Medicinal Plant Biology

ARTICLE

Open Access

https://doi.org/10.48130/mpb-0026-0012

Medicinal Plant Biology **2026**, 5: e017

Biotransformation of characteristic saponins and pharmacological compounds in *Panax notoginseng* by the golden flower fungus *Aspergillus cristatus*

Qi Wei[1#], Jialong Song[1#], Lei Wang[2#], Meiyi Li[1], Hang Ji[1], Chuhan He[1], Huiyan Ju[1*] and Shouan Liu[1*]

[1] *Laboratory of Tea and Medicinal Plant Biology, College of Plant Sciences, Jilin University, Changchun 130062, China*
[2] *Institute of Chemical and Industrial Bioengineering, Jilin Engineering Normal University, Changchun 130052, China*
[#] Authors contributed equally: Qi Wei, Jialong Song, Lei Wang
[*] Correspondence: juhy@jlu.edu.cn (Ju H); shouan@jlu.edu.cn (Liu S)

Abstract

Panax notoginseng, an important traditional Chinese medicine with a long history of clinical application, possesses significant pharmacological value. *P. notoginseng* quality improvement has been extensively studied by chemical transformation methods; however, the bio-transformation method with beneficial microbes remains relatively underexplored. In this study, we first fermented *P. notoginseng* roots with the golden flower fungus *Aspergillus cristatus*, and then high-performance liquid chromatography (HPLC) was performed to reveal ginsenoside alterations in fungal treatment. To elucidate the underlying mechanisms, integrated transcriptome and metabolome methods were used to identify the differentially expressed genes of *A. cristatus* and the differentially accumulated metabolites in *P. notoginseng* roots after fermentation. Our study demonstrated that the *P. notoginseng* pharmacological compounds were dramatically changed, and the content of several important characteristic saponins, including ginsenosides R1, Re, Rb1, Rd, and Rf, was significantly increased. Concurrently, several metabolites associated with medicinal function were also significantly increased. These data further revealed the molecular characteristics of the beneficial fungus *A. cristatus* upon fermentation on the medicinal plant *P. notginseng* by reprogramming and activating fungal transcripts in the ribosome and oxidative phosphorylation-related events. This study provides new insights into the molecular mechanisms underlying *A. cristatus*-mediated enhancement of bioactive constituents in *P. notoginseng*, and establish a biotechnological foundation for improving the quality of cultivated *P. notoginseng* roots by controlled fermentation.

Keywords: *Panax notoginseng*, *Aspergillus cristatus*, Non-traditional commercial sterilization, Ginsenosides, Metabolome and transcriptome

Citation: Wei Q, Song J, Wang L, Li M, Ji H, et al. 2026. Biotransformation of characteristic saponins and pharmacological compounds in *Panax notoginseng* by the golden flower fungus *Aspergillus cristatus*. *Medicinal Plant Biology* 5: e017 https://doi.org/10.48130/mpb-0026-0012

Introduction

Panax notoginseng (Burk.) F.H. Chen, also named as Tianqi or Sanqi, is a traditional medicinal herb native to Asia and is now commonly used worldwide. *P. notoginseng* has been documented in Chinese *materia medica* for over 400 years. It is highly regarded for its dual effects in hemostasis and nourishing blood among traditional Chinese medicines[1]. Its therapeutic effects were first systematically reported by the famous Ming Dynasty (1368–1644 AD) pharmacologist Li Shizhen (李时珍) in his book *Compendium of Materia Medica*[2]. In this book, the pharmaceutical value of *P. notoginseng* was described as possessing detumescent, analgesic, and blood stasis alleviating properties[3]. The 2020 edition of the Chinese pharmacopoeia further characterizes Sanqi as 'warm in nature, sweet and slightly bitter in flavor, in the liver meridian and the blood system, is good at stopping bleeding, and the generation of new tissue'.

Numerous works have revealed that *P. notoginseng* has wide-reaching pharmacological activities, such as antioxidant, anti-inflammatory, antitumor, and cardiovascular protective effects[4]. It has been applied to treat cardiovascular, nervous system, and immune system diseases. Thus, *P. notoginseng* has been used as the main component in many classical herbal formulas[5], such as Hua Xue Dan, Yun Nan Bai Yao Xue Sai Tong, An Shen Zhi Tong Tang, Xue Shuan Tong, Xue Sai Tong, Lu Lu Tong, and Sanqi Tong Shu. Recently, *P. notoginseng* has also been recognized as a medicinal and edible resource, with increasing incorporation into dietary supplements[6].

P. notoginseng saponins, which are triterpenoid saponins, are characterized by a tetra-cyclic structural framework that serves as the principal bioactive ginsenoside. Based on this skeleton, ginsenosides are divided into dammarane-type and oleanane-type saponins[7]. Compared with other species in *Panax* spp., *P. notoginseng* only contains the dammarane-type saponins[8]. Besides, the total saponin content in *P. notoginseng* is higher than that in *P. ginseng*, which is the most famous medicinal plant, suggesting the special role of *P. notoginseng*[9]. Beyond saponins, *P. notoginseng* polysaccharides, which have both immunological-stimulating and inflammation-suppressing activities, are the second most important bioactive compounds[10]. To date, more than 20 homogeneous polysaccharides have been isolated and characterized from *P. notoginseng*, with nine of them having immunoregulatory efficacy. Additionally, more than 40 aromatic oils and over 10 flavonoid fractions were identified from *P. notoginseng*, which include the established anti-inflammatory substances kaempferol, liquiritigenin, quercetin, and andrographolide[10].

For humans, the absorption and bioavailability of saponins mainly rely on gastrointestinal biotransformation. Notably, minor saponins exhibit superior bioactivity due to their enhanced absorption into the human bloodstream compared to major saponins[11]. Presently, the main methods to produce minor ginsenosides include chemical conversion, physical processing, biological fermentation, and the

https://doi.org/10.48130/mpb-0026-0012

cloned ginsenoside enzymes-mediated transformation[12]. Among these, the bio-transformation method has more advantages, such as mild reaction conditions, less environmental pollution, stable product profiles, efficient transformation, minimal by-product formation, and simplified downstream separation[13]. For the bio-transformation, beneficial microorganisms and their inducible enzymes are used to modify the ginsenoside. For example, *Aspergillus niger* has been demonstrated to transform ginsenoside Rb1 into Rg3 or CK[14].

