Adv Biochem Eng Biotechnol
DOI 10.1007/10_2015_335

Cell Factories of Higher Fungi for Useful Metabolite Production

Hao Qin, Jun-Wei Xu, Jian-Hui Xiao, Ya-Jie Tang, Han Xiao and Jian-Jiang Zhong

Abstract Higher fungi or called as macro-fungi, consisting of the divisions ascomycetes, basidiomycetes, and imperfect fungi, are receiving great interest around the world, because studies of higher fungi help us not only to find new edible and officinal resources but also to understand their complicated biology. In recent decades, a large number of useful substances from higher fungi have been isolated, identified, and characterized, which have important biological functions, such as reducing blood pressure, enhancing immunity, and possessing anti-cancer and anti-HIV and other pharmacological activities. This chapter will review the genetic manipulation tools for higher fungi, omics analysis of higher-fungus cell factories, and production of useful metabolites by higher fungi, including those of terpenoids, heterocyclics, polysaccharides, and polyketides. Trends in future development of cell factories of higher fungi for useful metabolite production will also be analyzed.

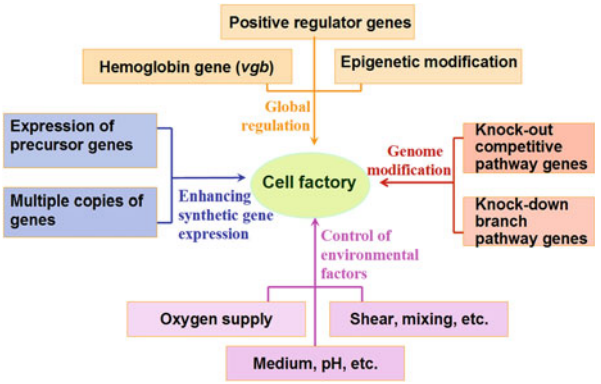
H. Qin · H. Xiao · J.-J. Zhong (✉)
State Key Laboratory of Microbial Metabolism, Joint International Research Laboratory of Metabolic & Developmental Sciences, and Laboratory of Molecular Biochemical Engineering, School of Life Sciences and Biotechnology, Shanghai Jiao Tong University, 800 Dong-Chuan Road, Shanghai 200240, China
e-mail: jjzhong@sjtu.edu.cn

J.-W. Xu
Faculty of Life Science and Technology, Kunming University of Science and Technology, Kunming 650500, China

J.-H. Xiao
Center for Translational Medicine, Affiliated Hospital of Zunyi Medical University, Zunyi 563000, China

Y.-J. Tang
Key Laboratory of Fermentation Engineering (Ministry of Education), Hubei Provincial Cooperative Innovation Center of Industrial Fermentation, Hubei University of Technology, Wuhan 430068, China

Graphical Abstract



Strategies for improving cell factories of higher fungi for useful metabolite production

Keywords Genetic manipulation · Higher fungi · Omics analysis · Cell factory · Medicinal mushroom · Fermentation technology · Metabolic engineering · Secondary metabolite production

Contents

1 Introduction

Higher fungi or macrofungi, consisting of the divisions ascomycetes, basidiomycetes, and imperfect fungi, refer to the fungi with hyphae well-developed, septate, and usually at some stage of development interwoven into a compact tissue especially in the fruiting body. Studies of higher fungi help us not only to find new edible and officinal resources but also to understand their complicated biology. Several thousand years ago, people used higher fungi as medicinal and food sources, but at that time, their functional components were unknown. In recent decades, a large number of useful substances from higher fungi have been isolated, identified, and characterized, which could reduce blood pressure, enhance immunity, and possess anticancer and anti-HIV and other pharmacological activities. Some new bioactive compounds reported in recent years are shown in Table 1, and most of them are secondary metabolites, which are organic compounds not directly involved in the normal growth, development, or reproduction of an organism but often play an important role in plant defense against herbivory and other interspecies defenses. These compounds can be used as potential lead compounds for new drug development. However, bioactive secondary metabolites produced by higher fungi are generally of very low productivity and are thus unable to meet the requirement for (pre-)clinical study and large market supply. Furthermore, these metabolites are usually of complicated chemical structures and very difficult to synthesize chemically at high efficiency. Higher fungus cell factories, therefore, have received increasing attention to achieve large-scale industrial production of their unique and important natural compounds.

Microbial cell factories are the basis of biochemical conversion from low-cost raw materials and/or agro-industrial by-products into valuable medicine, chemicals and energy, or detoxifying harmful/toxic chemicals. When a beneficial compound from higher fungi is identified, we need to reveal its biosynthetic pathway and to improve its biosynthesis by genetic or metabolic engineering. Also, its large-scale efficient production using fermentation technology is necessary for industrial application. The framework in constructing higher fungus cell factories is shown in Fig. 1.

Cell factories of higher fungi have been more and more widely applied to produce value-added compounds. For example, the higher fungus *Ganoderma lucidum* is used for the production of ganoderic acid, a potential antitumor terpenoid; the key biosynthetic genes in its pathway upstream were overexpressed, and the fermentation process parameters were optimized, and a new two-stage fermentation strategy was also developed to achieve a high yield of the product [41–44]. In order to better understand the significance of cell factories of higher fungi, in the following, this article is outlined from the construction and analysis of high-fungus cell factories to their production of useful metabolites.

Table 1 New bioactive compounds recently reported in higher fungi

Higher fungi	Compounds	Bioactivities	References
Terpenoids			
Ganoderma boninense	Ganoboninketals A-C	Anticancer	[1]
Stereum hirsutum	Three hirsutane-type sesquiterpenoids	Antimicrobial and anticancer	[2]
Inonotus rickii	Inonotic acid A		[3]
	3-O-Formyl inonotic acid A		
	Inonotic acid B		
	3,6-Dihydroxycinnamolide	Anticancer	
Pleurotus cornucopiae		Antibacterial	[4]
Granulobasidium vellereum	2-Hydroxycoprinolone		[5]
	8-Deoxy-4a-hydroxytsugicoline		
	8-Deoxydihydrotsugicoline		
Naematoloma fasciculare	Four new lanostane triterpenoids	Anticancer	[6]
Flammulina velutipes	Enokipodins E-J	Antifungal activity	[7]
	Sterpurols A	Anticancer and antioxidant	
Sarcodon scabrosus	Secoscabronine M	Anticancer	[8]
	Scabronine M	Anticancer	[9]
Neonothopanus nambi	Nambinones A-C	Anticancer	[10]
	1-Epi-nambinone B		
	Nambinone D		
	Aurisin K	Antimalarial and anticancer	
Armillaria	Melleolide sesquiterpene aryl esters	Anticancer	[11]
Ganoderma lucidum	Lucidenic acids A-C, N	Anticancer	[12]
	7-O-Ethyl ganoderic acid O	Anticancer	[13]
	3α,22β-Diacetoxy-7α-hydroxyl-5α-lanost-8, 24E-dien-26-oic acid	Anticancer	[14]
	3-O-Acetylganoderic acid B	Antimicrobial, anti-HIV, antitumor, antioxidation	[15]
	8β,9α-Dihydroganoderic acid C		
	3-O-Acetylganoderic acid K		
	Ethyl 3-O-acetylganoderate B		
	Ethyl ganoderate J		

(continued)

Table 1 (continued)

Higher fungi	Compounds	Bioactivities	References
Heterocyclics			
Hericium erinaceus	Erinacerins C-L	Anticancer	[16]
	Isohericenone	Anticancer	[17]
	3-Hydroxyhericenone F, Hericenone I and J	Endoplasmic reticulum (ER) stress-suppressive activity	[18]
Sarcodon leucopus	Sarcoviolin beta and episarcoviolin beta	Antioxidant and anticancer	[19]
Phellinus ribis	Phelliribsin A	Anticancer	[20]
Trifolium nigrescens	4‴,5,5″,7,7″-Pentahydroxy-3′,3‴-dimethoxy-3-O-beta-D-glucosyl-3 ″,4′-O-biflavone	Antioxidant and tyrosinase inhibitory activities	[21]
Neolentinus lepideus	5-Methoxyisobenzofuran-4,7(1H,3H)-dione, 1,3-Dihydroisobenzofuran-4,6-diol	Antibacterial	[22]
Lasiosphaera fenzlii	4,6-Dihydroxy-1H-isoindole-1,3(2H)-dione, 4,6-Dihydroxy-2,3-dihydro-1H-isoindol-1-one	Anticancer	[23]
Macrolepiota neomastoidea	Macrolepiotin	Anticancer	[24]
Amanita exitialis	N-2-(1-Methoxycarbonylethyl)guanosine	Anticancer	[25]
Ganoderma colossum	Ganomycin 1	Anti-HIV	[26]
Xylaria sp. PSU-F100	Xylarisin 132	Antibacterial	[27]
Xylaria sp. (#2508)	Xylopyridine A	DNA-binding affinity	[28]
Phellinus linteus	Phellifuropyranone A	Anticancer	[29]
Cortinarius brunneus	N-Glucosyl-1H-indole derivatives		[30]
Cortinarius subtortus	(Iso)-Quinoline alkaloids	Antioxidant	[31]
Miscellaneous			
Cordyceps taii	Deacetylcytochalasin C Zygosporin D	Anticancer	[32]
Lentinus polychrous	6-Methylheptane-1,2,3,4,5-pentaol	Anticancer	[33]
Tuber indicum	Four novel cerebrosides	Antifatty liver, antitumor	[34]
Cordyceps jiangxiensis	Jiangxienone	Anticancer	[35]
Hericium erinaceums	Hericenone L	Anticancer	[36]
Cantharellus cibarius	(10E, 14Z)-9-Oxooctadeca-10,14-dien-12-ynoicacid	Anticancer	[37]

(continued)

Table 1 (continued)

Higher fungi	Compounds	Bioactivities	References
Thelephora aurantiotincta	Thelephantin O	Anticancer	[38]
Tuber indicum	5α-Androst-16-en-3α-ol	Increase the sexual arousal of human female, adjust moods, mediate human menstrual synchrony	[39]
Thelephora vialis	Vialinin A	Antioxidant and anticancer	[40]

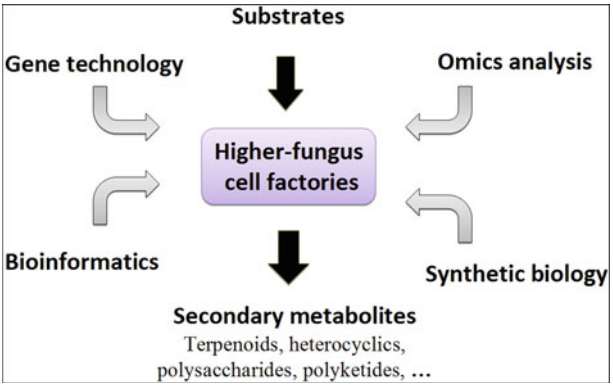


Fig. 1 Framework in constructing higher fungus cell factories for useful secondary metabolite production

2 Genetic Manipulations of Higher Fungi

Initially, the designing strategies of higher fungus cell factories by genetic engineering were mainly aimed at single gene modification and/or an individual pathway, which may not efficiently change the metabolic flux. With the advance of metabolic engineering and synthetic biology, the focus has gradually shifted to polygenic modifications and multimetabolic pathways. This has resulted in the shift in designing strategies of metabolic engineering from conventional deletion or overexpression of endogenous genes in individual metabolic pathways to current combinatorial approaches with manipulation of key gene expression of metabolic networks in an entire cell [45] (Fig. 2), while control of environmental factors (Fig. 2) could provide useful epigenetic information and cellular physiological and metabolic responses to cultivation conditions (especially in large-scale cultivation) with aid of omics analysis (Fig. 1). Recently, higher fungi as cell factories for the production of secondary metabolites have attracted extensive interests around the world. While compared with the achievement of the cell factories of *Escherichia*

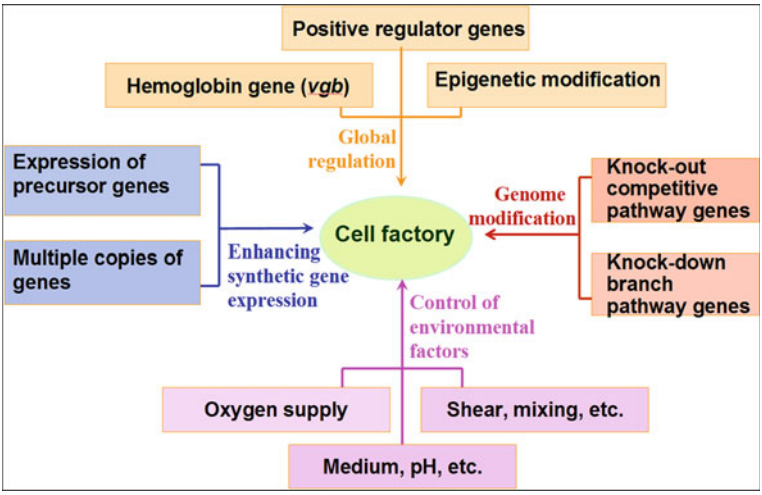


Fig. 2 Strategies for improving cell factories of higher fungi

coli and yeasts, the studies on higher fungi are obviously lagging behind, which requires more research inputs urgently. In this section, the tools for constructing high-fungus cell factories, including genetic transformation methods, gene over-expression, gene silence, and gene deletion, will be overviewed as follows.

2.1 Genetic Transformation Methods

The development of genetic transformation technology provides a powerful tool for the study of gene expression and gene function. Establishment of an efficient transformation system is an important premise for functional analysis of genes. The genetic transformation technology has been applied to various mushrooms, such as *Agaricus bisporus* [46], *Flammulina velutipes* [47], *Volvariella volvacea* [48], *Ganoderma weberianum* [49], *and Tremella fuciformis* [50]. Over the years, there have been great advances in transformation methods of higher fungi. For example, *Agrobacterium*-mediated transformation was successfully applied to higher fungi, which was originally used in plants. Here, the development of transformation technology in higher fungi is to be overviewed, including polyethylene glycol (PEG)-mediated transformation, electroporation transformation, *Agrobacterium tumefaciens*-mediated transformation, and restriction enzyme-mediated DNA integration. The comparison of those four methods is shown in Table 2.

