Contents lists available at SciOpen

Food Science and Human Wellness

journal homepage: https://www.sciopen.com/journal/2097-0765

Comparative proteomics analysis reveals the domesticated *Lepista sordida* primordium differentiation regulation mechanism and the subsequent different development patterns in the pileus and stipe

Xiuqing Yang[a], Meijie Liu[a], Yanjiao Zhang[a], Lizhong Guo[a], Junqi Wang[a], Sizhu Li[a], Yan Zhang[b], Xiaobo Li[c], Lili Xu[a,*], Hao Yu[a,*]

[a] *Shandong Provincial Key Laboratory of Applied Mycology, School of Life Sciences, Qingdao Agricultural University, Qingdao 266109, China*
[b] *Shandong Agricultural Technology Extending Station, Jinan 250100, China*
[c] *Shandong Mushroom Industrial Technology Innovation Research Institute, Jining 273500, China*

ARTICLE INFO

Article history:
Received 26 May 2023
Received in revised form 26 June 2023
Accepted 20 July 2023

Keywords:
Lepista sordida
Comparative proteomics
Primordial differentiation
Growth and development regulation
Pileus
Stipe

ABSTRACT

The wild *Lepista sordida* is a kind of precious and rare edible fungus. An excellent strain of it by artificial domestication was obtained, which was high-yield and high in iron content. In this study, high-throughput comparative proteomics was used to reveal the regulatory mechanism of its primordium differentiation in the early fruiting body formation. The mycelium before the primordium differentiation mainly expressed high levels of mitochondrial functional proteins and carbon dioxide concentration regulatory proteins. In young mushrooms, the highly expressed proteins were mainly involved in cell component generation, cell proliferation, nitrogen compound metabolism, nucleotide metabolism, glutathione metabolism, and purine metabolism. The differential regulation patterns of pileus and stipe growth to maturity were also revealed. The highly expressed proteins related to transcription, RNA splicing, the production of various organelles, DNA conformational change, nucleosome organization, protein processing, maturation and transport, and cell detoxification regulated the pileus development and maturity. The proteins related to carbohydrate and energy metabolism, large amounts of obsolete cytoplasmic parts, nutrient deprivation, and external stimuli regulated the stipe development and maturity. Multiple CAZymes regulated nutrient absorption, morphogenesis, spore production, stress response, and other life activities at different growth and development stages.

1. Introduction

Mushrooms are well-known functional foods with high protein, low fat, and low energy contents and the presence of a huge quantity of nutraceutical components. They are rich in minerals, bioactive constituents, and polysaccharides. Due to the occurrence of biologically active substances, mushrooms can serve as hepatoprotective, immune-potentiating, anti-cancer, anti-viral, and hypocholesterolemic agents[1]. *Lepista sordida*, commonly called the flesh-brown blewit, is one of the edible mushrooms with edible and medicinal values. It is a basidiomycete belonging to the Tricholomataceae and *Clitocyboid agaric* genus. It is characterized by a deep lilac or lilac-brown pileus color and pinkish spore prints. At present, it has been cultivated commercially[2].

L. sordida is very worthy of research and development. In several investigations, several significant amounts of compounds were mentioned to be present in its fruiting body, such as phenols, flavonoids, and ascorbic acid, which presented excellent free

* Corresponding authors at: Shandong Provincial Key Laboratory of Applied Mycology, School of Life Sciences, Qingdao Agricultural University, Qingdao 266109, China.
E-mail address: ellyxu@163.com (L.L. Xu); yuhao@qau.edu.cn, yuhaosunshine@163.com (H. Yu)
Peer review under responsibility of Tsinghua University Press.

Publishing services by Tsinghua University Press

http://doi.org/10.26599/FSHW.2023.9250051

radical scavenging potency showing potential as functional foods or additives[3]. Some new chlorinated sesquiterpenes and diterpenoids isolated from the mycelial culture broth of *L. sordida* could be used as inducers of the differentiation of human leukemic cells[4-5]. It was proved that lipopolysaccharides (LSP) from *L. sordida* could disturb the laryngocarcinoma HepG2 cell cycle and act on the process of cancer apoptosis induced by intrinsic mitochondrial caspase[6]. LSP exerted antitumor effects on HepG2 cells by inhibiting indolamide 2,3-dioxygenase via Janus kinase-protein kinase C-δ-signal transducer and activator of transcription (JAK-PKC-δ-STAT1) signaling pathway[7]. The intracellular polysaccharides (CLSP) and a novel alkali-soluble polysaccharide (LSAP) extracted from *L. sordida* mycelium in submerged culture could significantly inhibit the formation of malondialdehyde (MDA) and raise the activities of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px), which showed antioxidative and hepatoprotective activities[8-9]. The polysaccharide-iron (III) chelates isolated from the co-culture products of *L. sordida* and *Pholiota nameko* exhibited stronger hydroxyl radical and superoxide radical scavenging activity than the polysaccharides. Therefore, these chelates could be used as a potential better antiaging food and iron supplement[10]. *L. sordida* has potential application value in agriculture. After the treatments of the Cd and Cu to *L. sordida*, it could significantly increase the antioxidant enzyme activities in resisting stress. So *L. sordid* could be used to improve soil[11].

But now, the mechanism of *L. sordida* primordial differentiation and fruiting body growth and development is unclear. Some studies can provide references for this. Developmental morphology was used to summarize the developmental processes, time courses, and tissue structures of a few model species and some species of commercial interest to show the style of *Agaricomycetes* shaping their fruiting bodies[12]. Lower nitrogen and carbon concentration, low temperature or temperature downshift, starvation, and light can accelerate fruiting body induction, development, and maturation in some mushroom-forming fungi. Moreover, carbon dioxide concentrations can also affect fruiting body development[13]. Genome survey and transcriptome analysis reveal the genes regulating the mycelia growth and the primordia development in fruiting body formation, these genes are mainly associated with response to stress, ribosome biogenesis, arginine biosynthesis, and steroid biosynthesis pathway[14].

With the development of science and technology, omics technology has gradually walked into people's vision. Now omics technology in edible fungi has been flexibly combined with other research methods, involving multiple studies of edible fungus, such as genetic breeding, growth and development, stress resistance, and the use of special components in edible fungus as pharmaceutical additives[15-16]. Comparative transcriptomics of *Pleurotus eryngii* reveals blue-light regulation of carbohydrate-active enzymes (CAZymes) expression at primordium differentiated into fruiting body stage. And the CAZymes play important roles in the degradation of renewable lignocelluloses to provide carbohydrates for fungal growth, development, and reproduction[17].

The two-dimensional liquid chromatography-tandem mass spectrometry (LC-MS/MS) with comparative proteomic analysis has been used in many studies to analyze proteomic changes during the complex development of edible fungi[18]. For example, the proteomes covering the three key stages of *Ophiocordyceps sinensis* fruiting body development and the underlying biological causes of *O. sinensis* fruiting body development and plateau adaptation have been analyzed with the aid of this technique[19]. The great metabolic differences between the stipe and cap of the *Leurotus ostreatus* fruiting body have also been revealed by high-throughput proteomics[20].

At present, there was no report on proteomic research about *L. sordida* till this research. The genome information resources of *L. sordida* can provide a research basis for the deep interpretation of some physiological and biochemical phenomena, and it could be found in the database called "F-RINGS" (http://bioinf.mind.meiji.ac.jp/f-rings/)[21]. The complete mitochondrial genome sequence (57 375 bp) of *L. sordida* containing 20 protein-coding genes, 2 ribosomal RNA genes, and 26 transfer RNA genes was also used as a research basis[22]. In this study, we used LC-MS/MS to detect the domesticated *L. sordida* samples of different growth stages. The differentially expressed proteins (DEPs) between samples could be found through comparative proteomic analysis to study the mechanism of regulating mycelial maturation, primordium differentiation, fruiting body growth, and development of *L. sordida*. This study is expected to find out the key regulatory factors of its development and reproductive process, which provides a reliable reference for improving the production stability of edible fungi and broadening the development path of emerging varieties of edible fungi. This study also is helpful to further promote the popularizing and production of *L. sordida*.

2. Materials and methods

2.1 L. sordida strain domestication and cultivation

The initial *L. sordida* was collected from the grassland of Inner Mongolia, China, and tissue separation and purification were carried out in a sterile environment. After screening various nutritional factors, the most suitable medium for mycelia growth was finally determined as Medium A. After more than 20 times optimal mycelia transfer, the optimal mother species was obtained. After the culture in Medium B, the strong, dense, and snow-white mycelia with fast germination speed was obtained, which was the original species. After the culture in Medium C, the cultivated species were obtained. Medium D was 18 cm thick with 1.5% cultivated species. After inoculation for 14–20 days, loam or humus was covered on it once with a thickness of 3–4 cm.

Medium A: glucose 30 g, yeast powder 3 g, $MgSO_4$ 0.5 g, agar 20 g, water, each 1 L medium; Medium B: wheat kernel 87 g, bran 10 g, $(NH_4)_2SO_4$ 1 g, $CaSO_4$ 2 g, water, each 1 L medium; Medium C: 70% wheat straw and 30% cow manure, fermented for about 4 weeks before use; Medium D: 40% grass, 30% cow manure, 17% corn cob, 10% wheat bran, 2.5% gypsum, 0.5% $(NH_4)_2SO_4$, pH 6.4.

2.2 Nutrient composition detection of the domesticated strain fruiting body

The detection methods of various growth factors and trace elements were listed in Table S1.

2.3 Sample Material, total proteins acquisition, and Mass Spectrometry detection

L. sordida mycelium vegetative growth to reproductive growth, to fruiting body maturation process was divided into four growth time nodes: the mycelium (Myc), the young fruiting body (YFr), the small fruiting body (SFr), and the middle fruiting body (MFr). Each node corresponded to a relatively clear morphology of *L. sordida*. We obtained samples of 4 different time nodes according to different morphology on the same *L. sordida* strain once and for all. Finally, 6 test samples were obtained and named Myc, YFr, pileus of SFr (SFrP), stipe of SFr (SFrS), pileus of MFr (MFrP), stipe of MFr (MFrS). Three biological replicates were taken for each sample.

The proteome detection was performed by label-free LC-MS/MS Quantitative Proteomics Analysis technology (Benagen Co., Ltd., Wuhan, China). All the methods for the processes of protein extraction and digestion and the MS detection for total proteins could be found in the previous work content[23].

2.4 Protein identification and DEPs screening

The total protein sequences from these raw files were screened out with a false discovery rate (FDR) lower than 0.05 mapping to the *L. sordida* genome and proteome databases. Raw data were normalized by Proteome Discovery software suite version 2.0 (Thermo Fisher Scientific, San Jose, CA, USA)[23]. Firstly, missing values were supplemented by the k proximity method. And then, median standardization was executed on intensity data. After that, EdgeR was used to screen DEPs with $|\log_2 (\text{fold change})| > 1$ and P-value < 0.05. The DEPs expression difference was shown by a heatmap constructed by using TB tools.

2.5 Functional annotation of the DEPs

We performed Gene Ontology (GO) analysis by using the GO tool (http://geneontology.org; accessed on March 2023). The DEPs were annotated and enriched to different terms according to 3 classes cellular component (CC), molecular function (MF), and biological process (BP). The result was presented by using the TBtools.

The DEPs were also annotated and functionally enriched using UniProt and Pfam. The candidate DEPs mapping to the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways were retrieved by blasting against the KEGG database (https://www.genome.jp/kegg/pathway.html; accessed on March 2023). Some of the DEPs participated in several pathways after mapping in the KEGG database. The pathways were attributed to Metabolism (A09100), Brite hierarchies (A09180), Genetic information processing (A09120), and Cellular processes (A09140).

2.6 DEPs co-expression analysis

The String database was used to accurately identify and annotate all expressive interacting DEPs (https://cn.string-db.org/cgi/). The co-expression network of the DEPs in each sample could be constructed by Cytoscape. The minimum required interaction score of the confidence was set to be 0.1. Proteins within the network that were highly correlated to each other tend to be aggregated together. Some proteins with high connectivity degrees in the network were identified as hub DEPs, which were believed to play core roles in the co-regulation processes. The weight value of the hub DEPs edge was set to be greater than 1. The DEPs closely contacted with the hubs were hub-surrounded DEPs, which might assist the hubs.

2.7 Analysis of CAZymes

DbCAN database for CAZymes families (based on CAZyDB 07/15/2016) was used to annotate the identified proteins. And the result was exhibited by the script of hmmscan-parsersh (https://github.com/carden24/Bioinformatics_scripts/blob/master/hmmscan-par-se.sh). All the CAZyme sequences were put together to construct phylogenetic trees by using MEGA7 software and the Neighbor-Joining method.

2.8 The proteomic data were validated by real-time quantitative polymerase chain reaction (RT-qPCR)

Total RNA and cDNA were prepared according to the description of the previous job[24]. The threshold cycle values were normalized by the expression level of the 18S rRNA gene[25]. RT-qPCR reactions were performed in a volume of 15 μL on a LightCycler® 96 Real-Time PCR System (F. Hoffmann-La Roche Ltd., Basel, Switzerland) with ChamQ Universal SYBR qPCR Master Mix (Sparkjade, Shandong, China) according to the manufacturer's instruction. Expression levels were calculated according to the $2^{-\Delta\Delta Ct}$ method.

3 Results and discussion

3.1 The domesticated L. sordida strain and its nutrient composition compared with Agaricus bisporus

After artificial domestication, we finally obtained a strain with an excellent appearance (Fig. 1A) and was named *L. sordida* (Schum.:Fr.) Sing strain MCCWT230001. *L. sordida* is a grass-rotting edible fungus same to *A. bisporus*, which is different from wood-rotting edible fungi consuming a large number of forest resources every year, such as *Lentinus edodes*, *Auricularia auricula*, *Ganoderma lucidum*, etc. This domesticated strain tastes delicious and is rich in a variety of nutrients needed by the human body. We have detected various growth factors, trace elements, and crude polysaccharides in its fruiting bodies and their contents were listed (Table S1). The iron (Fe) content was 122.682 μg/g dry weight (DW) sample, which was higher than that of commercial *A. bisporus* (49.300 μg/g DW)[26]. For the first time, we realized the large-scale cultivation of *L. sordida*. The tested yield was as high as 27 000–30 000 kg/hectare per batch. Two batches could be executed in a year.

