymethyl cellulase and laccase	*Coprinus comatus*, *Hericium erinaceus* and *Pleurotus nebrodensis* 10 strains	Highest activity of 33.92 U/L (*C. comatus*, 9th day) and 22.58 U/L (*H. erinaceus*, 10th day)	Potato dextrose agar	Li et al., 2014

incubation time at 25°C and pH 5.0 when pressmud for SSF was pretreated with H_2O_2+NaOH (2%) and thickness of 0.8 cm (10 g) (Pandit and Maheshwari, 2012). Cotton-waste was also used for *Pleurotus sajor-caju* growth yet yielded relatively low levels of three components of cellulases (Tan and Wahab, 1997). The maximum production of recombinant exoglucanase was obtained by *P. ostreatus* (Jacq.) P. Kumm. (type NRRL-0366) with 6% avicel PH101 for 12 days after incubation (Daba et al., 2011).

The production of cellulase and laccase by *Oudemansiella radicata* (Relhan ex Fr.) was investigated under submerged (SMF) and solid-state (SSF) fermentations. Higher levels of enzyme activity were observed in SSF than SMF fermentation (Balaraju et al., 2010).

The mycelia of *Sparassis crispa* DSMZ 5201 were cultivated at 24°C for 15 days and resulted high activities of EG, CBH and β-glucosidase while little activity of xylanase (Kim et al., 2004).

Complete cellulolytic enzymes of *Stereum ostrea* were evaluated by filter paper assay, carboxymethyl cellulase and β-D-glucosidase under submerged conditions. *S. ostrea* exhibited activities of cellulolytic enzymes 6th day of incubation higher than the reference culture *Phanerochaete chrysosporium* (Praveen et al., 2012).

The ability of *Ganoderma* to produce extracellular enzymes, including β-glucosidase, cellulase, avicelase, pectinase, xylanase, protease, amylase, and ligninase was tested in chromogenic media. β-Glucosidase showed the highest activity among the eight tested enzymes. In particular, *G. neo-japonicum* showed significantly stronger activity for β-glucosidase than that of the other enzymes (Jo et al., 2011).

The multicomponent enzyme system by *Volvariella volvacea* was found consisting of endo-1,4-β-glucanase,

cellobiohydrolase, and β-glucosidase for the conversion of cellulose to glucose (Cai et al., 1999). Further, an endoglucanase EG1 was isolated from culture fluid of *V. volvacea* grown on crystalline cellulose. EG1 showed a molecular mass of 42 kDa and an isoelectric point of 7.7 and was assigned to glycoside hydrolase family 5 (Ding et al., 2001). A 95 kDa β-glucosidase was also purified from extracts of *V. volvacea* mycelium with N-terminal sequences of PPSDFAKANIDEIVEQLTLD (Ding et al., 2007).

Ten strains of mushrooms (*Lentinus edodes* 868, *L. edodes* 939, *Pholiota nameko*, *Coprinus comatus*, *Macrolepiota procera*, *Auricularia auricula*, *Hericium erinaceus*, *Grifola frondosa*, *Pleurotus nebrodensis*, and *Shiraia bambusicola*) were inoculated onto CMC agar-Congo red plates to evaluate their ability to produce carboxymethyl cellulase (CMCase). The filter paper culture screening test showed that *H. erinaceus* and *M. procera* grew well and showed extreme decomposition of the filter paper. CMCase activity determination indicated that *C. comatus* and *H. erinaceus* had the ability to produce CMCase, while *C. comatus* and *P. nebrodensis* had the ability to produce laccase (Li et al., 2014).

Fruiting bodies

Once mushroom mycelium is inoculated to the prepared growth substrate, it starts utilizing the nutrients available in the growth substrate, presupposed by the release and activity of cellulolytic enzymes including cellulases. The yield of mushroom fruiting bodies on lignocellulosic wastes can be expressed as dry weight of the fruiting bodies to the dry growth substrate termed as bioconversion efficiency. After harvesting, the spent mushroom compost (SMC) still contains plenty of extracellular enzymes. Some of cellulases isolated from mushroom mycelia were summarized in Table 3.

