Gene Expression Analyses of Mutant Flammulina velutipes (Enokitake Mushroom) with Clogging Phenomenon

Ju-Ri Woo, Doo-Ho Choi, Muhammed Taofiq Hamza, Kyung-Oh Doh, Chang-Yoon Lee, Yeon-Sik Choo, Sangman Lee, Jong-Guk Kim, Heeyoun Bunch and Young-Bae Seu

ABSTRACT
Regulation of proper gene expression is important for cellular and organismal survival, maintenance, and growth. Abnormal gene expression, even for a single critical gene, can thwart cellular integrity and normal physiology to cause diseases, aging, and death. Therefore, gene expression profiling serves as a powerful tool to understand the pathology of diseases and to cure them. In this study, the difference in gene expression in Flammulina velutipes was compared between the wild type (WT) mushroom and the mutant one with clogging phenomenon. Differentially expressed transcripts were screened to identify the candidate genes responsible for the mutant phenotype using the DNA microarray analysis. A total of 88 genes including 60 upregulated and 28 downregulated genes were validated using the real-time quantitative PCR analysis. In addition, proteomic differences between the WT and mutant mushroom were analyzed using two-dimensional gel electrophoresis and matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF). Interestingly, the genes identified by these genomic and proteomic analyses were involved in stress response, translation, and energy/sugar metabolism, including HSP70, elongation factor 2, and pyruvate kinase. Together, our data suggest that the aberrant expression of these genes attributes to the mutant clogging phenotype. We propose that these genes can be targeted to foster normal growth in F. velutipes.

1. Introduction
Flammulina velutipes, also known as velvet shank, has several names, including enokitake in Japan, golden needle mushroom in China, futu in India, and paengi beoseog in Korea. Because it is low in calories yet rich in savory flavors and nutrients, including essential amino acids, minerals, and polysaccharides, F. velutipes is one of the most popular edible mushrooms. This popularity has led to an increasing demand and the artificial cultivation of F. velutipes in recent years. Although wild F. velutipes grows on stumps of Celtis sinensis, commonly called Enoki, cultivated mushrooms are grown in a dark, carbon dioxide-rich environment. This peculiar environment induces the development of their long thin stems, which often cause overcrowding of mushrooms (referred to “mutants with clogging phenomenon” from this on). The overcrowdedness results in poor yields of F. velutipes. One of important steps to overcome this problem in the artificial cultivation of F. velutipes is to identify the genes or mutations responsible for the clogging phenomenon.

Since the initiation of the Human Genome Project in 1990s, the genomes of diverse fungal species have been sequenced. Recently, the Joint Genome Institute commenced the 1000-fungal genome project, which focuses mainly on plant feedstock health, biorefinery, and fungal diversity. Furthermore, with the ongoing development of omics analyses, high-throughput sequencing and proteomics analyses have been widely used. Using ESTs, precise probes can be designed for DNA microarrays, which can be used to analyze gene expression profiles. Since Chang introduced the concept and methodology of microarrays...
in 1983 [13], as a multiplex lab-on-a-chip, DNA microarrays have been widely used to test large amounts of biological materials using high-throughput miniaturized, multiplexed, and parallel processing [14]. In addition, Wilkins invented proteomics [15], a powerful tool for examining differences in gene expression in the protein level [11,16]. For example, specific growth stages at which some proteins are expressed could indicate morphological changes, unique functions, or developmental requirements [17]. This approach is complementary to the DNA microarray experiment in that both of these techniques examine which genes are activated or repressed under certain conditions. Furthermore, the information provided by these experiments is invaluable for constructing three-dimensional protein maps and investigating the regulation of protein function [18].

For example, metabolic changes of F. velutipes mycelia in response to cold and light stresses were examined through proteomics approaches [19,20]. In these studies, proteome changes in F. velutipes using iTRAQ-coupled two-dimensional liquid chromatography-tandem mass spectrometry identified differentially expressed proteins upon cold or light stresses. The identified proteins were analyzed through Kyoto encyclopedia of genes and genomics (KEGG) to identify the affected pathways under these conditions.