3.2 Label-free LC-MS/MS technology enabled the high-throughput proteomic analysis of the L. sordida mycelium and fruiting body samples.

To examine the key regulatory proteins in the transition from vegetative growth to reproductive growth of *L. sordida* and the formation of fruiting bodies, we collected 6 *L. sordida* samples (Figs. 1B–E) and extracted the total proteins, which would be detected by Label-free LC-MS/MS technology. Totally, 2 906 proteins were

identified (Table S2). Principal component analysis (PCA) showed the differences among the groups and the consistency of the biological duplications. The Myc was significantly different from the others, SFrP was close to MFrP, and SFrS was close to MFrS. These results indicated that the data was valid and could be used for further analysis (Fig. 2A).

3.3 Samples grouping and screening of the DEPs

We compared the protein expression of samples before primordium formation Myc with that of samples after primordium formation YFr, looking for key proteins that regulate primordium differentiation and form young fruiting bodies. In addition, the protein expression in the pileus and stipe samples from the same growth time node were compared to find the respective key regulating proteins for the growth, development, and maturation of the stalk and cap. The DEPs were screened out when YFr *vs*. Myc, SFrP *vs*. SFrS, and MFrP *vs*. MFrS separately (Fig. 2B). Totally 622 DEPs were obtained by gathering all the DEPs together (Table S3). There are 61 highly expressed DEPs in Myc and 275 in YFr (Table S4). There are 78 highly expressed DEPs in SFrP and 43 in SFrS (Table S5). There are 175 highly expressed DEPs in MFrP and 82 in MFrS (Table S6). The heatmap showed the distribution of DEPs in each group (Fig. 2C). Myc differed the most from the other groups. The similarity between SFrP and MFrP was high. 33 were present in both SFrP and MFrP, 45 were present only in SFrP, and 142 were present only in MFrP (Fig. 2D). SFrS and MFrS had a certain similarity. 12 were present in both SFrS and MFrS, 31 were present only in SFrS, and 70 were present only in MFrS (Fig. 2E).

3.4 Primordium differentiation in the early development of the fruiting body

3.4.1 The analysis for the highly expressed DEPs in Myc

The GO analysis of the DEPs in Myc was shown. There were 13 terms significantly enriched ($P < 0.05$), mainly including the BP terms of small molecule metabolic process, carboxylic acid/oxoacid/organic acid metabolic process, amino acid/alpha-amino acid metabolic process, and carboxylic acid/organic acid biosynthetic process ($P < 0.01$); and the CC term of the mitochondrion ($P < 0.01$). The corresponding DEPs and their annotations, expression ratio, and *P*-value were listed. (Fig. 3A and Table S7).

The KEGG pathway enrichment has been analyzed for Myc and 2 KEGG pathways ($P < 0.05$) were enriched. They were both involved in metabolism, including amino acid metabolism ($P < 0.01$) and energy metabolism ($P < 0.05$) pathways. The detailed KEGG information was listed. (Fig. 4A and Table S8).

For Myc, there were 26 DEPs were functionally related and connected into a network with the help of the co-expression analysis (Fig. 5A). Most of them were mitochondrion (GO: 0005739) or related components. It was known that mitochondria were organelles that played an important role in both the energetic and synthetic metabolism of eukaryotic cells. 9 DEPs were found to be associated with energy metabolism (KEGG: B 09102). The hub DEPs were mitochondrial ATP synthase subunit (MDBLsor1_04729) and glutamate synthase (NADH; GOGAT, MDBLsor1_05852). 33 DEPs were relevant to the amino acid metabolism process (KEGG: B 09105). The MDBLsor1_05852, aminotran domain-containing protein (MDBLsor1_00577), and aspartate aminotransferase (MDBLsor1_03207) were the hub. MDBLsor1_05852 was involved in both energy and amino acid metabolism, which played a crucial role in ammonium assimilation and glutamate biosynthesis[27]. Glutamic acid went through the gluconeogenic pathway to produce glucose or glycogen. MDBLsor1_05852 and its more closely related proteins were more likely to store energy. Most of the others were related to mitochondrial function, such as the proline-rich-polypeptides 1_N domain-containing protein (MDBLsor1_00184)[28], armadillo repeat-containing protein (MDBLsor1_03585)[29], and DNA damage repair (MDBLsor1_02970)[30]. The DEPs involved in all networks were marked in yellow in Table S3.

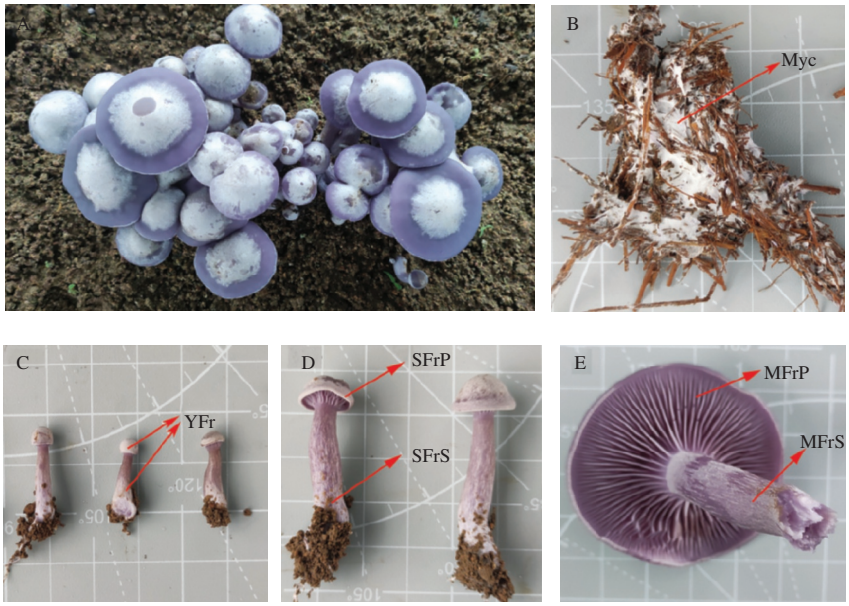

Fig. 1 Six *L. sordida* samples were collected in 4 growth time nodes and used for the proteomic study. (A) Fresh *L. sordida* in cultivation. (B) Myc. (C) YFr. (D) SFr, SFrP, and SFrS. (E) MFr, MFrP, and MFrS. The square size of the background is 1.0 cm × 1.0 cm.

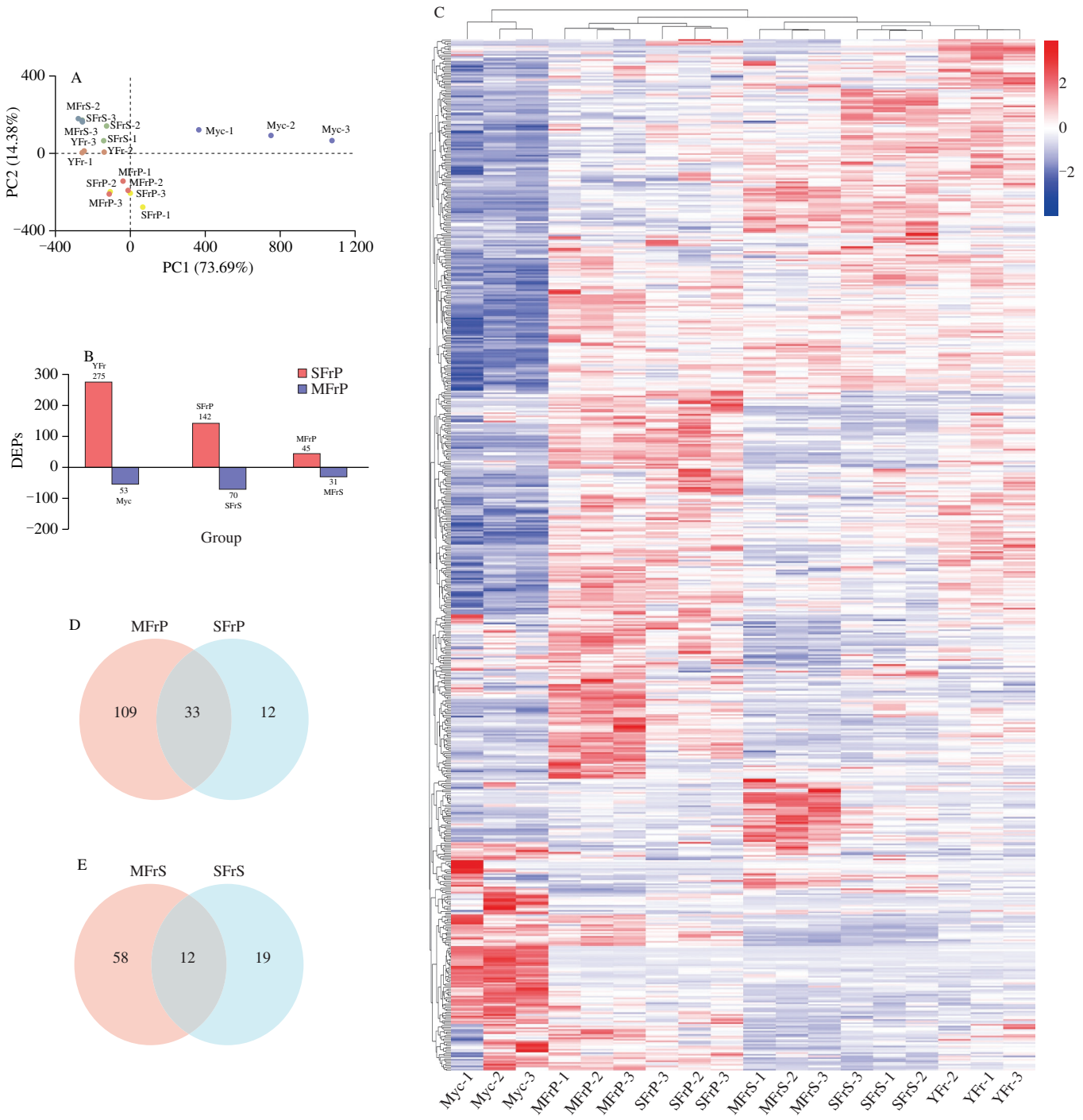

Fig. 2 PCA and statistical overview of the DEPs. (A) PCA plot showed the clusters of 6 samples based on their similarity. (B) The bar chart showed the highly expressed DEPs number of 6 samples obtained by YFr *vs*. Myc, SFrP *vs*. SFrS, and MFrP *vs*. MFrS. (C) The heatmap showed the different expressions of all the DEPs in each group. The Venn diagrams showed the number of the same and the different highly expressed DEPs in (D) SFrP *vs*. MFrP and (E) SFrS *vs*. MFrS.

3.4.2 The analysis for the highly expressed DEPs in YFr

In YFr, 34 highly expressed GO terms ($P < 0.05$) were enriched. The most significant terms ($P < 0.01$) distributed to BP were organonitrogen compound/amide metabolic process and biosynthetic process, protein metabolic process and regulation, small molecule/carboxylic acid metabolic or biosynthetic process, cellular component/protein-containing complex organization or assembly, organic substance/macromolecule catabolic process, regulation of molecular function/atalytic activity, and mitotic cell cycle/process; to CC were cytoplasm, cytosol, site of polarized growth, and obsolete cell cortex part ($P < 0.01$); to MF were molecular function regulator activity and enzyme regulator activity terms ($P < 0.01$; Fig. 3A and Table 9).

Totally 20 KEGG pathways ($P < 0.05$) were obtained from the DEPs in YFr. It was found that 10 of them were involved in metabolism, mainly including amino acid/other amino acids/alanine, aspartate, and glutamate metabolism, nucleotide metabolism, amino