Table 2 Comparison of four transformation methods

Method	Applicability	Efficiency	Operability	References
PEG-mediated transformation	Ordinary	Ordinary	Ordinary	[51]
Electroporation transformation	High	Ordinary	High	[52]
Agrobacterium tumefaciens-mediated transformation	Ordinary	Low	Low	[44]
Restriction enzyme-mediated DNA integration	Low	High	Ordinary	[53]

2.1.1 PEG-Mediated Transformation

The PEG protoplast transformation method has been applied to *G. lucidum* [51], *Pleurotus nebrodensis* [54], *Lentinus edodes* [55], etc. In the past, the efficiency of this transformation process was generally low and the exogenous gene was difficult to integrate into the genome by this method, and many transformants lost their resistance phenotype after several-week growth in the absence of selection pressure [56]. However, after many efforts, this method has been improved and become one of main ones in higher fungi.

Li et al. [51] developed an efficient PEG-mediated transformation method for *Pleurotus ostreatus*. Heparin, ATA, and spermidine were used to improve the transformation efficiency. As a result, 80–180 colonies could be obtained per μg of DNA per 10^7 protoplasts with the hygromycin B phosphotransferase gene (*hph*) as a selection marker, which was much higher than previously reported [57]. And 120–150 and 85–100 transformants per μg of DNA per 10^7 protoplasts were also obtained in *G. lucidum* and *L. edodes*, respectively. That means the PEG-mediated transformation could be a useful tool for genetic engineering in mushrooms.

Yu et al. [58] transformed a homogenous 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR) gene into *G. lucidum* by PEG-mediated transformation method. The gene *sdhB* mutation was used as a selection marker. A total number of 15–20 transformants per μg plasmid DNA were obtained. What's more, they found that the transformants could maintain the resistance phenotype after five passages of cultivation on a non-selection medium. Southern blot analysis confirmed that the *sdhB* gene was stably integrated at multiple sites in the genome.

High efficiency, convenience, and genome integration are the merits of the PEG-mediated transformation method in the genetic engineering of higher fungi. However, the genes integrated into the genome are usually of multiple copies, and the protoplast preparation is also time-consuming.

2.1.2 Electroporation Transformation

Electroporation transformation has also been used in higher fungi, such as *T. fuciformis* [59], *L. edodes* [60], *F. velutipes* [61], and *G. lucidum* [62]. Compared with other transformation methods, it is simple, rapid, and of wide application range. In

the past, electroporation transformation required protoplast preparation, which was tedious, but recently, scientists have developed a modified method for basidiospores or mycelial fragments. Kuo et al. [63] did transformation by electroporation of basidiospores or mycelial fragments in *F. velutipes*. While the basidiospores or mycelial fragments also need lysing enzymes to incubate for 2 h, it is much easier than protoplasting. The efficiency using basidiospores could be 30–150 transformants per μg DNA in *L. edodes* [60]. The resistance was stable for at least 6 months during subcultivation. Southern blotting analysis confirmed that the gene was stably integrated into the genome.

The modified electroporation transformation has many advantages. It is simple and cheap and does not require protoplasts. For some mushrooms, it is difficult to obtain a sufficient number of protoplasts and the regeneration efficiency from protoplasts was also very low. Thus, this method was more widely applicable than others under these circumstances. However, the method also has limitations [61]. During basidiospore isolation, contamination frequently happens. Moreover, multiple copies may be generated via random integration. It is not surprising that electroporation transformation is yet to be improved.

2.1.3 *Agrobacterium Tumefaciens*-Mediated Transformation

In 1995, Bundock used *A. tumefaciens*-mediated transformation (ATMT) in *Saccharomyces cerevisiae* for the first time [64]. De Groot et al. [65] applied ATMT to filamentous fungi—*Dictyostelium discoideum*. Recently, some higher fungi have been transformed by this method, including *A. bisporus* [66, 67], *F. velutipes* [68], *Heterobasidion annosum* [69], *P. nebrodensis* [70], *V. volvacea* [71], and *G. lucidum* [44]. The ATMT method has the following advantages: a wide range of transformation recipients, high degree of stability, and high proportion of single-copy transformants [44]. For the fungi lacking sexual stages, the exogenous gene single-copy transformation is very important. This made ATMT become one of the common higher fungal transformation methods.

Xu et al. [44] applied the *Agrobacterium*-mediated transformation to *G. lucidum* and obtained 10–15 transformants per 10^7 protoplasts. All tested transformants maintained resistance stably and the transformants showed 100 % mitotic stability for the *sdhB* selection marker. Most of the integrated DNA had a single copy in the genome.

The efficiency of ATMT was dependent on species, and this method was also reported to be unable to obtain transformants [72]. Many factors can affect the efficiency of this transformation, such as the conditions of protoplasts and *A. tumefaciens*, the ratio of the number of bacteria to protoplasts, and cocultivation temperature. Zhang et al. [73] tested different transformation parameters in *Hypsizygus marmoreus*. Their results showed that 25 °C and 2 days of coculture was best. Kemppainen et al. [74] studied the mechanism of ATMT and found no obvious sequence similarities between genomic sites and T-DNA (the transferred DNA of the tumor-inducing (Ti) plasmid of *A. tumefaciens*). About 75 % of the

integrations took place at the predicted locus in the model mushroom *Laccaria bicolor*.

ATMT has the irreplaceable advantage in the single-copy DNA integration, so this method has been developed rapidly these years [75]. But it should also be improved due to limitations such as low applicability and fluctuant transformation efficiency. As a transformation method affects the fate of transformed DNA, it should be paid attention to during the design of metabolic engineering strategies. For example, the PEG method could be best in the case where multiple copies of a gene should be randomly integrated into the genome [76]. On the other hand, when targeted integration or gene deletion is expected, the ATMT method would be the choice [77].

2.1.4 Restriction Enzyme-Mediated DNA Integration (REMI)

The mechanism of REMI is that added restriction enzymes in the mixture enter recipient cells, recognize genome/plasmid enzyme cutting sites, and realize the cutting and integration, and then, incisions are connected by DNA ligase. The earliest REMI was established in yeasts [78], and it has a wide range of application [79]. Recently, with increasing studies on higher fungi, REMI has also been applied to *G. lucidum* [80], *C. cinereus* [81], *L. edodes* [82], *Pleurotus eryngii* [53], and others. For example, Noh et al. [53] transformed the enhanced cyan fluorescent protein (ECFP) gene in *P. eryngii* via REMI, resulting in 10–40 hygromycin-resistant transformants per μg of the Hind III-digested DNA and 10^6 protoplasts. Southern blot analysis revealed that the gene was integrated to the genome.

One of the advantages of REMI is that it can generate various mutants [83]. This advantage has been used to analyze the genes related to the mutant characteristics. For example, by using REMI, Nakazawa et al. [84, 85] obtained a lot of mutants and found that the *Cc.rmt1* gene encoded a putative arginine methyltransferase and the *Cc.ubc2* gene affected clamp cell morphogenesis and nuclear migration of *C. cinerea*.

REMI can also be used for the site of insertion and selection marker and provides great convenience for screening. But, it also needs protoplast preparation and requires the addition of PEG and $CaCl_2$ for higher transformation efficiency. Cutting sites of the restriction enzyme are random; at the same time, to obtain sufficient number of transformants, the REMI system needs to select the optimal transformation enzyme, plasmid, and recipient cells. Thus, this method has not been widely applied, compared to the ATMT and PEG methods described above.

As another transformation method, the particle bombardment method has also been used as a transformation tool in higher fungi, as reported by Sunagawa and Magae [86] and Sunagawa et al. [87]. But, because of requiring a special instrument —particle bombardment gun—this method is not the first choice.

2.2 Selectable Marker

A resistance marker is generally used for selection of positive transformants. The commonly used resistance markers in higher fungi are of drug resistance, so that the positive transformants could grow in the culture medium with the presence of drug (s). Until now, the most widely used marker is hygromycin B phosphotransferase (*hph*) gene derived from bacteria and the transformants would obtain hygromycin resistance. Interestingly, our recent work [44] found that the mutated *sdhB* gene encoding an iron-sulfur protein subunit of succinate dehydrogenase, which successfully conferred carboxin resistance upon transformation, was a suitable resistance marker, and the efficiency of transformants screening in carboxin resistance was within the range of other reported cases. But, the use of hygromycin resistance was found unsuccessful to obtain a positive transformant in the system.

2.3 Gene Overexpression

The exogenetic genes may exist stably in cells through transformation. However, the low gene expression level is a bottleneck to the construction of cell factories. Thus, in this case, the gene overexpression is critical to successful metabolic engineering. Upregulation expression of key genes can effectively improve the level of downstream metabolite synthesis. Recently, gene overexpression has been applied to gene engineering of higher fungi in developing useful cell factories.

Xu et al. [44] overexpressed the HMGR gene which is a key enzyme in the synthetic pathway of ganoderic acids in *G. lucidum*, by using a homogenous *gpd* as a promoter. As shown in Fig. 3, the ganoderic acid content reached twofold in the overexpressed cells compared to the wild type (control). The accumulation of intermediates, such as squalene and lanosterol, was also increased. The results showed the promising potential of metabolic engineering of the ganoderic acid pathway via the transgenic system.

Zhou et al. [88] improved the production of individual ganoderic acids by engineering the biosynthetic pathway of ganoderic acids in *G. lucidum* through overexpressing the gene of squalene synthase (SQS), which catalyzes the following reaction: 2 farnesyl diphosphate + NAD(P)H $\leftrightarrows$ squalene + 2 diphosphate + NAD(P)(+). The constructed SQS strain may be a suitable basis for further development of an industrial process for hyperproduction of the antitumor secondary metabolite (Fig. 4).

The direct expression of a target gene is one way of overexpression, while the promoter engineering is another way to optimize the expression of target genes. Chai et al. [89] adopted promoter engineering to enhance the biosynthesis of β-glucans in *P. ostreatus*, in which β-glucan synthase is its key enzyme. After changing the β-glucan synthase promoter into a strong one from *Aspergillus nidulans*, the product level was increased up to 32 %, which is the first report of successful swapping of promoters in higher fungi.

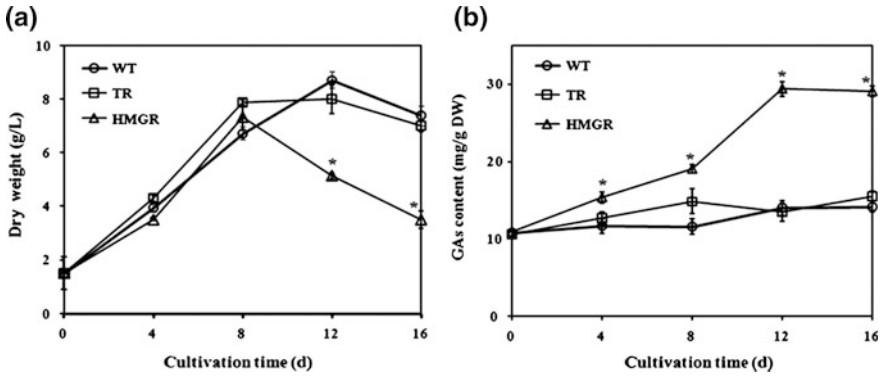


Fig. 3 Kinetic profiles of cell growth (**a**) and ganoderic acid content (**b**) in the wild-type strain (*open circle*), the strain transformed with a void plasmid (*open square*), and the tHMGR-overexpressed strain (*open triangle*). The error bars indicate the standard deviations from three independent samples. *Asterisks* statistical significance ($P < 0.05$) compared to the WT strain, *d* days [44]

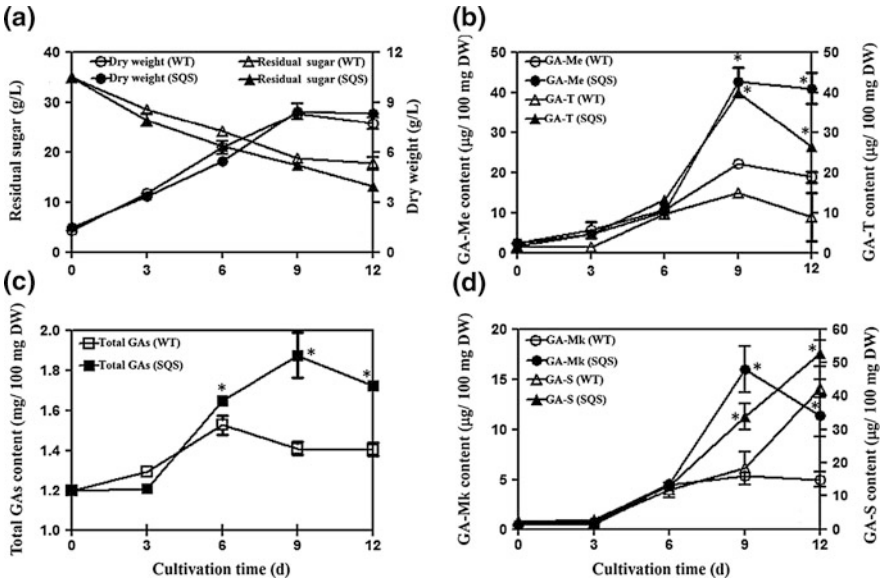


Fig. 4 Kinetic profiles of cell growth and residual sugar (**a**), content of total GAs (**c**), accumulation of GA-Me and GA-T (**b**), and GA-S and GA-Mk (**d**) in the WT strain and the SQS-overexpressed strain. The *error bars* indicate the standard deviations from three independent samples, *d* days [88]

The above-mentioned strategies are mainly related to changing promoters by introducing endogenous or heterogonous promoters to increase the expression of key genes in biosynthetic pathways. In another aspect, Lin et al. [90] reported that heterogonous protein expression in the enoki mushroom *F. velutipes* was notably enhanced by polycistronic strategy to express multiple copies of a single gene. This strategy can realize not only single gene copy but also coexpression of multiple genes.

Many efforts have been made to enhance the expression of targeted genes in higher fungi by promoter engineering, increasing gene copy number, etc. Coconi-Linares et al. [91] overexpressed three peroxidases in a single Phanerochaete chrysosporium strain, which increased the production yield of lignolytic enzymes up to 4 times. However, the gene expression level is still difficult to control precisely, making the regulation of the metabolic flux of higher fungus cell factories not so easy.