During the research for the enhanced production of artificially grown F. velutipes, the traits specific to the clogging phenomenon in the mushrooms have been identified [6,21,22]. Compared with wild type (WT) F. velutipes, mutant mushrooms exhibit changes in their fruiting body, featuring irregular lengths or shapes of the cap, forming colonies with a fluffy surface, and abnormal growth with less density and weight. Due to these unfavorable traits, the mutant mushroom apparently fails to grow appropriately. To prevent and cure this mutation, identifying the genetic problems underlying the clogging phenomenon is necessary [21]. Therefore, we performed the gene expression profiling and identified differentially expressed genes (DEGs), comparing the WT and mutant mushrooms in this study. We believe that our genomic and proteomic analyses identified the genes involved in growth and fruiting body formation.

2. Materials and methods

2.1. Sample collection

We obtained the mycelia of WT and mutant F. velutipes from Green-Peace Mushroom Farm (Cheongdo, Republic of Korea). The samples were cultivated in potato dextrose broth (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) at 25°C for 15 d. After cultivation, the clogging phenomenon was observed in the mycelia of mutant F. velutipes.

2.2. Total RNA isolation

To isolate total RNA from mycelium and fruiting body of F. velutipes, RNeasy Maxi kit (Qiagen, Valencia, CA, USA) was used. All isolated mycelium and fruiting body samples were freeze-dried to facilitate RNA isolation results due to large amounts of sugars [4,22]. One gram of mycelium and fruiting body tissues of F. velutipes were grinded with liquid nitrogen. The samples were dissolved in the buffer with 2-mercaptoethanol and grinded by homogenizer (Daihan, Gangwon, Republic of Korea). Then, samples were centrifuged at 3000 g for 10 min at 15 degree and the supernatant was separated. Remaining pellets were mixed with 70% ethanol and the mixture was centrifugated at 3000 g for 5 min at 15 degree. After washing twice, the total RNA was eluted.

2.3. Array hybridization and data analysis

Total RNAs were mixed with Bacterial alkaline phosphatase (TakaRa, Tokyo, Japan) and Tabacco acid pyrophosphatase (TakaRa). Then, synthetic oligomer that specifically reacts to uncleaved mRNA having phosphate group was added. From the total RNA mixture, mRNA was isolated using Oligotex mRNA purification kit (Qiagen, Valencia, CA) [23,24]. cDNA was synthesized from the isolated mRNA by SuperScript double-stranded cDNA synthesis kit (Invitrogen, Waltham, MA) and amplified by XL PCR kit (PerkinElmer, Waltham, MA). cDNA from mycelium was labeled with Cy3 and cDNA from fruiting body was labeled with Cy5 by 3 DNA Array 50 kit (Genisphere, Hatfield, PA). The labeled genes were hybridized to NimbleGen expression customized arrays that had 135,000 probes (12 probes for each gene) for 10,405 target genes, with a 60 mer probe length and feature size of 13 x 13 μl. The first hybridization was performed for 16 h and second hybridization was followed after washing and fixation. After hybridization, the sample was centrifuged at 1000 g for 3 min. Results of the array were scanned by Scan–array 5000 microarray scanner (Packard BioScience, Meriden, CT) at 532 nm for Cy3 and 635 nm for Cy5. Gene PIX program (Axon Instruments, Burlingame, CA) was used to analyze the scanned images and normalized. The results were analyzed by Significance analysis of microarray (SAM) and genes with increased or decreased expression levels were identified.
2.4. Real-time PCR

Based on the microarray results, DEGs in mutant mushrooms, either upregulated or downregulated, were validated in the mycelia of *F. velutipes*. Primers for real-time PCR were designed based on these genes using Primer version 3 (Whitehead Institute for Biomedical Science) [25,26]. The sequences of primers are listed in Supplementary Data 1 and 2. For the reference gene, glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used. The GAPDH forward primer was 5'-GCCGTCACAACAA CATCATTCCCT-3', and the GAPDH reverse primer was 5'-CGGAAACATCGAGGGACA-GGACA-3'. A Takara real-time PCR system was used (Takara, Otsu, Japan). Cycling conditions were as follows: initial denaturation at 95 degree for 10 min followed by 40 cycles of denaturation at 95 degree for 10 s, and annealing and elongation at 60 degree for 15 s.

2.5. Protein sample preparation

The mycelia of WT and mutant *F. velutipes* were washed twice in ice-cold phosphate-buffered saline. The washed samples were then homogenized with liquid nitrogen. The homogenized powder was lysed in the sample buffer containing 7 M urea, 1 mM benzamidine, and 2 M thiourea with 4% (w/v) 3-[(3-cholamidopropy) dimethyammonio]-1-propane-sulfonate (CHAPS), 1% (w/v) dithiothreitol (DTT), and 2% (v/v) pharmalyte [27]. Samples were vortexed for 1 h at room temperature to extract the proteins. After centrifugation at 15,000 g for 1 h at 15 degree, the insoluble material was discarded, and the soluble fraction was used for two-dimensional gel electrophoresis. Protein loading was normalized, based on the result from the Bradford assay [28].

