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Senescence of the *Lentinula edodes* Fruiting Body After Harvesting

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1. Introduction

Lentinula edodes, or shiitake mushroom as it is more popularly known, is one of the most economically important edible cultivated mushrooms. However, postharvest spoilage, such as browning of the gills and softening of the fruiting body, results in loss of freshness and consequent loss of food value (Minamide et al.; 1980a, b).

Fig. 1. Fruiting bodies a): just after harvest (fresh fruiting body) and b): at 4 days after harvest (senescent fruiting body).

Numerous studies addressing the mechanisms of quality loss during postharvest storage have revealed that browning of the *L. edodes* fruiting body is associated with increased activities of tyrosinase (Tyr; Kanda et al., 1996a, b) and laccase (Lcc; Nagai et al., 2003) after harvest. In addition to being an important fresh food source, shiitake mushrooms also have medicinal value. Lentinan, a β-1,3-glucan used for tumor immunotherapy (Chihara et al., 1969), is purified from fresh shiitake mushrooms. However, lentinan content decreases during postharvest storage (Minato et al., 1999). The reported structure of lentinan is a β-1,3-linked-$_D$-glucan with β-1,6 branches (Chihara et al., 1969), and it appears that postharvest degradation of lentinan during mushroom storage is mediated by β-1,3-glucanase (Minato et al., 1999). Four glucanases have been reported in *L. edodes* fruiting bodies, two exo-β-1,3-glucanases, EXG1 (Sakamoto et al., 2005a) and EXG2 (Sakamoto et al., 2005b), and two endo-β-1,3-glucanases, TLG1 (Sakamoto et al., 2006) and GLU1 (Sakamoto et al., 2011). An endo-β-1,6-glucanase, Pus30, was also purified from *L. edodes* fruiting body (Konno and Sakamoto, 2011). Except for EXG1, these glucanases are involved in lentinan degradation after harvesting.

Postharvest changes are considered to be complex, and there is currently little information about the changes in gene transcription following harvest of the *L. edodes* fruiting bodies. It has been reported that the expression of several genes increases during the postharvest period in *Agaricus bisporus* (button mushroom, Eastwood, 2001). Eastwood et al., (2001) revealed that argininosuccinate lyase increases after harvest, and the relationship between elevated levels of argininosuccinate lyase and postharvest physiological changes of the *A. bisporus* fruiting body has been investigated in detail (Wagemaker et al., 2007; Eastwood et al., 2008). Several studies have analyzed changes in gene transcription during development of the fruiting body of *L. edodes* (Hirano et al., 2004; Miyazaki et al., 2005). More recently, EST analysis (Suizu et al., 2008) and SAGE analysis (Chum et al., 2008, 2011) were carried out on *L. edodes*. Changes in gene transcription after harvest of the *L. edodes* fruiting body were investigated, revealing that many genes were newly expressed after harvest, such as putative chitinases, chitosanase, and a transcription factor (Sakamoto et al., 2009). This chapter will discuss about genes involved in fruiting body senescence in mushrooms, especially in *L. edodes*.

2. Phenol oxidases involved in browning of fruiting body after harvesting

The postharvest preservation of *L. edodes* fruiting bodies causes gill browning, which is commercially undesirable since it causes an unpleasant appearance. In general, melanins involved in gill browning are considered to be synthesized from β-(3,4-dihydroxyphenyl)alanine (DOPA), derived from tyrosine. DOPA can be oxidized enzymatically to quinones, which polymerize nonenzymatically to form the melanin pigments. Oxidation of tyrosine is commonly catalyzed by tyrosinasae (Tyr: EC1.14.18.1). The mechanisms of mushroom browning have been investigated extensively in *A. bisporus* (Burton, 1988; Espín et al., 1999). Browning in this species is mainly due to DOPA and c-glutaminyl-3,4-dihydroxy- benzene (GDHB) melanins (Jolivet et al., 1998), and Tyr seems to play the most important role in their synthesis (Turner, 1974). Burton (1988) reported that epidermal tissues of *A. bisporus* had a greater activity of non-latent Tyr and a greater concentration of phenols than did the fresh. It has been reported that Tyr activity of *L. edodes* fruiting bodies increases after harvest, and a Tyr has been purified (Kanda et al., 1996a).

Laccases (Lccs: EC 1.10.3.2) catalyze the single-electron oxidation of phenols or aromatic amines to form different products via various pathways. Lcc belongs to a group of polyphenol oxidases that contain copper atoms in their catalytic center; thus, they are typically referred to as multicopper oxidases. Lcc catalyzes the single-electron oxidation of phenolic substrates or aromatic amines to form different products via a variety of biochemical pathways (Leonowicz et al., 2001). There are many reports in the literature of the purification and characterization of Lcc isoforms from white-rot fungi, and several Lcc-encoding genes have been isolated (reviewed by Kumar et al., 2003; Baldrian, 2006). A correlation between melanin synthesis and intracellular Lcc in *Cryptococcus neoformans* has been reported (Ikeda et al., 2002). Lcc in *Aspergillus nidulans* is considered to form L-DOPA to synthesize melanins in conidia (Aramayo and Timberlake, 1990). Activity of Lcc increases after harvest of the *L. edodes* fruiting body, and a Lcc in *L. edodes* purified from fruiting bodies after harvest can oxidize DOPA (Nagai et al., 2003). Therefore, it is considered that Tyr and Lcc are involved in melanin synthesis after harvest of *L. edodes* fruiting bodies.

2.1 Tyr involvement in gill browning after harvest

Tyr (EC 1.14.18.1) is an ubiquitous enzyme in nature and the key enzyme in the process of melanin biosynthesis (van Gelder et al., 1997). Tyr catalyzes oxidation of phenolic substrates to quinone, which spontaneously polymerizes into dark-colored pigments known as melanin in the presence of nucleophilic moieties. Tyrs are found in a wide range of organisms, including prokaryotic and eukaryotic microorganisms, plants, invertebrates, and mammals. Tyrs are involved in a variety of biological functions, for example skin pigmentation in mammals and browning in plants and mushrooms. In mushrooms, browning after harvest has been investigated, especially in *A. bisporus* and *L. edodes*. Browning after harvest is considered a consequence of the Tyr-catalyzed oxidation of phenolic substrates (e.g., DOPA) into quinones leading to the formation of dark pigments of melanins. The enzymatic pigmentation of mushrooms is mediated largely by Tyr (Jolivet et al., 1998).

Several reports revealed that Tyr is related to gill browning after harvest of *L. edodes* fruiting bodies. It was reported that Tyr activity increases in the gills during postharvest preservation (Kanda et al., 1996a). A Tyr (LeTyr) was purified and characterized as the *L. edodes* Tyr, and LeTyr can catalyze tyrosine to DOPA (Kanda et al. 1996a, 1996b). The gene encoding LeTyr (*Letyr*) was isolated, and anti-serum was synthesized to LeTyr (Sato et al., 2009). Sato et al., (2009) showed that LeTyr increased after harvest, suggesting that LeTyr catalyzes DOPA synthesis for melanin formation after harvest in *L. edodes*. *Letyr* is the only Tyr encoding gene in *L. edodes* (Sato et al., 2009). On the other hand, Tyr is involved in browning of the surface of vegetative mycelia (Sano et al., 2010), suggesting that LeTyr is involved in melanin synthesis in different tissues. Browning of the surface of vegetative mycelia is regulated by light, and the blue light receptor PHRB regulates *Letyr* (Sano et al., 2009). This suggests that melanin synthesis in vegetative mycelium under light is caused by an increase of LeTyr via PHRB. PHRB is a homolog of WC2 in *Neurospora crassa,* and expression of the WC2 homolog in *L. edodes* is low in the *L. edodes* fruiting body after harvest (Sakamoto et al., 2009). This suggests that expression of *Letyr* in the fruiting body after harvest is regulated by transcription factor(s) other than PHRB.

2.2 Lcc involvement in gill browning after harvest

White-rot fungi produce several isoforms of extracellular lignin degrading enzymes, including lignin peroxidase, manganese peroxidase, and Lcc. These lignin degrading enzymes are considered as secreted enzymes, but several Lccs are intracellular enzymes.