As one of the most cultivated mushroom, *Pleurotus*

Table 3. Cellulase isolated from mushroom fruiting bodies

Enzyme	Mushroom	Characterization	Main medium ingredients	Reference
Cellulase and β-glucosidase	*Pleurotus* spp.	Maximum activities of 3.31 and 121.13 U g^{-1} at 4 months	Sawdust bags	Singh et al., 2003
Carboxymethyl cellulase	*Pleurotus ostreatus* (Jacq.:Fr.) Kumm	Maximum activity of 140 U g^{-1} after 49 days	Cotton wastes	Elisashvili et al., 2003
Cellulase	Lentinus tigrinus	Maximum activity around 90 days	Wheat straw	Lechner and Papinutti, 2006
Endoglucanase, exoglucanase and β-glucosidase	Grifola frondosa	Highest activities of 12.3, 16.2 and 2.3 U/g	Oak sawdust	Montoya et al., 2012
Endo-1,4-β-glucanase EG1 and β-glucosidase BG1	Piptoporus betulinus	EG1 of 62 kDa with a pI of 2.6–2.8, K_m for CMC of 3.5 g l^{-1}; BG1 of 36 kDa with a pI around 2.6, K_m for *p*NPG of 1.8 mM	Dead birch trees	Valášková and Baldrian, 2006
Endoglucanase	Lepista flaccida	Maximum activity of 25 U/mg protein at pH 4.0 and 50°C	-	Elvan et al., 2010
Endoglucanase	Agaricus bisporus	Three main polypeptides of 38, 58, and 60 kDa immune-precipitated	-	Raguz et al., 1992
α-Amylase, cellulase and β-glucosidase	*Pleurotus ostreatus*, *Lentinula edodes*, *Flammulina velutipes* and *Hericium erinaceum*	Highest cellulase activity 759 nkat/g and β-glucosidase 767 nkat/g	Spent mushroom compost	Ko et al., 2005
Amylase, cellulase, laccase and xylanase	*Pleurotus ostreatus*, *P. eryngii*, and *P. cornucopiae*	Highest cellulase activity 1.67 U/g	Spent mushroom compost	Lim et al., 2013

spp. were reported with high cellulase and β-D-glucosidase productivity at 5 to 10-month period in bags of sawdust (Singh et al., 2003). CMCase from *P. ostreatus* (Jacq.:Fr.) Kumm gradually increased during the 49 days after inoculation and then gradually declined towards the end of mushroom cultivation (Elisashvili et al., 2003). There was significant decrease in cellulose content when *P. ostreatus* grew on cotton seed hulls, corresponding more cellulose consumed during fruiting phase (Li et al., 2001).

During 110 days of solid state fermentation (SSF), *Lentinus tigrinus* showed the capacity to degrade wheat straw, causing a 21.49% decrease in lignin content and a 53.26% decrease in cellulose. All enzyme activities were high during colonization and cellulases showed the maximum of activity around 90 days post inoculation (Lechner and Papinutti, 2006). After inoculation of *Lentinus edodes* the crystallinity value of waste oak logs dropped dramatically 33% compared to 49% of normal wood (Lee et al., 2008).

White-rot fungi *Grifola frondosa* is active producer of cellulases and xylanases, while not able to use lignin. The growth of *G. frondosa* on oak-sawdust plus corn bran yielded highest activities of endoglucanase, exoglucanase and β-glucosidase at 12.3, 16.2 and 2.3 U/g dry substrate (Montoya et al., 2012).

When grown on wheat straw, *Piptoporus betulinus* caused 65% loss of dry mass within 98 days, and produced endo-1,4-β-glucanase, endo-1,4-β-xylanase, 1,4-β-glucosidase and cellobiohydrolase. The major glycosyl hydrolases, endoglucanase EG1 and β-glucosidase BG1, were purified. EG1 was a protein of 62 kDa with a pI of 2.6–2.8, K_m for CMC of 3.5 g l^{-1}. BG1 was a protein of 36 kDa with a pI around 2.6, K_m for *p*NPG of 1.8 mM (Valášková and Baldrian, 2006).

A crude extract was prepared from the fruiting body of *Lepista flaccida*, an edible mushroom and endoglucanase was partially purified and characterized (Elvan et al., 2010). The extracellular endoglucanase from *Agaricus bisporus* was found to be induced by cellulose and repressed by fructose, and regulated in activity during fruiting body development (Raguz et al., 1992).

The potential of using the enzymes from spent mushroom compost (SMC) as an industrial enzyme was evaluated for the production of α-amylase, cellulase, β-glucosidase, laccase and xylanase. Four edible mushroom species (*Pleurotus ostreatus*, *Lentinula edodes*, *Flammulina velutipes* and *Hericium erinaceum*) were tested and *L. edodes* showed the highest enzyme activity in α-amylase, cellulase and β-glucosidase in 0.5% Triton X-100 (Ko et al., 2005). Another efficient extraction of lignocellulolytic enzymes amylase, cellulase, laccase and xylanase from SMC of *Pleurotus ostreatus*, *P. eryngii*, and *P. cornucopiae*. The synthetic dyes remazol brilliant blue R and Congo red were decolorized completely by the SMC extract of *P. eryngii* within 120 min which was comparable to that of commercial laccase (Lim et al., 2013).

