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Gene expression analysis for stem browning in the mushroom *Lentinula edodes*

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ABSTRACT

The mushroom *Lentinula edodes,* is consumed worldwide and has high industrial value because of its rich content of bioactive compounds such as ergothioneine and eritadenine. Currently, mainstream artificial cultivation methods for this mushroom typically use synthetic logs. However, browning of the stem's interior (stem browning) has been observed during the cultivation in some *L. edodes* strains. Although browning does not affect the taste or other qualities of the mushroom, it gives consumers a perception of "poor quality", and is a major challenge for producers. To identify the genes responsible for stem browning in this mushroom, we performed differential gene expression analysis during stem browning development and quantified it using real-time PCR. Our results indicated that certain oxidoreductases, such as tyrosinase and laccase, were significantly upregulated during the progression of stem browning. The results obtained in the present study provide valuable insights to address the problem of stem browning in mushroom *L. edodes*.

Keywords: Discoloration, Fungi, RNA-seq

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1. Introduction

Lentinula edodes, which belong to the family Tricholomataceae, are mushrooms that are consumed worldwide (Kirk et al., 2010). *L. edodes* produces useful bioactive compounds such as ergothioneine and eritadenine that exhibit anti-oxidant and hypocholesterolemic activities, respectively (Dubost et al., 2007; Kim et al., 2020). Considering its nutritional value, health benefits, and industrial value, a wide range of studies have been conducted using this mushroom. For example, extensive research has been conducted on the functionality of the crude extract of this mushroom such as its anti-inflammatory activity and anti-oxidant action (Gao et al., 2006; Ignacio et al., 2015; Nogusa et al., 2009; Ritz et al., 2006).

Cultivation using synthetic logs is the main method for artificial cultivation of *L. edodes*. This cultivation method involves inoculating *L. edodes* into a synthetic log made by adding rice bran, wheat bran, and water to powdered wood and cultivating them in a facility with controlled humidity and temperature (Wei et al., 2020). A notable issue encountered during the cultivation is the browning of the fungal stem interior in some fruiting bodies of *L. edodes*, a phenomenon called stem browning. Although stem browning does not affect taste, it is a serious problem for producers as it creates a perception of poor quality among customers.

Discoloration of foods over time has been a persistent problem in the food industry, with the enzymatic browning of food being a prime example. Martinez and Whitaker reported that enzymatic browning occurs when polyphenols in food are enzymatically oxidized by polyphenol oxidases (PPO), and the resultant quinone is subsequently polymerized to produce melanin, a relatively insoluble brown polymer (Martinez & Whitaker, 1995). PPO has also been implicated in mushroom browning. The measurement of total phenolic content and some enzyme activities, including PPO activity, of *Flammulina filiformis* revealed that storage of this mushroom for 1 d led to the accumulation of phenylpropanoid phenolic substrates and increased activity of PPO and tyrosinase (Fu et al., 2022). Similarly, Xu et al. reported that the increased PPO activity was responsible for browning in the fresh-cut mushroom *Agaricus bisporus* (Xu et al., 2021).

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The PPO family contains three types of enzymes: tyrosinases, catechol oxidases, and aurone synthases (Kampatsikas et al., 2019). Tyrosinases have both monophenolase and diphenolase activities, whereas catechol oxidases have only diphenolase activity. Tyrosinase first catalyzes the oxidation of monophenols to *o*-diphenols (monophenol oxidase activity; EC 1.14.18.1), followed by the conversion of *o*-diphenols to *o*-quinones (diphenol oxidase activity; EC 1.10. 3.1). In contrast, catechol oxidases are specific to *o*-diphenol and can only catalyze the oxidation of *o*-diphenol to *o*-quinone (Sommer et al., 1994). Aurone synthases are a new class of PPO that play a crucial role in the formation of aurones, which are plant pigment flavonoids. Laccases are enzymes belonging to the oxidoreductase group and are produced by filamentous fungi, and are classified as a subclass of multicopper oxidases also exhibiting PPO activity (Dong et al., 2023; Martin et al., 2024). Mushrooms are highly susceptible to enzymatic browning because of their high tyrosinase and phenol contents (Aguirre et al., 2009). Enzymatic browning is usually detrimental because it causes discoloration and foul odors and leads to the nutritional degradation of fresh fruits, vegetables, nuts, and crustaceans, and various techniques have been used to prevent it (Vhangani & Van Wyk, 2021). Additionally, bacterial infection have been reported to cause browning of the its fruiting bodies. Tolaasin, a toxin specifically produced by *Pseudomonas tolaasii*, forms pores in the mushroom cell membrane and disrupts the tissue structure, resulting in yellow or brown spots on the mushroom's umbrella surface (Nguyen et al., 2012). Moreover, the application of tolaasin to the fruiting bodies of *A. bisporus* caused tyrosinase-induced browning (Soler-rivas et al., 1997).