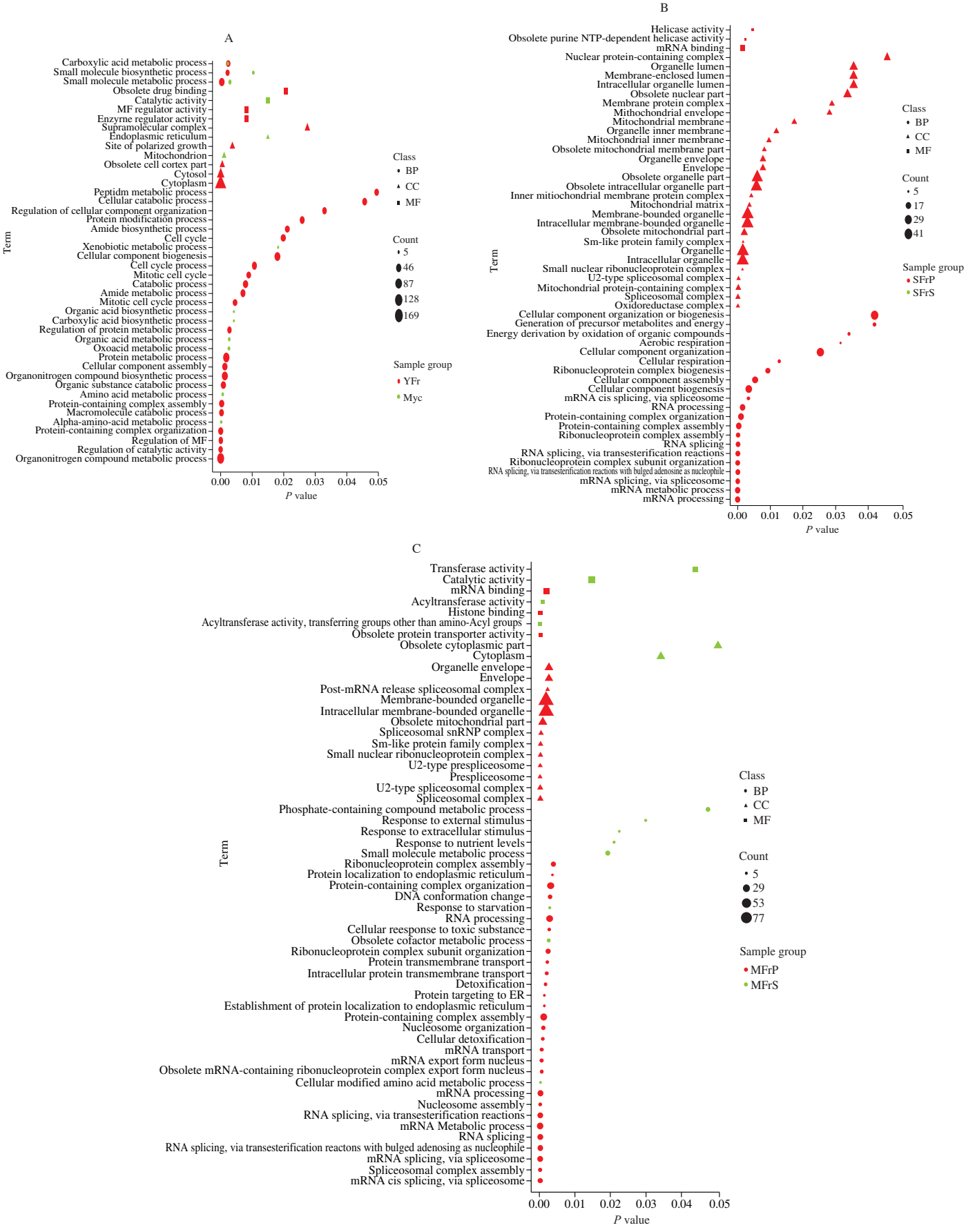

Fig. 3 The GO analysis of the highly expressed DEPs. (A) Myc and YFr. (B) SFrP and SFrS. (C) MFrP and MFrS. The *P*-value ranges from 0.00 to 0.05 and the closer it is to 0, the more significant.

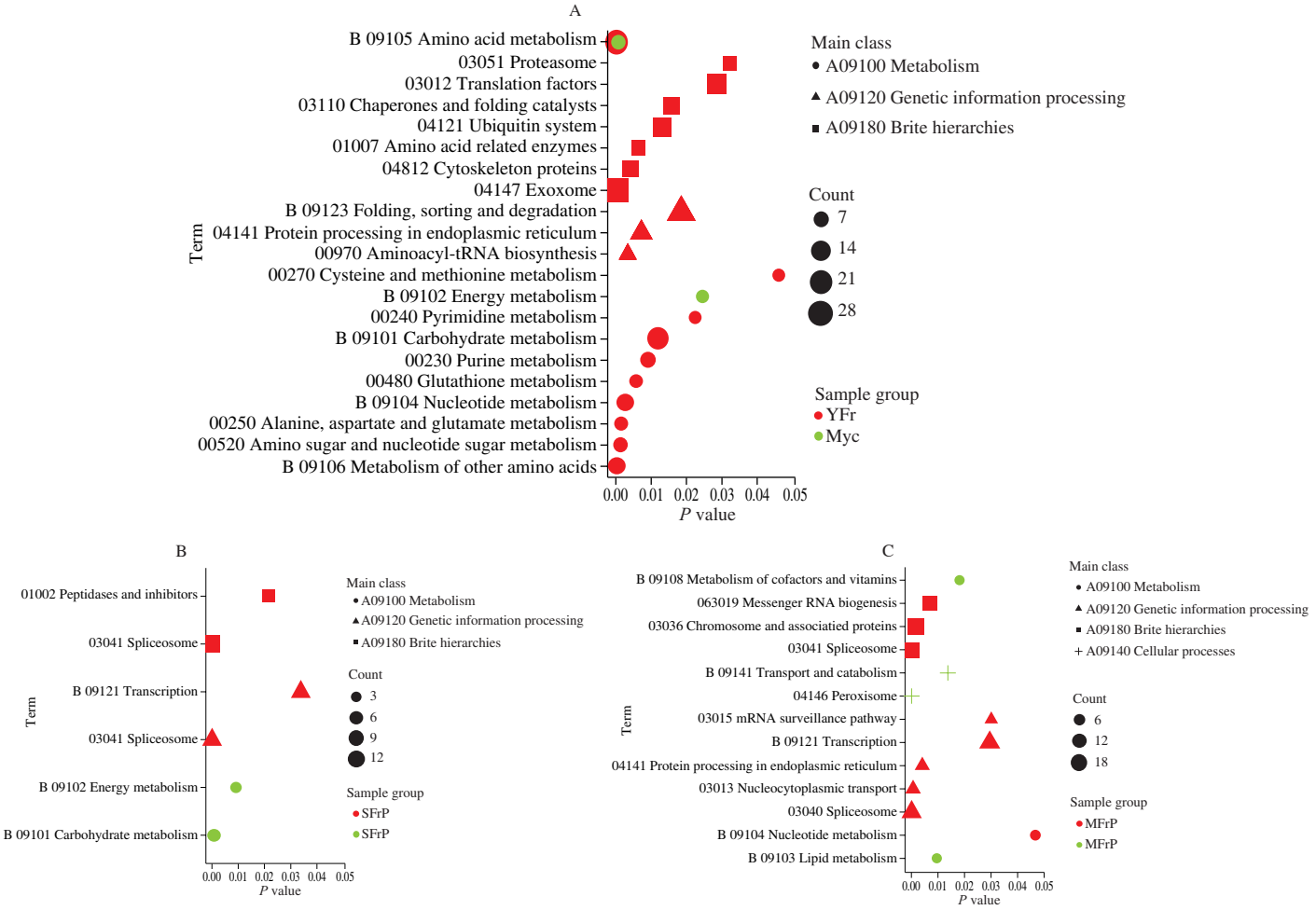

Fig. 4 The KEGG pathway analysis of the highly expressed DEPs. (A) Myc and YFr. (B) SFrP and SFrS. (C) MFrP and MFrS. The *P*-value ranges from 0.00 to 0.04 and the closer to 0, the more significant it is.

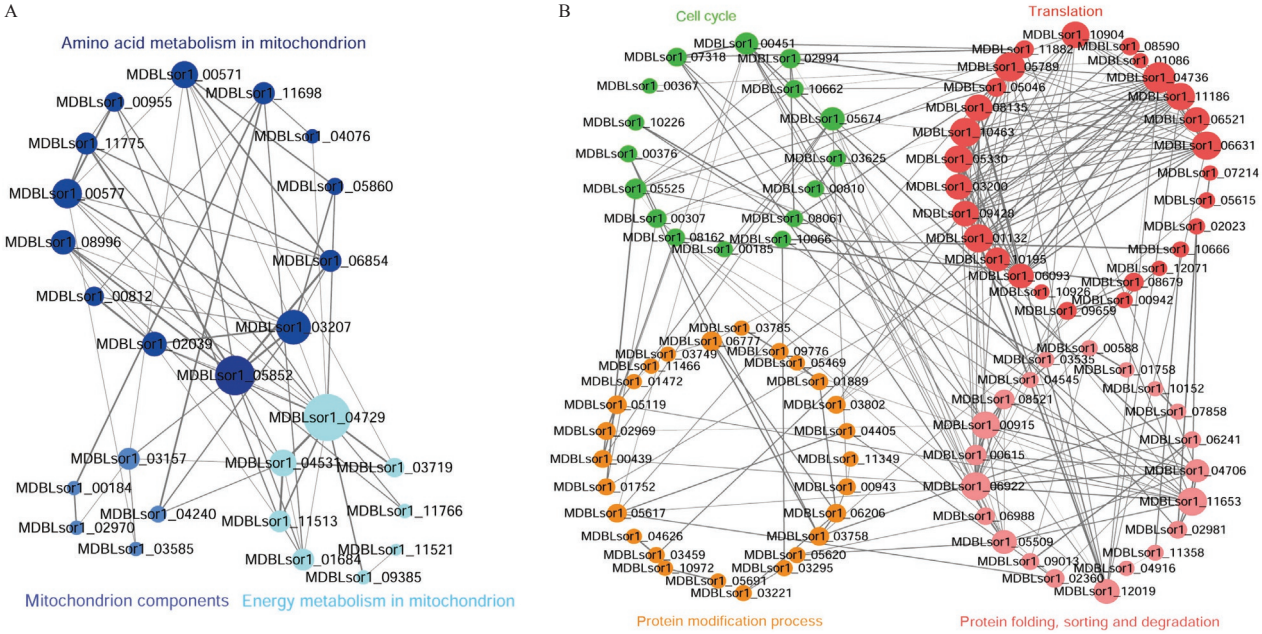

Fig. 5 The co-expression network diagrams of the functionally related DEPs from the (A) Myc, (B) YFr, (C) SFrS, (D) SFrP, (E) MFrP, and (F) MFrS. The circle nodes represent DEPs and the node size stands for the connectivity value of proteins. The DEPs with high connectivity value could be identified as hub DEPs. The straight lines represent edges and the width of a straight line stands for the weight value. The larger the weight value between two DEPs, the closer the relationship between them.

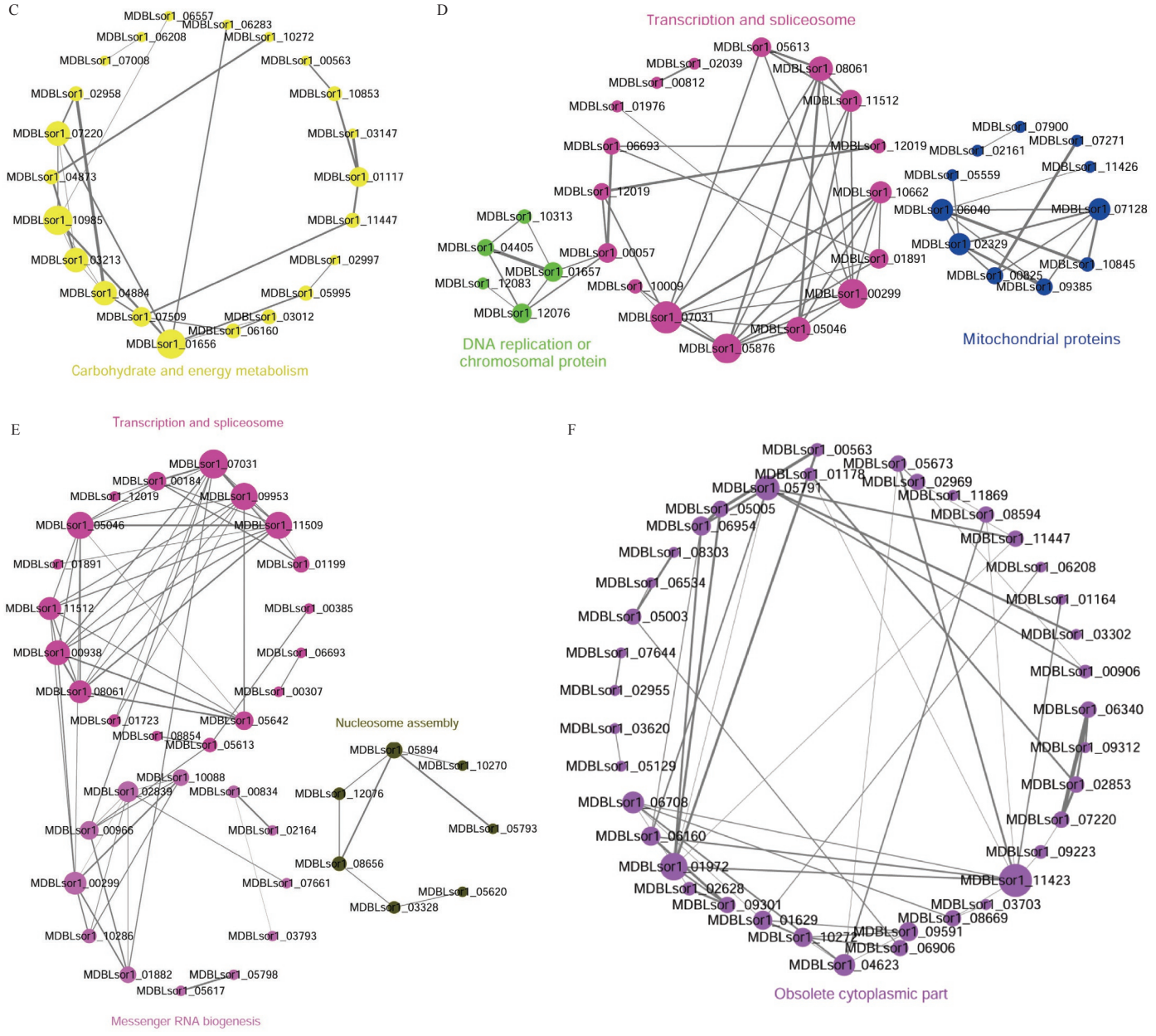

Fig. 5 (*Continued*)

sugar and nucleotide sugar metabolism, glutathione metabolism, and purine metabolism pathways ($P < 0.01$). Moreover, 3 of them were related to genetic information processing, dominantly containing Protein processing in the endoplasmic reticulum (ER) and aminoacyl-tRNA biosynthesis pathways ($P < 0.01$), 7 of them were related to brite hierarchies, primarily comprising exosomes, amino acid-related enzymes, and cytoskeleton proteins pathways ($P < 0.01$; Fig. 4A and Table S10).