2.4 Gene Silencing

Gene silencing, or gene knockdown, reduces the expression of a targeted gene [92]. By this, we can downregulate the metabolic branch pathway and decrease the flux to unfavorable metabolic pathway, and ultimately enhance the metabolic flux toward the targeted product biosynthesis. These years, gene silencing has been applied to *C. cinereus* [93], *A. bisporus* [94], *L. bicolor* [95, 96], *L. edodes* [97], and *G. lucidum*62. The results show that the gene silencing technology is important to efficient biosynthesis of useful metabolites and it also helps the characterization of gene functions in higher fungi [98].

RNA interference (RNAi) is an RNA-dependent gene silencing process that could inhibit the expression of specific genes at the posttranscriptional stage by introducing small double-stranded interfering RNAs [99]. Mu et al. [62] cosilenced the genes of orotidine 5′-monophosphate decarboxylase (URA3, as a reporter) and laccase in *G. lucidum*. Their results showed that the highest rate of URA3 silencing, reaching 81.9 %, was obtained by using the dual promoter vector.

RNAi is usually used for gene functional analysis. Godio et al. [100] proved that a squalene epoxidase gene (*erg1*) from basidiomycete *Hypholoma sublateritium* was involved in the biosynthesis of clavaric acid (an antitumor triterpenoid) and ergosterol. When *erg1* was cloned and expressed with the *gpdA* promoter from *A. bisporus*, the production of clavaric acid was increased, while silencing *erg1* gene by antisense RNA resulted in the reduction of clavaric acid production and appeared an ergosterol-dependent phenotype (Fig. 5).

Different from gene knockout, the efficiency of gene silencing cannot reach 100 %, but at least 70 % of the targeted RNA could be depleted. However, this ‘limitation’ also has an advantage over the gene knockout in cases where essential genes under investigation are still required [101].

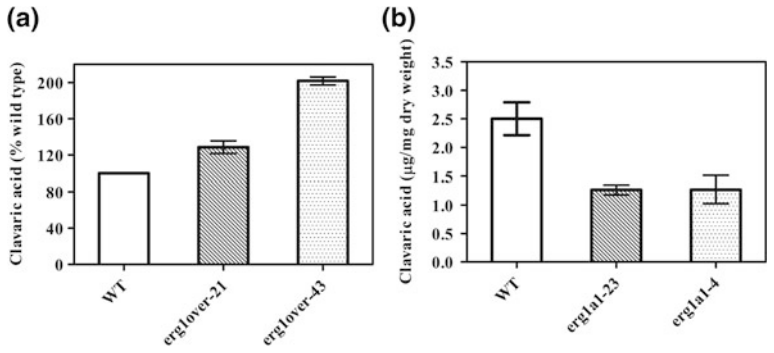


Fig. 5 Production (**a**) and content (**b**) of clavaric acid in cultures of *H. sublateritium* and the different transformants in minimal medium containing asparagine/glutamate as nitrogen source. WT, the wild-type strain; erg1over-21, the erg1-overexpressed strain 21; erg1over-43, the erg1-overexpressed strain 43; erg1a1-23, the erg1-silenced strain 23; erg1a1-4, the erg1-silenced strain 4 [100]

2.5 Gene Deletion

Gene deletion, the same as gene knockout or gene inactivation, is an important tool in learning about genes with unknown or incompletely known functions. This method usually occurs by homologous recombination. As a result, after recombination, no expression of targeted genes can be detected.

Gene deletion strategy has made a breakthrough in mushroom-forming fungus in recent years. In the past, gene inactivation was only reported in the mushroom *Schizophyllum commune* by homologous recombination, where the deletion efficiency was only 3.25 % for most targeted genes [102]. The low efficiency of homologous integration is the main obstacle to apply this method to other higher fungi. Recent progresses were made by the group of de Jong [103] and Ohm [104]. At first, they constructed a dedicated deletion vector pDelcas consisting of two antibiotic resistance cassettes. The nourseothricin resistance was located between the genes to be deleted, while the phleomycin resistance was an amplification of an ectopic integration. These transformants were screened out by using a fast colony PCR to confirm gene knockouts. Other scientists found that the inactivation of *ku80* and/or *ku70* which was related to the non-homologous end joining (NHEJ) could increase the frequency of the targeted gene knockout by homologous recombination [105]. Then, de Jong et al. [103] developed a constructive method to delete *ku80* gene and used the resulting strain for inactivation; finally, 7 out of 10 transformants were deleted, in which the efficiency of their gene disruption was greatly increased.

This improved system has been used in functional gene analysis in *S. commune*. van Peer et al. [106] deleted the *spc14* gene, which is related to the septal pore cap (SPC), and found that SPC was an organelle functioning in vegetative growth and mushroom formation. Ohm et al. [107] found that 5 transcription factor genes were related to the regulation of mushroom formation by deleting these genes in Δ*ku80*

strain with pDelcas vector. In addition, Berends et al. [108] inactivated the *alg3* gene in *S. commune* by introducing pDelcas vector, and as a result, Man(3)GlcNAc (2) protein-linked N-glycans was predominantly produced.

Nakazawa et al. [109] also found the frequency of gene disruption was enhanced by inactivation of *ku70* gene in *C. cinerea*. Salame et al. [110] disrupted a targeted gene based on the Δ*ku80* strain and found the redundancy among genes of manganese peroxidases (MnPs) in *P. ostreatus* [111, 112].

3 Omics Analysis of Higher Fungus Cell Factories

Omics analysis, including genomics, transcriptomics, proteomics, and metabolomics, is an important approach to find new functional genes, proteins, metabolic networks, and metabolic products. Combining with bioinformatics, it has a great advantage to identify gene functions and to understand metabolite biosynthetic mechanisms. As we know, there are still many bioactive compounds of higher fungi, whose biosynthetic pathways are not yet identified. The development of omics analysis plays a significant role in elucidating biosynthetic pathways of metabolic products, and it is complementary to the traditional methods of drug candidate screening and identification. By omics technologies, scientists can further design, modify, and reconstruct higher fungus cell factories based on the obtained findings and implications, which can improve the rationality and validity of metabolic engineering and synthetic biology.

3.1 Genomic Analysis

More and more higher fungi genomes have been released due to technological progress. The development of genome sequencing in higher fungi provides opportunities for research and development of their metabolic products, as the genome information facilitates the discovery and biosynthetic study on bioactive compounds from higher fungi.

Recently, genomic information of a couple of higher fungi is obtained, including common edible mushroom such as *A. bisporus* [113], *F. velutipes* [114], *V. volvacea* [115], *Omphalotus olearius* [116], *S. commune* [117], *Lignosus rhinocerotis* [118], *Mycena chlorophos* [119], medicinal mushroom *G. sinese* [120], *G. lucidum* [121], and *Cordyceps militaris* [122].

F. velutipes, as an important edible mushroom, is also a rich source of secondary metabolites and enzymes which affect wood-degrading machinery and ethanol production. By sequencing and analyzing *F. velutipes* genome, 58 potential enzymes for ethanol production were identified, which provided new possibilities to use *F. velutipes* for ethanol fermentation [114].

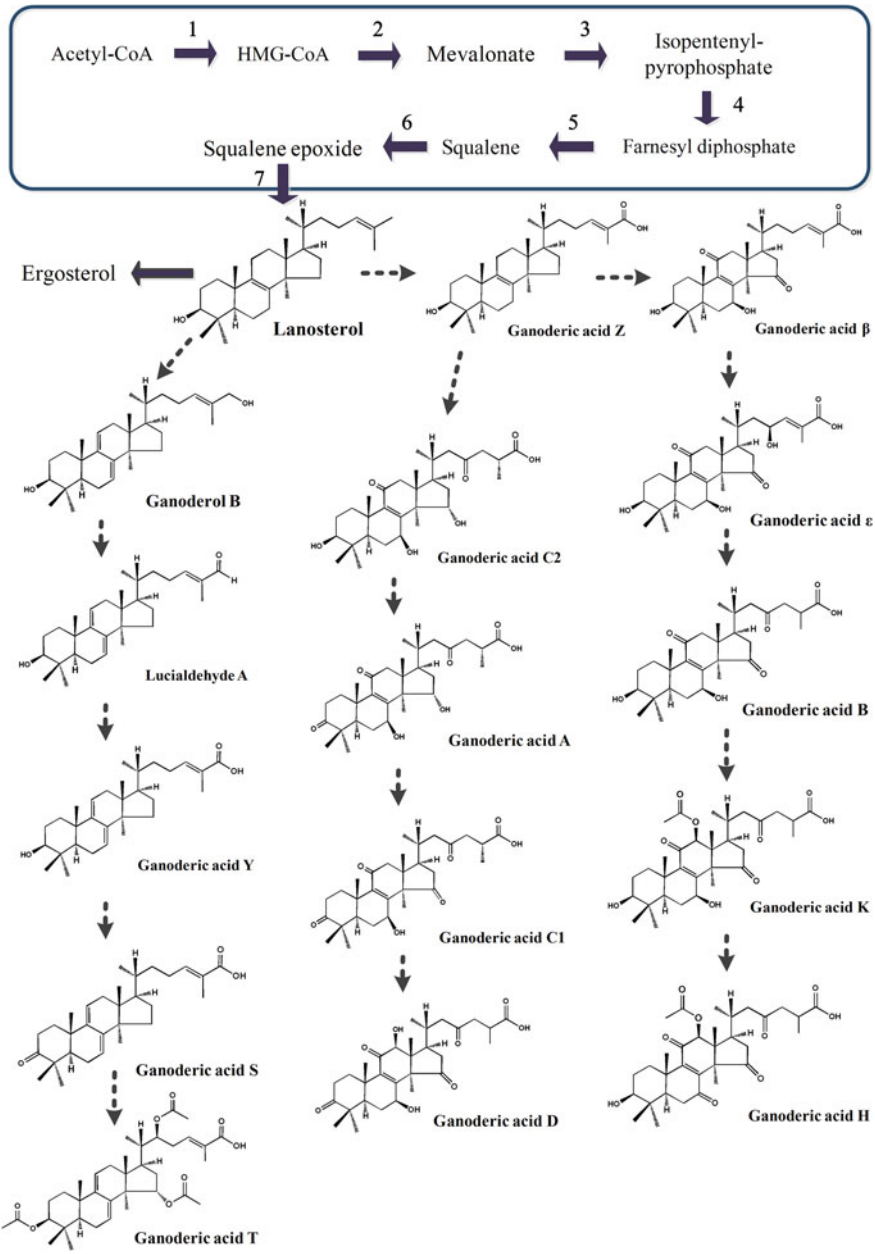


Fig. 6 Possible synthetic pathway of ganoderic acids [121]. *1* HMG-CoA synthase (*hmgs*); *2* HMG-CoA reductase (*hmgr*); *3* mevalonate-5-pyrophosphate decarboxylase (*mvd*); *4* farnesyl pyrophosphate synthase (*fps*); *5* squalene synthase (*sqs*); *6* squalene epoxidase (*se*); *7* lanosterol synthase (*ls*)

Zhu et al. reported the genome sequence of *Ganoderma sinense* and found that most of *G. sinense* gene clusters were silenced under common culture conditions. DNA methylation and small RNA-mediated reversible gene silencing tightly maintained this control, suggesting epigenetics may play critical roles in the regulation of *G. sinense* secondary metabolism [120].

Chen et al. [121] analyzed the genome of *G. lucidum*, which has been used as a drug with antitumor, antiaging, antiviral, and immunomodulatory activities since ancient times in East Asia [123]. Its triterpenoids—ganoderic acids and ganoderan—are known as main bioactive compounds [124]. Although the ganoderic acids are known to be synthesized via the mevalonate pathway (MVP) [125], it remains a mystery about the detailed modification of the lanosterol skeleton, which may be related to cytochrome P450 superfamily (CYPs). The predicted biosynthetic pathway of ganoderic acids is shown in Fig. 6. Sequencing analysis of *G. lucidum* genome revealed possible 16 CYPs involved in the terpenoid synthesis, which was favorable to the identification of ganoderic acid synthetic pathway and to the massive production of the triterpenoids as well as to the heterogonous expression via synthetic biotechnology. Then, Liu et al. [126] performed comprehensive annotation for these genes, which were analyzed from the genome of *G. lucidum*. Their work showed the genes related to triterpene biosynthesis and wood degradation. Recently, Qian et al. [127] identified simple sequence repeats (SSRs) or microsatellites in *G. lucidum* and analyzed their frequency and distribution in different genomic regions. Xu et al. [128] used qRT-PCR in *G. lucidum* gene analysis. Li et al. [129] identified and characterized long intergenic noncoding RNAs (lincRNA) in the mushroom. The study about the genome of *G. lucidum* will definitely promote the future R&D toward pharmacological and industrial applications.

3.2 Transcriptomic Analysis

Transcriptome is the set of all RNA molecules—mRNA, rRNA, tRNA, and other noncoding RNA transcribed in one cell or a cell population. It can be applied to the total set of transcripts in a given organism or to the specific subset of transcripts present in a particular cell type. Different from the genome which is roughly fixed for a given cell line, the transcriptome may vary significantly with external environmental conditions. As all mRNA transcripts in a cell are included, the transcriptome reflects the genes being actively expressed at any given time, except for mRNA degradation phenomena. The study of transcriptomics, also called as expression profiling, examines the mRNAs expression level in a given cell population, often using high-throughput techniques based on DNA microarray technology, or using next-generation sequencing technology known as RNA sequencing (RNA-Seq) to study the transcriptome at the nucleotide level. Transcriptomic analysis has been used in higher fungi *C. militaris* [130], *G. lucidum* [131], *L. edodes* [132], *V. volvacea* [133], and *P. ostreatus* [134].

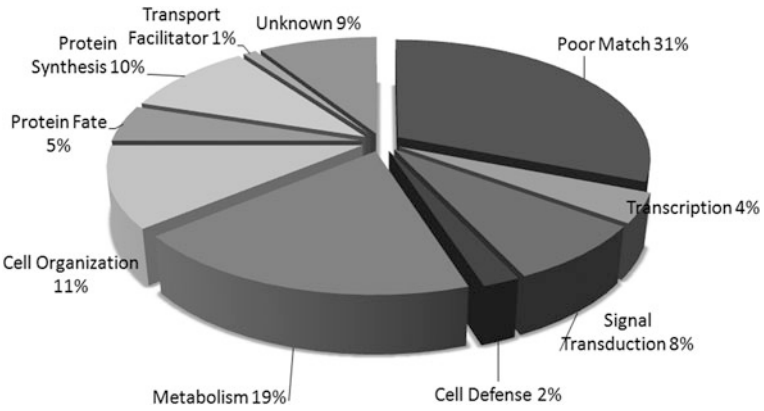


Fig. 7 Functional categories of 147 cDNA sequences in the *G. lucidum* SSH-cDNA library [135]

Xu et al. [135] did comparative transcriptome analysis of *G. lucidum* using suppression subtractive hybridization (SSH) technique to identify differentially expressed genes in liquid static culture versus shaking culture. As a result, 147 unigenes significantly expressed in static culture were identified, including those related to asexual sporulation and signal transduction (Fig. 7). Zhu et al. [136] screened for RNA editing sites at the genomic level in *G. lucidum* and revealed the role of transcriptional plasticity in the mushroom growth and development and in the regulation of secondary metabolic pathway.