In this study, we first incubated non-traditional commercially sterilized *P. notoginseng* (115 °C, 15 min) with the golden flower fungus *A. cristatus* and subsequently analyzed the changes in certain ginsenosides after *A. cristatus* treatment. Furthermore, integrated metabolome and transcriptome analyses were employed to elucidate differentially accumulated metabolism and differentially expressed genes during the *P. notoginseng*–*A. cristatus* interaction. These findings provide mechanistic insights into the role of beneficial microbes, i.e., *A. cristatus*, in mediating medicinal plants, i.e., *P. notoginseng*.

Materials and methods

Materials and treatments

Three-year-old *P. notoginseng* (Sanqi) roots were collected from Wenshan, Yunnan Province, China, in 2023 and 2025. The roots were cut into small pieces (~10 × 5 mm) before sterilization. The sterilized Sanqi roots were kept in glass bottles for fungal fermentation. Approximately 250 g of fresh Sanqi roots were placed in a 500-mL glass bottle. The sterilization was done under modified conditions (115 ˚C, 15 min) compared with traditional commercial conditions (121 ˚C, 30 min). The golden flower fungus *A. cristatus* was used for fermentation. Fungal spores were harvested (1 × 10^6/mL) and sprayed on the sterilized *P. notoginseng* root surface, and then incubated at 25 ˚C for 0, 3, 6, 9, and 12 d. *P. notoginseng* roots without fungal incubation (only sprayed with dd H_2O) were used as the control. After inoculation, the roots of *P. notoginseng* were dried, ground into powder, and screened through 40-mesh sieves. The powder was used for chemical measurements and the analysis of metabolites. Three to six replicates were done for each measurement. For RNA-sequencing, the *A. cristatus* was harvested from *P. notoginseng* roots with three replicates. The fungi without interaction with *P. notoginseng* roots were used as a control.

High Performance Liquid Chromatography (HPLC) analysis of certain ginsenosides with or without fungal treatment

Before HPLC analysis, a 0.5 g Sanqi powder sample was accurately weighed and extracted with 80% methanol. The solution was sonicated for 30 min and then kept undisturbed overnight. The sonication was repeated twice. After the final sonication, 80% methanol was added, and the mixture was left to stand overnight. To get a clear sample for HPLC analysis, the supernatant was collected and then filtered through a 0.22 μm filter membrane.

For HPLC analysis of the Sanqi ginsenosides, the chromatographic conditions are set as follows. (i) Column: ZORBAX Eclipse Plus C18 column (4.6 × 250 mm, 5 μm). (ii) Mobile phase: water (A) - acetonitrile (B). (iii) The gradient elution: 0–15 min, 19% B; 15–25 min, 19% to 29% B; 25–35 min, 29% to 40% B; 35–65 min, 40% to 100% B; 65–75 min, 100% to 19% B. (iv) Detection wavelength: 203 nm. (v) Column temperature: 35 °C. (vi) Flow rate: 1 mL/min. The injection volume: 5 μL.

Liquid Chromatograph Mass Spectrometer (LC-MS) analysis of *P. notoginseng* metabolites

For the extraction of *P. notoginseng* metabolites, root powder was first extracted with 120 μL of precooled 50% methanol buffer as indicated previously[15]. In short, the extraction mixture was vortexed for 1 min, stood on the bench and incubated for 10 min, centrifugated at 4,000 g for 20 min, and finally transferred the supernatants into a new 96-well plate. The QC samples were included by combining 10 μL of each extraction mixture. All root samples were measured and analyzed by the LC-MS system according to machine orders (LC-Bio, Technology Co., Ltd, Hangzhou, China). Metabolomic profiling was carried out by an UltiMate 3000 UPLC system (Thermo Fisher Scientific, Bremen, Germany) coupled with the high-resolution tandem mass spectrometer Q-Exactive (Thermo Fisher Scientific, Bremen, Germany). Chromatographic separation was achieved on the ACQUITY UPLC T3 column (100 mm × 2.1 mm, 1.8 μm, Waters, Milford, USA) and kept at 40 °C. The Q-Exactive mass spectrometer was operated in positive and negative ion modes, respectively.

After detection, the LC-MS data were analyzed as follows. First, the LC-MS data were transformed into mzXML format by MSConvert. Second, the formats were processed by XCMS, CAMERA, and metaX toolbox, respectively. Finally, the data was incorporated into R software. The combined retention time (RT) and m/z data were used to identify each ion[15].

Transcriptome analysis

For transcriptome analysis, *A. cristatus* mRNA was used, sequenced, and analyzed. In short, total RNA identification, purification, monitoring, cDNA library construction, sequencing, and the raw data cleaning and analysis were performed as previously indicated[15]. Clean reads were observed by removing lower-quality reads from the raw data, including (i) reads containing adaptors, (ii) reads containing primers, and (iii) nucleotides with lower Q quality scores. Q20, Q30, and GC content of the clean data were then calculated.

After cleaning, the higher-quality reads were used for downstream analysis. The *A. cristatus* reference genome and fungal gene annotation files were obtained from the reference[16]. For mapping fragments to the genome and quantification of gene levels, the index of the fungal genome reference was built, paired-end clean reads were aligned to the reference, and the FPKM of each gene was then calculated. The *A. cristatus* reference genome and fungal gene annotation files were obtained from the NCBI genome datasets with assembly number ASM4470619v1[16].

For analysis of differentially expressed genes (DEGs), DEGs in all samples (EcSQ, EcCK) were analyzed according to the study by Chen et al.[15]. The DEGs were selected with $\log_2$ (fold change) ≥ 1 or $\log_2$ (fold change) ≤ 1 and with statistical significance (p-value ≤ 0.05) by the R package.

Gene Ontology (GO)/Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis

GO enrichment and KEGG analysis of DEGs were performed as previously described[15]. Gene ontology (GO) enrichment analysis of DEGs was implemented by the Goseq R package, in which gene length bias was corrected. Kyoto Encyclopedia of Genes and Genomes (KEGG) is a database resource based on large-scale molecular datasets generated by genome sequencing or metabolite technologies. GO/KEGG analysis was performed as previously indicated[15,17].