2.6. 2D-PAGE

To separate the proteins by isoelectric point and molecular weight, two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) was used to identify differentially expressed proteins [29]. IPG dry strips were equilibrated for 12–16 h with 7 M urea, loaded with 200 μg of sample and 2 M thiourea containing 2% CHAPS, 1% DTT, and 1% pharmalyte. Isoelectric focusing (IEF) was performed at 20 degree using a Multiphor II electrophoresis unit and EPS 3500 XL power supply (Amersham Biosciences, Amersham, UK), according to the manufacturer’s instructions [30]. For IEF, the voltage was linearly increased from 150 to 3500 V over 3 h for sample entry, followed by a constant 3500 V, focusing completely after 96 kVh. Before the second dimension, strips were incubated for 10 min in an equilibration buffer (50 mM Tris–Cl, pH 6.8, containing 6 M urea, 2% sodium dodecyl sulfate [SDS], and 30% glycerol), first with 1% DTT and then with 2.5% iodoacetamide. Then, equilibrated trips were inserted in SDS–PAGE gels (20–24 cm, 10–16%). SDS–PAGE was performed using the Hoefer DALT 2 D system (Amersham Biosciences), following the manufacturer’s instructions. The 2 D gels were run at 20 degree for 1.7 kVh. The 2 D gels were silver stained as described by Oakley et al. [31,32].

2.7. Image analysis

Quantitative analysis of digitized images was performed using PDQuest version 7.0 (Bio-Rad, Hercules, CA) according to the protocol provided by the manufacturer. The total valid spot intensity was normalized for the quantity of each spot. Protein spots were selected based on significant expression variation greater than two-fold compared with the WT samples [33].

2.8. In-gel digestion and mass spectrometry

Protein spots were enzymatically digested in-gel as previously described by Shevchenko et al., using modified porcine trypsin [34]. Gel pieces were washed with 50% acetonitrile to remove SDS. They were then salted and stained, and dried to remove the solvent. This was followed by rehydration with trypsin (8–10 ng/μl) and incubation for 8–10 h at 37 degree. The proteolytic reaction was terminated by adding 5 μl of 0.5% trifluoroacetic acid [35]. Tryptic peptides were recovered by combining the aqueous phase from several extractions of gel pieces with 50% aqueous acetonitrile. Next, the peptide mixture was desalted using C18ZipTips (Millipore, Billerica, MA) and acetonitrile. Finally, an aliquot of this solution was mixed with an equal volume of a saturated solution of α-cyano-4-hydroxycinnamic acid in 50% aqueous acetonitrile and the mixture was spotted onto a target plate [35]. Protein analyses were performed using Ettan matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) (Amersham Biosciences). First, peptides were evaporated with an N2 laser at 337 nm using a delayed extraction approach. Then, they were accelerated with 20 Kv injection pulse for the time of flight analysis. Each spectrum was the cumulative average of 300 laser shots. The search program ProFound, developed by the Rockefeller University (http://129.85.19.192/profound_bin/ WebProFound.exe), was used for protein identification by peptide mass fingerprinting. Spectra were calibrated with trypsin auto-digestion ion peak m/z (842.510, 2211.1046) as internal standards [36–38].
2.9. Visualization methods of DEGs

The DEGs that were up or down-regulated from mycelia and fruiting bodies were compared and visualize in Venn diagram. Scattered plots of DEGs in mycelia or fruiting bodies were visualized using “EnhancedVolcano” package version 1.10.0 (https://bioconductor.org/packages/release/bioc/html/EnhancedVolcano.html) within R program version 4.1.1 (The R Foundation for Statistical Computing, Vienna, Austria).

3. Results

3.1. RNA profiling of WT vs. mutant F. velutipes

To identify DEGs in clogging mutant F. velutipes, DNA microarray was performed, comparing WT and mutant mushrooms. The results showed that a total of 371 genes were differential expressed (log2 fold change, FC > 2; p value < 0.05) from the mycelia of the mutant mushroom, including 156 upregulated genes and 215 downregulated genes respectively (Figure 1(A); Supplementary Data 1). In addition, a total of 161 DEGs were identified in the fruiting bodies (FC > 2; p < 0.05): 135 upregulated genes and 26 downregulated genes (Figure 1(B); Supplementary Data 1). The DEGs from mycelia and fruiting bodies were compared and visualized in a venn diagram (Figure 2; Table 1).