RNA-Seq is a recently developed method for transcriptome profiling by deep-sequencing technologies [137], which has advantages: detected transcripts correspond to genomic sequences and it has a low background signal. Thus, RNA-Seq has become a main method for transcriptomic analysis. Yu et al. [138] analyzed the transcriptome of *G. lucidum* via Illumina high-throughput technology. Their studies performed the functional genes involved in the terpenoid biosynthesis pathway and wood degradation. Plaza et al. [139] reported that the exposure of different fungi tissues to different types of antagonists shaped the expression patterns of defense loci in a tissue-specific manner. Yang et al. [140] found enzymes related to saponin biosynthesis in the termite mushroom *Termitomyces albuminosus*, including 22 glycosyltransferase and 6 cytochrome P450 s genes by de novo sequencing and transcriptome analysis.

Ophiocordyceps sinensis, or called *Cordyceps sinensis*, has thousands of years of history as a traditional Chinese medicine because of its regulation function on human body [141]. In recent years, the main active ingredients of *O. sinensis* were identified. Transcriptome analysis by Xiang et al. [142] found that 121 genes might be involved in the regulation of signal transduction and transcription level of *O. sinensis*. They also analyzed the adenosine kinase, adenylate kinase, and 5′-nucleotidase probably related to the phosphorylation and dephosphorylation in the cordycepin biosynthesis, and the work provided useful information for identifying the cordycepin biosynthetic pathway.

3.3 Proteomic Analysis

Proteomic analysis is the systematic identification and quantification of the complete complement of proteins of a biological system such as a cell, tissue, or organism at a specific time point. Cell metabolic activities are directly/indirectly regulated by proteins. Therefore, proteomic analysis can help us better understand the cellular metabolism. But not all of the protein spots can be identified by proteomic analysis. Proteome study is still under development in higher fungi although some proteomic analyses were conducted in recent years in *Termitomyces heimii* [143], *A. bisporus* [144], *Pleurotus tuber-regium* [145], *Antrodia cinnamomea* [146], and *G. lucidum* [147].

Zhang et al. [148] investigated the mechanism of the effect of Tween 80 on the exopolysaccharide production by *P. tuber-regium* using proteomic analysis. They identified 32 differentially expressed proteins by one-dimensional gel electrophoresis, and the ATP:citrate lyase isoform 2 could stimulate exopolysaccharide production. Wang et al. [149] used high-throughput sequencing analysis to obtain the transcriptome and proteome of *Agrocybe aegerita* mycelia and fruiting bodies. *A. aegerita* possesses multiple pharmacological activities such as antitumor, antiaging, and reducing blood lipids [150]. The work helped in revealing the polysaccharide and sterol synthetic pathway, and it was also found that the polysaccharide was highly biosynthesized in fruiting bodies. The information provided important clues for establishing the mushroom cell factories toward future application.

3.4 Metabolomic Analysis

Metabolome refers to the complete set of small-molecule metabolites (usually less than 1 kDa in size), such as metabolic intermediates, hormones and other signaling molecules, and secondary metabolites, to be found in a biological sample. Like transcriptome and proteome, metabolome is dynamic and changing from second to second. Metabonomics, as a scientific study of chemical processes involving metabolites, is the quantitative measurement of dynamic multiparametric metabolic responses of living systems to pathophysiological stimuli or genetic modifications. Thus, metabolic profiling can give an instantaneous snapshot of the cell physiology. One of the challenges of systems biology and functional genomics is to integrate proteomic, transcriptomic, and metabolomic information to provide a better understanding of cellular biology.

The metabolomic study can not only find difference in external and internal environment disturbance response, but also distinguish different phenotypes; thus, it is an important technology in omics research. With technology development, such as sophisticated nuclear magnetic resonance (NMR), gas chromatography–mass spectrometry (GC/MS), and high-performance liquid chromatography–mass

spectrometry (HPLC-MS), high-throughput metabolomics analysis has become possible on higher fungi metabolites.

Some scientists used metabolite profiles for the chemotaxonomy of higher fungi [151]. What's more, metabolomic study was also used for the analysis of different developmental stages or growth environments of higher fungi. For example, Park et al. [152] investigated the metabolic profiling of mycelia and fruiting bodies of *Cordyceps bassiana* by H-1 NMR spectroscopy and multivariate data analysis.

Metabolomic analysis is usually done by combining with other omics technologies such as transcriptomic and proteomic analyses to obtain more in-depth results. Matsuzaki et al. [153] performed the proteomic and metabolomic analyses of the benzoic acid metabolism of *Phanerochaete chrysosporium*. Bak et al. [154] used a polyomics-based analysis including metabolomics, proteomics, and transcriptomics of *P. chrysosporium* and tried to understand the metabolic and regulatory mechanisms of lignocellulose depolymerization.

4 Production of Useful Metabolites by Higher Fungus Cell Factories

As we know, during the long-term evolution, higher fungi have formed a special mechanism of metabolism in resisting unfavorable environments and becoming self-defense and survival during the entire life cycle. Diversified secondary metabolites with various bioactivities could be produced by higher fungi, such as terpenoids, heterocyclics, polysaccharides, polyketides, and polyphenols, which provide an abundant resource for drug discovery.

4.1 Terpenoids

Terpenes, as main bioactive compounds isolated from mushrooms, are known as an important category of naturally occurring bioactive metabolites produced by many higher fungi [116]. In particular, sesquiterpenoid, diterpenoid, and triterpenoid are typical representatives of terpenes with various interesting biological activities.

Ganoderic acids (GAs), a kind of highly oxygenated lanostane-type tetracyclic triterpene, are important secondary metabolites of *G. lucidum*. The production titer and productivity of GAs have reached great advancement in recent decade, as shown in Table 3. For example, Zhang and Tang [155] developed a novel three-stage light irradiation strategy for the efficient production of GAs and *Ganoderma* polysaccharides in submerged fermentation of *G. lucidum*. The maximal GA production was 69 % higher than the two-stage culture. Tang et al. [156] enhanced the GA production up to 754.6 mg/L through a pH-shift and DO-shift integrated fed-batch fermentation process. Liang et al. [42] found phenobarbital, a

Table 3 Typical studies on fermentation production of ganoderic acids

Strain	Ganoderic acid	Titer (mg/L)	Productivity [mg/(L h)]	Reference
G. lucidum CGMCC 5.616	Total GAs	210–1900	21–160	[158, 160–162]
G. lucidum CGMCC 5.616	Mk	0.71–260	0.088–22	[88, 158–160, 162]
G. lucidum CGMCC 5.616	T	0.71–240	0.089–20	[88, 158–160, 162]
G. lucidum CGMCC 5.616	S	0.81–270	0.10–17	[88, 158–160, 162]
G. lucidum CGMCC 5.616	Me	0.37–110	0.046–9.3	[88, 158–160, 162]
G. lucidum SB97	Total GAs	500	110	[163]
Ganoderma sinense SCIM 0701	Total GAs	260	37	[164]
Ganoderma applanatum ACCC-52297	Total GAs	293	65	[165]
G. lucidum SCIM 0006	Me	12	0.69	[166]
G. lucidum 5.26	Total GAs	680	75	[167]
G. lucidum 5.26	T	19	2.1	[167]

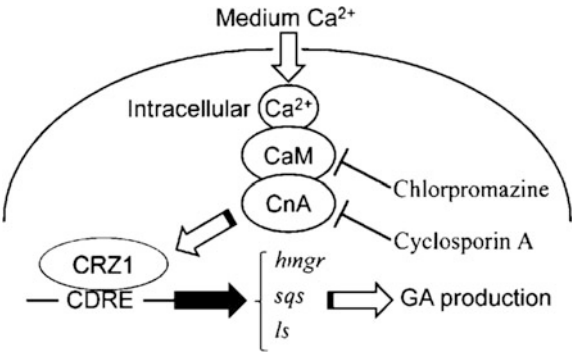


Fig. 8 Proposed Ca^{2+} induction mechanism in GA biosynthesis via calcineurin signaling [158]

P450 inducer, could enhance the production of total and individual GAs in two-stage cultivation. Zhao et al. [157] reported that nitrogen limitation led to more accumulation of GAs and upregulation of the biosynthetic genes.

Recently, signal transduction engineering of fungal secondary metabolism is receiving great interest for efficient production of valuable metabolites. Xu and Zhong [158] found that the calcineurin signal transduction was significant to GAs biosynthesis by *G. lucidum* (Fig. 8). Addition of calcium ion to static liquid cultures of *G. lucidum* resulted in the enhanced production of GAs, and the total GAs and individual GA-Mk, GA-S, GA-T, and GA-Me reached 3.7-, 2.6-, 3.2-, 4.5-, and 3.8-fold improvement compared to control, respectively. The group further reported that Na^{+} addition [159] and Mn^{2+} addition [160] could both enhance the GAs accumulation.

Dou et al. [168] studied the oxygen supply effect on the biomass and helvolic acid production in submerged fermentation of *C. taii*. Helvolic acid belongs to a member of fusidane skeleton triterpenoid family, which has a significant bactericidal activity, but few studies were performed about its fermentation production. The results showed that the value of initial volumetric oxygen transfer coefficient (K_La) greatly affected the production of both biomass and helvolic acid.

4.2 Heterocyclics

Heterocyclic compounds, or heterocyclics, are those whose one or more of the ring carbon atoms are replaced with a different element such as oxygen, nitrogen, and sulfur. Many types of heterocyclics from higher fungi have been isolated, and their structures and biological activities have been analyzed. These include indoles, pyridines, cytochalasins, quinolines, flavonoids, and nucleosides, and their anticancer, anti-HIV, antibacterial, and other pharmacological activities have been reported [169].

Cordycepin, 3-deoxyadenosine, is a major bioactive compound of *C. militaris*, which has various pharmacological activities, including antitumor, immunomodulatory, anti-inflammatory, and antibacterial ones [170]. The biosynthetic pathway of cordycepin has not been completely elucidated; however, many efforts have been made to enhance its production. Mao et al. [171] found that NH_4^+ had a significant effect on cordycepin production. Das et al. [172] improved the productivity of cordycepin in *C. militaris* by mutation using high-energy ion beam irradiation. In the work by Fan et al. [173], the influence of ferrous sulfate on the production of cordycepin was studied in shake flask cultures. The results indicated that the highest cordycepin titer was about 70 % higher than that without ferrous sulfate addition. This work might also be useful for further understanding the cordycepin biosynthesis.

Lovastatin is a member of the drug class of statins found in oyster mushrooms [174], used in combination with diet, weight loss, and exercise for lowering cholesterol in those with hypercholesterolemia to reduce risk of cardiovascular disease. Statistical experimental designs were used to optimize the lovastatin production by submerged fermentation of *P. ostreatus* [175]. The maximum lovastatin production reached 114.82 mg/L, which was 50 times higher than that obtained under the conditions without optimization.

Flavonoids, a class of secondary metabolites which have antioxidant effects and inhibitory activities on melanin biosynthesis, mostly exist in plants but have also been reported in higher fungi [176]. The *Vitreoscilla* hemoglobin, an oxygen-binding protein, could enhance cell growth, and it was used to alleviate oxygen limitation during submerged fermentation. Zhu et al. [177] expressed the *Vitreoscilla* hemoglobin gene (*vgb*) by REMI in *Phellinus igniarius*, which resulted in the improved growth and production of both total flavones. The metabolites reached 11.43 and 1.33 g/L, respectively, in bioreactor cultivations.

4.3 Polysaccharides

Macrofungal polysaccharides have been well known as part of traditional diet and medicine. The polysaccharides comprise a variety of biopolymers, such as β-glucans, providing a mechanism for cell protection or attachment to others [178]. The fungal polysaccharides mainly include exopolysaccharides (EPS) and intracellular polysaccharides (IPS), which could be produced by submerged mycelial cultures with a variety of medical applications. Until now, many polysaccharides have been isolated from various mushrooms, such as *Morchella conica* [179], *G. applanatum* [180], *Laetiporus sulphureus* [181], and *C. taii* [182], which have many bioactivities including antitumor [183], antioxidant [184], immunomodulatory [185], cytostatic, and antibacterial properties. Large-scale production of polysaccharides is very important for application, and some reports have been published on how to enhance its production such as optimization of culture conditions [186], addition of metabolic inducers [189], and genetic modifications [89].

Hwang et al. [187] investigated the optimum culture conditions in submerged culture of *L. sulphureus* var. *miniatus*, an edible mushroom. Interestingly, the most suitable initial pH for the metabolite synthesis was 2.0, which is rare in submerged cultures of macrofungi. In addition, supplementation of deep seawater (DSW) was also used for cultivation of higher fungi, and DSW was found to efficiently increase the mycelial growth and EPS production. As a result, the maximum mycelial growth (4.1 g/L) and EPS production (0.6 g/L) were achieved. Their work also showed that the EPS of *L. sulphureus* could increase cell proliferation and promote insulin secretion. Cui and Zhang [188] found that Mg^{2+}, Mn^{2+}, sodium dodecyl sulfate (SDS), and Tween 80 significantly enhanced the EPS production during two-stage submerged cultivation of *C. militaris*. The highest EPS production reached 3.28 g/L under the optimal condition. The results showed that additions of metal ion and surfactant could be used for enhancing the EPS production by *C. militaris*.