Statistical analysis

The analysis of variance used Statistical Product and Service Solution software (IBM). The considered significance was indicated at * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$. All data are represented as the mean ± SEM of at least three independent replicates.

Results

Fermentation of *A. cristatus* on the *P. notoginseng* roots

The golden flower fungus *A. cristatus* was first cultivated on PDA plates for 2 weeks. For fermentation, roots of *P. notoginseng* were cut into pieces approximately 10 mm in length and 5 mm in thickness (Fig. 1a, b; Supplementary Fig. S1, 0 d). To eliminate the potential contamination from endogenous or rhizosphere microbes, roots were sterilized by autoclave under the conditions of 115 °C for 15 min (Fig. 1c; Supplementary Fig. S1, 0 d). This mild sterilization condition was deliberately chosen to minimize thermal degradation of bioactive constituents while ensuring microbial decontamination, which differs from traditional commercial sterilization conditions (121 °C, 30 min). Non-traditional commercial sterilization is deliberately employed to reduce the damage to the active compounds in *P. notoginseng* under the elevated temperature and pressure. Fermentation was carried out by adding spores of *A. cristatus* (Fig. 1d) into each bottle and followed by incubation at 25 °C (Fig. 1e).

To determine the proper incubation time, we fermented Sanqi roots for about 12 d (Supplementary Fig. S1; 0–12 d). Dense mycelial coverage was visually observed on the *P. notoginseng* root surface. Within 1 week after inoculation (Fig. 1e; Supplementary Fig. S1), all fermented *P. notoginseng* samples showed robust fungal growth without any contamination over the 12 d (Supplementary Fig. S1). These results confirm the efficacy of the non-traditional commercial sterilization approach and demonstrate that golden flower fungal *A. cristatus* successfully grows on the roots of *P. notoginseng*. After fermentation, the samples were dried at 80 °C for 5 h. Visual comparison revealed distinct color differences: the untreated Sanqi control (SCK, Fig. 1f) appeared light brown, whereas the *A. cristatus* treated samples (SJ, Fig. 1g) displayed dark brown. This chromatic transformation indicates the successful development of a novel *P. notoginseng* based on the non-traditional commercial sterilization combined with the golden flower fungus *A. cristatus* fermentation (Fig. 1f, g).

Effects of *A. cristatus* fermentation on *P. notoginseng* saponins accumulation

To evaluate if *A. cristatus* treatment could improve *P. notoginseng* quality, we measured and compared the selected ginsenosides in the control (SCK), and fermented (SJ) samples. Both samples were ground into powder and extracted for HPLC analysis. The HPLC grade of ginsenoside compounds (Yuanye, Shanghai, China) was used as a standard. HPLC analysis revealed that many of the measured saponins were significantly increased in SJ except Rg3 and F2 (Fig. 2a). The accumulation of Rg3 was significantly decreased, but the content of F2 was not significantly increased (Fig. 2a). Notably, the induced ginsenoside compounds included R1, Re, Rb1, Rd, and Rf (Fig. 2b). This data indicated that the golden flower fungus is involved in *P. notoginseng* saponins transformation, which enhances the production of both major and rare ginsenosides in *P. notoginseng*.

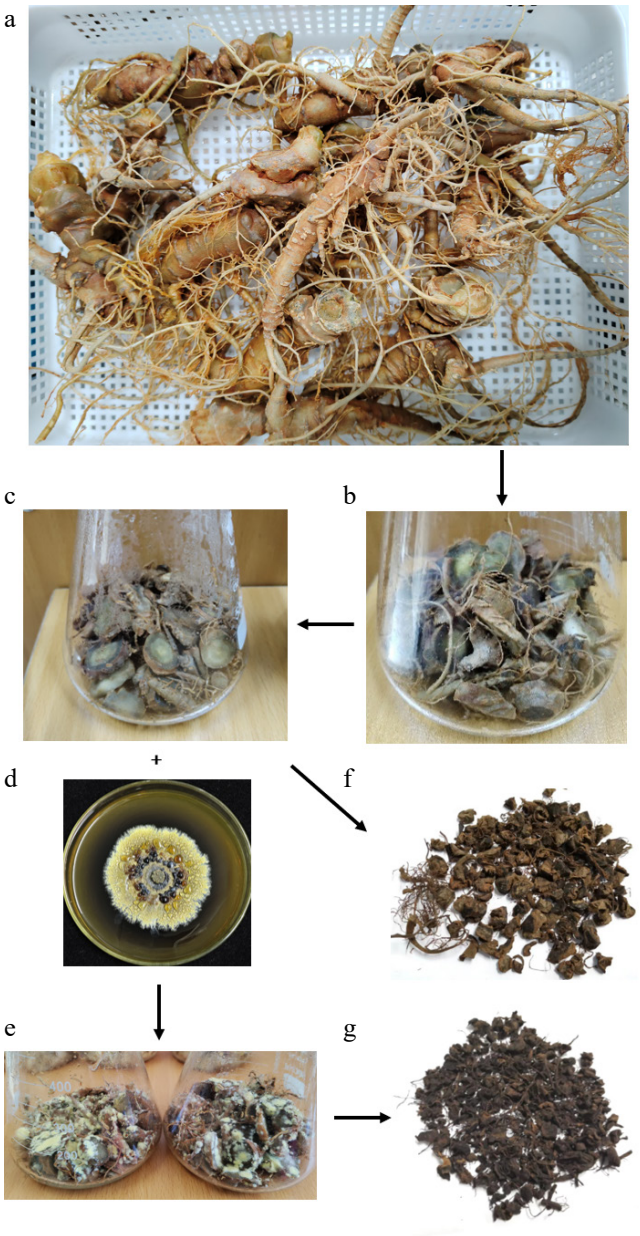

Fig. 1 The production process and characteristics of *A. cristatus* fermented with *P. notoginseng*. (a)–(g) Schematic of the production process of *P. notoginseng* upon *A. cristatus* treatment. (g) Fermented (SJ), and (f) the untreated control samples (SCK).