Two Lccs (Lcc1 and Lcc2) have been purified from *L. edodes* (Nagai et al., 2002 and 2003), an Lcc-encoding gene has been cloned and characterized, and six Lcc encoding genes (*lcc1-lcc6*; lcc1: AB035409; lcc2: AB035410; lcc3: AB046713; Lcc4: AB446445; Lcc5: AB543788; Lcc6: AB543787) have been deposited in the DNA data bank (DDBJ) (Zhao and Kwan, 1999; Sakamoto et al., 2008, 2009; Yano et al., 2010). Lcc1 is secreted from vegetative mycelia in culture (Nagai et al., 2002) and is encoded by *lcc1* (Sakamoto et al., 2008). Lcc2 is expressed in the brown gills of fruiting bodies after harvesting (Nagai et al., 2003). The putative amino acid sequence of *lcc4* includes identical amino acid sequences to the N-terminal amino acid sequence of an enzymatically digested peptide of Lcc2 (Sakamoto et al., 2009). We expressed the *lcc4* gene heterologously in *Aspergillus oryzae*, and observed Lcc activity of the recombinant enzyme (Yano et al. 2009). These data suggest that *lcc4* encodes Lcc2, as designated by Nagai et al. (2003), and the gene *lcc2* (Accession No. AB035410) does not. Therefore, the Lcc2 purified by Nagai et al. (2003) is designated Lcc4 in this paper. Expression of *lcc1* is high in vegetative mycelia (Sakamoto et al., 2008), but *lcc4* is expressed only in the fruiting body and increases after harvest (Sakamoto et al., 2009). Furthermore, Lcc1 cannot oxidize DOPA (Nagai et al., 2002), but Lcc4 can (Nagai et al., 2003; Yano et al., 2009). These observations collectively suggest that Lcc4 is involved in melanin synthesis after harvesting by catalyzing L-DOPA to DOPA quinone. Gill browning after harvesting of *L. edodes* fruiting bodies is considered to be caused by melanin synthesis due to cooperation of LeTyr and Lcc4 and their increased levels after harvest.

3. Cell wall degrading enzymes involved in fruiting body autolysis

Fruiting body softening occurs due to cell wall degradation. The cell wall of *L. edodes* is constructed of several polysaccharides, such as β-1,3-glucan, β-1,6-glucan, chitin, and chitosan (Shida et al. 1981). Thus, β-1,3-glucanase, β-1,6-glucanase, chitinase, chitosanase are involved in cell wall degradation after harvesting. Cell wall degrading enzymes found in the *L. edodes* fruiting body after harvest will be introduced. One of the cell wall components, β-1,3-1,6-glucan, called lentinan, is used for antitumor therapy. Lentinan degradation after harvesting is caused by an increase in β-1,3-glucanase activity after harvesting. Thus, controlling β-1,3-glucanase expression is very important to keep the lentinan content in the *L. edodes* fruiting body after harvest. There are few reports on cell wall degrading enzymes, such as endoglucanase and exoglucanase encoding in basidiomycetous fungi, such as in *A. bisporus*. However, there were fewer reports on glucanases related to senescence of mushrooms when we started our research on senescence in *L. edodes*. There were several studies on chitinase activity in several mushrooms, but there was no evidence of a relationship between chitinases and mushroom senescence at that time. Cell wall degrading enzymes in *L. edodes* fruiting bodies after are discussed in this section.

3.1 β-glucanases

The cell wall structure of fungi is constantly changing during mycelial growth and the cell cycle. Morphological changes involving synthesis, reorienting and lysis of the cell wall structure are an essential process in fungi (Enderlin and Selitrennikoff, 1994; Seiler and Plamann, 2003). The cell wall forms a multilayered complex of polysaccharides, glycoproteins and proteins with cross-linkages. The polysaccharides consist of β-glucans (mainly β-1,3-glucan and β-1,6-glucan), chitin, chitosan, mannans and α-glucans (Aimanianda et al., 2009; Fontaine et al., 2000; Kollár et al., 1997). Fungi such as ascomycetes and basidiomycetes produce enzymes associated with these polysaccharides. Some of these fungal glycoside hydrolases (GH) act on cell wall components and are responsible for morphological changes (Adams, 2004; Fukuda et al., 2008; Mahadevan and Mahadkar, 1970; Wessels and Niederpruem, 1967).

Most basidiomycetes form a fruiting body (mushroom) for sporulation. The cell walls of the fruiting body are constructed mainly from chitin, β-1,3-glucan and β-1,6-glucan. Several cell wall polysaccharides extracted from basidiomycetes, such as *Schizophyllum commune* (Tabata et al., 1990), *Agaricus blazei* (Ohno et al., 2001), *Coprinopsis cinerea* (Bottom and Siehr, 1979), *Grifola frondosa* (Ishibashi et al., 2001) and *L. edodes* (Chihara et al., 1969), show bioactive (antitumor) activity, and some of these antitumor compounds are β-1,3-glucans with β-1,6-linked branches, i. e., lentinan isolated from *L. edodes* (Chihara et al., 1969) and schizophyllan from *S. commune* (Ooi and Liu, 2000). Studies of these mushroom polysaccharides have indicated that their content decreases during storage after harvest, suggesting that polysaccharides of the cell wall are self-degraded by enzymes associated with cell wall autolysis during fruiting body senescence (Minato et al., 2004). Two types of exo-β-1,3-glucanases, EXG1 (Sakamoto et al., 2005a) and EXG2 (Sakamoto et al., 2005b), and two endo-β-1,3-glucanases, TLG1 (Sakamoto et al., 2006) and GLU1 (Sakamoto et al., 2011) have been reported in *L. edodes* fruiting bodies. EXG1 is classified in GH family 5 and EXG2 is classified in GH family 55. TLG1 is not classified in the GH family, so far, and TLG1 is similar to thaumatin-like protein (Sakamoto et al. 2006). GLU1 is classified in a new GH family, GH128 (Sakamoto et al. 2011).

β-1,6-glucan is thought to be a unique and essential component of fungal cell walls. Whereas β-1,3-glucan forms a microfibrillar structure, β-1,6-glucan forms a branched amorphous structure (Kollár et al., 1997). Lichen *Umbilicaria* species produce a linear glucan composed of only β-1,6-linkages (pustulan; Nishikawa et al., 1970). Many fungi are considered to secrete β-1,6-glucanases, some of which have been purified and characterized (Bryant et al., 2007; Oyama et al., 2002; Moy et al., 2002; Pitson et al., 1996). All of the isolated enzymes are β-1,6-glucan endohydrolases (EC 3.2.1.75) classified into GH family 5 or 30 of the GH-A clan in the CAZy database. Konno and Sakamoto (2011) first reported a β-1,6-glucanase in basidiomycetes, which is classified in the GH30 family, from *L. edodes* fruiting body.

3.1.1 exo-β-1,3-glucanase

Several exo-β-1,3-glucanases genes of fungi have been isolated and characterized. In *Saccharomyces cerevisiae*, three exo-β-1,3-glucanase encoding genes (*EXG1*, *EXG2*, *SSG1*) have been cloned and characterized (Larriba et al., 1995). The *EXG1* gene encodes two main extracellular exo-β-1,3-glucanases (Kuranda and Robbins, 1987; Vázquez de Aldana et al., 1991), *EXG2* encodes an exo-β-1,3-glucanase attached to the plasma membrane (Correa et al.,

1992; Larriba et al., 1995) and *SSG1* (also known as *SPR1*) encodes a sporulation-specific exo-β-1,3-glucanase (Muthukumar et al., 1993). Several other exo-β-1,3-glucanase genes have been isolated from yeasts, including *Candida albicans* (Chambers et al., 1993), *Kluyveromyces lacis*, *Hansenula polymorpha* and *Schwanniomyces occidentalis* (Esteban et al., 1999). In filamentous fungi, an exoglucanase encoding gene, *EXG2,* was isolated from the plant pathogenic fungus *Cochliobolus carbonum* (Kim et al., 2001). In basidiomycetous mushrooms, there is little information about exo-β-1,3-glucanases encoding genes such as a report of two open reading frames (ORF) for exo-β-1,3-glucanase sequences in *A. bisporus* (van den Rhee et al., 1996). Sakamoto et al., (2005b) purified and characterized an exo-β-1,3-glucanase from *L. edodes*, designated EXG1. EXG1 is a β-1,3-glucanase classified in the GH5 family. EXG1 has high similarity to exo-β-1,3-glucanase sequences in *A. bisporus* (van den Rhee et al., 1996). EXG1 in *L. edodes* is specifically expressed in fruiting bodies but not in vegetative mycelia, and decreases after harvesting. EXG1 can degrade a β-1,3-glucan, laminarin, but not lentinan (Sakamoto et al., 2005a; Table 1). These observations suggest that EXG1 is not involved in lentinan degradation or fruiting body senescence. On the other hand, EXG1 is expressed abundantly in growing stipes. Cell wall degrading and rearranging enzymes are important for stipe elongation; therefore, EXG1 could have a function in stipe elongation in *L. edodes* fruiting bodies.

	EXG1[1)]	EXG2[2)]	TLG1[3)]	GLU1[4)]	PUS30[5)]
cleavage type	exo	exo	endo	endo	endo
cleavage linkage	β-1,3	β-1,3	β-1,3	β-1,3	β-1,6
GH family	GH5	GH55	–	GH128	GH30
Lentinan (β-1,3-1,6)	× [a)]	○[b)]	○	○	×
laminarin (β-1,3-1,6)	○	○	○	○	○
pusturan (β-1,6)	×	×	×	×	○
expression after harvesting	decrease	increase	increase	increase	increase

1) Sakamoto et al. 2005a; 2) Sakamoto et al. 2005b; 3) Sakamoto et al. 2006; 4) Sakamoto et al. 2011; 5) Konno and Sakamoto 2011
a) not degraded b) degraded

Table 1. Summary of glucanases (EXG1, EXG2, TLG1, GLU1, and PUS30) purified from *L. edodes*.