Xu et al. [189] overexpressed the gene of phosphoglucomutase (PGM) which is a key enzyme in the biosynthetic pathway of nucleotide sugar precursors. This enzyme catalyzes the conversion of glucose-6-phosphate into glucose-1-phosphate representing a branch point in carbohydrate metabolism. The maximum IPS content and EPS production in *G. lucidum* overexpressing the PGM gene reached 23.67 mg/100 mg dry weight and 1.76 g/L, respectively, which was higher by 40.5 % and 44.3 % than by the wild-type strain (Fig. 9). Ji et al. [190] improved the polysaccharide production by engineering the biosynthetic pathway in *G. lucidum* by overexpressing the homologous UDP glucose pyrophosphorylase (UGP) gene. The maximum IPS content and EPS production in the strain were 24.32 mg/100 mg dry weight and 1.66 g/L, respectively (Fig. 10). Their results showed the feasibility to enhance polysaccharide production by altering the expression of genes involved in precursor supply.

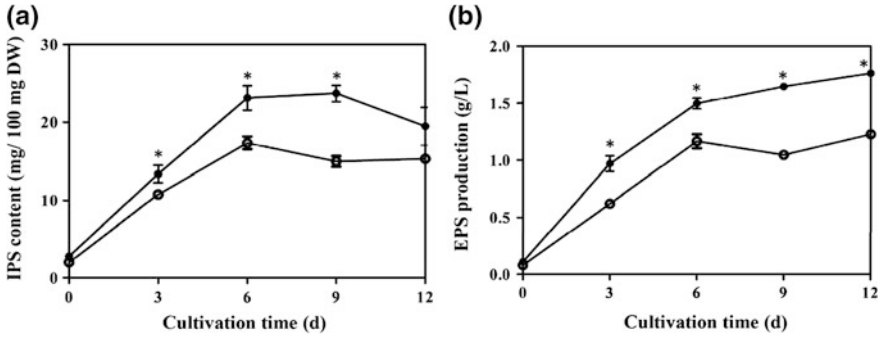


Fig. 9 Kinetic profiles of IPS content (**a**) and EPS production (**b**) in fermentation of the WT strain (*blank circles*) and the PGM strain (*dark circles*). The error bars indicate the standard deviations from three independent samples [189]

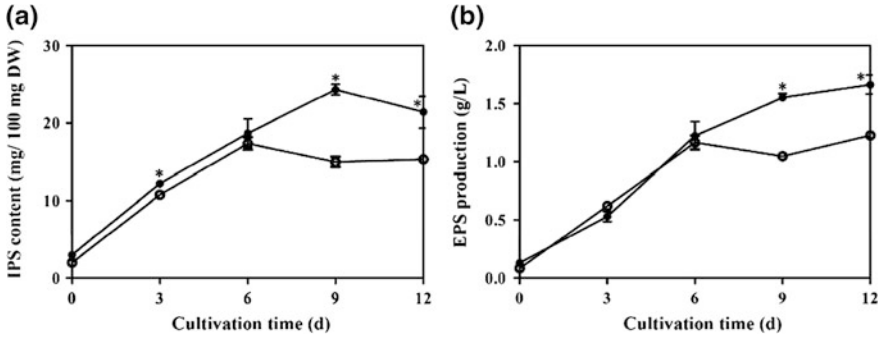


Fig. 10 Kinetic profiles of IPS content (**a**) and EPS production (**b**) in fermentation of the WT strain (*blank circles*) and the UGP strain (*dark circles*). The error bars indicate the standard deviations from three independent samples [190]

4.4 Polyketides

Polyketides (PKs), as a class of secondary metabolites, are structurally a very diverse family of complex organic compounds produced by certain living organisms in order to impart to them some survival advantages. They often possess biological activities and pharmacological properties such as antibacterial, anti-cancer, and antimalarial activities [191]. Polyketides are biosynthesized by polyketide synthases (PKSs) through the decarboxylative condensation of malonyl-CoA-derived extender units in a similar process to fatty acid synthesis [192]. In recent years, polyketides have been isolated from many higher fungi such as *Cordyceps* species and *Cortinarius purpurascens* [193].

Many fungal secondary metabolites are regulated by epigenetic modification, which not only affects metabolite titers, but also activates cryptic gene clusters

[194]. The application of epigenetic modification to higher fungi is becoming a new strategy for strain improvement and a powerful method to obtain novel natural products. For example, Strauss et al. [195] significantly enhanced the production of bisabolene-type sesquiterpenoids and xanthones analogs from *Aspergillus sydowii* by addition of 5-azacytidine, a chemical epigenetic modifier. As higher fungi can produce a variety of unique secondary metabolites including polyketides, the epigenetic modification could help to enhance the production titer and efficiency of those interesting metabolites.

Asai et al. [196] found that addition of epigenetic modification chemicals such as histone deacetylase (HDAC) inhibitor or DNA methyltransferase inhibitor could significantly enhance polyketide production by *Cordyceps annullata*, and a couple of new aromatic polyketides were isolated, such as indigotides C-F, 13-hydroxyindigotide A and 8-O-methylindigotide B [197].

5 Summary and Future Perspectives

Higher fungi have been used as both medicinal and edible materials for thousands of years in East Asia and many other regions around the world. Nowadays, with the quick development of biological science and related engineering fields, the research on higher fungi is deepening rapidly. As described above, many unique important bioactive components have been found, which is important to the development of higher fungus cell factories for industrial applications.

The technologies such as gene transformation, overexpression, silencing, and deletion are gradually applied to the studies of the construction of higher fungus cell factories. The combination of different gene editing methods will help to explore gene functions and promote the identification and optimization of metabolic pathways. However, some problems also need to be solved. The gene transformation methods have not yet mature, and their transformation efficiency and transformant stability still need improvement as well. The gene deletion and silencing have to be extended to more species. Meanwhile, the rapid development of bioinformatics will help to understand molecular characteristics of higher fungi and biosynthesis pathways of various secondary metabolites, which is important to large-scale commercial production.

Recently, omics technologies including genomics, transcriptomics, proteomics, and metabolomics play an important role in studies on behaviors and mechanisms of higher fungi. Whole genome transcription analysis will enable researchers to accurately evaluate the relationship between phenotype and expression of genes, helping understand the cellular metabolism. It also contributes to the identification of target genes for strain improvement and accelerates the rational design and construction of higher fungus cell factories. Because the regulation of all levels of cellular activity/metabolism is interacted with each other, single omic analysis technology has apparent limitations. Thus, the integrated utilization of omics technologies is important in obtaining complete information, which will deepen the

understanding of complex biological systems and speed up the identification of target sites in metabolism. In order to have more rational and efficient improvement on cell/organism breeding, scientists of different professional backgrounds need to cooperate intimately with each other, especially with tools of bioinformatics and mathematics to simulate and design new biosynthesis systems, so as to enhance the existing bioproduct production and new bioproduct synthesis.

Without doubt, the establishment of cell factory platform has a big impact on large-scale production of useful metabolites by higher fungi. Through gene transformation, gene overexpression and other biological technologies, we can deepen our understanding on the synthetic mechanism and pathways of secondary metabolites, so as to realize the reconstruction of metabolic pathways and the massive accumulation of targeted metabolites. However, due to the short history of higher fungi research, their genetic technologies have yet to be improved to ensure exogenous genes or homologous genes expressed efficiently and stably in higher fungal cells; gene overexpression, silencing, and knockout technology also need to be applied widely in higher fungi. Furthermore, the construction of higher fungal cell factories needs the integration of genomics, gene technology platforms, and bioinformatics technologies, to have a better understanding about metabolic pathways and cellular metabolic mechanisms. Here, many new bioactive products may be found through these approaches, and the metabolic pathway can be improved to achieve efficient biomanufacturing of valuable metabolites. The expression of heterogonous genes in higher fungi, which resulted in the synthesis of other products, is also under development. Synthetic biology as an emerging discipline has become more and more popular due to its enormous potential, and how to better apply synthetic biology into higher fungi is becoming one of the targets in our future research. As the higher fungi are getting more and more global attention in recent years with their expanding markets, therefore research and development on higher fungal cell factories are anticipated to meet the growing demand of market by further promoting useful metabolites production.

Acknowledgements JJZ thanks the National 973 program (No. 2012CB721006) and 863 program (No. 2012AA021701) from MoST and the National Natural Science Foundation of China (No. 20776084) for financial support. JHX appreciates the Guizhou High-Level Innovative Talent Support Program (No. QKH-RC-20154028).

References

1. Ma K, Ren J, Han J, Bao L, Li L, Yao Y, Sun C, Zhou B, Liu H (2014) Ganoboninketals A-C, antiplasmodial 3,4-seco-27-norlanostane triterpenes from *Ganoderma boninense* Pat. J Nat Prod 77:1847–1852
2. Ma K, Bao L, Han J, Jin T, Yang X, Zhao F, Li S, Song F, Liu M, Liu H (2014) New benzoate derivatives and hirsutane type sesquiterpenoids with antimicrobial activity and cytotoxicity from the solid-state fermented rice by the medicinal mushroom *Stereum hirsutum*. Food Chem 143:239–245

3. Chen HP, Dong WB, Feng T, Yin X, Li ZH, Dong ZJ, Li Y, Liu JK (2014) Four new sesquiterpenoids from fruiting bodies of the fungus *Inonotus rickii*. J Asian Nat Prod Res 16:581–586
4. Wang S, Bao L, Zhao F, Wang Q, Li S, Ren J, Li L, Wen H, Guo L, Liu H (2013) Isolation, identification, and bioactivity of monoterpenoids and sesquiterpenoids from the mycelia of edible mushroom *Pleurotus cornucopiae*. J Agric Food Chem 61:5122–5129
5. Nord CL, Menkis A, Vasaitis R, Broberg A (2013) Protoilludane sesquiterpenes from the wood decomposing fungus *Granulobasidium vellereum* (Ellis & Cragin) Julich. Phytochemistry 90:128–134
6. Kim KH, Moon E, Choi SU, Kim SY, Lee KR (2013) Lanostane triterpenoids from the mushroom *Naematoloma fasciculare*. J Nat Prod 76:845–851
7. Wang YQ, Bao L, Yang XL, Li L, Li SF, Gao H, Yao XS, Wen HA, Lie HW (2012) Bioactive sesquiterpenoids from the solid culture of the edible mushroom *Flammulina velutipes* growing on cooked rice. Food Chem 132:1346–1353
8. Shi XW, Zhang AL, Pescitelli G, Gao JM (2012) Secoscabronine M, a novel diterpenoid from the Chinese bitter mushroom *Sarcodon scabrosus*. Chirality 24:386–390
9. Liu L, Shi XW, Zong SC, Tang JJ, Gao JM (2012) Scabronine M, a novel inhibitor of NGF-induced neurite outgrowth from PC12 cells from the fungus *Sarcodon scabrosus*. Bioorg Med Chem Lett 22:2401–2406
10. Kanokmedhakul S, Lekphrom R, Kanokmedhakul K, Hahnvajanawong C, Bua-art S, Saksirirat W, Prabpai S, Kongsaeree P (2012) Cytotoxic sesquiterpenes from luminescent mushroom *Neonothopanus nambi*. Tetrahedron 68:8261–8266
11. Bohnert M, Miethbauer S, Dahse HM, Ziemen J, Nett M, Hoffmeister D (2011) In vitro cytotoxicity of melleolide antibiotics: structural and mechanistic aspects. Bioorg Med Chem Lett 21:2003–2006
12. Weng CJ, Chau CF, Chen KD, Chen DH, Yen GC (2007) The anti-invasive effect of lucidenic acids isolated from a new *Ganoderma lucidum* strain. Mol Nutr Food Res 51:1472–1477
13. Wang JL, Li YB, Liu RM, Zhong JJ (2010) A new ganoderic acid from *Ganoderma lucidum* mycelia. J Asian Nat Prod Res 12:727–730
14. Li YB, Liu RM, Zhong JJ (2013) A new ganoderic acid from *Ganoderma lucidum* mycelia and its stability. Fitoterapia 84:115–122
15. Li YY, Mi ZY, Tang Y, Wang G, Li DS, Tang YJ (2009) Lanostanoids isolated from *Ganoderma lucidum* mycelium cultured by submerged fermentation. Helv Chim Acta 92:1586–1593
16. Wang K, Bao L, Qi Q, Zhao F, Ma K, Pei Y, Liu H (2015) Erinacerins C-L, isoindolin-1-ones with alpha-glucosidase inhibitory activity from cultures of the medicinal mushroom *Hericium erinaceus*. J Nat Prod 78:146–154
17. Kim KH, Noh HJ, Choi SU, Lee KR (2012) Isohericenone, a new cytotoxic isoindolinone alkaloid from *Hericium erinaceum*. J Antibiot (Tokyo) 65:575–577
18. Ueda K, Tsujimori M, Kodani S, Chiba A, Kubo M, Masuno K, Sekiya A, Nagai K, Kawagishi H (2008) An endoplasmic reticulum (ER) stress-suppressive compound and its analogues from the mushroom *Hericium erinaceum*. Bioorg Med Chem 16:9467–9470
19. Ma K, Han J, Bao L, Wei T, Liu H (2014) Two sarcoviolins with antioxidative and alpha-glucosidase inhibitory activity from the edible mushroom *Sarcodon leucopus* collected in Tibet. J Nat Prod 77:942–947
20. Kubo M, Liu Y, Ishida M, Harada K, Fukuyama Y (2014) A new spiroindene pigment from the medicinal fungus *Phellinus ribis*. Chem Pharm Bull (Tokyo) 62:122–124
21. Demirkiran O, Sabudak T, Ozturk M, Topcu G (2013) Antioxidant and tyrosinase inhibitory activities of flavonoids from *Trifolium nigrescens* Subsp. petrisavi. J Agric Food Chem 61:12598–12603
22. Li Y, Bao L, Song B, Han J, Li H, Zhao F, Liu H (2013) A new benzoquinone and a new benzofuran from the edible mushroom *Neolentinus lepideus* and their inhibitory activity in NO production inhibition assay. Food Chem 141:1614–1618