To explain the temporal dynamics of Sanqi ginsenoside accumulation during fermentation, selected saponins were quantified at different time points (0, 3, 6, 9, and 12 d). At 3 d after fungal incubation, the accumulation of R1, Re, Rb1, and Rd was significantly decreased, while the accumulation of Rg3 showed a notable increase compared with day 0 (Supplementary Fig. S2). At 6 d, most of the tested ginsenosides (R1, Re, Rd, and Rg3) were significantly increased, with Rb1 slightly increased (Supplementary Fig. S2). At 9 d, the accumulation of Rg3 declined significantly, while the other ginsenosides continued to increase (Supplementary Fig. S2). These temporal patterns indicated that *A. cristatus* indeed modulated Sanqi ginsenosides biosynthesis, and that the changes strictly depended on incubation times.

https://doi.org/10.48130/mpb-0026-0012

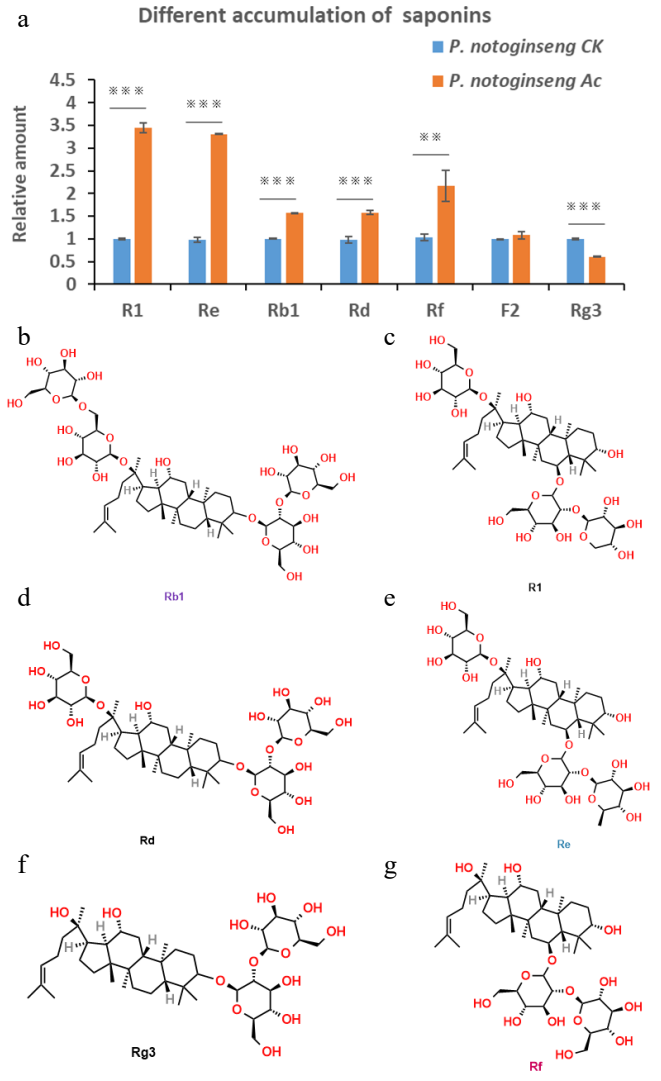

Fig. 2 Differentially accumulated Sanqi saponins in *P. notoginseng* CK (SCK) and *P. notoginseng* Ac (SJ). (a) Relative amount of selected Sanqi saponins between SCK and SJ; ** $p < 0.01$; *** $p < 0.001$. (b)–(g) Structure of indicated Sanqi saponins.

Metabolites analysis of *P. notoginseng* upon *A. cristatus* fermentation and un-fermentation

To comprehensively assess the quality improvement in *P. notoginseng* after *A. cristatus* fermentation, an untargeted metabolome approach was performed by LC-MS. The identified metabolites were annotated against the KEGG databases (www.kegg.jp/), revealing several significantly enriched pathways (Supplementary Fig. S3, KEGG pathway 3). Principal component analysis (PCA) and partial least squares-discriminant analysis (PLS-DA) were performed to get a deep overview of the metabolic changes between fermented and control samples (Supplementary Fig. S4). The quantification finally identified 11,738 metabolic features in *P. notginseng*, with 6,272 of them increased and 5,466 of them decreased following *A. cristatus* treatment (Supplementary Fig. S5a). Heatmap analysis of all quantified features in *P. notoginseng* is shown in Supplementary Fig. S6 with different levels. Subsequently, 832 high-quality MS2 metabolites were used for differential analysis (Supplementary Data 1), identifying 412 significant differentially accumulated metabolites (DAMs) in *P. notoginseng*. In the comparison between *A. cristatus*-infected *P. notginseng* and the control, a total of 233 and 179 metabolites were presented as being upregulated and downregulated, respectively (Fig. 3a).

All DAMs were functionally assigned to various major metabolic categories. Supplementary Fig. S5b indicates the significantly enriched metabolic pathways. These include glycerolipid metabolism, glycerophospholipid metabolism, linoleic acid metabolism, ABC transporters, fat digestion and absorption, steroid hormone biosynthesis, glycosylphosphatidylinositol (GPI)-anchor biosynthesis, nucleotide metabolism (particularly purine metabolism), etc. It suggests that inoculation of *P. notoginseng* with *A. cristatus* changed the metabolites. Notably, 28 metabolites are enriched in lipid metabolism upon *A. cristatus* incubation, with approximately half exhibiting increased abundance and half exhibiting decreased abundance (Supplementary Fig. S7a). Five metabolites associated with the isoflavonoid biosynthesis pathway (daidzin, pseudobaptigenin, biochanin A, pratensein, and coumesterol) are increased upon *A. cristatus* treatment (Supplementary Figs S7b, S8). Interestingly, 12 metabolites are significantly enriched in human disease-associated pathways, with six of them increased and six decreased upon *A. cristatus* treatment (Supplementary Fig. S7c), suggesting potential modulation of bioactive compounds with pharmacological relevance.

Several metabolites associated with pharmacological effects are dramatically increased during *P. notginseng* - *A. cristatus* interaction

We next analyzed the top DAMs in *P. notoginseng* with or without *A. cristatus* treatment (Fig. 3b). Heatmap analysis clearly revealed the DAMs between SJ and SCK. Among the significantly upregulated metabolites, several metabolites associated with pharmacological effects are dramatically increased during *P. notginseng*–*A. cristatus* interaction (Fig. 3c–h). These include euparin, prostaglandin A2, pseudobaptigenin, uridine 5'-diphospho-N-acetylglucosamine, 19-norandrosterone, and 19-oxotestosterone. However, there are several compounds that are decreased, such as Hemi-bis (monoacylglycero) phosphate (HemiBMP 62:23; HemiBMP18:3/22:5/22:5), ginsenoside compound K, and 7,2-Dimethoxy-3-hydroxyflavone (Fig. 3i–k).