There were 90 DEPs connected to a network (Fig. 5B), and 27 of them were related to protein translation. They were translation factors (KEGG: 03012) and amino acid-related enzymes (KEGG: 01007). The hub DEPs were eukaryotic translation initiation factor (MDBLsor1_03200, MDBLsor1_11186, MDBLsor1_06631, MDBLsor1_01132), SUI1/S1 motif domain-containing protein (MDBLsor1_06093, MDBLsor1_10463), and ATP-dependent RNA helicase eIF4A (MDBLsor1_05330). Besides, 26 DEPs mainly participated in protein modification process (BP; GO: 0036211), 21 were related to protein folding, sorting, and degradation (KEGG: B 09123), and some of them were proteasome (KEGG: 03051) and ubiquitin system proteins (KEGG: 04121). The hub DEPs were associated with diverse cellular activity (AAA) domain-containing protein (MDBLsor1_06922), Mpr1/Pad1 N-terminal (MPN) domain-containing protein (MDBLsor1_00915), and AAA ATPase (MDBLsor1_11653). The other 16 DEPs were mainly taken part in the cell cycle (BP; GO: 0007049, 0022402).

3.4.3 The key regulatory protein for primordium differentiation in the early development of the fruiting body

These results indicated that there were great catalytic differences within the cells of the YFr and Myc. The mitochondrial function was especially active in Myc, which indicated that mycelium growth was

exuberant at this time. The involved proteins very likely affected the initiation differentiation of the primordium. Interestingly, another 2 DEPs outside the networks were related to response to the fluctuation of intracellular carbon dioxide concentration also highly expressed, which could help *L. sordida* adapt to the high carbon dioxide concentration due to efficient aerobic respiration. One is the carbonic anhydrase (MDBLsor1_11521), which catalyzes the interconversion of carbon dioxide and bicarbonate[31]. The other is the glycosylphosphatidylinositol (GPI)-anchored serine-rich protein (MDBLsor1_07792), which could be induced by low temperature and anaerobic growth conditions[32]. High ventilation and low carbon dioxide concentration can stimulate the primordium to differentiate toward the cap and inhibit the growth of the stipe[33]. Thus these 2 proteins might also play key regulatory roles in the initial differentiation of the primordium. Another 4 DEPs in Myc were related to answering the oxidative stress. The glyoxalases (MDBLsor1_06276, MDBLsor1_11689) were involved in the detoxification of methylglyoxal (MG) into *D*-lactic acid through a single-step process. MG could cause oxidative damage by triggering the generation of reactive oxygen species (ROS)[34]; the aldehyde dehydrogenase (MDBLsor1_04012) was closely related to the metabolism of aldehydes produced under oxidative stress[35]; the organic hydroperoxide resistance protein (MDBLsor1_05045) could reduce fatty acid peroxides and peroxynitrite, 2 oxidants relevant in host-pathogen interactions[36]. These proteins also laid the foundation for primordial differentiation.

After the primordium differentiation in the early development of the fruiting body, the YFr formed. This process was complex and more proteins were involved. The activities of protein synthesis, protein processing in the ER, protein exocrine, cell components assembly, cytoskeletal protein synthesis, organonitrogen compound and amide metabolism, amino acids metabolism, cell cycle, nucleotide metabolism, amino and nucleotide sugar metabolism, glutathione metabolism, and purine metabolism pathway were especially active. All these related proteins played important roles in the primordium differentiation into the pileus and stipe of the young fruiting body. Some other proteins were also noteworthy. For example, the BTB domain-containing proteins (MDBLsor1_06155, MDBLsor1_07416) and subtilisin-like protease (MDBLsor1_05000) had effects on sclerotial formation, conidiation, and virulence[37-38]. The BTB domain-containing proteins could also regulate melanin and toxin synthesis. The *L*-dihydroxyphenylalanine 4,5-dioxygenase (MDBLsor1_06284) was a key enzyme in the biosynthesis of betaine, a reddish purple and yellow pigment derived from tyrosine[39]. The flavin adenine dinucleotide/nicotinamide adenine dinucleotide (phosphate) [FAD/NAD(P)]-binding domain-containing protein (MDBLsor1_10058) was involved in the morphological development of primordium, and cell adhesion and affect the structure of cellulosic and non-cellulosic cell walls of fruit body development, and acted as a photoreceptor of blue light signaling for fruit body and pigment development, respectively[40]. These 3 proteins could not only affect the development of primordium and fruiting body but also might form fruiting body color. After all, anthocyanidin was not detected in the fruiting body (Table S1). Another protein serine/threonine-protein phosphatase (MDBLsor1_10926) could participate in several cellular processes, transcription, translation, differentiation, cell cycle, signal transduction, and Ca^{2+} dependent signal transduction pathways[41]. Ca^{2+} had a potential role in primordium differentiation and cap and stipe development by affecting calcineurin, a serine/threonine protein phosphatase[42]. In addition, the cerato-platanin (MDBLsor1_07644) could induce necrosis, and change chemotaxis and locomotion[43]. The CCHC-type domain-containing protein (MDBLsor1_01202) was involved in the positive regulation of signaling[44]. And there was an autophagy-related protein 29 (MDBLsor1_01144). These 3 proteins might be related to the growth and development of *L. sordida*. Another 3 DEPs in FYr were related to answering OS and detoxification. The glutathione *S*-transferase (GST, MDBLsor1_00291) was involved in the detoxification of noxious compounds and protection against oxidative damage[45]. The cyanate hydratase (MDBLsor1_07649) participated in cyanate detoxification[46]. The haloacid dehalogenase (HAD)-like protein (MDBLsor1_02909) was implicated in the detoxification of phosphorylated compounds and pseudouridine[47]. They might also be vital regulatory proteins.

Next, we continued to explore the different differentiation patterns between the pileus and stipe.

3.5 Regulation of pileus growth and development

3.5.1 The analysis for the highly expressed DEPs in SFrP

In SFrP, there were 52 GO terms ($p < 0.05$) were enriched from the highly expressed DEPs compared with SFrS. The BP terms mainly consisted of the cellular component biogenesis/assembly, mRNA splicing/*cis* splicing/processing/metabolic process, RNA splicing via transesterification reactions, Protein-containing complex organization/assembly, ribonucleoprotein complex assembly/organization/biogenesis ($P < 0.01$). The CC mainly distributed to a lot of terms ($P < 0.01$), such as organelles-related components (especially membrane components), mitochondrial-related components (especially membrane components), spliceosomal complex, Sec1/Munc18 (Sm)-like protein family complex, oxidoreductase complex, and small nuclear ribonucleoprotein complex. The MF was concentrated in the mRNA binding and obsolete purine NTP-dependent helicase activity terms ($P < 0.01$; Fig. 3B and Table S11).

From the highly expressed DEPs in SFrP *vs*. SFrS, there were 4 pathways significantly enriched ($P < 0.05$). They were involved in genetic information processing or Brite hierarchies, dominantly comprising spliceosome pathways ($P < 0.01$; Fig. 4B and Table S12).

There were 33 DEPs connected from SFrP (Fig. 5D), 17 of them were also mainly related to transcription (KEGG: B 09121) and spliceosome (KEGG: 03040, 03041). The hub DEPs were MPN domain-containing protein (MDBLsor1_07031), pre-mRNA-splicing factor (MDBLsor1_05876, MDBLsor1_05046), ruvB-like helicase (MDBLsor1_00057), and RNA-recognition motif (RRM) domain-containing protein (MDBLsor1_08061). Another 11 were mitochondrial proteins (CC; GO: 0098798), especially the components on the mitochondrial membrane (CC; GO: 0005740, 0140513, 0043227, 0043231). The other 5 were proteins related to DNA replication or chromosomal protein.

3.5.2 The analysis for the highly expressed DEPs in MFrP

In MFrP, there were 85 GO terms ($P < 0.05$) were enriched compared with MFrS. The BP chiefly consisted of the terms

($P < 0.01$) of protein-containing complex organization/assembly, mRNA metabolic process/splicing (via spliceosome)/*cis* splicing/processing/export from nucleus/transport, RNA processing/splicing (via transesterification reactions), spliceosomal complex assembly, ribonucleoprotein complex biogenesis/assembly/organization, Nucleosome organization/assembly/export from nucleus, protein localization and targeting to ER, protein transmembrane transport, cellular detoxification, DNA conformation change, and negative regulation of chromosome organization; the CC terms were included the terms ($P < 0.01$) of intracellular/membrane-bounded organelle, (organelle) envelope, mitochondrial membrane/envelope/protein-containing complex, obsolete mitochondrial part; U2-type/post-mRNA release/catalytic step 2 spliceosomal complex, U2-type prespliceosome, Sm-like protein family complex, small nuclear ribonucleoprotein complex, condensed chromosome, nuclear replication fork; The MF terms primarily projected to mRNA binding, histone binding, and obsolete protein transporter activity (Fig. 3C and Table S13).

In MFrP, there were 9 pathways ($P < 0.05$) were screened out for the highly expressed DEPs compared with MFrS. It was found that 5 of them were related to genetic information processing, dominantly containing the pathways of the spliceosome, protein processing in the ER, and nucleocytoplasmic transport ($P < 0.01$); 1 pathway involved in metabolism was nucleotide metabolism ($P < 0.05$); 3 pathways belonging to Brite hierarchies were spliceosome, chromosome and associated proteins, and messenger RNA biogenesis ($P < 0.01$); (Fig. 4C and Table S14).

From MFrP, 37 DEPs were connected to a network (Fig. 5E), 18 of them were components of the spliceosome (KEGG: 03040, KEGG: 03041) or related to transcription (KEGG: B 09121) pathways. The hub DEPs were the MPN domain-containing protein (MDBLsor1_07031), pre-mRNA-splicing factors (MDBLsor1_05046, MDBLsor1_09953), U1 small nuclear ribonucleoprotein C (MDBLsor1_00938), SF3b1 domain-containing protein (MDBLsor1_11509), and RRM domain-containing protein (MDBLsor1_08061). Besides, 12 were related to messenger RNA biogenesis (03019). The hub DEPs were the nuclear-cap-binding protein subunit 1 (MDBLsor1_00299) and nuclear pore protein (MDBLsor1_02839). The last 7 DEPs participated in nucleosome assembly (BP; GO: 0006334).

3.5.3 The key regulatory protein for pileus development

There were 33 DEPs both highly expressed in SFrP and MFrP (Fig. 2D), which might play continuous regulatory roles in the growth and development of pileus, 10 of them were related to spliceosome (KEGG: 03040, 03041) and transcription (KEGG: B 09121) pathways (Table S15). Additionally, 11 of them were components on mitochondria membrane, which acted on the energy derivation by oxidation of organic compounds or cellular respiration (BP; GO: 0045333, 0015980) processes (Table S16). Another 4 were correlated to the pathogenicity or defense of *L. sordida*. The peptidase A1 domain-containing protein (MDBLsor1_00812) and aspzincin_M35 domain-containing protein (MDBLsor1_08202) were peptidase or inhibitor (KEGG: 01002), which were virulence protein used to attack the host[48]. The heat shock protein Hsp90 (MDBLsor1_00768) played a role in fungal growth, cell survival, and pathogenicity[49]. The expression of carboxylic ester hydrolase (MDBLsor1_07701) along with several thioredoxin- and glutathione-dependent oxidoreductases participated in the biodegrading the toxicity of triphenyl phosphate (TPhP)[50]. Another 4 of them were nucleus components (CC; GO: 0005634) or nuclear-protein-containing complex (CC; GO: 0140513), including the nop domain-containing protein (MDBLsor1_06693), histone H2B and H4 (MDBLsor1_10313, MDBLsor1_12076), and chromo domain-containing protein (MDBLsor1_12083), which participated in the processes of the nucleosome organization (BP; GO: 0034728) or DNA conformation change (CC; GO: 0071103). The rest DEPs were mainly related to the modification and processing of proteins. The L-type lectin-like domain-containing protein (MDBLsor1_04233) played crucial functions in the trafficking, sorting, and targeting of maturing glycoproteins in the secretory pathway[51]. The disruption of this protein might weaken the plant's responses to abiotic and biotic stress[52].

The highly expressed DEPs only in SFrP were mainly related to the processes of transcription and RNA splicing, mitochondria and the other membrane organelles assembly, and epigenetic modification. There were another 3 DEPs related to vesicle function and exocytosis. The prenylated rab acceptor 1 (PRA1) family protein (MDBLsor1_04515) facilitated the Rab GTase delivery to the membrane by the dissociation of the Rab-GDP dissociation inhibitor (Rab-GDI) complex during vesicle transport[53]. The CAP64 protein (MDBLsor1_02336) might be involved in intracellular acidification and vesicle secretion through exocytosis[54]. The exocyst complex component Sec8 (MDBLsor1_03412) was a component of the extracapsular complex involved in exocytosis[55]. Interestingly, there were 4 DEPs correlated to pathogenicity or defense prominently expressed in SFrP. The P-loop containing nucleoside triphosphate hydrolase protein (MDBLsor1_04084) had a role in pathogen recognition and disease resistance linked to the significantly associated genomic regions in wheat to resist the leaf rust disease[56]. The subtilisin-like protease (MDBLsor1_05000) related to virulence was also highly expressed in SFrP. The J domain-containing protein (MDBLsor1_11756, MDBLsor1_00854) was a functional chaperone of the 70 kDa heat shock protein (Hsp70) and mediated various cellular processes, including stress tolerance, by regulating the activity of Hsp70[57].

More complex regulatory processes arose in MFrP than SFrP and more proteins were involved. There were up to 33 mitochondrial parts DEPs, mainly including the membrane (CC; GO: 0031966), protein-containing complex (CC; GO: 0098798), obsolete parts (CC; GO: 0044429), or matrix (CC; GO: 0005759) (Table S17). In addition to the assembly of membrane organelles, transcription, and RNA splicing, the processes of protein processing in the ER and transport, cellular detoxification, and DNA and chromosome conformation changes were also more active. Up to 37 were nucleus components (CC; GO: 0005634), nuclear-protein-containing complex (CC; GO: 0140513), or chromosomes, which participated in the processes of the nucleosome organization (BP; GO:0034728), DNA conformation change (CC; GO: 0071103), DNA mismatch repair, or epigenetic modification (Table S18). Another 6 DEPs were correlated to the pathogenicity or defense of MFrP and participated in the processes of detoxification (BP, GO: 0098754; BP, GO: 0097237; BP, GO: 0009636; Table S19). Another 6 DEPs were related to protein synthesis, stability, modification, and transport.