Several ascomycetous fungi, including *Aspergillus saitoi* (Oda et al., 2002), and the mycoparasitic fungi *Trichoderma harzianum* (Cohen-Kupiec et al., 1999) and *Ampelomyces quisqualis* (Rotem et al., 1999) express exo-β-13-glucanases other than the GH5 type of exo-β-1,3-glucanases. These exo-β-13-glucanases are classified in the GH55 family. An second exo-β-1,3-glucanase EXG2 was purified and characterized from *L. edodes* fruiting body after harvest (Sakamoto et al. 2005b). EXG2 is expressed in growing stipe but only weakly

expressed in fresh gills. Expression of EXG2 increases immediately after harvest, and then becomes abundant in fruiting bodies 3 days after harvest. EXG2 has a high ability for degrading lentinan (Sakamoto et al. 2005b; Table 1), producing mainly glucose and gentiobiose (Fig. 2A). This suggests that EXG2 can hydrolyze β-1,3-glucan linkages in spite of the existence of a β-1,6-glucan linkage in lentinan (Fig. 2A). This hydrolyzing activity is demonstrated in a GH55 enzyme in *Phanerochaete chrysoporium* (Ishida et al., 2010). These observations suggest that EXG2 in *L. edodes* is mainly related to lentinan degradation after harvest. EXG2, like EXG1, is also abundantly expressed in growing stipes. This suggests that EXG2 has a dual function in stipe elongation and fruiting body senescence.

3.1.2 endo-β-1,3-glucanase

A. bisporus produce an endo-β-1,3-glucanase (Galán et al., 1999) and an endo-β-1,3-glucanase has also been reported from *L. edodes* (Grienier et al., 2000) that exhibits similarities to the anti-fungal thaumatin-like (TL) proteins that are highly conserved in plants. Plants accumulate a large number of pathogenesis-related (PR) proteins, which are divided into five families (PR1-PR5), and TL proteins share sequence homology with the thaumatin isoforms from arils of *Thaumatococcus danielli* (Dudler et al., 1994) are members of the PR5 family (van Loon and van Strien, 1999). Some TL proteins exhibit both β-1,3-glucan binding (Tundel et al., 1998), and endo-β-1,3-glucanase activities (Grenier et al., 1999). Proteins with endo-β-1,3-glucanase activities in mushrooms that have glucan binding activity were purified (Grienier et al., 2000), and their N-terminal amino acid sequences were similar to TL proteins. Sakamoto et al. (2006) purified an endo-β-1,3-glucanase, TLG1, and isolated the encoding gene *tlg1*, which has high similarity to TL proteins. TL protein encoding genes have been found in organisms outside of the plant kingdom, such as in the nematode *Caenorhabditis elegans* (Kitajima and Sato, 1999) and in the locust *Schistocerca gregaria* (Brandazza et al., 2004). It is reported that genes with similarity to TL proteins are highly conserved in fungi (Sakamoto et al., 2006). Fungal TL proteins also exhibit both β-1,3-glucan binding and β-1,3-endoglucanase activities (Grenier et al., 2000). Expression of TLG1 in *L. edodes* is significantly weak in vegetative mycelia, growing stipes and fresh fruiting bodies (Sakamoto et al. 2006). TLG1 is only expressed abundantly in fruiting bodies after harvest. TLG1 has lentinan and cell wall degrading activity (Sakamoto et al., 2006; Table 1), suggesting that TLG1 is specifically involved in lentinan degradation and fruiting body senescence.

GLU1 was purified as an endo-β-1,3-glucanase from *L. edodes* fruiting bodies after harvest, and was separated from TLG1. GLU1 showed greater hydrolyzing activity against laminarin than against lentinan by endo lytic manner (Fig. 2A; Sakamoto et al., 2011). The gene encoding GLU1 was isolated (*glu1*), and recombinant GLU1 in *Pichia pastoris* had endo- β-1,3-glucanase activity (Sakamoto et al., 2011). However, GLU1 did not have significant similarity to known β-1,3-glucanases or to any glycoside hydrolases. Endo- β-1,3-glucanases are divided into two classes, EC 3.2.1.6 [endo-1,3(4)- β-glucanase] and EC 3.2.1.39 (endo-1,3- β-glucanase), based mainly on substrate specificity. Because GLU1 did not degrade β-1,3-linkages within β-1,3-1,4-glucans such as barley glucan, the enzyme was categorized into EC 3.2.1.39. A phylogenetic tree was drawn based on sequences; GLU1 obviously does not belong to existing GH families containing EC 3.2.1.39 glucanases on the CAZy server (Sakamoto et al., 2011). Moreover, amino acid sequence analysis of GLU1 revealed no significant homology with any previously described functional proteins, the enzyme and

other similar proteins were classified in a new GH family, GH128. Expression of *glu1* is weak in vegetative mycelia and growing fruiting bodies, but increases after harvest (Fig. 3). Its expression pattern is very similar to TLG1

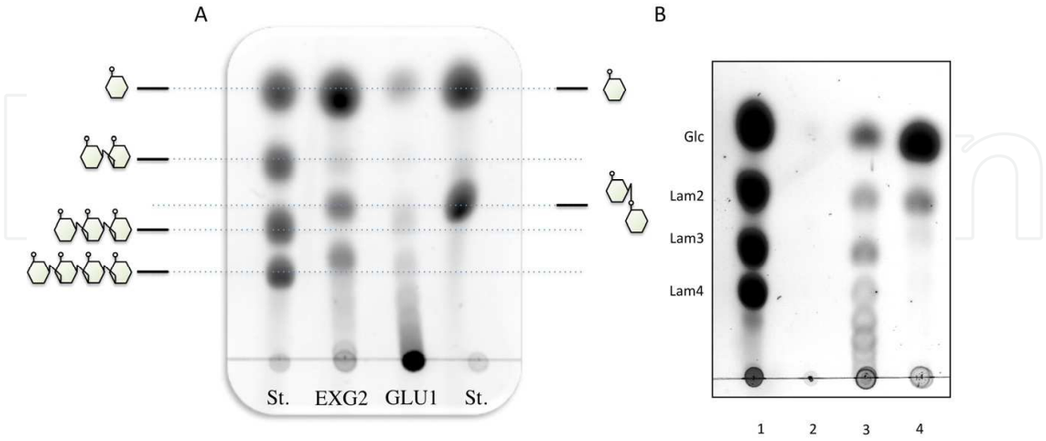

Fig. 2. A Degradation of β-1,3-1,6-glucan (lentinan) by EXG2 and GLU1. Left standards (St.) indicates β-1,3-glucan oligosaccharides, and right St. indicates β-1,6-glucan oligosaccharides. B Combination of GLU1 and EXG2 from *L. edodes* on the β-1,3-glucan hydrolysis. Pachyman (1%, w/v) (lane 2, untreated substarate) was firstly treated with GLU1 (lane3), and the GLU1-treated pachyman was further incubated with of EXG2 (lane 4). Standards (lane 1) are glucose (Glc), laminaribiose (Lam2), laminaritriose (Lam3) and Laminaritetraose (Lam4).

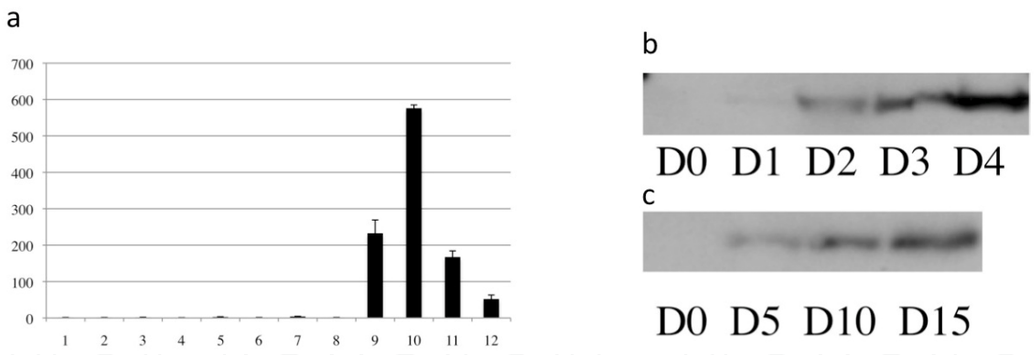

Fig. 3. Expression pattern of the *glu1* a: transcription levels of the *glu1*. 1: mycelium from liquid culture. 2: young fruiting bodies under 1 cm. 3: young fruiting bodies, 1-2 cm. 4: stipe of young fruiting bodies, 2-3 cm. 5: pileus of young fruiting bodies, 2-3 cm. 6: stipe of young fruiting bodies, 3-5 cm. 7: pileus of young fruiting bodies, 3-5 cm. 8: gill of mature fruiting body; 9: gill of fruiting body at 1 day after harvest; 10: gill of fruiting body at 2 days after harvest; 11: gill of fruiting body at 3 days after harvest; 12: gill of fruiting body at 4 days after harvest. b: Western blot analysis of GLU1 after harvest. D0, D1, D2, D3 and D4 indicate day 0 (fresh fruiting body), day 1 day 2, day 3, day 4 after harvesting, respectively. c: Western blot analysis of GLU1 after spore dispersal. D0, D5, D10, D15 indicates day 0, day 5 day 10, day 15 after cap veil open.