Euparin has many activities such as anti-bacterial, anti-diabetes, anti-viral, anti-inflammatory, anti-tumor, and antidepressant[18]. Euparin and its derivatives from *Eupatorium chinense* acted as dual inhibitors of protein tyrosine phosphatase and α-glucosidase, highlighting their therapeutic potential for metabolic disorders[18]. Prostaglandin A2 affected various biological processes, including leukemic cell differentiation, cell cycle progression, and the induction of apoptosis in various cancer cell lines[19]. The anabolic androgenic steroid, 19-nortestosterone, was first produced for androgen replacement therapy as a testosterone analog, while 19-oxotestosterone is an androstanoid that is testosterone with an oxo-group at the C-19 position. The enrichment of these compounds suggests that *A. cristatus* fermentation enhances Sanqi pharmacological activity. Uridine 5'-diphospho N-acetylglucosamine was reported to serve as the high-value nucleotide sugar donor for the synthesis of various glycans in *in vitro* glycol-engineering[20]. This compound may be catalyzed by fungal N-glucan biosynthesis-related genes during fermentation of Sanqi roots.

Among the decreased compounds in Sanqi under this condition, the ginsenoside compound K is characterized as a deglycosylated metabolite derived from protopanaxadiol-type ginsenoside[21]. The

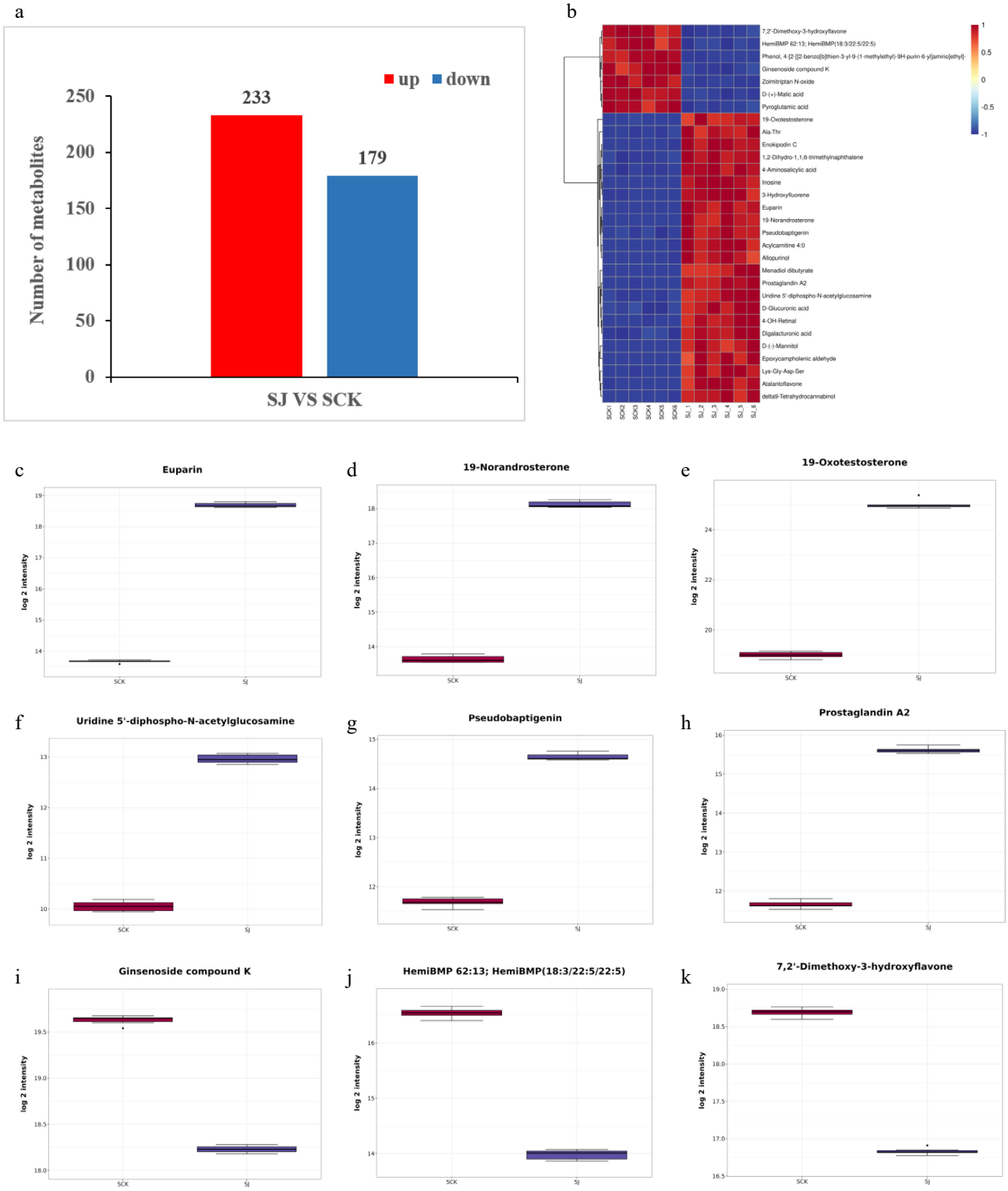

Fig. 3 Differentially accumulated metabolites in *P. notoginseng* during interaction with *A. cristatus*. (a) Numbers of differentially changed metabolites in *A. cristatus* treated *P. notoginseng* (SJ) and the untreated control (SCK). (b) The top differentially accumulated metabolites in *P. notoginseng* with or without *A. cristatus* treatment. (c)–(k) Differentially accumulated compounds in SCK and *A. cristatus*-treated *P. notoginseng* (SJ).