Since more genes were identified in the mycelia than in the fruiting bodies, DEGs with significant fold changes in the mycelia were selected and validated by real-time PCR. Among the upregulated genes, the gene with FC > 2 were analyzed (Supplementary Table 1; Supplementary Data 2). We also validated the downregulated genes with FC < −2 (Supplementary Table 2; Supplementary Data 2). The data showed that 6 genes including cytochrome p450 and hydrophobin were differentially expressed between WT and mutant F. velutipes, consistently to the DNA microarray results (Supplementary Data 1; Table 2). Other four genes were selected from genes identified as being down-regulated in the mutant [39,40]. The representative ones and their differential expressions are summarized in Table 2. It is noted that cytochrome p450 and hydrophobin have been reported to play important roles in both the growth and development of fruits [41]. Therefore, our mRNA analysis results suggest that cytochrome p450 and hydrophobin could be implicated with the clogging phenomenon in F. velutipes.

3.2. Protein profiling of WT vs. mutant F. velutipes

Total proteins were collected from both WT and mutant F. velutipes and analyzed using 2 D-PAGE. Representative gels of 2D-PAGE were shown in Supplementary Figure 1. After gel electrophoresis, gel images were obtained, and image analysis was performed using the PDQuest image analysis software. The first 2D-gel included 1313 and 1008 spots, the second 2D-gel 1764 and 1789 protein spots, and the third 2D-gel 1211 and 1345 from normal and clogging mutant mycelium mushrooms (Supplementary Figure 1; Figure 3). Different protein spots between the WT and mutant samples

Figure 1. Differentially expressed genes in the mycelia and fruiting body. (A) Scatter plots of the DEGs in the mycelia, comparing the WT and mutant. N = 371 (upregulated, 156; downregulated, 215), log₂FC > 2, p value < 0.05; (B) Scatter plots of the DEGs in the fruiting body, comparing the WT and mutant. N = 161 (upregulated, 135; downregulated, 26), log₂FC > 2, p value < 0.05.

Figure 2. Summary of DNA microarray. Venn diagram summarizing DEGs identified through the DNA microarray analysis.
were screened and measured for their intensity. Most protein spots were concentrated between 10 and 96 kDa, with pI ranging from pH 6–9 (Figure 3). A total of 79 spots showed significantly increased or decreased protein expression and these were selected for mass spectrometry (MS) analysis (Supplementary Table 3).

### 3.3. Identification of differentially expressed proteins by mass spectrometry

The differential protein spots from the 2D-PAGE analyses were subjected to MALDI-TOF MS analysis, and the resulting tandem MS/MS scans with the protein spots were searched against the NCBI Fungi database using the Mascot Server. Although some preoteins have not been annotate, the results were determined using a cutoff value of $p < 0.05$ and categorized into upregulated and downregulated protein groups. Among the upregulated proteins in mutant mushrooms, 14 protein spots were identified, including heat shock protein 70 and 90 kDa, glyceraldheyde-3-phosphate dehydrogenase, phosphopyruvate hydratase, transaldolase, 20S proteasome subunit, actin and enolase (Table 3). We identified three proteins in the downregulated protein samples, including HSP70-domain-containing protein, elongation factor 2, and pyruvate kinase (Table 4).

### 4. Discussion

This study compared WT and mutant *F. velutipes* for gene expression using genomics and proteomics analyses. The DNA microarray and real-time PCR analyses showed that cytochrome p450 and hydrophobin were among the most significantly downregulated genes in mutant *F. velutipes* than in the WT (Table 1; Supplementary Table 2; Supplementary Data 1 and 2). As these two proteins participate in the construction of mushroom fruiting bodies [40], deregulation of these two proteins in the mutant mushrooms might lead to a poor quality of fruiting bodies. The expression of other genes such as C4-dicarboxylate transporter/malic acid transport protein, glyceralate- and formate-dehydrogenase, malate dehydrogenase, and endo-beta-1,3-glucanase was also decreased in mutant *F. velutipes*. These genes suggest that altered energy and glucose