In this study, we observed a phenomenon wherein the interior of the stem of the mushroom *L. edodes* M113 strain turned brown when the culture temperature was raised during cultivation (Fig. 1). Therefore, to identify genes involved in stem browning in *L. edodes*, we performed a differential gene expression analysis at each stage of stem browning development.

2. Materials and Methods

2.1. Fungal materials

The fruiting bodies of *L. edodes* (strain M113) used in this study were provided by Mori & company (Gunma, Japan). The following experimental conditions were used to induce stem browning in *L. edodes*: After observing the development of primordium, the primordia were initially incubated at 17 °C for 6 d and subsequently subjected to a higher temperature of 25 °C for 24 h on day 7. Following high temperature treatment for 24 h, these primordium were again incubated at 17 °C for an additional 2 d. The fruiting bodies that showed stem browning (BR) were then collected on day 9. For the control, the primordia were incubated at 17 °C for 9 d, the fruiting bodies that did not show browning (NL) were collected. All the collected samples were immediately stored at -80 °C until use.

2.2. DNA extraction and genome sequencing

For the genome sequencing analysis of the *L. edodes* XR1 strain, we initially aimed to isolate a monokaryotic strain from the dikaryotic XR1 strain. Spores were collected from the fruiting bodies of this strain and suspended in sterile water. These collected spores were then spread onto potato dextrose agar (PDA) media and incubated at 22 °C for 2–3 d. Following spore germination, the resulting mycelia were excised under a microscope and transferred to fresh PDA media. After an additional 4-5 d of incubation, mycelia without clamp connections were identified and confirmed as the monokaryotic strain of XR1 (XR1-m1) . Genomic DNAs were extracted using the C-TAB method from XR1-m1 and M113 strains as described previously (Agnestisia et al., 2023; Doyle, 1991). First, the extracted genomic DNA from XR1-m1, an industrial reference strain, was sequenced using a PacBio RS II (Pacific Bioscience, Menlo Park, CA, USA). The resulting long-reads were subjected to *de novo* assembly using Falcon (ver. 0.4.0). The draft genome sequences of the XR1-m1 obtained in this study were deposited in the DNA Data Bank of Japan (DDBJ) Mass Submission System (MSS) under accession numbers BAACCF010000001—BAACCF010000263.