The phenylalanine-tRNA ligase (MDBLsor1_05782) was an essential enzyme for protein biosynthesis[58]. The STAS domain-containing protein (MDBLsor1_04221) was vital for transporter activity and biosynthesis/stability[59]. The ER or vesicle proteins, tetratricopeptide repeat (TPR)_region domain-containing protein (MDBLsor1_07537), *S*-adenosyl-*L*-methionine methyltransferase ERG6 *S*-adenosylmethionine: sterol C24-methyltransferase (SAM_MT_ERG6_SMT) domain-containing protein (MDBLsor1_10152), ER-derived vesicles protein ERV14 (MDBLsor1_04580), and protein SEY1 (MDBLsor1_04639), were involved in protein processing and modification[60-62]. The urothelial bladder cancer core domain-containing protein (MDBLsor1_00763), BTB domain-containing protein E3 ligase (MDBLsor1_06155), homologous to E6AP C-terminus (HECT)-type E3 ubiquitin transferase (MDBLsor1_06470), cullin_2 domain-containing protein (MDBLsor1_01198), B box-type domain-containing protein (MDBLsor1_01106) were ubiquitination modification proteins, which were involved in the regulation of protein stability, subcellular localization, activity, and interaction. They were widely involved in physiological processes such as immune response regulation, mitochondrial autophagy, DNA damage repair, cell cycle regulation, epigenetic regulation, cell apoptosis, and protein degradation[63-67]. In addition, another 5 DEPs also participated in a variety of life processes. The bromodomain-containing protein (MDBLsor1_03055) was involved in the regulation of cell apoptosis[68]. The non-specific serine/threonine protein kinase (MDBLsor1_03636) participated in cell growth, metabolism, differentiation, and cell death[69]. The F-actin-capping protein subunit alpha (MDBLsor1_07369) was a component of the cytoskeleton that permitted eukaryotic cell growth, intracellular movement, and cytoplasmic division[70]. The protein kinase domain-containing protein (MDBLsor1_01282) was related to signal transduction through the mitogen-activated protein kinase (MAPK) pathway and morphogenesis through the regulation of cytokinesis and actin-dependent polarized growth[71]. The phosphatidyl ethanolamine binding protein (PEBP)-like protein (MDBLsor1_02849) might be involved in the differentiation of pileus and play a central role in the transition from the vegetative to the reproductive stage[72]. Three DEPs were related to abiotic stress. The Pirin (MDBLsor1_05709) was an iron-dependent redox regulator of nuclear factor κB to deliver the oxidative stress signal[73]. The *N*-acetyltransferase domain-containing protein (MDBLsor1_01288, MDBLsor1_01551) reduced intracellular oxidation levels and protect cells from oxidative stress[74]. Two DEPs might be related to virulence and they were the CFEM domain-containing protein (MDBLsor1_02250)[75] and carboxylic ester hydrolase (MDBLsor1_07701).

3.6 Regulation of stipe growth and development

3.6.1 The analysis for the highly expressed DEPs in SFrS

There were no GO terms ($P < 0.05$) enriched but 2 KEGG pathways ($P < 0.05$) were enriched in SFrS *vs*. SFrP. They were both involved in metabolism, including carbohydrate metabolism and energy metabolism pathways ($P < 0.01$; Figs. 4B, 5C and Table S20). Moreover, 22 DEPs in SFrS were connected into a network (Fig. 5C), mainly involved in carbohydrate metabolism (B 09101) and energy metabolism (B 09102). The hub DEPs were the dihydroxyacetone kinase 1 (MDBLsor1_10985), fructose-bisphosphate aldolase (MDBLsor1_01656), WD40 repeat-like protein (MDBLsor1_04884), UPF0103-domain-containing protein (MDBLsor1_03213), and carn_acyltransf domain-containing protein (MDBLsor1_07220).

3.6.2 The analysis for the highly expressed DEPs in MFrS

There were 14 highly expressed GO terms ($P < 0.05$) enriched in MFrS *vs*. MFrP. The BP terms ($P < 0.05$) included the small molecule metabolic process, cellular modified amino acid metabolic process, obsolete cofactor metabolic process, phosphate-containing compound metabolic process, response to starvation/nutrient levels, and response to an extracellular/external stimulus; the CC terms ($P < 0.05$) contained the cytoplasm and obsolete cytoplasmic part; the MF terms ($P < 0.05$) mainly covered the acyltransferase activity, transferase activity, and catalytic activity (Fig. 3C and Table S21).

From the DEPs in MFrS, 4 KEGG pathways ($P < 0.05$) were enriched. Two pathways were involved in metabolism, mainly including lipid metabolism ($P < 0.01$); the other 2 pertained to cellular processes, primarily comprising of peroxisome pathway ($P < 0.01$; Fig. 4C and Table S22).

There were 39 highly expressed DEPs in MFrS were connected to a network (Fig. 5F). Most of them were obsolete cytoplasmic parts (GO: 0044444) and some of them were related to acyltransferase activity (GO: 001674), lipid metabolism (B 09103), peroxisome (04146), and transport and catabolism (B 09141; Tables S21 and S22). The hub DEPs were the dynein light chain (MDBLsor1_11423), prefoldin-domain-containing protein (MDBLsor1_01972), signal recognition particle 54 kDa protein (MDBLsor1_05791), NAD^+ diphosphatase (MDBLsor1_04623), and carbohydrate-binding module family 48 protein (MDBLsor1_06708).

3.6.3 The key regulatory protein for stipe development

There were 12 DEPs both highly expressed in SFrS and MFrS (Fig. 2E), which might continuously regulate the stipe growth and development. They were the protein kinases (MDBLsor1_05818, MDBLsor1_06208), α-amylase (MDBLsor1_10272), heparinase II/III family protein (MDBLsor1_11869), aldo/keto reductase (MDBLsor1_07220), maintenance of telomere capping protein 1 (MDBLsor1_06160), nuclear transport factor 2 (MDBLsor1_06557), molybdopterin molybdenum transferase (MDBLsor1_03558), amino acid (AA)_trna_ligase_II/phosphofructokinase gene (*pfkB*) domain-containing protein (MDBLsor1_00563, MDBLsor1_11447), and SSCRP protein (MDBLsor1_08215).

The regulation of growth and development of SFrS and MFrS was very different. Carbohydrate conversion and energy metabolism were vigorous in SFrS, which was consistent with the rapid growth of the stalk at this stage. Two highly expressed proteins were also noteworthy and played critical roles in response to biotic and abiotic stress. One was the Pirin (MDBLsor1_05709). The other was the ankyrin repeat-containing protein (MDBLsor1_00081), which was identified to contribute to increased resistance to salt stress in eukaryotes[76]. Interestingly, the type III secretion system effector protein (MDBLsor1_06414) was also highly expressed in SFrS and was secreted out of cells in a low-calcium environment, it might relate to virulence or nutrient acquisition[77].

In MFrS, the highly expressed proteins were mainly used in the activities of small molecular acids synthesis and metabolism, respondence to adverse external environments, such as nutrient deficiency, etc. More cytoplasm and obsolete cytoplasmic parts were generated in MFrS, which predicted the maturation and aging of the stipe. And also more nutrients accumulated. Some other highly expressed proteins in MFrS were also notable. Four proteins might be also indicative of cellular aging and repair. The maintenance of telomere capping protein 1 (MDBLsor1_03037) could limit cell division and was essential to the survival of eukaryotic cells[78]. The visual word form area (VWFA) domain-containing proteins (MDBLsor1_02352, MDBLsor1_07454) were necessary for biofilm enhancement by the extracellular peptides in the growth medium[79]. The GPI-anchored wall transfer protein (MDBLsor1_02372) was involved in cell wall biogenesis and remodeling[80]. Another 4 proteins were fungi virulence-related and they were the carboxylic ester hydrolase (MDBLsor1_07608)[81], DUF2235 domain-containing protein (MDBLsor1_04003)[82], nudix hydrolase domain-containing protein (MDBLsor1_02155)[83], and F-box domain-containing protein (MDBLsor1_03424) [84]. Two proteins were related to stress response and detoxification. They were the tryptophan-rich sensory protein (MDBLsor1_04908) [85] and proto globin domain-containing protein (MDBLsor1_03895)[86]. Two phosphatases were involved in the inactivation of intracellular signaling pathways, the phosphoglycerate mutase-like protein (MDBLsor1_04065) [87] and protein-tyrosine phosphatase (MDBLsor1_06812) [88].

3.7 The roles of CAZymes in the growth, development, and differentiation of L. sordida

A series of CAZymes were selected from the DEPs list and were usually classified into different families in the CAZyme database, including glycoside hydrolases (GHs), auxiliary activities (AAs), glycosyl transferases (GTs), carbohydrate esterases (CEs), and polysaccharide lyases (PLs). Most of them were secreted enzymes and could efficiently deconstruct rice straws to help *L. sordida* get nutrients. We analyzed their genetic relationships (Fig. 6). All the CAZymes were marked in green in Table S4-6. These enzymes played universal regulatory roles during the growth process of *L. sordida*, and their expression levels were different according to the different needs at each stage. There were several GHs highly expressed in each sample. They played key roles in the metabolic process of hydrolyzing macromolecular carbohydrates into monomers of glucose in preparation for glycolysis to produce energy. The common GHs included glycoside hydrolase family protein, glucoamylase, β-galactosidase, 1,4-α-glucan-branching enzyme, β-glucosidase, α-amylase, etc.[89-93]. GTs, mainly including glycosyl transferases family proteins, were usually used for natural product modification with sugar moieties[94].

In Myc, there were 5 highly expressed CAZymes marked in yellow, including 3 GHs (ratio: 60%), 1 AA (20%), and 1 GT (20%). The multicopper oxidases (MCOs; AA, MDBLsor1_11822), were confirmed to be involved in various physiological processes such as morphogenesis, lignin degradation, and defense against environmental stress[95]. In YFr, 10 highly expressed CAZymes were compared out and marked in green, including 6 GHs (60%), 2 AAs (20%), and 2 GTs (20%). In addition to the common energy production-related enzymes of GHs, it was worth noting that the RNase_PH domain-containing protein (GH, MDBLsor1_01955), which was a core subunit of RNA exosome, could participate in the degradation and processing of cellular RNA[96]. The oxidoreductase (MDBLsor1_00585) highly expressed in YFR could spatially protect glycosylase from oxidative damage caused by REDOX enzyme reactions[97], which might assist the GHs. AAs contained glutamine-fructose-6-phosphate transaminase (MDBLsor1_10226) and copper radical oxidase (MDBLsor1_11752). The former was an essential enzyme in yeast that catalyzes the first and rate-limiting step in hexosamine biosynthesis[98]. The latter was functionally similar to MCOs. Except for a GT family protein, there was a chitin synthase (MDBLsor1_00718) in GTs, which could exert diverse functions to regulate the fungal growth and development, conidial germination, conidial production, stress response, penetration, and virulence[99].

Compared with Myc, there were more CAZymes involved in energy metabolism in YFr, and the participation of the hexosamine metabolic pathway indicated accelerated glucose utilization and energy productivity. The chitin synthase in YFr could promote spore production, which might be a key regulatory enzyme in primordium differentiation in the early development of the fruiting body.

There were 3 highly expressed CAZymes marked in pink in SFrP compared with SFrS, including 2 GHs (66.7%) and 1 GT (33.3%). There was a dolichyl-phosphate-mannose-protein mannosyltransferase (GT, MDBLsor1_11523), responsible for initiating protein *O*-mannosylation in the ER. It could regulate fungal development and cell wall stability[100]. There were 8 highly expressed CAZymes in MFrP compared with MFrS, including 4 GHs (50%), 2 AAs (25%),1 GT (12.5 %), and 1 CE (12.5 %), which were marked in blue. GHs also contained an RNase_PH domain-containing protein. The carbohydrate esterase family 16 protein (fragment) (CE, MDBLsor1_04010) could participate in plant hemicellulose saccharification[101], indicating that it could provide monomers of glucose together with GHs. GT was a dolichyl-phosphate-mannose-protein mannosyltransferase same to the GT in SFrP, which showed this enzyme played a continuous regulatory role in *L. sordida* stipe development.

Four CAZymes with high expression in SFrS compared with SFrP were marked in red, including 2 AAs (50%), 1 GH (25%), and 1 CE (25%). The chitin deacetylase (CE, MDBLsor1_02997) could catalyze the deacetylation of chitin to produce chitosan. Chitosan was the only basic amino polysaccharide that exists in large quantities among natural polysaccharides and had the function of antibacterial, anticancer, and antiviral[102]. Four highly expressed CAZymes in MFrS compared with MFrP were marked in grey, including 2 GHs (50%), 1 AA (25%), and 1 PL (25%). Besides the common GHs, the 1,3-β-glucanosyltransferase (MDBLsor1_02955) was worth noting and played an active role in the biosynthesis of the fungal cell wall[103]. Peroxidase (AA, MDBLsor1_05005) was a kind of enzyme mainly involved in plant lignin degradation to gain carbohydrate accessibility and fungal growth[104]. This result indicated that the digestion and utilization of lignin were enhanced at the later growth stage for the

grass rot fungus *L. sordida*. The heparinase II/III family protein (PL; MDBLsor1_03384) could produce low molecular weight heparin oligosaccharides for utilization as anticoagulants[105].