---

**Table 1.** Commonly expressed DEGs in Mycelia (Myc) or fruiting bodies (Fruit).

| Gene expression | Gene ID | Annotation                  |
|-----------------|---------|-----------------------------|
| Fruit up vs. Myc up | EIW64561 | ATP-dependent DNA helicase |
| Fruit down vs. Myc down | XP_001876254 | Aspartic peptidase A1 |
| Fruit up vs. Myc down | EFY95978 | Metal dependent phosphohydrolase |

**Table 2.** Differentially expressed genes validated by real-time PCR.

| Gene name                               | Primers                     | Phenotype | Relative expression |
|-----------------------------------------|-----------------------------|-----------|---------------------|
| Cytochrome p450                          | Forward: TTGAAAGGTGTCGAGGAGGAGATG | WT        | 1                   |
| Hydrophobin                              | Reverse: TGAGGATATTGGGGGAAGATGGT | Mutant    | 0.3404972           |
| C4-dicarboxylate transporter/malic acid transport protein | Forward: CCGCTGGCCAGTGCAATGTT | WT        | 1                   |
| Glycerate-and formate-dehydrogenase      | Reverse: TGGATATAAGCCCCGGCCAGAA | Mutant    | 0.402826            |
| Malate dehydrogenase                     | Forward: CCGCTGGCCAGTGCAATGTT | WT        | 1                   |
| Endo-beta-1,3-glucanase                  | Reverse: CCGCTGGCCAGTGCAATGTT | WT        | 1                   |

**Figure 3.** In silico identification of differentially expressed genes. (A) 2D-PAGE image showing proteins that are differentially expressed in the mycelia of mutant mushroom and were subjected to MALDI-MS-MS analysis. Marked with red dots ($n = 79$). Significant expression variation $> 2$. 

---

*J.-R. WOO ET AL.*
metabolisms might be responsible for causing the clogging phenomenon in the mutant mushrooms.

In MALDI-TOF and bioinformatics analyses, unexpectedly, the DEGs identified through transcriptome analysis data were not detected. It is thought that the proteins for these genes have not been sequenced-determined, small, or could be difficult to be solubilized for separation. For example, hydrophobin is about 10 kDa and highly hydrophobic, appearing difficult to be solubilized and MS-determined. Malic acid transport protein is located in the mitochondria membrane that is also proposedly less soluble. Instead, we identified 14 upregulated and 3 downregulated proteins in the mutant mushroom (Tables 3 and 4). Heat shock proteins were the most prominent among the upregulated proteins: homologous to heat shock protein 90 kDa (HS90) from *Trametes versicolor*, cytochrome p450, hydrophobin, pyruvate kinase, and HSP70-domain-containing protein 90 kDa (HS70) from *Cladosporium cladosporioides* and *Coccidioides immitis*; and heat shock protein 70 kDa (HS70) from *Aspergillus nidulans*, *FGSCA4*, and *Trametes versicolor* (Table 3). Because heat shock protein expression is promoted in various stressful conditions, such as heat shock, cold, UV light, infection, inflammation, exercise, exposure of the cell to toxins, starvation, hypoxia, nitrogen deficiency, and dehydration, high levels of heat shock proteins could be evidence of more challenging mycelium environments or ineffective management/resolution of cellular stresses in the mutant *F. velutipes* with the clogging phenomenon than in the WT mushrooms [42]. In addition, actin and tubulin proteins are upregulated in the mutant mushroom (Table 3), suggesting that the over-production of skeletal proteins might be important for the clogging phenomenon. Another interesting protein, elongation factor 2, is an essential factor for protein synthesis. It promotes the GTP-dependent translocation of ribosomes in eukaryotes [43]. Pyruvate kinase also participates in translation, being involved in the last step of glycolysis, which produces ATP and pyruvate [44]. These proteins, elongation factor 2, pyruvate kinase, and HSP70-domain-containing proteins were identified to be downregulated in mutant mushrooms compared with the WT (Table 4). This suggests that the mutant mushrooms could have impaired translation and carbohydrate metabolism.

In this study, we suggest several deregulated genes in the clogging phenomenon of mushrooms, including cytochrome p450, hydrophobin, pyruvate kinase, and elongation factor 2 to be downregulated and heat-shock proteins and skeletal proteins to be increased in the clogging phenomenon of mushrooms. We suggest that the clogging phenomenon attributes to the deregulation of these genes in *F. velutipes*. Therefore, it might be possible to cure the clogging disease by restoring these genes for proper fruiting body formation in *F. velutipes*.