(Sakamoto et al. 2006). GLU1 can degrade lentinan, suggesting that GLU1 is involved in lentinan degradation after harvest. Enzymatic properties and expression pattern of GLU1 and

TLG1 are very similar; therefore, it is considered that these two enzymes have a redundant function in senescence of the *L. edodes* fruiting body.

It has been reported that some of the enzymes in the GH16 family have endo- β-1,3-glucanase activity. One GH16 family gene, *mlg1*, was found in fruiting bodies after harvest (Sakamoto et al., 2009). Furthermore, several other GH16 family genes, *mlg2* (DJ432070), *ghf16.1* (DJ432068), and *ghf16.2* (DJ432069) were isolated from *L. edodes* fruiting bodies. However, the enzymatic activities of the proteins encoded by these genes are not known; therefore, there is no clear evidence for a relationship between GH16 family enzymes and autolysis of the *L. edodes* fruiting body so far.

3.1.3 endo-β-1,6-glucanase

Some mycoparasitic fungi such as *Trichoderma* species produce an extracellular β-1,6-glucanase, member of GH30, for attack and degradation of host cell walls during their mycoparasitic action (De la Cruz and Llobell, 1999; Djonović et al., 2006; Montero et al., 2005). However, little information is known about the physiological function and role of the fungal β-1,6-glucanases. Sakamoto et al. (2009) found one GH30 protein, *ghf30* is up-regulated after harvest. Furthermore, LePus30A, an endo-type β-1,6-glucan hydrogenase, and classified as a member of GH family 30, was purified from *L. edodes* (Konno and Sakamoto 2011). LePus30A was the first purified basidiomycetous protein characterized as a GH 30 member (Konno and Sakamoto 2011). LePus30A has high levels of similarity to proteins from basidiomycetous species such as *L. bicolor*, *S. commune* and *C. cinerea*, suggesting that β-1,6-glucanases widely conserved in basidiomycetous fungi. The transcript level of *lepus30a* in fruiting bodies undergoing postharvest preservation for 2-4 days was also significantly higher than at other stages of the life cycle. This result supported an important role for LePus30A in the degradation of the cell wall's complex structure during fruiting body senescence after harvest. LePus30 has no activity toward lentinan, but the enzyme showed activity for the cell wall glucans from *L. edodes* fruiting bodies (Konno and Sakamoto 2011). In addition, LePus30A degrades cell wall glucans producing glucose and β-1,6-linked oligoglucosides. Therefore, LePus30A is mainly contribute to a degradation of the β-1,6-glucan rich content, during fruiting body senescence. The expression of *lepus30a* was also observed in mycelium and young fruiting body. This result implicates that the β-1,6-glucanase also contributes in hyphal growth and branching, or development (Djonović et al. 2006; Moy et al. 2002).

3.1.4 lentinan degradation

Cell wall degrading enzymes, namely exo-β-1,3-glucanase EXG2 and endo-β-1,3-glucanases TLG1 and GLU1 are involved in lentinan degradation after harvesting (Table 1). To study the cooperation of *L. edodes* endo- and exoglucanases during β-1,3-glucan hydrolysis, pachyman (a linear β-1,3-glucan from the basidiomycete *Poria cocos*) hydrolysates obtained after treatment with GLU1 were further treated with EXG2, and the products were analyzed (Fig. 2B). As a result, formed various length of oligosaccharides through the endo-type manner of GLU1 were further degraded to glucose and disaccharide (laminaribiose) by the exo-type manner of EXG2 (Fig. 2B). Thus, the cell wall after harvesting of *L. edodes* fruiting

bodies appear to be degraded by combination of endo-type glucanases (GLU1 and TLG1), and exo- β-1,3-glucanase (EXG2). GLU1 and TLG1 were suggested to have similar biological functions in the process of lentinan degradation after harvest. LePus30A showed its high substrate specificity for a linear β-1,6-glucan polysaccharide (pustulan) and very low activity toward a branched β-1,3/1,6-glucan (laminarin). Since mainly glucose was liberated when laminarin was treated with LePus30A, the enzyme presumably cut the β-1,6-linked side chains in laminarin (DP range 20-30, β-1,3/1,6=7/1). TLG1 and GLU1 shorten main chain length of lentinan, therefore, side chain of partially degraded lentinan would be cut by PUS30A. After all, lentinan is degraded immediately after harvest by cooperation of increased EXG2, TLG1, GLU1, PUS30A after harvest to oligo-saccharides and glucose (Fig. 4).

3.2 Chitinase

As chitin is one of the fungal cell wall components, chitinase and chitin synthase are very important for morphogenesis of fungi. There are several reports on chitinase activities in basidiomycetous mushrooms (for example, Kamada et al. 1981), but few on the relationship between chitinase and fruiting body senescence. Higher chitinase activity is observed in fruiting bodies following harvest compared to the activity just after harvest (Fig. 5). Genes encoding chitin degrading enzymes were identified in fruiting bodies after harvest (*chi1,*

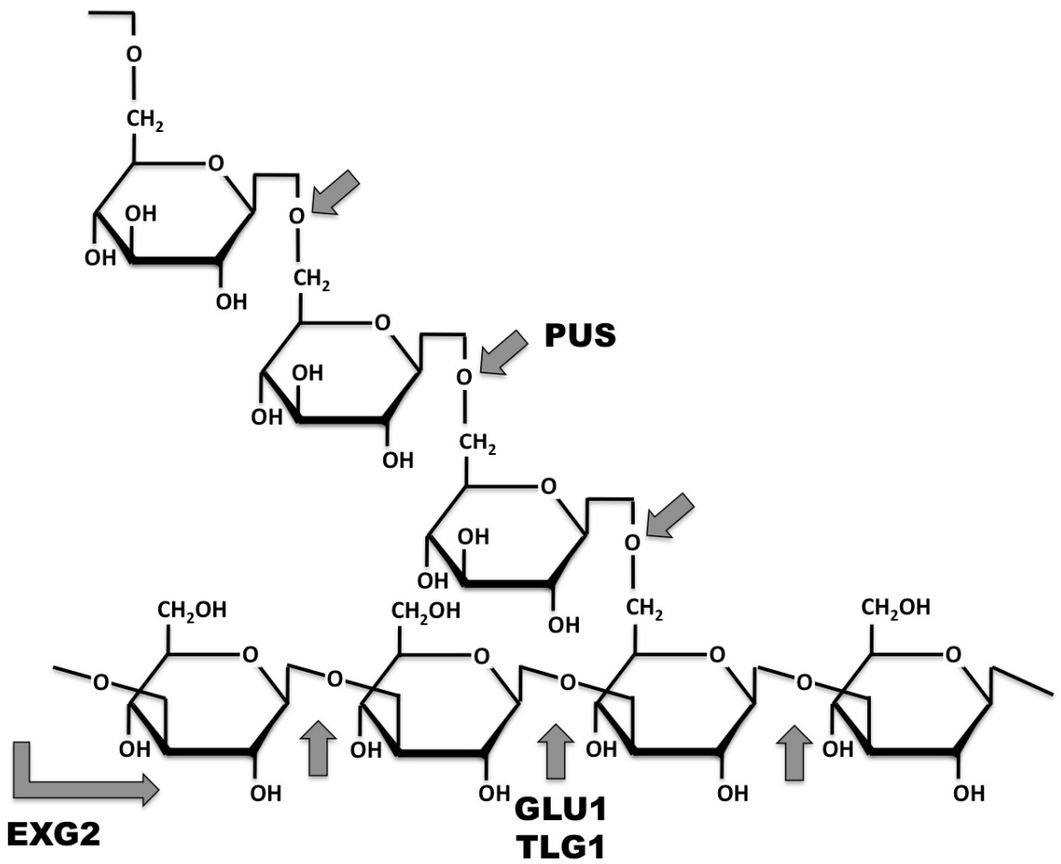

Fig. 4. Scheme of degradation of β-1,3-1,6-glucan after harvest of the *L. edodes* fruiting body

chi2; Sakamoto et al., 2009). The putative amino acid sequences of *chi1* and *chi2* contain a motif found in the GH18 family (Fig. 6). These genes do not have significant similarity to known chitinase encoding genes, but have significant similarities to hypothetical genes in basidiomycetous genomes such as *S. commune*, *Serpula lacrymans* and *Postia placenta*.