More highly expressed energy productivity-related proteins were detected in the mushroom pileus, especially the mature pileus, than that in the stipe. They might play important roles in the sexual reproduction and sporulation of the mushroom. A wider range and larger quantities of more active ingredients, such as chitosan and heparin, might be accumulated in the stipes. Interestingly, there were 2 GMC oxidoreductase highly expressed in both SFrS and MFrP. They could generate extracellular H_2O_2 acting as the ultimate oxidizer in the degradation of plant polymers in Basidiomycota[106]. It was related to the digestion and absorption of nutrients in the root of the stalk. Their low expression levels in MFrS indicated that in the MFr stage the depletion of nutrients and the mature tendency of the mushroom. The detection of the GMC oxidoreductase could be used as a mushroom harvest index.

3.8 RT-qPCR Validation of the DEPs expression

The gene expression level is largely consistent with its protein expression level. So we used RT-qPCR to detect the gene expression levels (mRNA content) of 6 key DEPs, including 3 hub DEPs in the spliceosome and transcription pathways, and 3 hub DEPs in the carbohydrate and energy metabolism pathways, to verify the accuracy of our proteomic results. Primers for RT-qPCR analysis were listed (Table S23). The results showed that the changes in gene expression were basically consistent with the protein expression indicating the reliability of our proteomic results and the accuracy of the DEPs screening (Fig. 7).

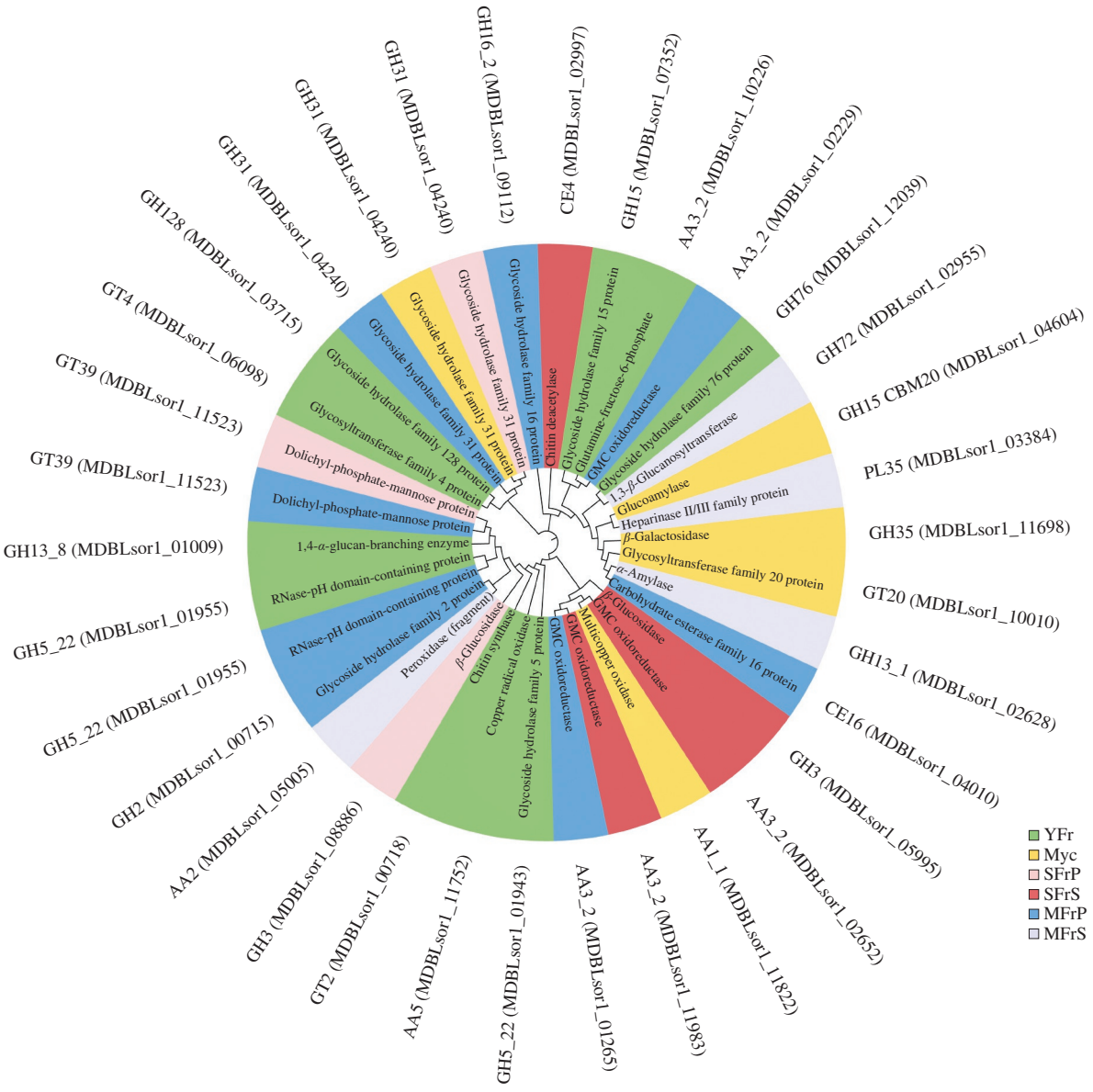

Fig. 6 The highly expressed CAZymes analysis among 6 samples. The phylogenetic tree shows the kinship of CAZymes. The outer circle represents the protein IDs and their family annotations for each protein. The inner circle shows the highly expressed CAZymes in different samples.

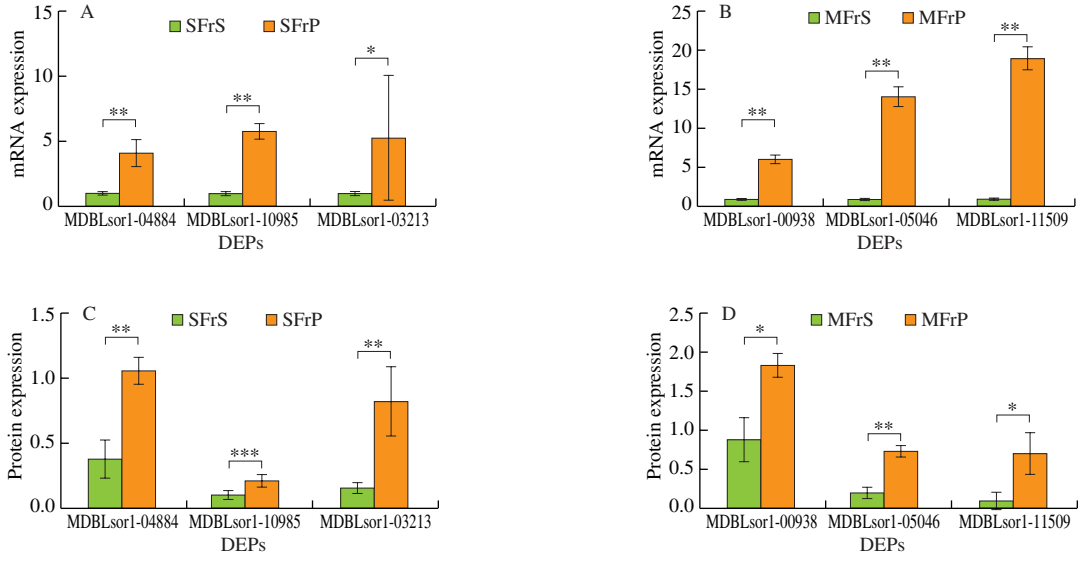

Fig. 7 *RT-qPCR* validation of the proteomic results. (A) The mRNA expression of the 3 hub DEPs in SFrP and SFrS are all in the carbohydrate and energy metabolism pathways. (B) The mRNA expression of the 3 hub DEPs in MFrP and MFrS are all in the transcription and spliceosome pathways. (C) The protein expression of the same 3 hub DEPs in SFrP and SFrS. (D) The protein expression of the same 3 hub DEPs in MFrP and MFrS. ** $P < 0.01$; * $P < 0.05$.

4. Conclusion

We domesticated an excellent strain of *L. sordida*, which was rich in nutrition, good flavor, and high iron content. We revealed the regulatory mechanism of primordial differentiation in the early fruiting body formation of this domesticated strain. After the primordium differentiation, young mushrooms were formed. The results showed that the mycelium samples before the primordium differentiation mainly expressed high levels of mitochondrial functional proteins and carbon dioxide concentration regulatory proteins, while the proteins highly expressed in young mushroom samples were mainly involved in cell component generation, cell proliferation, nitrogen compound metabolism, nucleotide metabolism, glutathione metabolism, and purine metabolism. At the same time, the differential regulation pattern of pileus and stipe growth to maturity was revealed. The analysis results showed that the highly expressed proteins regulating the continued growth and development of the pileus were mainly related to transcription, RNA splicing, and the production of various organelles, especially the components on the mitochondrial membrane. In addition to more of these proteins, the process of maturation was also involved in the highly expressed proteins related to DNA conformational change, nucleosome organization, protein processing, maturation and transport, cell detoxification, and so on. During stipe growth and development, proteins related to carbohydrate metabolism and energy metabolism were prominent. By the time the stalk matures, large amounts of obsolete cytoplasmic parts were accumulated, and proteins associated with nutrient deprivation and external stimuli were also highly expressed. CAZymes regulated multiple life activities in the growth and development process. The GHs were mainly the hydrolysis enzymes of macromolecular carbohydrates to glucose monomers, which helped energy substances absorption in the whole process; in the mycelium, AAs participated in mycelium formation, lignin degradation, and defense against environmental stress. In the young fruiting body, AAs and GTs were mainly involved in morphogenesis, spore production, and stress response; for the pileus, GTs regulated the development of fungi and the stability of cell wall, and CEs participated in hemicellulose saccharification to provide energy substances. For the stipe, CEs and PLs participated in the synthesis of medicinal ingredients, such as chitosan and heparin oligosaccharides, and AA was involved in the degradation of lignin to obtain more energy substances.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability statement

The raw data for the proteomic analysis reported in this paper have been deposited in the OMIX, China National Center for Bioinformation/Beijing Institute of Genomics, Chinese Academy of Sciences (https://ngdc.cncb.ac.cn/omix: Accession No. OMIX004078).

Acknowledgement

This research was funded by the Shandong Edible Fungus Agricultural Technology System (SDAIT-07-02), the National Natural Science Foundation of China (Grant No. 32000041 and 32272789), the Shandong Provincial Natural Science Foundation, China (ZR2020QC005), the Qingdao Agricultural University Scientific Research Foundation (6631120076), and horizontal project: Breeding and property protection of new varieties of factory produced *Hypsizygus marmoreus* (20183702012614).

Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version at http://doi.org/10.26599/FSHW.2023.9250051.

References

[1] K. Kumar, R. Mehra, R.P.F. Guiné, et al., Edible mushrooms: a comprehensive review on bioactive compounds with health benefits and processing aspects, Foods 10 (2021) 2996. https://doi.org/10.3390/foods10122996.

[2] B. Thongbai, K. Wittstein, C. Richter, et al., Successful cultivation of a valuable wild strain of *Lepista sordida* from Thailand, Mycol. Prog. 16 (2017) 311-323. https://doi.org/10.1007/s11557-016-1262-0.

[3] M. Aranha, G. Contato, S. Salgado, et al., Biochemical characterization and biological properties of mycelium extracts from *Lepista sordida* GMA-05 and *Trametes hirsuta* GMA-01: new mushroom strains isolated in Brazil, Braz. J. Microbiol. 53 (2022) 349-358. https://doi.org/10.1007/s42770-021-00670-5.

[4] H. Kang, S. Ji, H. Park, et al., Lepistatins A–C, chlorinated sesquiterpenes from the cultured basidiomycete *Lepista sordida*, Phytochemistry 143 (2017) 111-114. https://doi.org/10.1016/j.phytochem.2017.08.003.

[5] X.L. Chen, M. Wu, H.H. Ti, et al., Three new 3,6-dioxygenated diketopiperazines from the basidiomycete *Lepista sordida*, Helv. Chim. Acta 94 (2011) 1426-1430. https://doi.org/10.1002/hlca.201000455.

[6] S.S. Miao, R. Mao, R.H. Pei, et al., Antitumor activity of polysaccharides from *Lepista sordida* against laryngocarcinoma *in vitro* and *in vivo*, Int. J. Biol. Macromol. 60 (2013) 235-240. https://doi.org/10.1016/j.ijbiomac.2013.05.033.

[7] Q. Luo, L. Yan, P. Xu et al., Discovery of a polysaccharide from the fruiting bodies of *Lepista sordida* as potent inhibitors of indoleamine 2, 3-dioxygenase (IDO) in HepG2 cells via blocking of STAT1-mediated JAK-PKC-δ signaling pathways, Carbohydr. Polym. 197 (2018) 540-547. https://doi.org/10.1016/j.carbpol.2018.05.052.

[8] W.Q. Zhong, N. Liu, Y.G. Xie, et al., Antioxidant and anti-aging activities of mycelial polysaccharides from *Lepista sordida*, Int. J. Biol. Macromol. 60 (2013) 355-359. https://doi.org/10.1016/j.ijbiomac.2013.06.018.

[9] Y.Y. Xu, Y.H. Li, Y.X. Lu, et al., Antioxidative and hepatoprotective activities of a novel polysaccharide (LSAP) from *Lepista sordida* mycelia, Food Sci. Hum. Wellness 10 (2021) 536-544. https://doi.org/10.1016/j.fshw.2021.04.016.

[10] S.P. Yu, J.C. Jiang, W.X. Li, Co-cultured *Lepista sordida* and *Pholiota nameko* polysaccharide-iron(iii) chelates exhibit good antioxidant activity, RSC Adv. 10 (2020) 27259-27265. https://doi.org/10.1039/d0ra03258a.

[11] D.C. Yin, J.Y. Qi, The physiological response of ectomycorrhizal fungus *Lepista sordida* to Cd and Cu stress, PeerJ 9 (2021) e11115. https://doi.org/10.7717/peerj.11115.

[12] U. Kües, M. Navarro-González, How do Agaricomycetes shape their fruiting bodies? 1. Morphological aspects of development, Fungal Biol. Rev. 29 (2015) 63-97. https://doi.org/10.1016/j.fbr.2015.05.001.