### Table 3. Identification of upregulated proteins between the WT and mutant *Flammulina velutipes* by MALDI-TOF mass spectrometry.

| SSP    | M/N   | Identification                                      | Species similarity     | Accession       |
|--------|-------|-----------------------------------------------------|------------------------|-----------------|
| 0011R  | 5.7371| Uncharacterized protein SCHEDRAFT_75321             | Schizophyllum commune H4-8 | XP_003054427   |
| 0013R  | 4.959 | H5T0_TRIRU Heat shock protein 70kDa                  | Aspergillus nidulans FGSCA4 | XP_662733      |
| 8112R  | 3.2005| Glyceraldehyde-3-phosphate dehydrogenase            | Flammulina velutipes    | AAQ08201       |
| 0513R  | 2.6972| Heat shock protein 70kDa, partial                   | Cladosporium cladosporioides | ACZ95780       |
| 0112R  | 2.1063| Rab GDP-dissociation inhibitor                      | Trametes versicolor FP-101664 SS1 | EIW63882       |
| 1011R  | 2.0033| Heat shock 70-kDa protein                           | Coccidioides immittis RS | XP_001248723   |
| 3007R  | 1.8245| Predicted protein                                   | Laccaria bicolor S238N-H82 | XP_001877157   |
| 0011R  | 1.7994| Beta-tubulin                                        | Suillus bovinus         | AAM49060       |
| 0503R  | 1.6934| Heat shock protein 90-kDa, protein                  | Trametes versicolor FP-101664 SS1 | EIW51690       |
| 5001R  | 1.6162| Glyceraldehyde-3-phosphate dehydrogenase            | Flammulina velutipes    | AAQ08201       |
| 1112R  | 1.5906| Actin 1                                             | Dichtomitussoequales LYAD-421 SS1 | EFS1774        |
| 0503R  | 1.5366| Predicted protein                                   | Laccaria bicolor S238N-H82 | XP_001874825   |
| 3406R  | 1.5282| Phosphoprophylatasehydratase                       | Laccaria bicolor S238N-H82 | XP_001874138   |
| 5513R  | 1.4497| Mov34-domain-containing protein                     | Flomtioprima mediterranea MF3/22 | EJD05421       |

### Table 4. Identification of downregulated proteins by MALDI-TOF mass spectrometry.

| SSP    | M/N   | Identification                                      | Species similarity     | Accession       |
|--------|-------|-----------------------------------------------------|------------------------|-----------------|
| 2906R  | 0.33  | HSP70-domain-containing protein                      | Coniothoraputea RWD-64-598 SS2 | EIW83554       |
| 6804R  | 0.30  | Elongation factor 2                                  | Schizosaccharomyces pombe | BAA23590       |
| 8703R  | 0.33  | Pyruvate kinase                                     | Trametes versicolor FP-101664 SS1 | EIW63538       |

Finally, this study was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education [2016R1A6A1A05011910] and Research Institute for Dokdo and Ulleung-do Island of Kyungpook National University, Korea.

**Ethical standards**

Not applicable.

**Disclosure statement**

No potential conflict of interest was reported by the author(s).

**Funding**

This study was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education [2016R1A6A1A05011910] and Research Institute for Dokdo and Ulleung-do Island of Kyungpook National University, Korea.


References

[1] Chen G, Fu Y, Yang W, et al. Effects of polysaccharides from the base of Flammulina velutipes stipe on growth of murine RAW264.7, B16F10 and L929 cells. Int J Biol Macromol. 2018;107(Pt B):2150–2156.

[2] Zhang T, Ye J, Xue C, et al. Structural characteristics and bioactive properties of a novel polysaccharide from Flammulina velutipes. Carbohydr Polym. 2018;197:147–156.

[3] Shi C, Wu Y, Fang D, et al. Nanocomposite packaging regulates extracellular ATP and programed cell death in edible mushroom (Flammulina velutipes). Food Chem. 2020;309:125702.

[4] Chen X, Fang D, Zhao R, et al. Effects of ultrasound-assisted extraction on antioxidant activity and bidirectional immunomodulatory activity of Flammulina velutipes polysaccharide. Int J Biol Macromol. 2019;140:505–514.

[5] Wu Z, Peng W, He X, et al. Mushroom tumor: a new disease on Flammulina velutipes caused by Ochrobasctrum pseudagrinognense. FEMS Microbiol Lett. 2016;363(2):fnv226.