observed decrease of CK might be due to the degradation by the fungus at this time point, which might be increased at other time stages. This temporal dependency underscores the dynamic nature of microbial biotransformation. Hemi-bis(monoacylglycero)phosphate (Hemi-BMP) contains a glycerophosphoglycerol backbone with three fatty acyl chains esterified to the glycerol hydroxy[22]. 7,2-Dimethoxy-3-hydroxyflavone has been identified as a potent inhibitor of human phenylethanolamine N-methyltransferase (hPNMT), a target implicated in the treatment of human mental processes[23]. Its reduced abundance suggests that *A. cristatus*

https://doi.org/10.48130/mpb-0026-0012

fermentation may change the flavonoid glycosylation profile or shift phenylpropanoid metabolism towards other branch pathways. In golden flower fungal-treated Sanqi, the increase or the decrease of these compounds suggests that *A. cristatus* changes the transformation of the pharmacological chemicals in *P. notoginseng*. These transformations are strictly dependent on incubation duration, reflecting the stage-specific interplay between fungal enzymatic activity and host plant biosynthetic capacity.

mRNA profiling of golden flower fungal *A. cristatus* genes upon interaction with *P. notoginseng*

The differentially accumulated metabolites in *P. notoginseng* were due to the fermentation by the golden flower fungus *A. cristatus*. We observed that *A. cristatus* grew well on *P. notoginseng* roots, which indicates that the fungus could use the medicinal plant as a nutritional substrate for growth (Fig. 1; Supplementary Fig. S1). To elucidate the molecular mechanism of *A. cristatus* in fermenting *P. notoginseng* roots, mRNA sequencing was performed. We obtained 243 million validated high-quality reads from six cDNA libraries (Supplementary Table S1). A total of 2,429 SSTF ($|\log_2 FC| \geq 1$, $p \leq 0.05$, SSTF) genes were finally identified between EcSQ and EcCK, with 1,357 genes being upregulated and 1,072 genes being downregulated (Fig. 4a; Supplementary Data 2).

To identify the functional categories of genes involved in *A. cristatus-P. notoginseng* interaction, we analyzed the differentially expressed genes (DEGs) by the GO and KEGG methods, respectively. Significantly enriched GO terms were predominantly associated with translation machinery, ribosome biogenesis (ribosome, cytosolic large ribosomal subunit, structural constituent of ribosome, cytosolic small ribosomal subunit), and rRNA export from the nucleus, etc. (Fig. 5a, d, e; Supplementary Fig. S9a). KEGG enrichment revealed significant activation of multiple metabolic and biosynthetic pathways; genes associated with ribosome, oxidative phosphorylation, indole diterpene alkaloid biosynthesis, various types of N-glucan biosynthesis, butanoate metabolism, ether lipid metabolism, and linoleic acid metabolism are significantly enriched (Fig. 5b, c; Supplementary Figs S9b, S10). The GO and KEGG analysis of differentially expressed genes indicated that ribosome, translation, and oxidative phosphorylation-related genes are enriched in *A. cristatus* when interacting with *P. notoginseng*, particularly at the translational and post-translational levels. While the ribosome and oxidative phosphorylation-related events are activated (Fig. 5a, b; Supplementary Fig. S10), the N-glucan biosynthesis-related genes were differentially regulated (Fig. 5c), suggesting *P. notoginseng* might trigger fungal protein glycosylation and cell wall remodeling during the interaction.

We further analyzed the top induced genes in SSTFs and identified several DEGs that encoded fungal carbohydrate-active enzymes (CAZYmes) (Fig. 4b). Notably, *SI65_10113* encodes endopolygalacturonase I, while *SI65_09823* encodes endoglucanase A, and both of them are upregulated in *A. cristatus* by *P. notoginseng* with 100-fold or 208-fold induction, respectively. Two genes, *SI65_04327* and *SI65_05244*, encode putative endo-1,4-beta-xylanase, which are 300-fold or 33.6-fold higher in EcSQ than EcCK. Both *SI65_00176* and *SI65_00180* encode glucoamylase with the induction folds of 48 and 36.8, respectively. Three genes, *SI65_07774*, *SI65_05321*, and *SI65_09820*, all of which encode lytic polysaccharide monooxygenase, were significantly induced in *A. cristatus* after fermentation, with 163-, 35.6-, and 101-fold induction in each. *SI65_09821* encodes putative feruloyl esterase A, which is 956.5-fold higher in EcSQ than

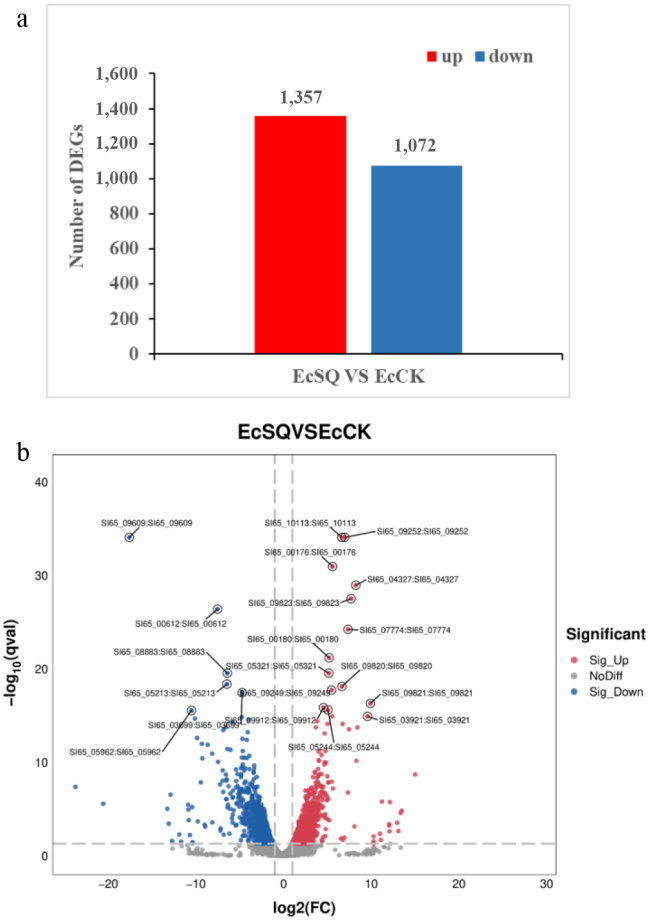

Fig. 4 Identification of the differentially expressed genes (DEGs) between EcCK and EcSQ. (a) Numbers of significantly differentially expressed genes ($|\log_2 FC| \geq 1$; $p \leq 0.05$) in *A. cristatus* after 1 week interaction with *P. notoginseng* roots by RNA-seq. (b) Volcano plot of differentially expressed genes with the labelled top genes of upregulated or downregulated genes.

in EcCK. The significantly higher induction of these genes in the CAZY family indicated their catalyzing function in *A. cristatus-mediated* biotransformation of *P. notoginseng* polysaccharides and saponins, thus contributing to Sanqi metabolites biosynthesis and degradation.