[13] Y. Sakamoto, Influences of environmental factors on fruiting body induction, development and maturation in mushroom-forming fungi, Fungal Biol. Rev. 32 (2018) 236-248. https://doi.org/10.1016/j.fbr.2018.02.003.

[14] Y.P. Lu, J.H. Liao, Z.J. Guo, et al., Genome survey and transcriptome analysis on mycelia and primordia of *Agaricus blazei*, BioMed Res. Int. 14 (2020) 1824183. https://doi.org/10.1155/2020/1824183c.

[15] L.P. Cao, Q. Zhang, R.Y. Miao, et al., Application of omics technology in the research on edible fungi, Curr. Res. Food Sci. 27 (2022) 100430. https://doi.org/10.1016/j.crfs.2022.100430.

[16] K. Mohanta, S. Kamran, M. Omar, et al., Plant MWpIDB: a database for the molecular weight and isoelectric points of the plant proteomes, Sci. Rep. 12 (2022) 7421. https://doi.org/10.1038/s41598-022-11077-z.

[17] C.L. Xie, W.B. Gong, Z.H. Zhu, et al., Comparative transcriptomics of *Pleurotus eryngii* reveals blue-light regulation of carbohydrate-active enzymes (CAZymes) expression at primordium differentiated into fruiting body stage, Genomics 110 (2018) 201-209. https://doi.org/10.1016/j.ygeno.

[18] J.Y. Liu, M.C. Chang, J.L. Meng, 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. 66 (2018) 3716-3725. https://doi.org/10.1021/acs.jafc.8b00383.

[19] X.X. Tong, F. Wang, H. Zhang, et al., iTRAQ-based comparative proteome analyses of different growth stages revealing the regulatory role of reactive oxygen species in the fruiting body development of *Ophiocordyceps sinensis*, PeerJ 9 (2021) e10940. https://doi.org/10.7717/peerj.10940.

[20] W.W. Zhu, J.B. Hu, Y. Li, et al., Comparative proteomic analysis of *Plezaqurotus ostreatus* reveals great metabolic differences in the cap and stipe development and the potential role of Ca^{2+} in the primordium differentiation, Int. J. Mol. Sci. 20 (2019) 6317. https://doi.org/10.3390/ijms20246317.

[21] T. Takano, N. Yamamoto, T. Suzuki, et al., Genome sequence analysis of the fairy ring-forming fungus *Lepista sordida* and gene candidates for interaction with plants, Sci. Rep. 9 (2019) 5888. https://doi.org/10.1038/s41598-019-42231-9.

[22] H. Choi, T. Suzuki, A. Ono, et al., The complete mitochondrial genome sequence of the fairy ring-forming fungus *Lepista sordida*, Mitochondrial DNA B 7 (2022) 712-714. https://doi.org/10.1080/23802359.2022.2067496.

[23] X.Q. Yang, R.M. Lin, K. Xu, et al., Comparative proteomic analysis within the developmental stages of the mushroom white *Hypsizygus marmoreus*, J. Fungi. 7 (2021) 1064. https://doi.org/10.3390/jof7121064.

[24] H. Yu, S.X. Zhao, W.D. Lu, et al., A novel gene, encoding 3-aminobenzoate 6-monooxygenase, involved in 3-aminobenzoate degradation in *Comamonas* sp. strain QT12, Appl. Microbiol. Biotechnol. 102 (2018) 4843-4852. https://doi.org/10.1007/s00253-018-9015-4.

[25] J.Y. Liu, Y.C. Chang, J.L. Meng, 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. 66 (2018) 3716-3725. https://doi.org/10.1021/acs.jafc.8b00383.

[26] M. Mleczek, A. Budka, M. Siwulski, et al., Investigation of differentiation of metal contents of *Agaricus bisporus*, *Lentinula edodes* and *Pleurotus ostreatus* sold commercially in Poland between 2009 and 2017, J Food Compost. Anal. (2020) 103488. https://doi.org/10.1016/j.jfca.2020.103488.

[27] W. Zhou, W. Shi, X.W. Xu, et al., Glutamate synthase MoGlt1-mediated glutamate homeostasis is important for autophagy, virulence and conidiation in the rice blast fungus, Mol. Plant Pathol. 19 (2017) 564-578. https://doi.org/10.1111/mpp.12541.

[28] V.H. Knaryan, S. Samantaray, M. Varghese, et al., Synthetic bovine proline-rich-polypeptides generate hydroxyl radicals and fail to protect dopaminergic neurons against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced dopaminergic neurotoxicity in mice, Neuropeptides 40 (2006) 291-298. https://doi.org/10.1016/j.npep.2006.03.005.

[29] G. López-Doménech, R. Serrat, S. Mirra, et al., The Eutherian *Armcx* genes regulate mitochondrial trafficking in neurons and interact with Miro and Trak2, Nat. Commun. 3 (2012) 814. https://doi.org/10.1038/ncomms1829.

[30] X.N. Yu, G.C. Zhang, H.N. Liu, et al., Pre-mRNA processing factor 19 functions in DNA damage repair and radioresistance by modulating cyclin D1 in hepatocellular carcinoma, Mol. Ther. Nucleic Acids 27 (2021) 390-403. https://doi.org/10.1016/j.omtn.2021.12.002.

[31] R.M. Effros, R.S. Chang, P. Silverman, Acceleration of plasma bicarbonate conversion to carbon dioxide by pulmonary carbonic anhydrase, Science 199 (1978) 427-429. https://doi.org/10.1126/science.413195.

[32] M. Donzeau, J.P. Bourdineaud, G.J. Lauquin, Regulation by low temperatures and anaerobiosis of a yeast gene specifying a putative GPI-anchored plasma membrane protein, Mol. Microbiol. 20 (1996) 449-459. https://doi.org/10.1111/j.1365-2958.1996.tb02631.x.

[33] W.W. Zhu, J.B. Hu, Y. Li, et al., Comparative proteomic analysis of *Pleurotus ostreatus* reveals great metabolic differences in the cap and stipe development and the potential role of Ca^{2+} in the primordium differentiation, Int. J. Mol. Sci. 20 (2019) 6317. https://doi.org/10.3390/ijms20246317.

[34] M.V. Mohanan, A. Pushpanathan, S. Padmanabhan, et al., Overexpression of Glyoxalase III gene in transgenic sugarcane confers enhanced performance under salinity stress, J. Plant Res. 134 (2021) 1083-1094. https://doi.org/10.1007/s10265-021-01300-9.

[35] J. Gao, Y. Hao, X.S. Piao, et al., Aldehyde dehydrogenase 2 as a therapeutic target in oxidative stress-related diseases: post-translational modifications deserve more attention, Int. J. Mol. Sci. 23 (2022) 2682. https://doi.org/10.3390/ijms23052682.

[36] D.A. Meireles, J.F. da Silva Neto, R.M. Domingos, et al., Ohr-OhrR, a neglected and highly efficient antioxidant system: structure, catalysis, phylogeny, regulation, and physiological roles, Free Radic. Biol. Med. 185 (2022) 6-24. https://doi.org/10.1016/j.freeradbiomed.2022.04.001.

[37] J.X. Gao, J. Chen, The role of clt1-regulated xylan metabolism in melanin and toxin formation for the pathogenicity of *Curvularia lunata* in maize, Mol. Plant Microbe Interact. 34 (2021) 617-630. https://doi.org/10.1094/MPMI-08-20-0235-R.

[38] X.Q. Liu, J.T. Xie, Y.P. Fu, et al., The subtilisin-like protease bcser2 affects the sclerotial formation, conidiation and virulence of *Botrytis cinerea*, Int. J. Mol. Sci. 21(2020) 603. https://doi.org/10.3390/ijms21020603.

[39] A. Kasei, H. Watanabe, N. Ishiduka, et al., Comparative analysis of the extradiol ring-cleavage dioxygenase ligb from *Arabidopsis* and 3,4-dihydroxyphenylalanine dioxygenase from betalain-producing plants, Plant Cell Physiol. 62 (2021) 732-740. https://doi.org/10.1093/pcp/pcab031.

[40] J.Y. Kim, D.Y. Kim, Y.J. Park, et al., Transcriptome analysis of the edible mushroom *Lentinula edodes* in response to blue light, PLoS ONE 15 (2020) e0230680. https://doi.org/10.1371/journal.pone.0230680.

[41] F. Rusnak, P. Mertz, Calcineurin: form and function, Physiol. Rev. 80 (2000) 1483-1521. https://doi.org/10.1152/physrev.2000.80.4.1483.

[42] W.W. Zhu, J.B. Hu, Y. Li, et al., Comparative proteomic analysis of *Pleurotus ostreatus* reveals great metabolic differences in the cap and stipe development and the potential role of Ca^{2+} in the primordium differentiation, Int. J. Mol. Sci. 20 (2019) 6317. https://doi.org/10.3390/ijms20246317.

[43] H.Y. Yu, L. Li, Phylogeny and molecular dating of the cerato-platanin-encoding genes, Genet. Mol. Biol. 37 (2014) 423-427. https://doi.org/10.1590/s1415-47572014005000003.

[44] R. Zang, H. Lian, X. Zhong, et al., ZCCHC3 modulates TLR3-mediated signaling by promoting recruitment of TRIF to TLR3, J. Mol. Cell Biol. 12 (2020) 251-262. https://doi.org/10.1093/jmcb/mjaa004.

[45] J.P. Munyampundu, Y.P. Xu, X.Z. Cai, Phi class of glutathione *S*-transferase gene superfamily widely exists in nonplant taxonomic groups, Evol. Bioinform. 12 (2016) 59-71. https://doi.org/10.4137/EBO.S35909.

[46] B. Ranjan, S. Pillai, K. Permaul, et al., Expression of a novel recombinant cyanate hydratase (rTl-Cyn) in *Pichia pastoris*, characteristics and applicability in the detoxification of cyanate, Bioresour. Technol. 238 (2017) 582-588. https://doi.org/10.1016/j.biortech.2017.04.091.

[47] E. Kuznetsova, B. Nocek, G. Brown, et al., Functional diversity of haloacid dehalogenase superfamily phosphatases from *Saccharomyces cerevisiae*: biochemical, structural, and evolutionary insights, J. Biol. Chem. 290 (2015) 18678-18698. https://doi.org/10.1074/jbc.M115.657916.

[48] L. Silva, M. Marcet-Houben, A. Zerlotini, et al., Evolutionary histories of expanded peptidase families in *Schistosoma mansoni*, Mem. Inst. Oswaldo Cruz. 106 (2011) 864-877. https://doi.org/10.1590/s0074-02762011000700013.

[49] A. Roy, R. Tamuli, Heat shock proteins and the calcineurin-crz1 signaling regulate stress responses in fungi, Arch. Microbiol. 204 (2022) 240. https://doi.org/10.1007/s00203-022-02833-w.

[50] M. Feng, J.H. Zhou, X.L. Yu, et al., Insights into biodegradation mechanisms of triphenyl phosphate by a novel fungal isolate and its potential in bioremediation of contaminated river sediment, J. Hazard. Mater. 424 (2020) 127545. https://doi.org/10.1016/j.jhazmat.2021.127545.

[51] Y. Huang, J.M. Tan, Z. Wang, et al., Cloning and characterization of two different *L*-type lectin genes from the Chinese mitten crab *Eriocheir sinensis*, Dev. Comp. Immunol. 46 (2014) 255-266. https://doi.org/10.1016/j.dci.2014.04.015.

[52] P. Huang, H.W. Ju, J.H. Min, et al., Overexpression of *L*-type lectin-like protein kinase 1 confers pathogen resistance and regulates salinity response in *Arabidopsis thaliana*, Plant Sci. 203-204 (2013) 98-106. https://doi.org/10.1016/j.plantsci.2012.12.019.

[53] J.B. Heo, W.Y. Bang, S.W. Kim, et al., OsPRA1 plays a significant role in targeting of OsRab7 into the tonoplast via the prevacuolar compartment during vacuolar trafficking in plant cells, Planta 232 (2010) 861-871. https://doi.org/10.1007/s00425-010-1226-6.

[54] Y. Imanishi-Shimizu, Y. Kamogawa, Y. Shimada, et al., A capsule-associated gene of *Cryptococcus neoformans*, CAP64, is involved in pH homeostasis, MICROBIOL-SGM. 167 (2021) 001029. https://doi.org/10.1099/mic.0.001029.

[55] H.Y. Wang, X. Tang, J.H. Liu, et al., The multiprotein exocyst complex is essential for cell separation in *Schizosaccharomyces pombe*, Mol. Biol. Cell. 13 (2002) 515-29. https://doi.org/10.1091/mbc.01-11-0542.

[56] K. Vikas, K. Pradhan, N. Budhlakoti, et al., Multi-locus genome-wide association studies (ML-GWAS) reveal novel genomic regions associated with seedling and adult plant stage leaf rust resistance in bread wheat (*Triticum aestivum* L.), Heredity 128 (2022) 434-449. https://doi.org/10.1038/s41437-022-00525-1.

[57] M. Yamamoto, D. Maruyama, T. Endo, et al., *Arabidopsis thaliana* has a set of J proteins in the endoplasmic reticulum that are conserved from yeast to animals and plants, Plant Cell Physiol. 49 (2008) 1547-1562. https://doi.org/10.1093/pcp/pcn119.

[58] S.S. Elbaramawi, S.M. Ibrahim, E.M. Lashine, et al., Exploring the binding sites of Staphylococcus aureus phenylalanine tRNA synthetase: a homology model approach, J. Mol. Graph. 73 (2017) 36-47. https://doi.org/10.1016/j.jmgm.2017.02.002.

[59] N. Shibagaki, A.R. Grossman, The role of the STAS domain in the function and biogenesis of a sulfate transporter as probed by random mutagenesis, J. Biol. Chem. 281 (2006) 22964-22973. https://doi.org/10.1074/jbc.M603462200.