A putative chitinase encoding gene has also been found in an EST sequence from *L. edodes* (Suizu et al. 2008); the cloned full-length gene, *chi3*, has a GH18 domain and chitin binding domains (Fig. 6). Expression of *chi3* also increased after harvest (Fig. 7). These observations suggest that increased expression of chitinases has an important role in fruiting body

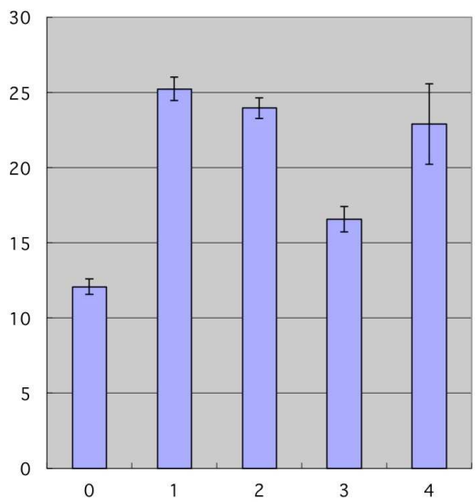

Fig. 5. chtinase activity after harvest measured by using 4-MUF-chitin (Hood 1991). The X axis indicates 0: fresh fruiting body; 1: fruiting body at day 1 after harvest ; 2 fruiting body at day 2 after harvest; 3 fruiting body at day 3 after harvest; 4: fruiting body at day 4 after harvest. The Y axis indicates unit/μg protein.

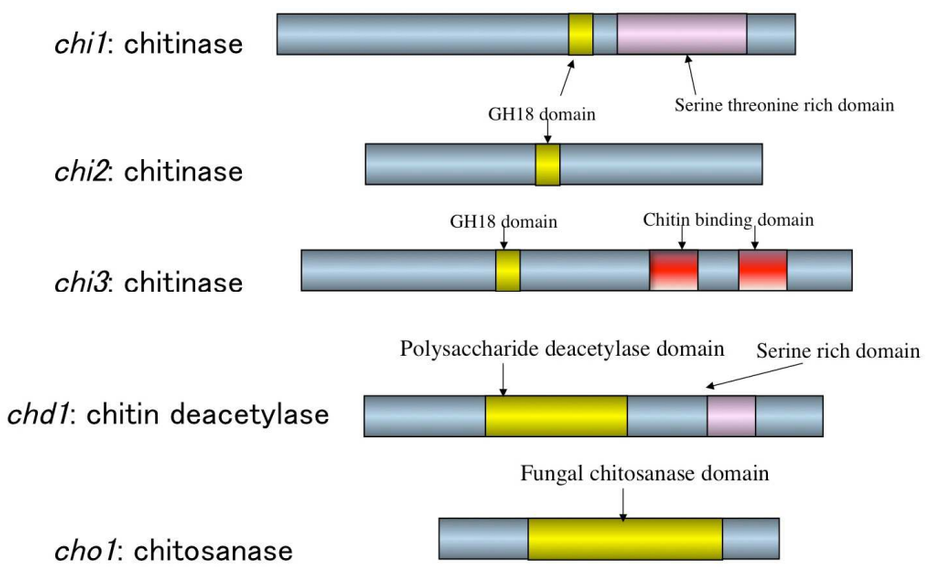

Fig. 6. Chitin related enzymes upregulated after harvest of the *L. edodes* fruiting body.

senescence after harvest. Other genes encoding putative enzymes related to chitin modification were cloned from fruiting bodies after harvest (Sakamoto et al., 2009), including chitin deacetylase (*chd1*) and chitosanase (*cho1*). The putative amino acid sequence of *chd1* has a polysaccharide deacetylase domain in the middle and a serine-rich region at the C-terminus that are present in the chitin deacetylase from *C. neoformans* (Levitz et al., 2001; Fig 6). The DNA sequence of the chitosanase from *L. edodes*, *cho1*, has high similarity with that of *A. oryzae*, but there are no significantly similar sequences in the basidiomycetous genome databases available so far. Chitosan is also a cell wall component of *L. edodes* (Pochanavanich et al., 2002). It was reported that chitin deacetylase (*chd1*) and chitosanase (*cho1*) were upregulated after harvesting of the *L. edodes* fruiting body (Sakamoto et al., 2009). The observation that the *chi1, chi2* and *cho1* genes do not have significant similarity to other basidiomycetous genes suggests that *L. edodes* has a unique chitin and chitosan metabolism system. These data indicate that the cell wall of the *L. edodes* fruiting body is possibly degraded as a result of increased glucanase and chitinase activity following harvest.

4. Regulation of senescence related genes

As shown above, many genes involved in fruiting body senescence increased after harvest of the *L. edodes* fruiting body, including those for phenol oxidases, involved in gill browning, and cell wall degrading enzymes involved in autolysis after harvest. Expression of many other genes increased after harvest of the *L. edodes* fruiting body (Sakamoto et al., 2009). The functions of these genes in senescence of the fruiting body are still unclear, but certainly gene expression is drastically changed after harvest. Transcription factors or chromatin remodelling could cause these drastic changes in gene expression. A gene encoding a putative transcription factor, *exp1*, was isolated from *L. edodes* fruiting bodies after harvest. The putative amino acid sequence of *exp1* displays high similarity with the sequence for *exp1* from *C. cinerea* (Accession No. AB363984; Muraguchi et al., 2008), and contains two HMG boxes in the C-terminus. Proteins that have HMG boxes have functions in transcription factor or chromatin remodeling. In this section, genes other than phenol oxidases and cell wall degradation enzymes, but expressed after harvest will be discussed.

4.1 Unknown genes increased after harvesting

Expression of numerous genes increases after harvest of *L. edodes* fruiting bodies, but their functions in senescence are still unknown. For example, genes encoding riboflavin forming enzyme (*baw28*) and malate dehydrogenase (*mdh*) were isolated from *L. edodes* fruiting bodies. The putative amino acid sequence of *baw28* gene displays 53% identity to the *C. albicans* riboflavin forming enzyme and 33% identity to the *L. edodes* riboflavin forming enzyme (Accession No. AB116639) that is expressed specifically in the fruiting body (Hirano et al., 2004). The putative amino acid sequence of *baw28* has a barwin-like endoglucanase domain in the N-terminus, and a serine-rich region in the C-terminus. The *mdh* gene identified in this study does not contain any significant motifs, but displays 34% identity to *Aspergillus fumigatus* malate dehydrogenase. Expression of the genes increased after harvest (Fig. 7); therefore, the genes presumably have some biological function in senescence, but their function is unclear.

Many genes upregulated after harvest in the *L. edodes* fruiting body have been reported but do not have significant similarities to other genes (Sakamoto et al., 2009; Fig. 8). On the other

hand, genome sequence information in basidiomycetous fungi is increasing, so extensive new information has been found from unknown genes. One of them, a gene that has similarity to glucoamylase (Fig. 8 *amy*). Expression of *amy1* is weak in vegetative mycelia and young fruiting bodies, but significantly increases after harvest. The *ghf79* has similarity to GH79 family which includes enzymes glucronidase. The gene *lup33* has high similarity to hypothetical proteins found in basidiomycetous genomes. The function of the hypothetical proteins is unclear, but expression of the gene specifically increases in the *L. edodes* fruiting body after harvest. On the other hand, there are several genes that have no similarity to any other genes, including hypothetical proteins in the basidiomycetous genomes available so far. These genes are *L. edodes* specific genes. Expression of these *L. edodes unknown protein* encoding genes, *lup23*, *lup410, lup66,* and *lup48*, increases significantly after harvest (Sakamoto et al., 2009, Fig. 8), but is weak in vegetative mycelia and young fruiting bodies. The function of these genes is still unknown, but these genes would have a specific role in senescence of the *L. edodes* fruiting body after harvest.

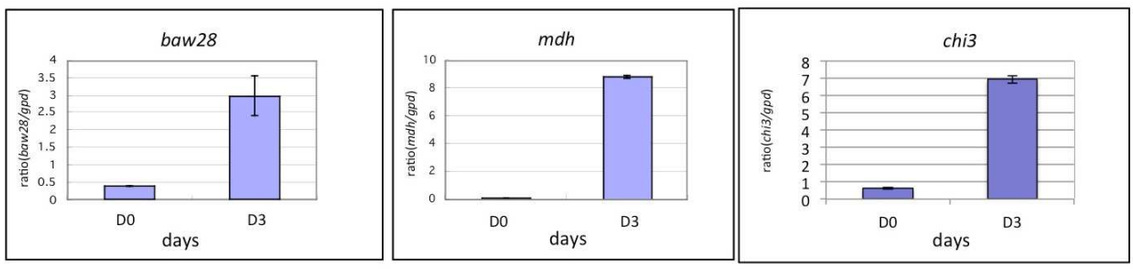

Fig. 7. Comparison of expression levels of *baw28, mdh* and *chi3* in fresh fruiting bodies and fruiting bodies (D0) at day 3 (D3) after harvest. The Y axis represents the ratio of mRNA levels of each gene to that of *gpd*.