[6] Liu ZH, Sossah FL, Li Y, et al. First report of Ewingella americana causing bacterial brown rot disease on cultivated needle mushroom (Flammulina velutipes) in China. Plant Dis. 2018;102(12):2633–2633.

[7] Oehmolt SW, Plathe E, Oyehaup I, et al. Gene regulatory networks generating the phenomena of additivity, dominance and epistasis. Genomics. 2000;155(2):969–980.

[8] Chen IMA, Chu K, Palaniappan K, et al. IMG/M v.5.0: an integrated data management and comparative analysis system for microbial genomes and microbiomes. Nucleic Acids Res. 2019;47(D1):D666–D677.

[9] Yamamoto K, Sasaki T. Large-scale Est sequencing in rice. Plant Mol Biol. 1997;35(1–2):135–144.

[10] Sim GK, Kafatos FC, Jones CW, et al. Use of a cDNA library for studies on evolution and developmental expression of the chorion multigene families. Cell. 1979;18(4):1303–1316.

[11] Gómez-Serrano M, Camafeita E, Loureiro M, et al. Mitoproteomics: tackling mitochondrial dysfunction in human disease. Oxid Med Cell Longev. 2018;2018:1435934.

[12] Whitfield CW, Band MR, Bonaldo MF, et al. Annotated expressed sequence tags and cDNA microarrays for studies of brain and behavior in the honey bee. Genome Res. 2002;12(4):555–566.

[13] Chang TW. Binding of cells to matrixes of distinct antibodies coated on solid surface. J Immunol Methods. 1983;65(1–2):217–223.

[14] Dudda-Subramanya R, Luccchese G, Kanduc D, et al. Clinical applications of DNA microarray analysis. J Exp Ther Oncol. 2003;3(6):297–304.

[15] Wu J, Baldwin IT. New insights into plant responses to the attack from insect herbivores. Annu Rev Genet. 2010;44:1–24.

[16] Chen Y, Barat B, Ray WK, et al. Membrane proteomes and ion transporters in Bacillus anthracis and Bacillus subtilis dormant and germinating spores. J Bacteriol. 2019;201:e00662–18.

[17] Dobson CM. 2000. The nature and significance of protein folding. In: Pain RH, editor. Mechanisms of protein folding. Oxford, Oxfordshire: Oxford University Press. P. 1–28.

[18] Koegl M, Uetz P. Improving yeast two-hybrid screening systems. Brief Funct Genomic Proteomic. 2007;6(4):302–312.

[19] Liu J, Chang M, Meng J, et al. A comparative proteome approach reveals metabolic changes associated with Flammulina velutipes mycelia in response to cold and light stress. J Agric Food Chem. 2018;66(14):3716–3725.

[20] Liu J, Men J, Chang M, et al. iTRAQ-based quantitative proteome revealed metabolic changes of Flammulina velutipes mycelia in response to cold stress. J Proteomics. 2017;156:75–84.

[21] Lin PI, Vance JM, Pericak-Vance MA, et al. No gene is an island: the flip-flop phenomenon. Am J Hum Genet. 2007;80(3):531–538.

[22] Fukushima M, Ohashi T, Fujiwara Y, et al. Cholesterol-lowering effects of maitake (Grifola frondosa) fiber, shiitake (Lentinus edodes) fiber, and enokitake (Flammulina velutipes) fiber in rats. Exp Biol Med. 2001;226(8):758–765.

[23] Rio DC, Ares M, Jr, Hannon GJ, et al. Purification of RNA using TRIzol (TRI reagent). Cold Spring Harb Protoc. 2010;2010(6):pdb.prot5439.

[24] Schroeder A, Mueller O, Stocker S, et al. The RIN: an RNA integrity number for assigning integrity values to RNA measurements. BMC Mol Biol. 2006;7:3.

[25] Magae Y, Sunagawa M. Characterization of a mycovirus associated with the brown discoloration of edible mushroom, Flammulina velutipes. Virol J. 2010;7:342.

[26] Rozen S, Skaltsky H. Primer3 on the WWW for general users and for biologist programmers. Methods Mol Biol. 2000;132:365–386.

[27] Min B, Yoon H, Park J, et al. Unusual genome expansion and transcription suppression in ectomycorrhizal Tricholoma matsutake by insertions of transposable elements. PLoS One. 2020;15(1):e0227923.