Cellulase genes

Although many cellulase genes have been cloned and characterized from bacteria and fungi, there were very few reports concerning mushroom cellulase genes (Yan and Wu, 2014). An organization of cellulase gene consists of catalytic domain and cellulose-binding domains (CBD) which both domain are separated by linker peptide. CBD is used to bind with cellulose, so they are essential for specific activities of the enzymes on soluble and insoluble cellulosic substrates. Some of mushroom cellulase genes were obtained from NCBI GenBank and listed in Table 4.

Two cellulase genes from *Lentinula edodes* were cloned employing degenerate primers directed at the cellulose-binding domain: cel7A encoded a 516-amino acid protein that belonged to glycosyl hydrolase family 7 and had sequence similarities to cbhI genes from other fungi; cel6B encoded a 444-amino acid protein that belonged to glycosyl hydrolase family 6 and had sequence similarities to cbhII genes (Lee et al., 2001).

A gene encoding for cellobiohydrolase PEcbh from *Pleurotus eryngii* was cloned by RT-PCR 3' and 5' RACE techniques. The result showed that PEcbh was 1377 bp encoding 459-deduced amino acid, belonging to glycosyl hydrolase family 7 (Romruen and Bangyeekhun, 2016).

Four previously unidentified cellulase genes cbhI-I, cbhI-II, cbhII-I and egII were cloned from *Volvariella volvacea* with catalytic domain (CD) and CBD located at the C-terminus in cbhI-I and egII (Ding et al., 2006).

Transcription of cellulase and laccase genes of *Lentinula edodes* was examined by competitive RT-PCR during growth and development under different temperature and moisture levels on a sawdust-based substrate. The cellulase transcript level peaked at the veil-break stage during fruit body development. (Ohga

Table 4. Mushroom cellulase genes

Gene name	Mushroom	GenBank number	Description
cec2	*Agaricus bisporus*	Z50094	Exocellobiohydrolase; 1-4-β-cellobio-hydrolase
cel3	*Agaricus bisporus*	P49075	Exoglucanase 3; 1,4-β-cellobiohydro-lase 3; Exocellobiohydrolase 3
cel3AC	*Agaricus bisporus*	AF244369	Cellobiohydrolase
cel6A	*Coniophora puteana*	AB501099	Glycoside hydrolase family 6 cellulase
Cel6B	*Coniophora puteana*	AB501100	Glycoside hydrolase family 6 cellulase
cel7A	*Coniophora puteana*	AB501101	Glycoside hydrolase family 7 cellulase
Cel7B	*Coniophora puteana*	AB501102	Glycoside hydrolase family 7 cellulase
CC1G_04059	*Coprinopsis cinerea* Okayama-7/130/ ATCC MYA-4618/FGSC 9003	XM_001836694	Glycosyl hydrolase family 5 protein/ cellulase
CcCel6C	*Coprinopsis cinerea*	AB433539	Glycoside hydrolase family 6 enzyme
CCM_06251	*Cordyceps militaris* CM01	XM_006671392	Cellulase, glycosyl hydrolase family 5
CCM_06716	*Cordyceps militaris* CM01	XM_006671857	Cellulase family protein
CCM_04551	*Cordyceps militaris* CM01	XM_006669699	Cellulase
DACRYDRAFT_20357	*Dacryopinax* sp. DJM 731	JH795857	Whole genome shotgun sequence
CGGC5_2851	*Flammulina velutipes* (*Agaricus velutipes*)	XM_007272982	Cellulase family protein
CEL6B	*Flammulina velutipes* (*Agaricus velutipes*)	GU169900	Cellulase
CMQ_6873	*Grosmannia clavigera* kw1407/UAMH 11150	XM_014320559	Extracellular cellulase
cel1	*Irpex lacteus*	AB019375	Cellulase
LACBIDRAFT_307733	*Laccaria bicolor* S238N-H82/ATCC MYA-4686	XM_001886199	Cellulase, exo-1,3-β-glucanase
cel7A	*Lentinus edodes*	AF411250	Cellulase
cel6B	*Lentinus edodes*	AF411251	Cellulase
cbhII-1	*Lentinus edodes*	AF244369	Cellobiohydrolase
CBHI.2	*Phanerochaete chrysosporium*	Z29653	Cellulase
PBH1-4	*Phanerochaete chrysosporium*	S76141	Exocellobiohydrolase
CBHI.1	*Phanerochaete chrysosporium*	Z22528	Cellulase
CBHI.2	*Phanerochaete chrysosporium*	Z22527	Cellulase
Pccbh1-1	*Phanerochaete chrysosporium*	X54411	Cellulase
PCBH1-5	*Phanerochaete chrysosporium*	Z11730	Cellobiohydrolase
PEcbh	*Pleurotus eryngii*	LC034188	Cellobiohydrolase
cbh II	*Pleurotus sajor-caju*	AY050518	Cellobiohydrolase II
cbhI-I	*Volvariella volvacea*	AY559102	Cellobiohydrolase I-I
cbhI-II	*Volvariella volvacea*	AY559103	Cellobiohydrolase I-II
cbhII-I	*Volvariella volvacea*	AY559104	Cellobiohydrolase II-I
egII	*Volvariella volvacea*	AY559101	Endoglucanase II

and Royse, 2001). Similar phenomenon was observed with cel3 gene (cellobiohydrolase) in *Agaricus bisporus* (Ohga et al., 1999).