4.2 putative senescence related gene transcription regulating factor *exp1*

Putative transcription factors, *exp1* which is up-regulated after harvest are isolated from *L. edodes*t. As the mRNA level of *exp1* was higher three days after harvest than on the day of harvest, *exp1* is likely to be involved in fruiting body senescence after harvesting. The *L. edodes exp1* gene, a homolog of *exp1* isolated from *C. cinerea* (Muraguchi et al., 2008), contains two HMG boxes in the C-terminus. The HMG1/2 class proteins have been considered architectural components of chromatin that have a general role in the regulation of chromosomal functions (Thomas et al., 2001). Several proteins that have an HMG box are considered to be transcription factors, or have been shown to interact with transcription factors (Wissmüller, 2006).

C. cinerea opens the cap of its fruiting body by lysing lamellae in the cap during spore diffusion, and the *C. cinerea exp1* mutant cannot open its cap (Muraguchi et al., 2008). This suggests that *exp1* in *C. cinerea* controls cap autolysis during spore diffusion by regulating the genes that encode cell wall lysing enzymes. For example, expression of *tlg1,* which is a homolog of one of the cell wall degrading enzymes for senescence in *L. edodes*, is suppressed

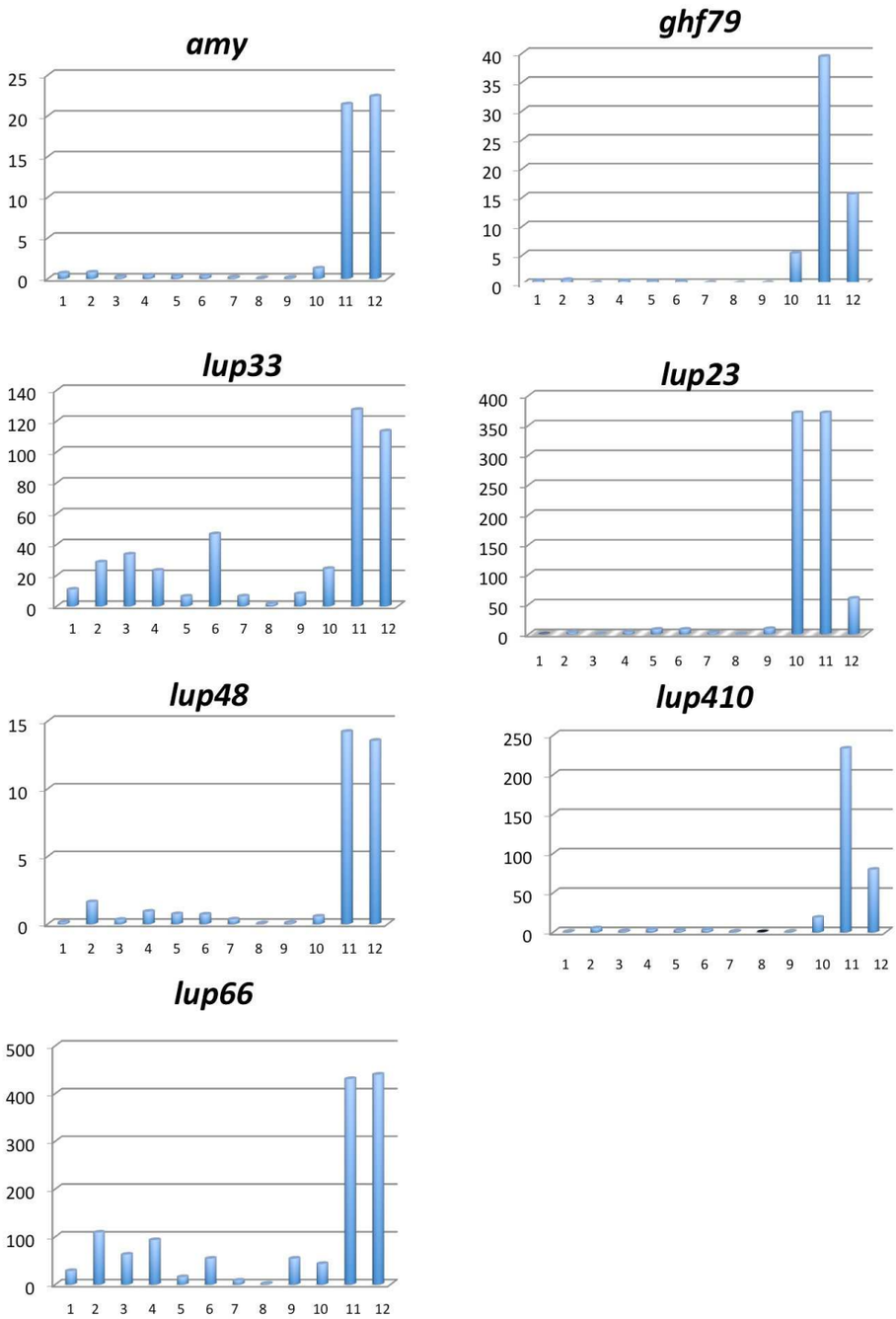

Fig. 8. Expression pattern of unknown genes found in the *L. edodes* fruiting body. The Y axis represents the ratio of mRNA levels of each gene to that of *gpd*. The X axis indicates 1: mycelium from liquid culture. 2: young fruiting bodies under 1 cm. 3: young fruiting bodies, 1-2 cm. 4: stipe of young fruiting bodies, 2-3 cm. 5: pileus of young fruiting bodies, 2-3 cm. 6: stipe of young fruiting bodies, 3-5 cm. 7: pileus of young fruiting bodies, 3-5 cm. 8: gill of mature fruiting body; 9: gill of fruiting body at 1 day after harvest; 10: gill of fruiting body at 2 days after harvest; 11: gill of fruiting body at 3 days after harvest; 12: gill of fruiting body at 4 days after harvest

in the *C. cinerea exp1* mutant (Fig. 9). Cell wall lysis after spore diffusion is observed in *L. edodes*, and expression of the cell wall degrading enzymes EXG2, TLG1 and GLU1 increases after spore diffusion (Sakamoto et al., 2005b, 2006, Fig. 3). Expression of these cell wall degrading enzymes also increased after harvest, suggesting that the systems for cell wall lysis in the fruiting body after harvesting and after spore diffusion are similar in *L. edodes*. These results suggest that *exp1* in *L. edodes* might control senescence of the fruiting body by regulating genes that are expressed after harvesting, such as genes that encode cell wall degrading enzymes.

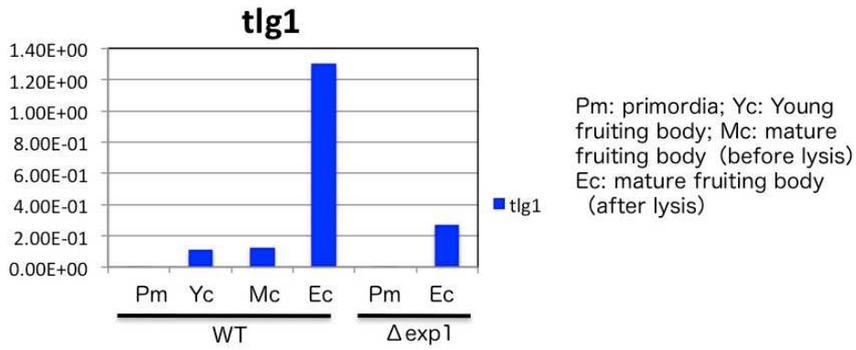

Fig. 9. expression pattern of the *tlg1* in Δexp1. The Y axis represents the ratio of mRNA levels of the *tlg1* to that of *gpd*.

5. Genes downregulated after harvesting

It has been reported that a large number of genes downregulated after harvest are likely involved in normal fruiting body formation and are needed to maintain freshness of the fruiting body (Sakamoto et al. 2009). Translation, transcription and protein metabolism related genes, in addition to those involved in spore formation, cytoskeleton, and cell cycle are downregulated after harvest, and many more transcription factors are also downregulated after harvest (Sakamoto et al. 2009). Interestingly, genes identified among those upregulated after harvest are completely different from those in the downregulated genes. This suggests that gene transcription is drastically altered after harvesting of the fruiting body.