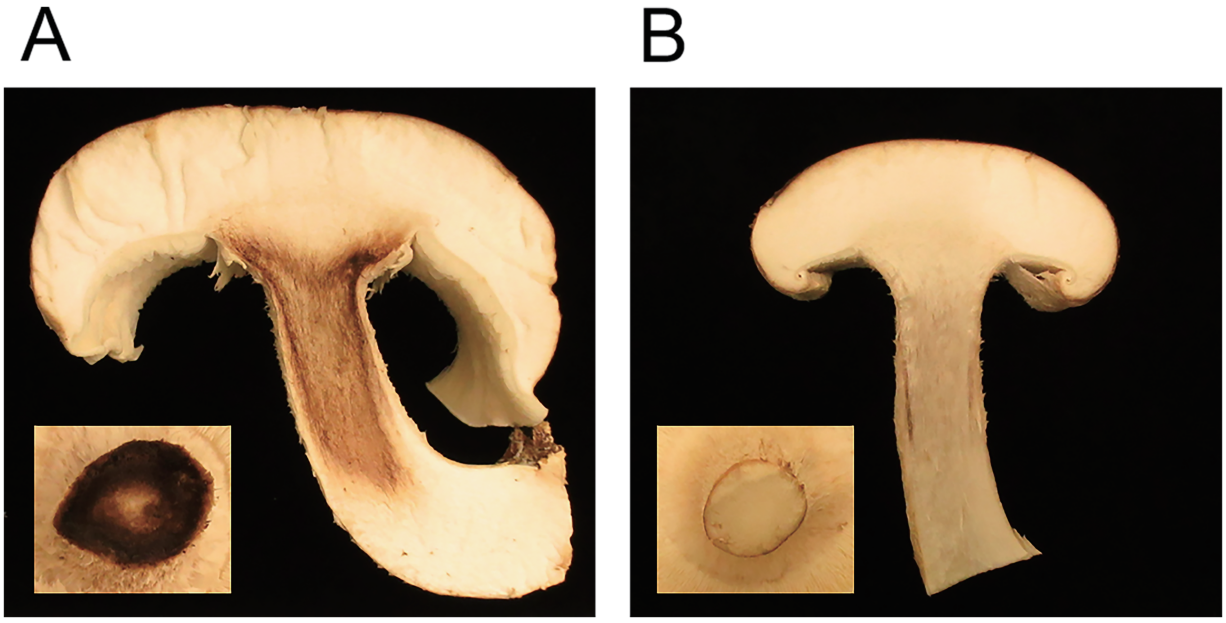

Fig. 1 – A: Cross-section of the fruiting bodies of *Lentinula edodes* M113 strain showing stem browning. B: Cross-section of the normal fruiting bodies of *L. edodes* M113 strain.

Furthermore, the error correction of the XR1-m1 draft genome was performed using short-read sequences from the M113 strain (dikaryotic strain) genome to perform more accurate differential gene expression analysis. Genomic DNA was extracted from the *L. edodes* M113 strain, followed by the acquisition of short-read sequences using Illumina Miseq. The contigs obtained through assembly with Falcon were corrected using Pilon (ver. 1.2) utilizing the short-read sequence obtained from *L. edodes* M113 strain. The resulting genomic contigs were used as reference sequences in this study.

2.3. RNA extraction and sequencing

Total RNA was extracted as previously described (Kanjana et al., 2016; Ono et al., 2019). Three biological replicate samples of BR and NL (control), which had been stored at -80 °C as descrived above were used. For RNA extraction, 100 mg of each stem was frozen and crushed into a powder in liquid nitrogen. The total RNA was extracted using TRIzol Reagent (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions. After treatment with the RNase-free DNase set (Qiagen, Hilden, Germany), the RNA obtained was purified using the RNeasy Plant Mini Kit (Qiagen). The quality of the extracted total RNAs was assessed by electrophoresis with a 2% agarose gel an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). Total RNA concentration was measured using a Quantus Fluorometer (Promega, Madison, WI, USA).

The preparation of the cDNA library and subsequent RNA-Seq were performed as previously reported (Araki et al., 2020; Yamazaki et al., 2020). Libraries were prepared using a KAPA Stranded mRNA-Seq Kit (Illumina, San Diego, CA, USA). According to the manufacturer's instructions, 4 μg of total RNA from each sample was used as a starting material. Following library construction, 151-bp paired-end sequencing was performed using HiSeq X (Macrogen Japan Corp., Tokyo, Japan). The raw sequence reads of the both BR and NL samples in this study were deposited in the DNA Data Bank of Japan (DDBJ) under accession no. DRR542112-DRR542117.

2.4. Bioinformatics analysis

Bioinformatic analysis was performed as previously described (Tanaka et al., 2018; Yamazaki et al., 2020). The genome sequence of *L. edodes* M113 strain used in this study was used as a reference genome. The raw reads (151 bp, paired-end) were initially quality-checked using FastQC v0.11.3. Next, the adapter sequences were removed, low-quality end sequences (quality scores < 15) trimmed, and the elimination of reads shorter than 80 bp and discarding of the last 151st base were performed using Trimmomatic 0.36 (Bolger et al., 2014). High-quality reads were mapped to the reference genome using Hisat2 ver. 2.1.0 (Kim et al., 2019) and the expression levels per transcript were calculated using StringTie version 1.3.4d (Pertea et al., 2015). Based on the resulting gene expression data, differentially expressed genes (DEGs) between normal and brown stems were screened using the Ballgown R package. The *p*-value was determined using the false discovery rate (FDR). Differentially expressed genes were set at > 2-fold up- or downregulated (FDR < 0.05).