23. Lu WW, Gao YJ, Su MZ, Luo Z, Zhang W, Shi GB, Zhao QC (2013) Isoindolones from *Lasiosphaera fenzlii* Reich. and their bioactivities. Helv Chim Acta 96:109–113
24. Kim KH, Park KM, Choi SU, Lee KR (2009) Macrolepiotin, a new indole alkaloid from *Macrolepiota neomastoidea*. J Antibiot (Tokyo) 62:335–338
25. Chi YL, Zhang HY, Xue JH, Hao J, Liu MF, Wei XY (2009) N-2-(1-Methoxycarbonylethyl) guanosine, a new nucleoside coupled with an amino acid derivative from *Amanita exitialis*. Chinese Chem Lett 20:830–832
26. El Dine RS, El Halawany AM, Ma CM, Hattori M (2009) Inhibition of the dimerization and active site of HIV-1 protease by secondary metabolites from the Vietnamese mushroom *Ganoderma colossum*. J Nat Prod 72:2019–2023
27. Rukachaisirikul V, Khamthong N, Sukpondma Y, Pakawatchai C, Phongpaichit S, Sakayaroj J, Kirtikara K (2009) An [11]cytochalasin derivative from the marine-derived fungus *Xylaria* sp. PSU-F100. Chem Pharm Bull (Tokyo) 57:1409–1411
28. Xu F, Pang JY, Lu BT, Wang JJ, Zhang Y, She ZG, Vrijmoed LLP, Gareth JEB, Lin YC (2009) Two metabolites with DNA-binding affinity from the mangrove fungus *Xylaria* sp (#2508) from the south China sea coast. Chinese J Chem 27:365–368
29. Kojima K, Ohno T, Inoue M, Mizukami H, Nagatsu A (2008) Phellifuropyranone A: a new furopyranone compound isolated from fruit bodies of wild *Phellinus linteus*. Chem Pharm Bull (Tokyo) 56:173–175
30. Teichert A, Schmidt J, Porzel A, Arnold N, Wessjohann L (2008) N-glucosyl-1H-indole derivatives from *Cortinarius brunneus* (Basidiomycetes). Chem Biodivers 5:664–669
31. Teichert A, Schmidt J, Porzel A, Arnold N, Wessjohann L (2008) (Iso)-quinoline alkaloids from fungal fruiting bodies of *Cortinarius subtortus*. J Nat Prod 71:1092–1094
32. Li XG, Pan WD, Lou HY, Liu RM, Xiao JH, Zhong JJ (2015) New cytochalasins from medicinal macrofungus *Crodyceps taii* and their inhibitory activities against human cancer cells. Bioorg Med Chem Lett 25:1823–1826
33. Fangkrathok N, Sripanidkulchai B, Umehara K, Noguchi H (2013) Bioactive ergostanoids and a new polyhydroxyoctane from *Lentinus polychrous* mycelia and their inhibitory effects on E2-enhanced cell proliferation of T47D cells. Nat Prod Res 27:1611–1619
34. Li YY, Liu P, Tang Y, Li HM, Tang YL, Liang XH, Tang YJ (2013) Novel cerebrosides isolated from the fermentation mycelia of *Tuber indicum*. Helv Chim Acta 96:702–709
35. Xiao JH, Sun ZH, Pan WD, Lu YH, Chen DX, Zhong JJ (2012) Jiangxienone, a new compound with potent cytotoxicity against tumor cells from traditional Chinese medicinal mushroom *Cordyceps jiangxiensis*. Chem Biodivers 9:1349–1355
36. Ma BJ, Ma JC, Ruan Y (2012) Hericenone L, a new aromatic compound from the fruiting bodies of *Hericium erinaceums*. Chin J Nat Medicines 10:363–365
37. Hong SS, Lee JH, Jeong W, Kim N, Jin HZ, Hwang BY, Lee HJ, Lee SJ, Jang DS, Lee D (2012) Acetylenic acid analogues from the edible mushroom Chanterelle (*Cantharellus cibarius*) and their effects on the gene expression of peroxisome proliferator-activated receptor-gamma target genes. Bioorg Med Chem Lett 22:2347–2349
38. Norikura T, Fujiwara K, Narita T, Yamaguchi S, Morinaga Y, Iwai K, Matsue H (2011) Anticancer activities of thelephantin O and vialinin A isolated from *Thelephora aurantiotincta*. J Agric Food Chem 59:6974–6979
39. Wang G, Li YY, Li DS, Tang YJ (2008) Determination of 5 alpha-androst-16-en-3 alpha-ol in truffle fermentation broth by solid-phase extraction coupled with gas chromatography-flame ionization detector/electron impact mass spectrometry. J Chromatogr B 870:209–215
40. Onose JI, Xie C, Ye YQ, Sugaya K, Takahashi S, Koshino H, Yasunaga K, Abe N, Yoshikawa K (2008) Vialinin A, a novel potent inhibitor of TNF-alpha production from RBL-2H3 cells. Biol Pharm Bull 31:831–833
41. Xu JW, Xu YN, Zhong JJ (2010) Production of individual ganoderic acids and expression of biosynthetic genes in liquid static and shaking cultures of *Ganoderma lucidum*. Appl Microbiol Biotechnol 85:941–948

42. Liang CX, Li YB, Xu JW, Wang JL, Miao XL, Tang YJ, Gu T, Zhong JJ (2010) Enhanced biosynthetic gene expressions and production of ganoderic acids in static liquid culture of *Ganoderma lucidum* under phenobarbital induction. Appl Microbiol Biotechnol 86:1367–1374
43. Xu JW, Zhao W, Zhong JJ (2010) Biotechnological production and application of ganoderic acids. Appl Microbiol Biotechnol 87:457–466
44. Xu JW, Xu YN, Zhong JJ (2012) Enhancement of ganoderic acid accumulation by overexpression of an N-terminally truncated 3-hydroxy-3-methylglutaryl coenzyme A reductase gene in the basidiomycete *Ganoderma lucidum*. Appl Environ Microbiol 78:7968–7976
45. Yadav VG, De Mey M, Lim CG, Ajikumar PK, Stephanopoulos G (2012) The future of metabolic engineering and synthetic biology: towards a systematic practice. Metab Eng 14:233–241
46. van de Rhee MD, Graca PM, Huizing HJ, Mooibroek H (1996) Transformation of the cultivated mushroom, *Agaricus bisporus*, to hygromycin B resistance. Mol Gen Genet 250:252–258
47. Cho JH, Lee SE, Chang WB, Cha JS (2006) *Agrobacterium*-mediated transformation of the winter mushroom. Flammulina velutipes. Mycobiology 34:104–107
48. Guo LQ, Lin JF, Xiong S, Chen SC (2005) Transformation of *Volvariella volvacea* with a thermal hysteresis protein gene by particle bombardment. Wei Sheng Wu Xue Bao 45:39–43
49. Zhou YP, Chen MH, Lu JJ, Kang X, Chen QH, Huang XL, Tian CE (2015) A simple and efficient genetic transformation method of *Ganoderma weberianum*. Folia Microbiol (Praha) 60:417–423
50. Shin DI, Park HS (2013) Mechanical wounding of yeast-like conidium cells of *Tremella fuciformis* makes them susceptible to *Agrobacterium*-mediated transformation. Biosci Biotechnol Biochem 77:2157–2159
51. Li G, Li R, Liu Q, Wang Q, Chen M, Li B (2006) A highly efficient polyethylene glycol-mediated transformation method for mushrooms. FEMS Microbiol Lett 256:203–208
52. Sun L, Cai HQ, Xu WH, Hu YL, Gao Y, Lin ZP (2001) Efficient transformation of the medicinal mushroom *Ganoderma lucidum*. Plant Mol Biol Rep 19:383
53. Noh W, Kim SW, Dong-Won B, Kim JY, Ro HS (2010) Genetic introduction of foreign genes to *Pleurotus eryngii* by restriction enzyme-mediated integration. J Microbiol 48:253–256
54. Lin JF, Zheng MY, Wang J, Shu W, Guo LQ (2008) Efficient transformation and expression of *gfp* gene in the edible mushroom *Pleurotus nebrodensis*. Prog Nat Sci 18:819–824
55. Sun L, Xu WH, Cai HQ, Hu YL, Lin ZP (2001) PEG-mediated transformation of *Lentinus edodes*. Acta Bot Sin 43:1089–1092
56. Peng M, Singh NK, Lemke PA (1992) Recovery of recombinant plasmids from *Pleurotus ostreatus* transformants. Curr Genet 22:53–59
57. Irie T, Honda Y, Hirano T, Sato T, Enei H, Watanabe T, Kuwahara M (2001) Stable transformation of *Pleurotus ostreatus* to hygromycin B resistance using *Lentinus edodes* GPD expression signals. Appl Microbiol Biotechnol 56:707–709
58. Yu XY, Ji SL, He YL, Ren MF, Xu JW (2014) Development of an expression plasmid and its use in genetic manipulation of lingzhi or reishi medicinal mushroom, *Ganoderma lucidum* (higher basidiomycetes). Int J Med Mushrooms 16:161–168
59. Guo L, Liu Y, Zhao S, Liu E, Lin J (2008) Highly efficient transformation of intact yeast-like conidium cells of *Tremella fuciformis* by electroporation. Sci China C Life Sci 51:932–940
60. Kuo CY, Huang CT (2008) A reliable transformation method and heterologous expression of beta-glucuronidase in *Lentinula edodes*. J Microbiol Methods 72:111–115
61. Kuo CY, Chou SY, Hseu RS, Huang CT (2010) Heterologous expression of EGFP in enoki mushroom *Flammulina velutipes*. Bot Stud 51:303–309
62. Mu D, Shi L, Ren A, Li M, Wu F, Jiang A, Zhao M (2012) The development and application of a multiple gene co-silencing system using endogenous *URA3* as a reporter gene in *Ganoderma lucidum*. PLoS ONE 7:e43737

63. Kuo CY, Chou SY, Huang CT (2004) Cloning of glyceraldehyde-3-phosphate dehydrogenase gene and use of the *gpd* promoter for transformation in *Flammulina velutipes*. Appl Microbiol Biotechnol 65:593–599
64. Bundock P, den Dulk-Ras A, Beijersbergen A, Hooykaas PJ (1995) Trans-kingdom T-DNA transfer from *Agrobacterium tumefaciens* to *Saccharomyces cerevisiae*. EMBO J 14:3206–3214
65. Hirth KP, Edwards CA, Firtel RA (1982) A DNA-mediated transformation system for *Dictyostelium discoideum*. Proc Natl Acad Sci US 79:7356–7360
66. Chen X, Stone M, Schlagnhaufer C, Romaine CP (2000) A fruiting body tissue method for efficient *Agrobacterium*-mediated transformation of *Agaricus bisporus*. Appl Environ Microbiol 66:4510–4513
67. Burns C, Leach KM, Elliott TJ, Challen MP, Foster GD, Bailey A (2006) Evaluation of *Agrobacterium*-mediated transformation of *Agaricus bisporus* using a range of promoters linked to hygromycin resistance. Mol Biotechnol 32:129–138
68. Okamoto T, Yamada M, Sekiya S, Okuhara T, Taguchi G, Inatomi S, Shimosaka M (2010) *Agrobacterium tumefaciens*-mediated transformation of the vegetative dikaryotic mycelium of the cultivated mushroom *Flammulina velutipes*. Biosci Biotechnol Biochem 74:2327–2329
69. Samils N, Elfstrand M, Czederpiltz DLL, Fahleson J, Olson A, Dixelius C, Stenlid J (2006) Development of a rapid and simple *Agrobacterium tumefaciens*-mediated transformation system for the fungal pathogen *Heterobasidion annosum*. FEMS Microbiol Lett 255:82–88
70. Lin JF, Zheng MY, Wang J, Shu W, Guo LQ (2008) Efficient transformation and expression of *gfp* gene in the edible mushroom *Pleurotus nebrodensis*. Prog Nat Sci 18:819–824
71. Wang J, Guo L, Zhang K, Wu Q, Lin J (2008) Highly efficient *Agrobacterium*-mediated transformation of *Volvariella volvacea*. Bioresour Technol 99:8524–8527
72. Michielse CB, Hooykaas PJJ (2005) van den Hondel CAMJJ, Ram AFJ: *Agrobacterium*-mediated transformation as a tool for functional genomics in fungi. Curr Genet 48:1–17
73. Zhang J, Shi L, Chen H, Sun Y, Zhao M, Ren A, Chen M, Wang H, Feng Z (2014) An efficient *Agrobacterium*-mediated transformation method for the edible mushroom *Hypsizygus marmoreus*. Microbiol Res 169:741–748
74. Kemppainen M, Duplessis S, Martin F, Pardo AG (2008) T-DNA insertion, plasmid rescue and integration analysis in the model mycorrhizal fungus *Laccaria bicolor*. Microb Biotechnol 1:258–269
75. Park HS, Choi JW (2014) Functional expression of bovine growth hormone gene in *Pleurotus eryngii*. Biotechnol Bioproc E 19:33–42
76. Kilaru S, Collins CM, Hartley AJ, Bailey AM, Foster GD (2009) Establishing molecular tools for genetic manipulation of the pleuromutilin-producing fungus *Clitopilus passeckerianus*. Appl Environ Microbiol 75:7196–7204
77. Meyer V (2008) Genetic engineering of filamentous fungi–progress, obstacles and future trends. Biotechnol Adv 26:177–185
78. Schiestl RH, Petes TD (1991) Integration of DNA fragments by illegitimate recombination in *Saccharomyces cerevisiae*. Proc Natl Acad Sci USA 88:7585–7589
79. Granado JD, Kertesz-Chaloupkova K, Aebi M, Kues U (1997) Restriction enzyme-mediated DNA integration in *Coprinus cinereus*. Mol Gen Genet 256:28–36
80. Kim S, Song J, Choi HT (2004) Genetic transformation and mutant isolation in *Ganoderma lucidum* by restriction enzyme-mediated integration. FEMS Microbiol Lett 233:201–204
81. Han F, Liu Y, Guo LQ, Zeng XL, Liu ZM, Lin JF (2010) Heterologous expression of the immunomodulatory protein gene from *Ganoderma sinense* in the basidiomycete *Coprinopsis cinerea*. J Appl Microbiol 109:1838–1844
82. Irie T, Sato T, Saito K, Honda Y, Watanabe T, Kuwahara M, Enei H (2003) Construction of a homologous selectable marker gene for *Lentinula edodes* transformation. Biosci Biotechnol Biochem 67:2006–2009
83. Riggle PJ, Kumamoto CA (1998) Genetic analysis in fungi using restriction-enzyme-mediated integration. Curr Opin Microbiol 1:395–399