5.1 Transcription, translation, and protein metabolism related genes

Many transcription, translation and protein metabolism related genes are down-regulated after harvesting. Several RNA related genes downregulated after harvest, such as the Pumilio family RNA binding protein, RNA binding protein 5-like protein, ATP-dependent helicase and pre-mRNA splicing factor (Sakamoto et al., 2009). These RNA related proteins are involved in transcription and translation (de Moore et al., 2005; Rogers et al., 2002); therefore, expression of many genes might be changed after harvesting as a result of suppression of these transcription and translation related genes. Heat shock proteins (i.e., Hsp70 and Hsp90), chaperonin and calnexin, which are involved in the proper translation and folding of proteins to control protein quality (Saibil, 2008; Spiess et al., 2004; Williams, 2006), were also down-regulated. This suggests that the protein quality control system in the

L. edodes fruiting body is less effective following harvest. Hsp70 and Hsp90 were especially highly expressed in young fruiting bodies (Fig. 10); therefore, these proteins have an important function in normal fruiting body development. Several proteases and peptidases, such as aspartic protease (*pro1*, *pep1*), metallopeptidase MepB and gamma-glutamyltranspeptidase were downregulated after harvest (Sakamoto et al., 2009), and proteasome-related protein encoding genes such as proteasome 26S, proteasome 26S subunit alpha type 6 and proteasome 26S ATPase subunit 3 were also downregulated after harvest (Sakamoto et al., 2009). These

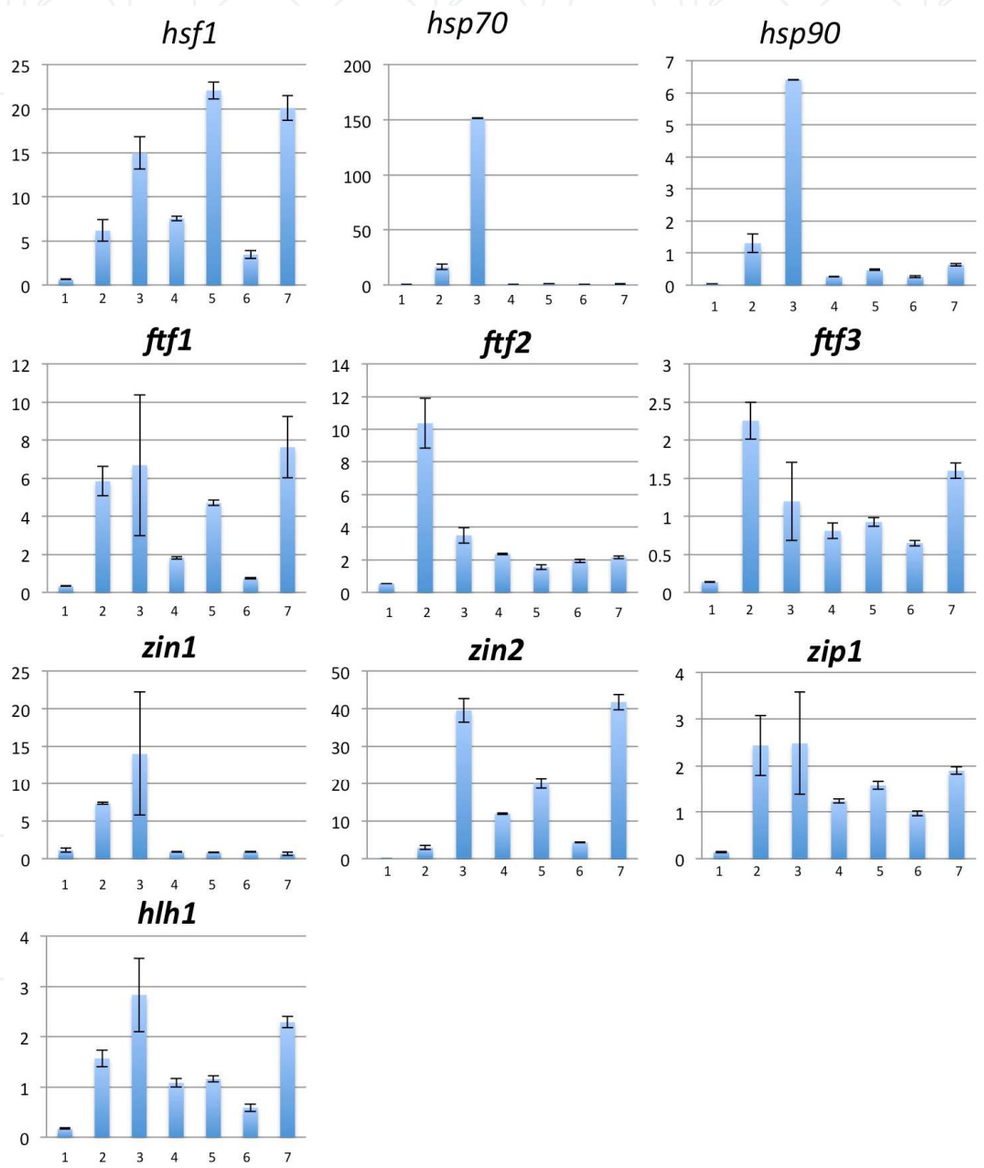

Fig. 10. Putative transcription factors found in the *L. edodes* fruiting body. The Y axis represents the ratio of mRNA levels of each gene to that of *gpd*. The X axis indicates 1: mycelium from liquid culture. 2: young fruiting bodies under 1 cm. 3: young fruiting bodies, 1-2 cm. 4: stipe of young fruiting bodies, 2-3 cm. 5: pileus of young fruiting bodies, 2-3 cm. 6: stipe of young fruiting bodies, 3-5 cm. 7: pileus of young fruiting bodies, 3-5 cm.

proteins comprise a proteasome complex that is involved in protein degradation (Wolf and Hilt, 2004). Decreased protease and proteasome activity would extend the life of proteins expressed after harvest. These data suggest that transcription, translation and posttranslational protein metabolism are drastically altered in fruiting bodies after harvest.

5.3 Mitosis and meiosis related genes

Many mitosis and meiosis related genes are down-regulated after harvesting. Several meiosis related proteins, such as a meiotic recombination related protein encoding gene, *dmc1*, show altered expression in harvested fruiting bodies (Sakamoto et al., 2009). DMC1 is a recombinase that is required for DNA pairing during recombination. Two DMC1-encoding genes were previously identified in *C. cinerea*, namely Rad51 (Stassen et al., 1997) and LIM15 (Namekawa et al., 2005). Whereas Rad51 is involved in both mitosis and meiosis, LIM15 is specifically involved in meiosis, being expressed during meiosis and disappearing immediately after meiosis (Nara et al., 1999). The putative amino acid sequence encoded by *dmc1* has significant similarity to LIM15/DMC1 in *C. cinerea*. The *dmc1* gene was found to be transcribed abundantly in the gills of fresh fruiting bodies and was downregulated after harvesting (Sakamoto et al., 2009). These data suggest that *dmc1* is specifically involved in meiosis, and is downregulated after spore formation. Expression of the cell division related genes septin and *cdc48* reportedly decreased after harvest (Sakamoto et al., 2009). Both of these genes have important functions during cell division (Lindsey and Momany, 2006; Cheeseman and Desai, 2004). Septins are GTPases that form filaments in fungi and animals and are involved in membrane trafficking, coordinating nuclear divisions, and organizing the cytoskeleton (Lindsey and Momany, 2006). cdc48 has a variety of cellular functions; for instance, it is implicated in the membrane fusions that occur after mitosis to reassemble the endoplasmic reticulum and the Golgi apparatus (Cheeseman and Desai, 2004). Genes encoding the cytoskeleton related proteins actin and beta-tubulin were also downregulated after harvest (Sakamoto et al., 2009). These genes are essential for progression of cytokinesis (Nanninga, 2001). This suggests that the frequency of cell division decreases after harvest.

5.4 Putative transcription factors identified by reverse subtraction

Several putative transcription factors are downregulated after harvest of the *L. edodes* fruiting body. One putative transcription factor gene, *hlh1*, which contains a basic helix-loop-helix motif involved in DNA binding (Fig. 11), was also cloned (Sakamoto et al., 2009). Basic helix-loop-helix proteins are a group of eukaryotic transcription factors that exert a determinative influence on a variety of developmental pathways (Littlewood and Evan, 1995). The putative amino acid sequence of *hlh1* contains a nuclear localization signal and displays high similarity to a hypothetical protein from *C. cinerea*. The *hlh* is specifically expressed in young fruiting bodies (Fig. 10). Expression of *ftf1, ftf2, ftf3*, which have a fungal specific transcription factor domain (Fig. 11), was observed in *L. edodes* fruiting bodies (Sakamoto et al., 2009). The putative amino acid sequences of these genes have a similar structure to the *priB* gene product, which is involved in fruiting body development in *L. edodes* (Miyazaki et al., 2004). The *priB* gene is transcribed specifically in the *L. edodes* fruiting body, and regulates fruiting body-specific genes (Kaneko et al., 2001; Miyazaki et al., 2004).

The *ftf1 ftf2*, and *ftf3* are highly transcribed in fresh fruiting bodies and their mRNA levels decreased after harvesting (Fig. 10; Sakamoto et al. 2009), suggesting that the genes regulates genes involved in fruiting body development, but does not regulate genes involved in fruiting body senescence.