To predict the function of DEGs, we annotated the sequences of DEGs as queries against the Universal Protein Resource Knowledge Base (UniProtKB)/Swiss-Prot database (NCBI Swissprot) and NCBI nt database (NCBI nucleotide collection) using BLASTx and BLASTn, respectively (the E-value cutoff was set to $1e^{-50}$ to increase annotation accuracy). In addition, TransDecoder (Ver. 5.5.0) was used to extract putative amino acid-coding regions from the transcripts. The estimated regions were then subjected to Pfam analysis against the Pfam database, and Gene Ontology (GO) enrichment analysis was performed as previously described (Kim & Volsky, 2005).

2.5. Quantitative gene expression analysis

Quantitative gene expression analysis was performed as previously described (Ono et al., 2019; Yamazaki et al., 2020). Quantitative reverse-transcription PCR (qRT-PCR) was performed to determine the expression levels of 17 DEGs annotated as transcription factors and oxidoreductases. Total RNAs used for this analysis were extracted from each of the five NL and BR samples (100 mg each) as described above. Gene-specific primers were designed using Primer3, which was integrated into Geneious Prime 2020.1.2, as shown in Supplementary Table S1 (Tomy Digital Biology, Tokyo, Japan). To synthesize cDNA, total RNA (250 ng) from each sample was reverse transcribed using a PrimeScript RT Reagent Kit (Clontech TaKaRa, Shiga, Japan) according to the manufacturer's instructions. qRT-PCR analysis was performed with a LightCycler 480 Instrument II (Roche, Basel, Switzerland) using FastStart Essential DNA Green Master (Roche,), according to the manufacturer's protocol. The expression level of the actin gene was used as an internal standard (Yu et al., 2020), and relative gene expression levels were calculated using the $\Delta\Delta$Ct method.

3. Results

3.1. DNA sequencing

The result of *de novo* assembly of long reads from PacBio sequencer (Pacific Bioscience) showed that the draft genome of XR1-m1 contained 263 contigs consisting of 47.3 Mb, with a G+C content of 45.9%. Next, error correction was performed using the Pilon software with short reads obtained from the *L. edodes* M113 strain to generate reference genome contigs (Table 1). Shim et al. (2016), reported that whole genome analysis of *L. edodes* strain B17 revealed 74 contigs with a total genome size of 46.1 Mb, and an average G+C content of 45.3% (Shim et al., 2016). The draft genome obtained in this study was similar to that obtained in a previous study.

3.2. Induction of stem browning

In the present study, *L. edodes* strain M113 was subjected to high-temperature treatment at 25 °C for 24 h on the sixth day after fruiting body formation. Following this treatment, the fruiting bodies were incubated at 17 °C for an additional 2 d, resulting in the observation of a stable BR strain phenotype. On the other hand, the primordia of this strain incubated at 17 °C for 8 d without high-tem-

Table 1. Results of genome assembly

	Lentinula edodes M113 strain
Total sequences	263
Total bases (bp)	47,303,853
Max sequence length (bp)	1,438,359
Average sequence length (bp)	179,862
Median sequence length (bp)	60,745
N50 length (bp)	416,453
(A + T)s	54.06 %
(G + C)s	45.94 %

perature treatment did not exhibit any stem browning (Fig. 1). Thus, we successfully established culture conditions that consistently induce system browning in the present study.

3.3. DEGs analysis

Next, RNA-Seq analysis was performed to identify the differentially expressed genes in BR and NL samples. RNA-Seq was carried out using a HiSeq system with 151-bp paired-end reads, resulting in 122,677,014 and 119,127,865 raw reads for NL and BR, respectively. After removing the low-quality ends (quality scores < 15) using Trimmomatic, 72,977,518 and 78,867,703 high-quality reads were obtained from the NL and BR samples, respectively (Table 2). The high-quality sequences obtained were used to calculate the expression levels of each sample using the HISAT2/StringTie/Ballgown pipeline, and DEGs analysis was performed using EdgeR (Pertea et al., 2016). Among the 8,815 genes used in the DEGs analysis, 366 upregulated and 54 downregulated genes were detected in the BR samples compared to the NL samples (Fig. 2; Supplementary Table S2–4).