Discussion

Sanqi, scientifically named *P. notoginseng*, has a long medicinal history and is used as one of the most highly regarded Chinese medicines. How to enhance or improve the pharmacological compounds of *P. notoginseng* has garnered considerable interest. Many works indicated that the extracted residues or ginsenoside mixture could be substrates for incubating different microbes to produce rare ginsenosides[24]. For instance, when using Rb1 as the substrate, Rh2 and Rd could be produced by the fungus *Trichoderma longibrachiatum* or other microbes[25,26], while *Aspergillus niger* XD101 efficiently transforms Rb1 to CK[14]. In another case, when *P. notoginseng* roots are fermented with *A. cristatus* Cosmax-GF, the protopanaxadiol and protopanaxatriol contents were higher and showed a multiplicative increase over the fermentation period[27]. Here, the golden flower fungus *A. cristatus* successfully grows on the *P. notoginseng* roots and produces more

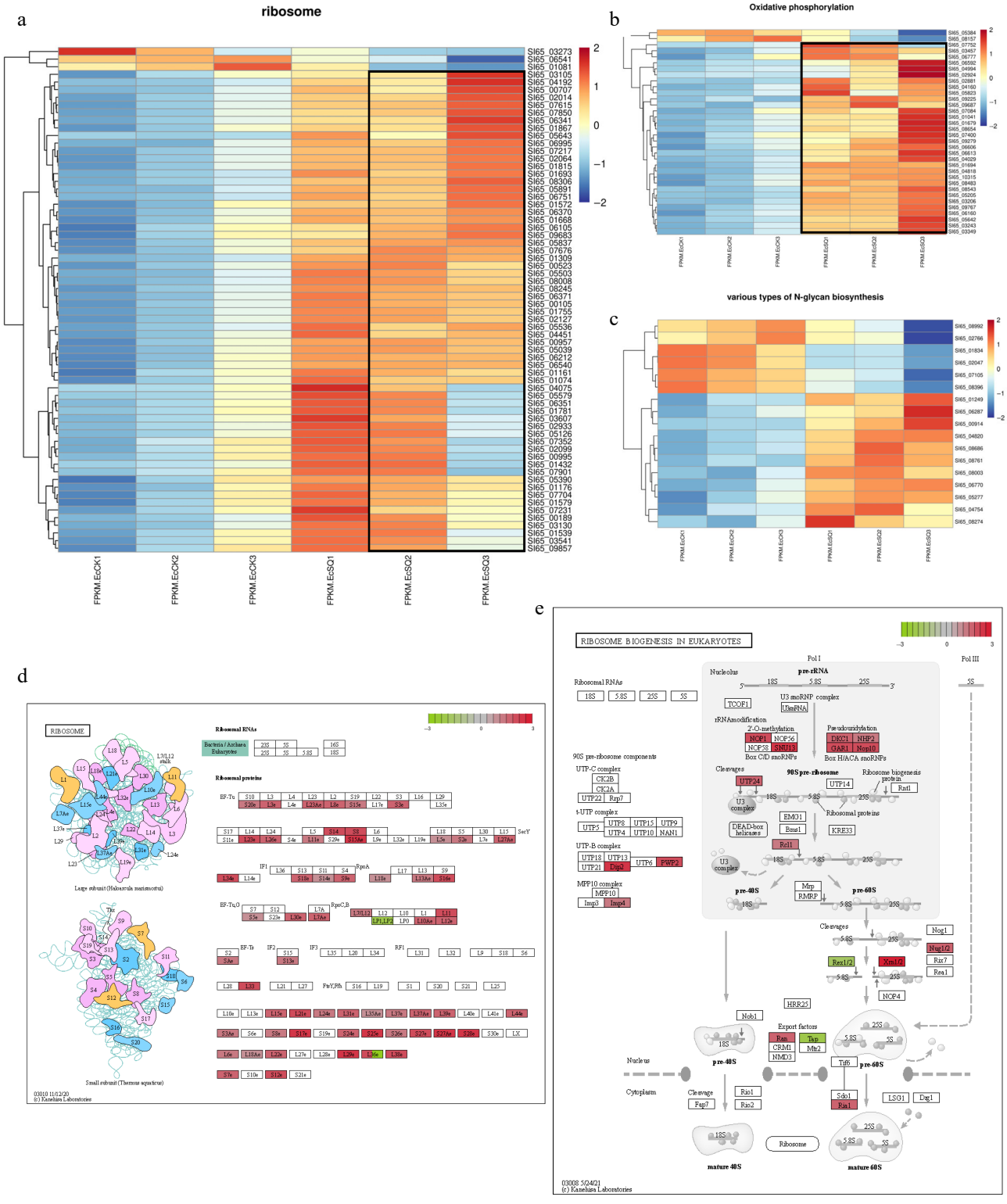

Fig. 5 Differentially expressed genes (DEGs) in *A. cristatus* during fermentation with *P. notoginseng*. (a) Heatmap analysis of DEGs in *A. cristatus* associated with the ribosome. (b) Heatmap analysis of DEGs in *A. cristatus* associated with oxidative phosphorylation. (c) Heatmap analysis of DEGs in *A. cristatus* involved in various type of N-glycan biosynthesis. (d) KEGG view of DEGs in ribosome pathway (map03010). (e) KEGG view of DEGs in ribosome biosynthesis pathway (map03008). KEGG pathway maps in (d) and (e)were reprinted with permission from Kanehisa Laboratories.