84. Nakazawa T, Tatsuta Y, Fujita T, Nakahori K, Kamada T (2010) Mutations in the *Cc.rmt1* gene encoding a putative protein arginine methyltransferase alter developmental programs in the basidiomycete *Coprinopsis cinerea*. Curr Genet 56:361–367
85. Nakazawa T, Kondo H, Nakahori K, Kamada T (2011) A mutation in the *Cc.ubc2* gene affects clamp cell morphogenesis as well as nuclear migration for dikaryosis in *Coprinopsis cinerea*. Fungal Genet Biol 48:519–525
86. Sunagawa M, Magae Y (2002) Transformation of the edible mushroom *Pleurotus ostreatus* by particle bombardment. FEMS Microbiol Lett 211:143–146
87. Sunagawa M, Murata H, Miyazaki Y, Nakamura M (2007) Transformation of the mycorrhizal basidiomycetes, *Suilllus grevillei* and *S. bovinus*, by particle bombardment. Biosci Biotech Bioch 71:47–50
88. Zhou JS, Ji SL, Ren MF, He YL, Jing XR, Xu JW (2014) Enhanced accumulation of individual ganoderic acids in a submerged culture of *Ganoderma lucidum* by the overexpression of squalene synthase gene. Biochem Eng J 90:178–183
89. Chai R, Qiu C, Liu D, Qi Y, Gao Y, Shen J, Qiu L (2013) beta-Glucan synthase gene overexpression and beta-glucans overproduction in *Pleurotus ostreatus* using promoter swapping. PLoS ONE 8:e61693
90. Lin YJ, Huang LH, Huang CT (2013) Enhancement of heterologous gene expression in *Flammulina velutipes* using polycistronic vectors containing a viral 2A cleavage sequence. PLoS One 8:e59099
91. Coconi-Linares N, Magana-Ortiz D, Guzman-Ortiz DA, Fernandez F, Loske AM, Gomez-Lim MA (2014) High-yield production of manganese peroxidase, lignin peroxidase, and versatile peroxidase in Phanerochaete chrysosporium. Appl Microbiol Biotechnol 98:9283–9294
92. Hood E (2004) RNAi: what's all the noise about gene silencing? Environ Health Perspect 112:A224–A229
93. Namekawa SH, Iwabata K, Sugawara H, Hamada FN, Koshiyama A, Chiku H, Kamada T, Sakaguchi K (2005) Knockdown of *LIM15/DMC1* in the mushroom *Coprinus cinereus* by double-stranded RNA-mediated gene silencing. Microbiology 151:3669–3678
94. Eastwood DC, Challen MP, Zhang C, Jenkins H, Henderson J, Burton KS (2008) Hairpin-mediated down-regulation of the urea cycle enzyme argininosuccinate lyase in *Agaricus bisporus*. Mycol Res 112:708–716
95. Kemppainen M, Duplessis S, Martin F, Pardo AG (2009) RNA silencing in the model mycorrhizal fungus *Laccaria bicolor*: gene knock-down of nitrate reductase results in inhibition of symbiosis with *Populus*. Environ Microbiol 11:1878–1896
96. Kemppainen MJ, Pardo AG (2010) Gene knockdown by ihpRNA-triggering in the ectomycorrhizal basidiomycete fungus *Laccaria bicolor*. Bioeng Bugs 1:354–358
97. Nakade K, Watanabe H, Sakamoto Y, Sato T (2011) Gene silencing of the *Lentinula edodes lcc1* gene by expression of a homologous inverted repeat sequence. Microbiol Res 166:484–493
98. Dang Y, Yang Q, Xue Z, Liu Y (2011) RNA interference in fungi: pathways, functions, and applications. Eukaryot Cell 10:1148–1155
99. Zhong S, Leng Y, Bolton MD (2012) Construction of hairpin RNA-expressing vectors for RNA-mediated gene silencing in fungi. Methods Mol Biol 835:623–633
100. Godio RP, Fouces R, Martin JF (2007) A squalene epoxidase is involved in biosynthesis of both the antitumor compound clavaric acid and sterols in the basidiomycete *H. sublateritium*. Chem Biol 14:1334–1346
101. Chang SS, Zhang Z, Liu Y (2012) RNA interference pathways in fungi: mechanisms and functions. Annu Rev Microbiol 66:305–323
102. Lugones LG, de Jong JF, de Vries OM, Jalving R, Dijksterhuis J, Wosten HA (2004) The SC15 protein of *Schizophyllum commune* mediates formation of aerial hyphae and attachment in the absence of the SC3 hydrophobin. Mol Microbiol 53:707–716

103. de Jong JF, Ohm RA, de Bekker C, Wosten HA, Lugones LG (2010) Inactivation of *ku80* in the mushroom-forming fungus *Schizophyllum commune* increases the relative incidence of homologous recombination. FEMS Microbiol Lett 310:91–95
104. Ohm RA, de Jong JF, Berends E, Wang F, Wosten HA, Lugones LG (2010) An efficient gene deletion procedure for the mushroom-forming basidiomycete *Schizophyllum commune*. World J Microbiol Biotechnol 26:1919–1923
105. Ninomiya Y, Suzuki K, Ishii C, Inoue H (2004) Highly efficient gene replacements in *Neurospora* strains deficient for nonhomologous end-joining. Proc Natl Acad Sci USA 101:12248–12253
106. van Peer AF, Wang FF, van Driel KGA, de Jong JF, van Donselaar EG, Mueller WH, Boekhout T, Lugones LG, Wosten HAB (2010) The septal pore cap is an organelle that functions in vegetative growth and mushroom formation of the wood-rot fungus *Schizophyllum commune*. Environ Microbiol 12:833–844
107. Ohm RA, de Jong JF, de Bekker C, Wosten HA, Lugones LG (2011) Transcription factor genes of *Schizophyllum commune* involved in regulation of mushroom formation. Mol Microbiol 81:1433–1445
108. Berends E, Lehle L, Henquet M, Hesselink T, Wosten HA, Lugones LG, Bosch D (2013) Identification of *alg3* in the mushroom-forming fungus *Schizophyllum commune* and analysis of the Delt*aalg3* knockout mutant. Glycobiology 23:147–154
109. Nakazawa T, Ando Y, Kitaaki K, Nakahori K, Kamada T (2011) Efficient gene targeting in Delta Cc.*ku70* or Delta *Cc.lig4* mutants of the agaricomycete *Coprinopsis cinerea*. Fungal Genet Biol 48:939–946
110. Salame TM, Knop D, Tal D, Levinson D, Yarden O, Hadar Y (2012) Predominance of a versatile-peroxidase-encoding gene, *mnp4*, as demonstrated by gene replacement via a gene targeting system for *Pleurotus ostreatus*. Appl Environ Microbiol 78:5341–5352
111. Salame TM, Knop D, Levinson D, Yarden O, Hadar Y (2013) Redundancy among manganese peroxidases in *Pleurotus ostreatus*. Appl Environ Microbiol 79:2405–2415
112. Salame TM, Knop D, Levinson D, Mabjeesh SJ, Yarden O, Hadar Y (2014) Inactivation of a *Pleurotus ostreatus* versatile peroxidase-encoding gene (*mnp2*) results in reduced lignin degradation. Environ Microbiol 16:265–277
113. Morin E, Kohler A, Baker AR, Foulongne-Oriol M, Lombard V, Nagy LG, Ohm RA, Patyshakuliyeva A, Brun A, Aerts AL et al (2012) Genome sequence of the button mushroom *Agaricus bisporus* reveals mechanisms governing adaptation to a humic-rich ecological niche. Proc Natl Acad Sci USA 109:17501–17506
114. Park YJ, Baek JH, Lee S, Kim C, Rhee H, Kim H, Seo JS, Park HR, Yoon DE, Nam JY et al (2014) Whole genome and global gene expression analyses of the model mushroom *Flammulina velutipes* reveal a high capacity for lignocellulose degradation. PLoS ONE 9: e93560
115. Bao D, Gong M, Zheng H, Chen M, Zhang L, Wang H, Jiang J, Wu L, Zhu Y, Zhu G et al (2013) Sequencing and comparative analysis of the straw mushroom (*Volvariella volvacea*) genome. PLoS ONE 8:e58294
116. Wawrzyn GT, Quin MB, Choudhary S, Lopez-Gallego F, Schmidt-Dannert C (2012) Draft genome of *Omphalotus olearius* provides a predictive framework for sesquiterpenoid natural product biosynthesis in Basidiomycota. Chem Biol 19:772–783
117. Ohm RA, de Jong JF, Lugones LG, Aerts A, Kothe E, Stajich JE, de Vries RP, Record E, Levasseur A, Baker SE et al (2010) Genome sequence of the model mushroom *Schizophyllum commune*. Nat Biotechnol 28:957–963
118. Yap HY, Chooi YH, Firdaus-Raih M, Fung SY, Ng ST, Tan CS, Tan NH (2014) The genome of the Tiger Milk mushroom, *Lignosus rhinocerotis*, provides insights into the genetic basis of its medicinal properties. BMC Genom 15:635
119. Tanaka Y, Kasuga D, Oba Y, Hase S, Sato K, Oba Y, Sakakibara Y (2014) Genome sequence of the luminous mushroom *Mycena chlorophos* for searching fungal bioluminescence genes. Luminescence 29:47–48

120. Zhu YJ, Xu J, Sun C, Zhou SG, Xu HB, Nelson DR, Qian J, Song JY, Luo HM, Xiang L et al (2015) Chromosome-level genome map provides insights into diverse defense mechanisms in the medicinal fungus *Ganoderma sinense*. Sci Rep 5:11087
121. Chen S, Xu J, Liu C, Zhu Y, Nelson DR, Zhou S, Li C, Wang L, Guo X, Sun Y et al (2012) Genome sequence of the model medicinal mushroom *Ganoderma lucidum*. Nat Commun 3:913
122. Zheng P, Xia Y, Xiao G, Xiong C, Hu X, Zhang S, Zheng H, Huang Y, Zhou Y, Wang S et al (2011) Genome sequence of the insect pathogenic fungus *Cordyceps militaris*, a valued traditional Chinese medicine. Genome Biol 12:R116
123. Boh B, Berovic M, Zhang J, Zhi-Bin L (2007) *Ganoderma lucidum* and its pharmaceutically active compounds. Biotechnol Annu Rev 13:265–301
124. Shiao MS (2003) Natural products of the medicinal fungus *Ganoderma lucidum*: occurrence, biological activities, and pharmacological functions. Chem Rec 3:172–180
125. Shi L, Ren A, Mu D, Zhao M (2010) Current progress in the study on biosynthesis and regulation of ganoderic acids. Appl Microbiol Biotechnol 88:1243–1251
126. Liu DB, Gong J, Dai WK, Kang XC, Huang Z, Zhang HM, Liu W, Liu L, Ma JP, Xia ZL et al (2012) The genome of *Ganderma lucidum* provide insights into triterpense biosynthesis and wood degradation. PLoS One 7:e36146
127. Qian J, Xu H, Song J, Xu J, Zhu Y, Chen S (2013) Genome-wide analysis of simple sequence repeats in the model medicinal mushroom *Ganoderma lucidum*. Gene 512:331–336
128. Xu J, Xu Z, Zhu Y, Luo H, Qian J, Ji A, Hu Y, Sun W, Wang B, Song J et al (2014) Identification and evaluation of reference genes for qRT-PCR normalization in *Ganoderma lucidum*. Curr Microbiol 68:120–126
129. Li J, Wu B, Xu J, Liu C (2014) Genome-wide identification and characterization of long intergenic non-coding RNAs in *Ganoderma lucidum*. PLoS ONE 9:e99442
130. Yin Y, Yu G, Chen Y, Jiang S, Wang M, Jin Y, Lan X, Liang Y, Sun H (2012) Genome-wide transcriptome and proteome analysis on different developmental stages of *Cordyceps militaris*. PLoS ONE 7:e51853
131. Li J, Zhang J, Chen H, Chen X, Lan J, Liu C (2013) Complete mitochondrial genome of the medicinal mushroom *Ganoderma lucidum*. PLoS ONE 8:e72038
132. Tang LH, Jian HH, Song CY, Bao DP, Shang XD, Wu DQ, Tan Q, Zhang XH (2013) Transcriptome analysis of candidate genes and signaling pathways associated with light-induced brown film formation in *Lentinula edodes*. Appl Microbiol Biotechnol 97:4977–4989
133. Tao Y, van Peer AF, Chen B, Chen Z, Zhu J, Deng Y, Jiang Y, Li S, Wu T, Xie B (2014) Gene expression profiling reveals large regulatory switches between succeeding stipe stages in *Volvariella volvacea*. PLoS ONE 9:e97789
134. Ramirez L, Oguiza JA, Perez G, Lavin JL, Omarini A, Santoyo F, Alfaro M, Castanera R, Parenti A, Muguerza E, Pisabarro AG (2011) Genomics and transcriptomics characterization of genes expressed during postharvest at 4 & #xB0;C by the edible basidiomycete Pleurotus ostreatus. Int Microbiol 14:111–120
135. Xu JW, Zhao W, Xu YN, Zhong JJ (2012) Isolation and analysis of differentially expressed genes during asexual sporulation in liquid static culture of *Ganoderma lucidum* by suppression subtractive hybridization. Mol Biol Rep 39:3603–3610
136. Zhu Y, Luo H, Zhang X, Song J, Sun C, Ji A, Xu J, Chen S (2014) Abundant and selective RNA-editing events in the medicinal mushroom *Ganoderma lucidum*. Genetics 196:1047–1057
137. Wang Z, Gerstein M, Snyder M (2009) RNA-Seq: a revolutionary tool for transcriptomics. Nat Rev Genet 10:57–63
138. Yu GJ, Wang M, Huang J, Yin YL, Chen YJ, Jiang S, Jin YX, Lan XQ, Wong BHC, Liang Y, Sun H (2012) Deep insight into the *Ganoderma lucidum* by comprehensive analysis of its transcriptome. PLoS One 7:e44031