[60] T. Kawano, M. Araseki, Y. Araki, et al., A small peptide sequence is sufficient for initiating kinesin-1 activation through part of TPR region of KLC1, Traffic 13 (2012) 834-848. https://doi.org/10.1111/j.1600-0854.2012.01350.x.

[61] S. Pagant, A. Wu, S. Edwards, et al., Sec24 is a coincidence detector that simultaneously binds two signals to drive ER export, Curr. Biol. 25 (2015) 403-412. https://doi.org/10.1016/j.cub.2014.11.070.

[62] P. Ngamskulrungroj, Y. Chang, B. Hansen, et al., *Cryptococcus neoformans* Yop1, an endoplasmic reticulum curvature-stabilizing protein, participates with Sey1 in influencing fluconazole-induced disomy formation, FEMS Yeast Res. 12 (2012) 748-754. https://doi.org/10.1111/j.1567-1364.2012.00824.x.

[63] F.R. Schumacher, G. Wilson, C.L. Day, The N-terminal extension of UBE2E ubiquitin-conjugating enzymes limits chain assembly, J. Mol. Biol. 425 (2013) 4099-4111. https://doi.org/10.1016/j.jmb.2013.06.039.

[64] P. Figueroa, G. Gusmaroli, G. Serino, et al., *Arabidopsis* has two redundant Cullin3 proteins that are essential for embryo development and that interact with RBX1 and BTB proteins to form multisubunit E3 ubiquitin ligase complexes *in vivo*, Plant Cell 17 (2005) 1180-1195. https://doi.org/10.1105/tpc.105.031989.

[65] V.S. Delvecchio, C. Fierro, S. Giovannini, et al., Emerging roles of the HECT-type E3 ubiquitin ligases in hematological malignancies, Discov. Oncol. 12 (2021) 39. https://doi.org/10.1007/s12672-021-00435-4.

[66] W.J. Cai, H.F. Yang, The structure and regulation of Cullin 2 based E3 ubiquitin ligases and their biological functions, Cell Div. 11 (2016) 7. https://doi.org/10.1186/s13008-016-0020-7.

[67] M.A. Massiah, B.N. Simmons, K.M. Short, et al., Solution structure of the RBCC/TRIM B-box1 domain of human MID1: B-box with a RING, J. Mol. Biol. 358 (2006) 532-545. https://doi.org/10.1016/j.jmb.2006.02.009.

[68] X.Y. Liu, H. Yang, X.H. Zhang, et al., Bdf1p deletion affects mitochondrial function and causes apoptotic cell death under salt stress, FEMS Yeast Res. 9 (2009) 240-246. https://doi.org/10.1111/j.1567-1364.2008.00469.x.

[69] Y. Wang, Y.Z. Deng, G. Cui, et al., The AGC kinase SsAgc1 regulates *Sporisorium scitamineum* mating/filamentation and pathogenicity, mSphere 4 (2019) e00259-19. https://doi.org/10.1128/mSphere.00259-19.

[70] V.E. González-Rodríguez, C. Garrido, J.M. Cantoral, et al., The F-actin capping protein is required for hyphal growth and full virulence but is dispensable for septum formation in *Botrytis cinerea*, Fungal Biol. 120 (2016) 1225-1235. https://doi.org/10.1016/j.funbio.2016.07.007.

[71] K.J. Boyce, A. Andrianopoulos, Ste20-related kinases: effectors of signaling and morphogenesis in fungi, Trends Microbiol. 19(2011) 400-410. https://doi.org/10.1016/j.tim.2011.04.006.

[72] N. Mimida, S. Komori, A. Suzuki, et al., Functions of the apple TFL1/FT orthologs in phase transition, Sci. Hortic. 156 (2013) 106-112. https://doi.org/10.1016/j.scienta.2013.04.001.

[73] F. Liu, I. Rehmani, S. Esaki, et al., Pirin is an iron-dependent redox regulator of NF-κB, Proc. Natl. Acad. Sci. U.S.A. 110 (2013) 9722-9727. https://doi.org/10.1073/pnas.1221743110.

[74] X. Du, H. Takagi, N-acetyltransferase Mpr1 confers freeze tolerance on *Saccharomyces cerevisiae* by reducing reactive oxygen species, J. Biochem. 138 (2005) 391-397. https://doi.org/10.1093/jb/mvi134.

[75] J. Ling, F. Zeng, Y.X. Cao, Identification of a class of CFEM proteins containing a new conserved motif in *Fusarium oxysporum*, Physiol. Mol. Plant Pathol. 89 (2015) 41-48. https://doi.org/10.1016/j.pmpp.2014.12.001.

[76] F.J. Zhang, Y.H. Xie, H. Jiang, et al., The ankyrin repeat-containing protein MdANK2B regulates salt tolerance and ABA sensitivity in *Malus domestica*, Plant Cell Rep. 40 (2021) 405-419. https://doi.org/10.1007/s00299-020-02642-9.

[77] S.K. Yadav, J. Das, R. Kumar, et al., Calcium regulates the mycophagous ability of *Burkholderia gladioli* strain NGJ1 in a type III secretion system-dependent manner, BMC Microbiol. 20 (2020) 216. https://doi.org/10.1186/s12866-020-01897-2.

[78] H.P. Ngo, D. Lydall, Survival and growth of yeast without telomere capping by Cdc13 in the absence of Sgs1, Exo1, and Rad9, PLoS Genet. 6 (2010) e1001072. https://doi.org/10.1371/journal.pgen.1001072.

[79] M. Puhm, J. Hendrikson, M. Kivisaar, et al., *Pseudomonas putida* biofilm depends on the vWFa-Domain of LapA in peptides-containing growth medium, Int. J. Mol. Sci. 23 (2022) 5898. https://doi.org/10.3390/ijms23115898.

[80] S.M. Bowman, A. Piwowar, M. Al Dabbous, et al., Mutational analysis of the glycosylphosphatidylinositol (GPI) anchor pathway demonstrates that GPI-anchored proteins are required for cell wall biogenesis and normal hyphal growth in *Neurospora crassa*, Eukaryot Cell. 5 (2006) 587-600. https://doi.org/10.1128/EC.5.3.587-600.2006.

[81] G. Xie, M.Y. Liu, H. Zhu, et al., Esterase SeE of *Streptococcus equi* ssp. *equi* is a novel nonspecific carboxylic ester hydrolase, FEMS Microbiol Lett. 289 (2008) 181-186. https://doi.org/10.1111/j.1574-6968.2008.01377.x.

[82] J.L. Ma, Z.H. Pan, J.H. Huang, et al., The Hcp proteins fused with diverse extended-toxin domains represent a novel pattern of antibacterial effectors in type VI secretion systems, Virulence 8 (2017) 1189-1202. https://doi.org/10.1080/21505594.2017.1279374.

[83] C.L. McCombe, A.M. Catanzariti, J.R. Greenwood, et al., A rust-fungus Nudix hydrolase effector decaps mRNA *in vitro* and interferes with plant immune pathways, New Phytol. 239 (2023) 222-239. https://doi.org/10.1111/nph.18727.

[84] T.B. Liu, C.Y. Xue, The ubiquitin-proteasome system and f-box proteins in pathogenic fungi, Mycobiology 39 (2011) 243-248. https://doi.org/10.5941/MYCO.2011.39.4.243.

[85] A.W. Busch, B.L. Montgomery, Distinct light-, stress-, and nutrient-dependent regulation of multiple tryptophan-rich sensory protein (TSPO) genes in the *Cyanobacterium Fremyella diplosiphon*, Plant Signal. Behav. 12 (2017) e1293221. https://doi.org/10.1080/15592324.2017.1293221.

[86] P. Ascenzi, A. Pesce, M. Nardini, et al., Reductive nitrosylation of *Methanosarcina acetivorans* protoglobin: a comparative study, Biochem. Biophys. Res. Commun. 430 (2013) 1301-1305. https://doi.org/10.1016/j.bbrc.2012.11.122.

[87] B. San Luis, B. Sondgeroth, N. Nassar, et al., Sts-2 is a phosphatase that negatively regulates zeta-associated protein (ZAP)-70 and T cell receptor signaling pathways, J. Biol. Chem. 286 (2011) 15943-15954. https://doi.org/10.1074/jbc.M110.177634.

[88] T. Jacoby, H. Flanagan, A. Faykin, et al., Two protein-tyrosine phosphatases inactivate the osmotic stress response pathway in yeast by targeting the mitogen-activated protein kinase, Hog1, J. Biol. Chem. 272 (1997) 17749-17755. https://doi.org/10.1074/jbc.272.28.17749.

[89] M.M. Chaudet, D.R. Rose, Divergent evolution for diverse substrate recognition by family 31 glycoside hydrolases, Int. J. Biochem. Cell Biol. 94 (2016) 323-330. https://doi.org/10.1139/bcb-2016-0022.

[90] X.L. Xu, J.M. Huang, J. Fang, et al., Expression of a fungal glucoamylase in transgenic rice seeds, Protein Expr. Purif. 61 (2008) 113-116. https://doi.org/10.1016/j.pep.2008.05.019.

[91] J.L. Xu, J. Zhao, L.F. Wang, et al., Enhanced *β*-galactosidase production from whey powder by a mutant of the psychrotolerant yeast *Guehomyces pullulans* 17-1 for hydrolysis of lactose, Appl. Biochem. Biotechnol. 166 (2012) 599-611. https://doi.org/10.1007/s12010-011-9451-4.

[92] D. Li, T. Fei, Y. Wang, et al., A cold-active 1,4-*α*-glucan branching enzyme from *Bifidobacterium longum* reduces the retrogradation and enhances the slow digestibility of wheat starch, Food Chem. 324 (2020) 126855. https://doi.org/10.1016/j.foodchem.2020.126855.

[93] F. Afzal-Javan, M. Mobini-Dehkordi, Amplification, sequencing and cloning of Iranian native *Bacillus subtilis* alpha-amylase gene in *Saccharomyces cerevisiae*, Jundishapur J Microbiol. 6 (2013). https://doi.org/10.5812/jjm.7371.

[94] A. Chang, S. Singh, K.E. Helmich, et al., Complete set of glycosyltransferase structures in the calicheamicin biosynthetic pathway reveals the origin of regiospecificity, Proc. Natl. Acad. Sci. 108 (2011) 17649-17654. https://doi.org/10.1073/pnas.1108484108.

[95] T. Chakraborty, R. Tóth, J.D. Nosanchuk, et al., Multicopper oxidases in *Saccharomyces cerevisiae* and human pathogenic fungi, J. Fungi 6 (2020) 56. https://doi.org/10.3390/jof6020056.

[96] Q. Liu, J.C. Greimann, C.D. Lima, Reconstitution, activities, and structure of the eukaryotic RNA exosome, Cell 127 (2006) 1223-1237. https://doi.org/10.1016/j.cell.2006.10.037.

[97] K. Umezawa, S. Itakura, Influence of carbon source on wood decay-associated gene expression in sequential hyphal zones of the brown rot fungus *Gloeophyllum trabeum*, Bioscience, Biosci. Biotechnol. Biochem. 85 (2021) 1782-1788. https://doi.org/10.1093/bbb/zbab080.

[98] B. Dummitt, W.S. Micka, Y.H. Chang, Yeast glutamine-fructose-6-phosphate aminotransferase (Gfa1) requires methionine aminopeptidase activity for proper function, J. Biol. Chem. 280 (2005) 14356-14360. https://doi.org/10.1074/jbc.M501059200.

[99] J. Qin, P. Zhao, Z. Ye, et al., Chitin synthase genes are differentially required for growth, stress response, and virulence in *Verticillium dahliae*, J. Fungi 8 (2022) 681. https://doi.org/10.3390/jof8070681.

[100] Z. He, L. Luo, N.O. Keyhani, et al., The C-terminal MIR-containing region in the Pmt1 *O*-mannosyltransferase restrains sporulation and is dispensable for virulence in *Beauveria bassiana*, Appl. Microbiol. Biotechnol. 101 (2017) 1143-1161. https://doi.org/10.1007/s00253-016-7894-9.

[101] V. Puchart, J.W. Agger, J.G. Berrin, et al., Comparison of fungal carbohydrate esterases of family CE16 on artificial and natural substrates, J. Biotechnol. 233 (2016) 228-236. https://doi.org/10.1016/j.jbiotec.2016.07.003.

[102] A. Mukherjee, S. Sarkar, S. Gupta, et al., DMSO strengthens chitin deacetylase-chitin interaction: physicochemical, kinetic, structural and catalytic insights, Carbohydr. Polym. 223 (2019) 115032. https://doi.org/10.1016/j.carbpol.2019.115032.

[103] I. Mouyna, T. Fontaine, M. Vai, et al., Glycosylphosphatidylinositol-anchored glucanosyltransferases play an active role in the biosynthesis of the fungal cell wall, J. Biol. Chem. 275 (2000) 14882. https://doi.org/10.1074/jbc.275.20.14882.

[104] J. Kuuskeri, M. Häkkinen, P. Laine, et al., Time-scale dynamics of proteome and transcriptome of the white-rot fungus *Phlebia radiata*: growth on spruce wood and decay effect on lignocellulose, Biotechnol Biofuels. 9 (2016) 192. https://doi.org/10.1186/s13068-016-0608-9.

[105] K. Balasubramaniam, K. Sharma, A. Rani, et al., Deciphering the mode of action, structural and biochemical analysis of heparinase II/III (PsPL12a) a new member of family 12 polysaccharide lyase from pseudopedobacter saltans, Ann. Microbiol. 68 (2018) 409-418. https://doi.org/10.1007/s13213-018-1347-x.

[106] P. Ferreira, J. Carro, A. Serrano, et al., A survey of genes encoding H_2O_2-producing GMC oxidoreductases in 10 polyporales genomes, Mycologia 107 (2015) 1105-1119. https://doi.org/10.3852/15-027.