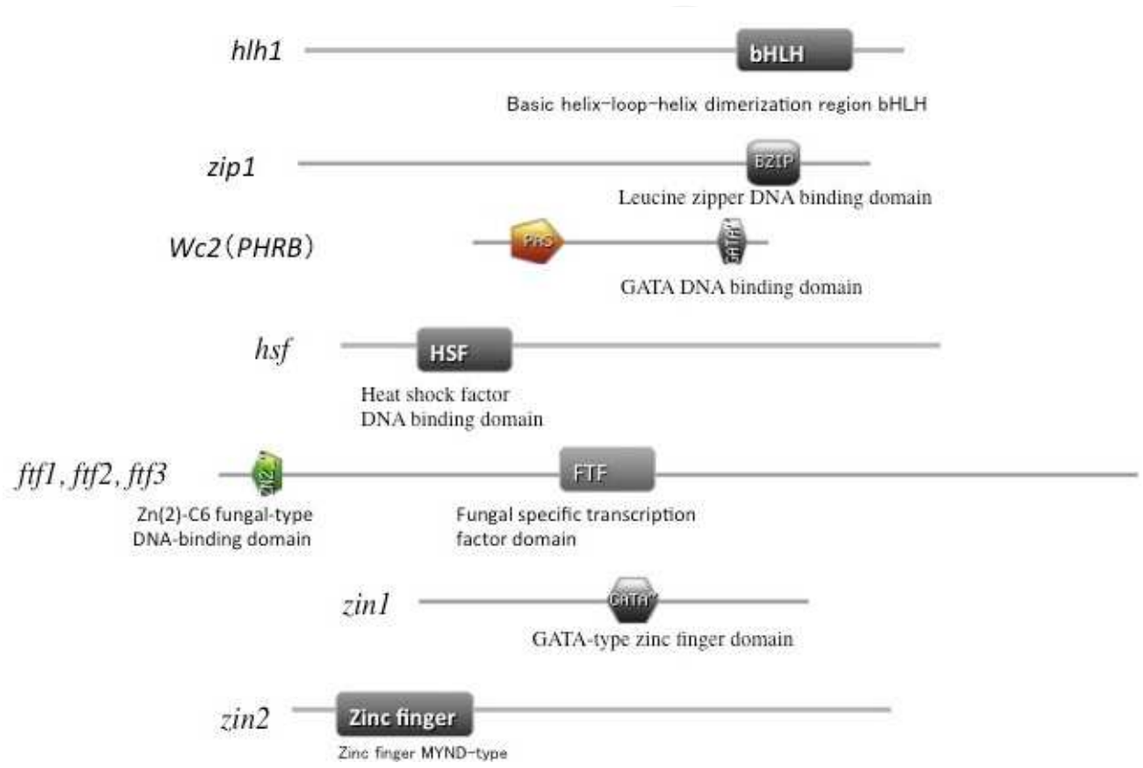

Fig. 11. Putative transcription factors found in the *L. edodes* fruiting body

The putative protein product of *hsf1* has no significant similarity to other known proteins, but has significant similarities to hypothetical proteins in several basidiomycetous fungi, such as *C. cinerea* and *Serpula lacryman*. The putative amino acid sequence of *hsf1* has a helix-turn-helix DNA binding domain for binding to a heat shock element (Fig. 11) that is conserved in the upstream region of heat shock proteins (Pirkkala et al., 2001). Its expression pattern was different from heat shock proteins (Fig. 10); therefore, *hsf* might not directly regulate the expression of heat shock proteins. The putative transcription factor, *zin1* and *zin2*, were also highly transcribed in fresh fruiting bodies, with a decline in expression after harvesting (Fig. 10; Sakamoto et al. 2009). The *zin1* and *zin2* gene products contain a zinc finger motif (Fig. 11). The putative amino acid sequence of *zin2* has a MYND-type zinc finger motif as well as significant similarity with the zf-MYND domain containing protein in *L. bicolor* that is upregulated by infection with ectomycorrhiza (Martin et al., 2008), but there is no significant similar sequence to the *zin2* gene sequences in other basidiomycetous genomes available so far. In contrast, there are other basidiomycetous proteins that have a MYND domain; for example, Fuz1 from *U. maydis* contains an MTND domain and is

involved in cell morphogenesis (Chew et al., 2008). These results suggest that *zin2* is involved in fruiting body development. *zip* is also specifically expressed in fruiting bodies but not in vegetative mycelia (Fig. 10). The putative amino acid sequence of *zip1* has a leucine zipper DNA sequence, which is found in several transcription factors, and also has an Aft osmotic stress domain (Fig. 11). The *zip* does not have any significant similarity to known transcription factors but has significant similarities to hypothetical proteins in several basidiomycetous fungi, such as *C. cinerea* and *S. lacrymans*.

A homolog of the blue light receptor white collar 2 (WC2) in *N. crassa* was also found in *L. edodes* fruiting bodies. In *N. crassa*, WC2 acts as a component of the blue light receptor by interacting with white collar protein 1 (WC1). WC2 in *L. edodes* was cloned and characterized; the protein has been designated PHRB; Sano et al., 2009). Genes similar to WC1 have also been reported in *L. edodes* (PHRA; Sano et al., 2008) and in *C. cinerea* (*dst1*; Terashima et al., 2005). The *C. cinerea dst1* mutant cannot form a mature cap under normal light/dark conditions, and the shape of the fruiting body of the *dst1* mutant is very similar to that formed by the wild-type mushroom when grown in complete darkness (Terashima et al., 2005). Since WC2 and PHRA in *L. edodes* interact with each other, they could act as a blue light receptor in *L. edodes* that regulates fruiting body development in the light (Sano et al., 2010). Gene disruptionof the WC2 homolog in *C. cinerea* results in a similar phenotype to that of *dst1* (Nakazawa et al. 2011).

These genes might not be directly involved in senescence of the *L. edodes* fruiting body after harvest, but these putative transcription factor genes presumably would be involved in normal fruiting body morphology. A decrease in expression of the putative transcription factor genes would influence morphology after harvest.

6. Future perspectives

As shown above, numerous genes are related to senescence of the *L. edodes* fruiting body after harvest. However, the mechanism of senescence of the *L. edodes* fruiting body after harvest is not fully understood. To understand the mechanism involved, an understanding of total gene expression changes after harvest is needed. Transcriptome analysis such as microarray and SAGE analysis is useful to understand total gene expression changes. In *Lentinula edodes*, there are several reports of transcriptome analysis, such as an EST study (Suizu et al., 2009) and SAGE analysis (Chem 2011). For SAGE analysis, genomic sequence data are needed to increase accuracy. Public genomic sequence data of *L. edodes* are not yet available so far, but genomic sequencing will be easier than before using a next-generation genome sequencer, such as the Illumina GA IIx. Furthermore, SAGE analysis is optimized for next-generation sequencing to obtain a larger amount of sequence tagged data (Matsumura et al. 2003). Therefore, gene expression profile changes after harvesting of the *L. edodes* fruiting body will be revealed in more detail by using a combination of genome sequence analysis and Super-SAGE (Matsumura et al. 2003) with a next-generation sequencer. In *C. cinerea,* autolysis after spore dispersion is similar to that of *L. edodes*, and public genomic sequence data of *C. cinerea* are available, so it is possible to compare gene expression profiles during autolysis in *C. cinerea* and *L. edodes*. Such research will provide a basic understanding of mushroom senescence.

To prove the function of genes, gene disruption or gene silencing studies will be needed. Gene disruption systems have been constructed in several mushrooms such as *C. cinerea* (Nakazawa et al. 2011). A homologous gene recombination system has also been constructed (Irie et al. 2003), but a gene disruption system by homologous recombination has not yet been constructed in *L. edodes*. However, gene silencing by RNAi has succeeded in *L. edodes* (Nakade et al., 2011). Therefore, genes upregulated after harvest will be knocked down by RNAi, which will reveal the functions of the genes, such as *exp1* and *exg2*, in senescence. This research will provide useful data for breeding strains with fruiting bodies that remain fresh for a longer period after harvest.

7. Conclusion

After harvesting of the *L. edodes* fruiting body, drastic gene expression changes occur. In particular, expression of phenol oxidases (Tyrs and Lccs) and cell wall enzymes (glucanases and chitinases) increase after harvest. This suggests that a gene regulation system for senescence exists in *L. edodes*. *exp1,* which is a putative transcription factor or chromatin remodeling related protein, is one of the candidates for regulation of the drastic gene expression changes involved in senescence. The process would include programmed cell death, but there are few studies on the relationship between programmed cell death and mushroom morphology. Therefore, studies on senescence in mushrooms will be important not only for applied agricultural science, but also for basic science.

8. Acknowledgment

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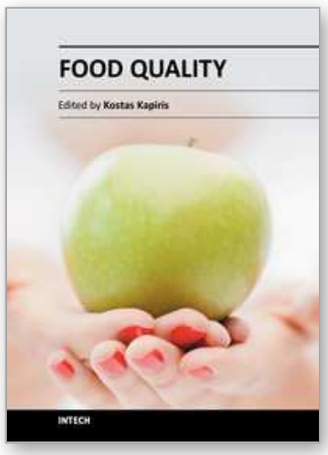

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The book discusses the novel scientific approaches for the improvement of the food quality and offers food scientists valuable assistance for the future. The detailed methodologies and their practical applications could serve as a fundamental reference work for the industry and a requisite guide for the research worker, food scientist and food analyst. It will serve as a valuable tool for the analysts improving their knowledge with new scientific data for quality evaluation. Two case study chapters provide data on the improvement of food quality in marine and land organisms in the natural environment.

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