139. Plaza DF, Lin CW, van der Velden NS, Aebi M, Kunzler M (2014) Comparative transcriptomics of the model mushroom *Coprinopsis cinerea* reveals tissue-specific armories and a conserved circuitry for sexual development. BMC Genom 15:492
140. Yang F, Xu B, Zhao S, Li J, Yang Y, Tang X, Wang F, Peng M, Huang Z (2012) De novo sequencing and analysis of the termite mushroom (*Termitomyces albuminosus*) transcriptome to discover putative genes involved in bioactive component biosynthesis. J Biosci Bioeng 114:228–231
141. Shashidhar MG, Giridhar P, Sankar KU, Manohar B (2013) Bioactive principles from *Cordyceps sinensis*: a potent food supplement—a review. J Funct Foods 5:1013–1030
142. Xiang L, Li Y, Zhu YJ, Luo HM, Li CF, Xu XL, Sun C, Song JY, Shi LC, He L et al (2014) Transcriptome analysis of the *Ophiocordyceps sinensis* fruiting body reveals putative genes involved in fruiting body development and cordycepin biosynthesis. Genomics 103:154–159
143. Rahmad N, Al-Obaidi JR, Al-Obaidi JR, Nor Rashid NM, Zean NB, Mohd Yusoff MH, Shaharuddin NS, Mohd Jamil NA, Mohd Saleh N (2014) Comparative proteomic analysis of different developmental stages of the edible mushroom *Termitomyces heimii*. Biol Res 47:30
144. Lau CC, Abdullah N, Shuib AS, Aminudin N (2012) Proteomic analysis of antihypertensive proteins in edible mushrooms. J Agric Food Chem 60:12341–12348
145. Chen L, Zhang BB, Cheung PC (2012) Comparative proteomic analysis of mushroom cell wall proteins among the different developmental stages of *Pleurotus tuber-regium*. J Agric Food Chem 60:6173–6182
146. Lin YL, Wen TN, Chang ST, Chu FH (2011) Proteomic analysis of differently cultured endemic medicinal mushroom *Antrodia cinnamomea* T.T. Chang et W.N. Chou from Taiwan. Int J Med Mushrooms 13:473–481
147. Yu GJ, Yin YL, Yu WH, Liu W, Jin YX, Shrestha A, Yang Q, Ye XD, Sun H (2015) Proteome exploration to provide a resource for the investigation of *Ganoderma lucidum*. PLoS ONE 10:e0119439
148. Zhang BB, Chen L, Cheung PCK (2012) Proteomic insights into the stimulatory effect of Tween 80 on mycelial growth and exopolysaccharide production of an edible mushroom *Pleurotus tuber-regium*. Biotechnol Lett 34:1863–1867
149. Wang M, Gu B, Huang J, Jiang S, Chen Y, Yin Y, Pan Y, Yu G, Li Y, Wong BH et al (2013) Transcriptome and proteome exploration to provide a resource for the study of *Agrocybe aegerita*. PLoS ONE 8:e56686
150. Petrovic J, Glamoclija J, Stojkovic D, Nikolic M, Ciric A, Fernandes A, Ferreira IC, Sokovic M (2014) Bioactive composition, antimicrobial activities and the influence of *Agrocybe aegerita* (Brig.) Sing on certain quorum-sensing-regulated functions and biofilm formation by *Pseudomonas aeruginosa*. Food Funct 5:3296–3303
151. Heinke R, Schone P, Arnold N, Wessjohann L, Schmidt J, Schmidt J (2014) Metabolite profiling and fingerprinting of *Suillus* species (Basidiomycetes) by electrospray mass spectrometry. Eur J Mass Spectrom (Chichester, Eng) 20:85–97
152. Park SJ, Hyun SH, Suh HW, Lee SY, Sung GH, Kim SH, Choi HK (2013) Biochemical characterization of cultivated *Cordyceps bassiana* mycelia and fruiting bodies by H-1 nuclear magnetic resonance spectroscopy. Metabolomics 9:236–246
153. Matsuzaki F, Shimizu M, Wariishi H (2008) Proteomic and metabolomic analyses of the white-rot fungus *Phanerochaete chrysosporium* exposed to exogenous benzoic acid. J Proteome Res 7:2342–2350
154. Bak JS (2015) Lignocellulose depolymerization occurs via an environmentally adapted metabolic cascades in the wood-rotting basidiomycete *Phanerochaete chrysosporium*. Microbiologyopen 4:151–166
155. Zhang W, Tang YJ (2008) A novel three-stage light irradiation strategy in the submerged fermentation of medicinal mushroom *Ganoderma lucidum* for the efficient production of ganoderic acid and *Ganoderma* polysaccharides. Biotechnol Prog 24:1249–1261
156. Tang YJ, Zhang W, Zhong JJ (2009) Performance analyses of a pH-shift and DOT-shift integrated fed-batch fermentation process for the production of ganoderic acid and

Ganoderma polysaccharides by medicinal mushroom *Ganoderma lucidum*. Bioresour Technol 100:1852–1859
157. Zhao W, Xu JW, Zhong JJ (2011) Enhanced production of ganoderic acids in static liquid culture of *Ganoderma lucidum* under nitrogen-limiting conditions. Bioresour Technol 102:8185–8190
158. Xu YN, Zhong JJ (2012) Impacts of calcium signal transduction on the fermentation production of antitumor ganoderic acids by medicinal mushroom *Ganoderma lucidum*. Biotechnol Adv 30:1301–1308
159. Xu YN, Xia XX, Zhong JJ (2013) Induced effect of Na(+) on ganoderic acid biosynthesis in static liquid culture of *Ganoderma lucidum* via calcineurin signal transduction. Biotechnol Bioeng 110:1913–1923
160. Xu YN, Xia XX, Zhong JJ (2014) Induction of ganoderic acid biosynthesis by Mn^{2+} in static liquid cultivation of *Ganoderma lucidum*. Biotechnol Bioeng 111:2358–2365
161. Fang QH, Zhong JJ (2002) Two-stage culture process for improved production of ganoderic acid by liquid fermentation of higher fungus *Ganoderma lucidum*. Biotechnol Prog 18:51–54
162. Zhang JM, Zhong JJ, Geng AL (2014) Improvement of ganoderic acid production by fermentation of *Ganoderma lucidum* with cellulase as an elicitor. Process Biochem 49:1580–1586
163. Xu P, Ding ZY, Qian Z, Zhao CX, Zhang KC (2008) Improved production of mycelial biomass and ganoderic acid by submerged culture of *Ganoderma lucidum* SB97 using complex media. Enzyme Microb Technol 42:325–331
164. Liu GQ, Ren GP, Wang XL, Zhao Y (2011) Statistical optimization of the key medium components by response surface methodology to promote ganoderic acid formation by medicinal mushroom *Ganoderma sinense* in submerged culture. J Med Plants Res 5:425–431
165. Zhang ZC, Shen WL, Liu D, Li JS (2011) Enhanced production of mycelial biomass and ganoderic acid in submerged culture of *Ganoderma applanatum* ACCC-52297 elicited by feeding rutin. Afr J Microbiol Res 5:3452–3461
166. Liu GQ, Wang XL, Han WJ, Lin QL (2012) Improving the fermentation production of the individual key triterpene ganoderic acid Me by the medicinal fungus *Ganoderma lucidum* in submerged culture. Molecules 17:12575–12586
167. Wei ZH, Duan YY, Qian YQ, Guo XF, Li YJ, Jin SH, Zhou ZX, Shan SY, Wang CR, Chen XJ et al (2014) Screening of *Ganoderma* strains with high polysaccharides and ganoderic acid contents and optimization of the fermentation medium by statistical methods. Bioprocess Biosyst Eng 37:1789–1797
168. Dou Y, Xiao JH, Xia XX, Zhong JJ (2013) Effect of oxygen supply on biomass and helvolic acid production in submerged fermentation of *Cordyceps taii*. Biochem Eng J 81:73–79
169. Jiang MY, Feng T, Liu JK (2011) N-containing compounds of macromycetes. Nat Prod Rep 28:783–808
170. Nakamura K, Yoshikawa N, Yamaguchi Y, Kagota S, Shinozuka K, Kunitomo M (2006) Antitumor effect of cordycepin (3′-deoxyadenosine) on mouse melanoma and lung carcinoma cells involves adenosine A3 receptor stimulation. Anticancer Res 26:43–47
171. Mao XB, Zhong JJ (2006) Significant effect of NH_4^+ on cordycepin production by submerged cultivation of medicinal mushroom *Cordyceps militaris*. Enzyme Microb Technol 38:343–350
172. Das SK, Masuda M, Hatashita M, Sakurai A, Sakakibara M (2008) A new approach for improving cordycepin productivity in surface liquid culture of *Cordyceps militaris* using high-energy ion beam irradiation. Lett Appl Microbiol 47:534–538
173. Fan DD, Wang W, Zhong JJ (2012) Enhancement of cordycepin production in submerged cultures of *Cordyceps militaris* by addition of ferrous sulfate. Biochem Eng J 60:30–35
174. Gunde-Cimerman N, Cimerman A (1995) *Pleurotus* fruiting bodies contain the inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A reductase-lovastatin. Exp Mycol 19:1–6
175. Atli B, Yamac M, Yildiz Z (2013) Optimization of submerged fermentation conditions for lovastatin production by the culinary-medicinal oyster mushroom, *Pleurotus ostreatus* (higher basidiomycetes). Int J Med Mushrooms 15:487–495

176. Lan WC, Tzeng CW, Lin CC, Yen FL, Ko HH (2013) Prenylated flavonoids from *Artocarpus altilis*: Antioxidant activities and inhibitory effects on melanin production. Phytochemistry 89:78–88
177. Zhu H, Sun S, Zhang S (2011) Enhanced production of total flavones and exopolysaccharides via *Vitreoscilla* hemoglobin biosynthesis in *Phellinus igniarius*. Bioresour Technol 102:1747–1751
178. Giavasis I (2014) Bioactive fungal polysaccharides as potential functional ingredients in food and nutraceuticals. Curr Opin Biotechnol 26:162–173
179. Su CA, Xu XY, Liu DY, Wu M, Zeng FQ, Zeng MY, Wei W, Jiang N, Luo X (2013) Isolation and characterization of exopolysaccharide with immunomodulatory activity from fermentation broth of *Morchella conica*. Daru J Pharm Sci 21:5
180. Osinska-Jaroszuk M, Jaszek M, Mizerska-Dudka M, Blachowicz A, Rejczak TP, Janusz G, Wydrych J, Polak J, Jarosz-WilkoBazka A, Kandefer-Szerszen M (2014) Exopolysaccharide from *Ganoderma applanatum* as a promising bioactive compound with cytostatic and antibacterial properties. Biomed Res Int :743812
181. Seo MJ, Kang BW, Park JU, Kim MJ, Lee HH, Choi YH, Jeong YK (2011) Biochemical characterization of the exopolysaccharide purified from *Laetiporus sulphureus* mycelia. J Microbiol Biotechnol 21:1287–1293
182. Xiao JH, Xiao DM, Chen DX, Xiao Y, Liang ZQ, Zhong JJ (2012) Polysaccharides from the medicinal mushroom *Cordyceps taii* show antioxidant and immunoenhancing activities in a D-galactose-induced aging mouse model. Evid-Based Compl Alt :273435
183. Ren L, Perera C, Hemar Y (2012) Antitumor activity of mushroom polysaccharides: a review. Food Funct 3:1118–1130
184. Xiang Y, Xu X, Li J (2012) Chemical properties and antioxidant activity of exopolysaccharides fractions from mycelial culture of *Inonotus obliquus* in a ground corn stover medium. Food Chem 134:1899–1905
185. Im SA, Wang W, Lee CK, Lee YN (2010) Activation of macrophages by exopolysaccharide produced by MK1 bacterial strain isolated from neungee mushroom, *Sarcodon aspratus*. Immune Netw 10:230–238
186. Zhu H, Cao CX, Zhang SS, Zhang Y, Zou WS (2011) pH-control modes in a 5-L stirred-tank bioreactor for cell biomass and exopolysaccharide production by *Tremella fuciformis* spore. Bioresour Technol 102:9175–9178
187. Hwang HS, Lee SH, Baek YM, Kim SW, Jeong YK, Yun JW (2008) Production of extracellular polysaccharides by submerged mycelial culture of *Laetiporus sulphureus* var. *miniatus* and their insulinotropic properties. Appl Microbiol Biotechnol 78:419–429
188. Cui JD, Zhang YN (2012) Evaluation of metal ions and surfactants effect on cell growth and exopolysaccharide production in two-stage submerged culture of *Cordyceps militaris*. Appl Biochem Biotechnol 168:1394–1404
189. Xu JW, Ji SL, Li HJ, Zhou JS, Duan YQ, Dang LZ, Mo MH (2015) Increased polysaccharide production and biosynthetic gene expressions in a submerged culture of *Ganoderma lucidum* by the overexpression of the homologous alpha-phosphoglucomutase gene. Bioprocess Biosyst Eng 38:399–405
190. Ji SL, Liu R, Ren MF, Li HJ, Xu JW (2015) Enhanced production of polysaccharide through the overexpression of the homologous UDP glucose pyrophosphorylase gene in a submerged culture of Lingzhi or Reishi medicinal mushroom, *Ganoderma lucidum* (higher basidiomycetes). Int J Med Mushrooms 17:435–442
191. Molnar I, Gibson DM, Krasnoff SB (2010) Secondary metabolites from entomopathogenic Hypocrealean fungi. Nat Prod Rep 27:1241–1275
192. Robinson JA (1991) Polyketide synthase complexes—their structure and function in antibiotic biosynthesis. Philos T Roy Soc B 332:107–114
193. Bai MS, Wang C, Zong SC, Lei M, Gao JM (2013) Antioxidant polyketide phenolic metabolites from the edible mushroom *Cortinarius purpurascens*. Food Chem 141:3424–3427

194. Strauss J, Reyes-Dominguez Y (2011) Regulation of secondary metabolism by chromatin structure and epigenetic codes. Fungal Genet Biol 48:62–69
195. Chung YM, Wei CK, Chuang DW, El-Shazly M, Hsieh CT, Asai T, Oshima Y, Hsieh TJ, Hwang TL, Wu YC, Chang FR (2013) An epigenetic modifier enhances the production of anti-diabetic and anti-inflammatory sesquiterpenoids from *Aspergillus sydowii*. Bioorg Med Chem 21:3866–3872
196. Asai T, Luo D, Obara Y, Taniguchi T, Monde K, Yamashita K, Oshima Y (2012) Dihydrobenzofurans as cannabinoid receptor ligands from *Cordyceps annullata*, an entomopathogenic fungus cultivated in the presence of an HDAC inhibitor. Tetrahedron Lett 53:2239–2243
197. Asai T, Yamamoto T, Oshima Y (2012) Aromatic polyketide production in *Cordyceps indigotica*, an entomopathogenic fungus, induced by exposure to a histone deacetylase inhibitor. Org Lett 14:2006–2009