3.4. Annotation of transcripts

To annotate all genes used in this study, Blastp searches against the Universal Protein Resource Knowledge Base (UniProtKB)/Swiss-Prot database (NCBI Swiss-Prot) and Blastn searches against the NCBI nr/nt database (NCBI nucleotide collection) were performed. Notably, 366 significantly upregulated genes in the BR samples encoded some oxidoreductases (cytochrome P450, tyrosinase, laccase, and cytochrome b5) and transcription factors (Supplementary Table S3). The Pfam analysis detected some genes with oxidoreductase domains such as FAD_oxidored (PF12831), Oxidored_FMN (PF00724), or genes related to oxidase (peroxidase; PF00724) upregulated in BR samples. Furthermore, 11 genes with transcription factor domains (Fungal_trans; pF04082 and pF11951, Elf1; PF05129), whose expression is upregulated by the development of stem browning, were also detected (Supplementary Table S3).

To analyze the functions of the DEGs in more detail, we performed a GO enrichment analysis. The results showed that some functional genes related to DNA replication (GO:0043631), RNA translation (GO:0006275), heme binding (GO:0020037), fatty acid biosynthesis process (GO:0006633), and monooxygenase activity (GO:0004497) were upregulated in the BR samples (Table 3). In contrast, genes related to signaling pathways (protein kinase activ-

Table 2. Number of reads obtained in this study

	Total reads	High quality reads
BR_1	40,773,175	25,046,746
BR_2	39,643,126	25,574,744
BR_3	35,802,142	22,356,028
NL_1	46,027,158	29,731,313
NL_2	38,711,564	25,234,933
NL_3	37,938,292	23,901,457

BR, fruiting bodies showing stem browning; NL, fruiting bodies that did not show stem browning

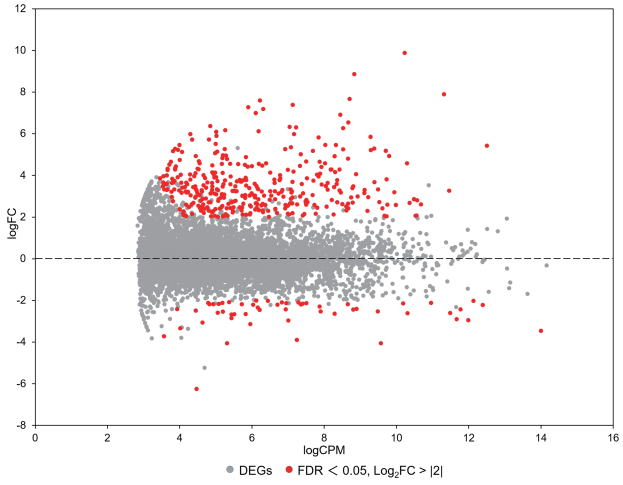

Fig. 2 – Distribution of differentially expressed genes. The DEGs shown in red log-FC > |2| and FDR ratio of < 0.05 for each gene in each pairwise comparison of NL and BR samples.

Table 3. Result of GO enrichment analysis.

GO name	Description	GO ID	*Z* Score	FDR
BP	RNA polyadenylation [1]	GO:0043631	8.47	0.00E+00
BP	regulation of DNA replication [1]	GO:0006275	7.46	2.16E-11
MF	protein binding	GO:0005515	-6.84	1.35E-09
MF	ATP binding	GO:0005524	-6.01	2.28E-07
CC	nucleus	GO:0005634	-4.84	1.31E-04
BP	regulation of DNA-templated transcription	GO:0006355	-4.45	7.03E-04
BP	mycotoxin biosynthetic process [1]	GO:0043386	4.41	7.47E-04
MF	heme binding [1]	GO:0020037	3.76	1.05E-02
MF	3-oxoacyl-[acyl-carrier-protein] synthase activity [1]	GO:0004315	3.65	1.28E-02
MF	protein kinase activity	GO:0004672	-3.66	1.28E-02
MF	zinc ion binding	GO:0008270	-3.58	1.30E-02
BP	viral process	GO:0016032	-3.58	1.30E-02
BP	protein phosphorylation	GO:0006468	-3.60	1.30E-02
MF	ATP hydrolysis activity	GO:0016887	-3.50	1.65E-02
BP	vesicle-mediated transport	GO:0016192	-3.33	2.72E-02
BP	proteolysis	GO:0006508	-3.33	2.72E-02
BP	signal transduction	GO:0007165	-3.30	2.79E-02
BP	fatty acid biosynthetic process[1]	GO:0006633	3.28	2.90E-02
MF	hydrolase activity	GO:0016787	-3.22	3.38E-02
BP	viral protein processing	GO:0019082	-3.12	4.44E-02
MF	acetyltransferase activity[1]	GO:0016407	3.11	4.47E-02
BP	cell adhesion	GO:0007155	-3.08	4.60E-02
MF	monooxygenase activity[1]	GO:0004497	3.06	4.75E-02