[28] Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 1976;72:248–254.

[29] Marcus K, Lelong C, Rabilloud T. What room for general users and for biologist programmers. Methods Mol Biol. 2000;132:365–386.

[30] Bandow JE, Baker JD, Berth M, et al. Improved large-scale 2-D gel-based proteomics image analysis workflow for 2-D gels enables twodimensional gel-based proteomics in a shotgun proteomics world? Proteomes. 2020;8(3):17.

[31] Bandow JE, Baker JD, Berth M, et al. Improved large-scale 2-D gel-based proteomics image analysis workflow for 2-D gels enables twodimensional gel-based proteomics in a shotgun proteomics world? Proteomes. 2020;8(3):17.

[32] Whitfield CW, Band MR, Bonaldo MF, et al. Annotated expressed sequence tags and cDNA microarrays for studies of brain and behavior in the honey bee. Genome Res. 2002;12(4):555–566.

[33] Chang TW. Binding of cells to matrixes of distinct antibodies coated on solid surface. J Immunol Methods. 1983;65(1–2):217–223.

[34] Dudda-Subramanya R, Lucchese G, Kanduc D, et al. Clinical applications of DNA microarray analysis. J Exp Ther Oncol. 2003;3(6):297–304.

[35] Wu J, Baldwin IT. New insights into plant responses to the attack from insect herbivores. Annu Rev Genet. 2010;44:1–24.

[36] Chen Y, Barat B, Ray WK, et al. Membrane proteomes and ion transporters in Bacillus anthracis and Bacillus subtilis dormant and germinating spores. J Bacteriol. 2019;201:e00662–18.

[37] Dobson CM. 2000. The nature and significance of protein folding. In: Pain RH, editor. Mechanisms of protein folding. Oxford, Oxfordshire: Oxford University Press. P. 1–28.
[34] Shevchenko A, Wilm M, Vorm O, et al. Mass spectrometric sequencing of proteins silver-stained polyacrylamide gels. Anal Chem. 1996;68(5): 850–858.

[35] Faserl K, Chetwynd AJ, Lynch I, et al. Corona isolation method matters: capillary electrophoresis mass spectrometry based comparison of protein Corona compositions following on-Particle versus in-Solution or in-Gel digestion. Nanomaterials. 2019;9(6):898.

[36] Welton JL, Khanna S, Giles PJ, et al. Proteomics analysis of bladder cancer exosomes. Mol Cell Proteomics. 2010;9(6):1324–1338.

[37] Sangha JS, Chen YH, Kaur J, et al. Proteome analysis of rice (Oryza sativa L.) mutants reveals differentially induced proteins during brown planthopper (Nilaparvata lugens) infestation. Int J Mol Sci. 2013;14(2):3921–3945.

[38] Sawahata T, Shimano S, Suzuki M. Tricholoma matsutake 1–ocean–3–ol and methyl cinnamate repel mycohagous Proisotoma minuta (Collembola: Insecta). Mycorrhiza. 2008;18(2):111–114.

[39] Vaario LM, Heinonsalo J, Spetz P, et al. The ectomycorrhizal fungus Tricholoma matsutake is a facultative saprotroph in vitro. Mycorrhiza. 2012; 22(6):409–418.

[40] Yamada M, Sakuraba S, Shibata K, et al. Isolation and analysis of genes specifically expressed during fruiting body development in the basidiomycete Flammulina velutipes by fluorescence differential display. FEMS Microbiol Lett. 2006;254(1): 165–172.

[41] Muraguchi H, Kamada T. A mutation in the eln2 gene encoding a cytochrome P450 of Coprinus cinereus affects mushroom morphogenesis. Fungal Genet Biol. 2000;29(1):49–59.

[42] Yang X, Zhao H, Wang Y, et al. The activation of Heat-Shock protein after copper (II) and/or arsenic (III)-induced imbalance of homeostasis, inflammatory response in chicken rectum. Biol Trace Elem Res. 2020;195(2):613–623.

[43] Rapp G, Klaudiny J, Hagendorff G, et al. Complete sequence of the coding region of human elongation factor 2 (EF-2) by enzymatic amplification of cDNA from human ovarian granulosa cells. Biol Chem Hoppe Seyler. 1989;370(10):1071–1075.

[44] Goodman HM. 2009. Basic medical endocrinology. 4th ed. Amsterdam, Netherlands: Elsevier; p. 132.