With the first genome sequence of the wood-rotting mushroom *Phanerochaete chrysosporium* available since 2004, more and more whole genomes of mushroom have provided with data for the analysis and search of cellulase genes (Martinez et al., 2004).

CBHs have been isolated and their enzyme properties characterized in several white-rot fungi, namely, in

Dichomeris squalens, *Ganoderma lucidum*, *Irpex lacteus*, *Phanerochaete chrysosporium*, and *Schizophyllum commune*, and in the brown-rot fungi *Coniophora puteana* and *Fomitopsis palustris* (Hatakka et al., 2014). The white-rot mushroom possess the same basic cellulose-degrading enzyme system as *Trichoderma reesei* including CBHs and EGs. The soil-inhabiting coprophilic mushroom *Coprinopsis cinerea* harbors 5 and 6 putative GH6- and GH7-encoding genes, respectively, thus pointing to its vital ability to degrade cellulose (Hatakka and Hammel, 2011). The first genome-level transcriptome of *P. chrysosporium* grown on red oak generated approximately 48,000 high quality sequence tags (246 bp average length) and demonstrated that four enzymes required for cellulose degradation: EG, exocellulase CBHI, CBHII, and β-glucosidase were all produced (Sato et al., 2009). The white rot basidiomycota *G. lucidum* secreted substantial amounts of hydrolytic and oxidative enzymes useful for the degradation of lignocellulosic biomass with sugarcane bagasse as substrate. 71 proteins were identified and sub-grouped into cellulases (24%), hemicellulases (5%), glycoside hydrolases (10%), lignin depolymerizing proteins (24%), a protease (2%), phosphatases (7%), transport proteins (10%) and hypothetical proteins (18%) (Manavalan et al., 2012).

The genome analysis of brown-rot wood decay *Postia placenta* revealed the presence of several hemicellulases and two EGs genes, but no CBHs or cellulose-binding domains. Also structurally divergent oxidases, analogous to the oxidative Fenton chemistry, were detected in *Postia placenta* which could be related to oxidative depolymerization of cellulose (Martinez et al., 2009). More recent studies have elucidated that cellulolytic systems of *P. placenta* depend on a combination of two mechanisms: lignocellulose oxidation (LOX) by reactive oxygen species (ROS) and polysaccharide hydrolysis by a limited set of glycoside hydrolases (GHs). Measured by whole-transcriptome shotgun sequencing (RNA-seq) and assayed relevant enzyme activities, up-regulation of GH5 endoglucanases and many other GHs clearly occurred with the notable exceptions of two likely expansins and a GH28 pectinase (Zhang et al., 2016). The gene expression of *P. placenta* and *Phanerochaete chrysosporium* was monitored by microarrays based on their annotated genomes to elucidate the enzymatic deconstruction of cell walls with different substrate compositions, revealing many of significantly expressed genes encode "proteins of unknown function" (Skyba et al., 2016).

Summary and future aspect

The use of lignocellulosic materials for the production of bio-fuel or other chemical feedstock is one of the most difficult tasks encountered in the history of biotechnology (Lin and Tanaka, 2006). The study of mushroom cellulase utilization, from quantification of enzymes in the cultures, purification, characterization to application of such enzyme, is one of the important aspects of microbial biotechnology.

With the rapidly changing situation especially since the 1000 Fungal Genomes Project (http://1000.fungalgenomes.org/home) and other efforts to sequence whole genomes of mushroom will provide new mega data and understanding of cellulase.

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Abbreviation

BG: β-glucosidase
CBD: cellulose-binding domains
CBH: cellobiohydrolases
CD: catalytic domain
CMC: carboxymethyl cellulose
CMCase: carboxymethyl cellulase
DNS: 3,5-dinitrosalicylic acid
EG: endoglucanase
GH: glycoside hydrolase
LOX: lignocellulose oxidation
PAHBAH: *p*-Hydroxybenzoic acid hydrazide
*p*NPG: *p*-nitrophenol-β-glucoside
ROS: reactive oxygen species
SMC: spent mushroom compost
SMF: submerged fermentation
SSF: solid-state fermentation

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