[1] represents significantly enriched in BR samples.

ity, GO:0004672; protein phosphorylation, GO:0006468; and signal transduction, GO:0007165) were downregulated (Table 3).

3.5. Quantitative gene expression analysis

As described above, DEGs analysis revealed that some oxidoreductases (cytochrome P450, tyrosinase, laccase, and cytochrome b5) and transcription factors were significantly upregulated in BR samples. Therefore, we designed primers for qRT-PCR of genes encoding the seven oxidoreductases, including tyrosinase, laccase, cytochrome P450 and cytochrome b5, and the 10 transcription factors whose expression was significantly upregulated in the BR samples (Supplementary Tables S2, S3).

Five biological replicates were used for each NL and BR sample, and the relative expression levels were calculated using the $\Delta\Delta$Ct method (Table 4). The qRT-PCR results revealed that the relative expression levels of almost all genes except MSTRG.3657 were increased compared to those in the NL samples. Among these 16 genes, 9 were found to be significantly upregulated (Table 4). Two of the four cytochrome P450s and four of the ten transcription factors were significantly upregulated ($p < 0.05$). Tyrosinase, laccase, and cytochrome b5 were also significantly upregulated in BR samples.

4. Discussion

In this study, we found that the M113 strain of *L. edodes*, a mushroom widely consumed as food, can stably exhibit both the BR and NL phenotypes, with and without heat treatment. Moreover, we used next-generation sequencing to detect DEGs between these two samples to identify potential genes involved in stem browning.

Differential gene expression analysis revealed that some oxidoreductases, such as laccase, tyrosinase, and cytochrome, were upregulated at the onset of stem browning. Seven genes were selected from these oxidoreductases and were subjected to qRT-PCR analysis. Among these, the expression levels of five genes, except for two cytochrome P450s (MSTRG.9532 and MSTRG.9486), were significantly upregulated ($p < 0.05$) (Table 4). Tyrosinases and laccases oxidize phenols to quinones, and the subsequent non-enzy-

Table 4. Gene expression levels determined by qRT-PCR for selected DEGs identified from the RNA-Seq results.

Cytochrome P450	RNA-Seq		qRT-PCR	
	logFC	FDR	Relative expression	*p*-value
MSTRG.8395 (Cytochrome P450, monooxygenase FCK2) [1]	3.78	1.38E-13	73.43	2.0E-04
MSTRG.7756 (Cytochrome P450 monooxygenase COX2) [1]	3.29	1.85E-07	17.02	8.0E-03
MSTRG.9532 (Cytochrome P450 67 (Fragment))	3.27	1.75E-06	28.00	3.0E-01
MSTRG.9486 (Cytochrome P450 52-E3)	3.24	5.90E-10	2.67	6.0E-02
Tyrosinase	RNA-Seq		qRT-PCR	
	logFC	FDR	Relative expression	*p*-value
MSTRG.3821 (Tyrosinase) [1]	2.57	1.18E-02	12.18	1.0E-02
Laccase	RNA-Seq		qRT-PCR	
	logFC	FDR	Relative expression	*p*-value
MSTRG.1879 (contains protein-coding region of laccase) [1]	3.10	1.40E-02	9.63	9.0E-03
Cytochrome b5	RNA-Seq		qRT-PCR	
	logFC	FDR	Relative expression	*p*-value
MSTRG.8344 (Acyl-lipid omega-3 desaturase (cytochrome b5), endoplasmic reticulum) [1]	3.31	1.70E-08	27.82	2.0E-02
Transcription Factors	RNA-Seq		qRT-PCR	
	logFC	FDR	Relative expression	*p*-value
MSTRG.4190 (Fungal specific transcription factor domain) [1]	3.52	5.29E-03	38.69	5.0E-03
MSTRG.7790 (Histone-like transcription factor (CBF/NF-Y) and archaeal histone) [1]	3.77	7.09E-04	8.47	5.0E-04
MSTRG.8657 (Transcription elongation factor Elf1 like) [1]	2.77	4.94E-06	3.36	3.0E-03
MSTRG.4872 (Transcription elongation factor Elf1 like) [1]	2.27	3.98E-02	14.37	2.0E-02
MSTRG.4913 (Transcription elongation factor Elf1 like)	3.21	2.79E-03	2.89	5.0E-01
MSTRG.3657 (Doublesex-and mab-3-related transcription factor C1 and C2)	4.80	5.77E-05	1.01	1.0E+00
MSTRG.3685 (Fungal specific transcription factor domain)	3.05	7.78E-04	6.19	1.0E-01
MSTRG.2708 (Fungal specific transcription factor domain)	2.32	1.70E-02	12.60	2.0E-01
MSTRG.3204 (Fungal specific transcription factor domain)	2.31	1.00E-02	2.66	1.0E-01
MSTRG.5775 (Fungal specific transcription factor domein)	2.13	1.00E-02	4.88	1.0E-01

[1] represents a significant difference between NL and BR samples ($p < 0.05$ by T-test).

matic polymerization of these quinones leads to the formation of melanin (Weijn et al., 2013). Previous studies have reported that tyrosinase and laccase are involved in the browning of *L. edodes* (Kanda et al., 1996; Nagai et al., 2003; Sato et al., 2009). Therefore, the increased expression of tyrosinase and laccase in BR samples observed in this study indicates that the melanin produced by these enzymes may be a contributing factor to stem browning. In contrast, cytochrome P450s, also detected as DEGs, are known to be active against a variety of substrates. The upregulation of certain cytochrome P450s in the BR samples and the enrichment of monooxygenase activity (GO:0004497) by GO enrichment analysis suggested that these enzymes were actively expressed in BR (Table 3). Cytochrome P450s are known to be upregulated during fruiting body formation, suggesting their involvement in fruiting body browning during these processes (Kim et al., 2020). However, the diverse cytochrome P450s that contribute to browning and the detailed molecular mechanisms remain unclear. Further studies are required to elucidate the effects of cytochrome P450s. Cytochrome b5 positively regulates the cytochrome P450 monooxygenase reaction *via* electron transfer (Schenkman & Jansson, 2003), indicating that increased expression levels of cytochrome P450s may be activated by cytochrome b5.

The GO term for heme-binding (GO:0020037) was enriched in BR samples, indicating the increased expression levels of these cytochromes within BR samples may be attributed to the heme present in the active site of cytochromes (Table 3). In the NL sample, enrichment of GO terms related to signaling pathways, such as protein kinase activity (GO:0004672), protein phosphorylation (GO:0006468), and signal transduction (GO:0007165), was observed compared to the BR sample (Table 3). These results imply that these signaling pathways were inactivated by the specific heat treatment in the BR samples. Moreover, several transcription factors (MSTRG.4190, MSTRG.7790, MSTRG.8657, and MSTRG.4872) were found to be specifically upregulated in the BR samples, and these transcription factors may be targets for future research (Table 4).

This study provides new information regarding the transcriptomics of stem browning in the mushroom *L. edodes*, which presents a major commercial problem. We searched for genes associated with stem browning by comparing the BR and NL samples of *L. edodes* M113 strain. Differential expression analysis revealed that the expression levels of oxidoreductases, including cytochrome, tyrosinase, and laccase, were significantly increased, which caused enzymatic browning. This suggests their crucial roles in stem browning. In addition, we successfully identified several transcription factors that are upregulated at the onset of stem browning, which will be targeted for our future research. These results provide valuable insights to address the problem of stem browning in mushroom *L. edodes* of high commercial value.

Disclosure

The authors declare no conflicts of interest.

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Supplementary Materials

Supplementary online materials are available at https://doi.org/10.69199/data.mycosci.26829796, https://doi.org/10.69199/data.mycosci.26829910, https://doi.org/10.69199/data.mycosci.26830036, https://doi.org/10.69199/data.mycosci.26830054

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