ginsenosides, such as R1, Re, Rb1, Rd, and Rf. Concomitantly, the accumulation of Rg3 was decreased in *P. notoginseng* upon *A. cristatus* treatment at the same time. One reason would be the transduction of Rg3 to the downstream compounds by the golden flower fungus; otherwise, the accumulation of Rg3 relies on fermentation time. Indeed, the concentration of Rg3 reached the highest level at the early stage, while it decreased at the late stage. These findings collectively demonstrate that fermentation with a beneficial microorganism is an accessible way to get the production of specific ginsenosides.

https://doi.org/10.48130/mpb-0026-0012

Many works focused on the molecular mechanisms of medicinal plant–microbe interactions, with particular emphasis on either identifying plant defense-related genes or trying to identify microbe-pathogen-related genes. When *P. ginseng* leaves are infected by the fungal microbe *Botrytis cinerea*, the ginseng decreases the expression of many defense-related genes and reduces the accumulation of many antifungal metabolites[15]. *B. cinerea* suppressed *P. ginseng* defense, and this might rely on the Zn_2Cys_6-type transcription factor BcSpd1[28]. BcSpd1 directly targeted the promoter of the gene encoding quercetin dioxygenase (*BcQdo*) and activated its function in catalyzing antifungal flavonoid degradation[28]. These data suggest that the fungi operate transcription reprogramming to degrade host antifungal metabolites.

One may imagine that fungi could operate the translational events or manipulate the translational events to adapt to their hosts. Here, to our excitement, the golden flower fungus *A. cristatus* adapted to live on the medicinal plant, *P. notoginseng*, indeed by profound modulation of its translational machinery (Fig. 5; Supplementary Fig. S11). Many genes involved in translational and post-translational events were co-regulated and dramatically increased in *A. cristatus* during interaction with *P. notoginseng*. Thus, *A. cristatus* actively engages in *P. notoginseng* chemical compound metabolism. To our surprise, many genes encoding basal transcription factors (TFs) were decreased, such as TFIIDs, TFIIFs, and TFIIHs (Supplementary Fig. S11). It has long been appreciated that eukaryotic RNA polymerase II (RNP II) requires numerous additional factors to recognize promoters and initiate RNA synthesis. TFIID is involved in the recognition of the gene core promoter sequences and neighboring chromatin marks. In addition, the pre-initiation complex (PIC) assembly at promoters containing a TATA box includes the TATA box binding protein, TFIIF, and TFIIH. Based on the above, one may hypothesize that the fungal transcription events reorganized during *A. cristatus* interaction with the medicinal plant *P. notoginseng*. With the costs of fungal basal TFs, the translation and post-translational events are activated. It can be concluded that a balanced existence between fungal transcription and translation under normal growth conditions, however, the balance changed when the fungi fed on the medicinal plants. *A. cristatus* translational events were activated at the cost of fungal transcriptional activity. Whether this represents a general adaptive mechanism among beneficial plant-associated fungi warrants further study.

To transform the Sanqi compounds, one of the strategies is to use fungal carbohydrate-active enzymes (CAZymes). CAZymes are responsible for the biosynthesis, modification, and degradation of glycans across all domains of life. The number of CAZyme families has been steadily increasing through both experimental and bioinformatics-driven approaches[29]. CAZymes from various microbes are commonly used to transform bioactive compounds which displayed excellent conversion yields; these include *Aspergillus*, *Beauveria*, *Cunninghamella*, and *Penicillium*[29]. Glycosyltransferases (GTs), a major CAZyme class, mediate regio- and stereospecific glycosylation of diverse scaffolds. For instance, the phenolic glycosyltransferase MhGT1 from *Mucor hiemalis* catalyzes O-glycosylation of 72 structurally diverse druglike compounds and sterols with UDP-glucose as a sugar donor[30]. GTs are typically involved in the catalyzing of glycosylation modification during secondary metabolites at the late stage. Although numerous GTs have been identified from plants and bacteria, a limited number of fungal GTs have been characterized. So far, it has been reported that around 15 fungal *O*-glycosyltransferases were verified in the biosynthesis of terpene *O*-glycosides or used as biocatalysts for the O-glycosylation of phenolic compounds[31]. More recently, a fungal C-glycosyltransferase, AuCGT, involved in the biosynthesis of stromemycin, was identified from *A. ustus*. Subsequently, based on aromatic polyketide GT-guided genome mining and MS analysis for the characteristic fragment ions of glycosides, 28 fungal aromatic polyketide C/O-glycosides, including 20 new compounds, were specifically discovered and isolated from the fungi, including *Talaromyces cellulolyticus*, *Phaeomoniella chlamydospora*, and *Verticillium dahliae*[31]. The novel fungal C/O-glycosyltransferases, especially three novel α-pyrone C-glycosyltransferases, were functionally characterized and verified[31]. In this study, we identified multiple highly-expressed genes in the CAZymes family, which likely play an important function in *A. cristatus* during the fermentation of *P. notoginseng*. Further study will illustrate which special genes of *A. cristatus* in the CAZymes family are involved in the production of pharmacological compounds. Integrated genetic and biochemistry studies would be essential to fully decipher the molecular mechanisms underlying this *A. cristatus*–*P. notoginseng* phytomedicine system.

Conclusions

In summary, this study revealed that the quality of the cultivated medicinal plant *P. notoginseng* was improved by the medicinal fungus *A. cristatus* fermentation. The fungus *A. cristatus* successfully colonized the roots of *P. notoginseng,* forming the typical 'golden flower' mycelial structures. *A. cristatus* fermentation increases ginsenosides accumulation. Untargeted metabolomic analysis of *A. cristatus*-fermented *P. notoginseng* revealed hundreds of significant DAMs. Specifically, several metabolites associated with pharmacological effects are dramatically changed after *A. cristatus* treatment. The study elucidated the molecular characteristics of *A. cristatus* during interaction with *P. notoginseng*. Genes associated with ribosome and oxidative phosphorylation are activated, suggesting *P. notoginseng* root colonization might trigger fungal genes changes, especially in translational and post-translational events. The study establishes that *A. cristatus* had a beneficial effect on the quality of *P. notoginseng*, providing a promising biotechnological strategy for the sustainable utilization and quality enhancement of this important medicinal plant.

Author contributions

The authors confirm contribution to the paper as follows: study conception and design, methodology, funding acquisition: Liu S; data collection, analysis and interpretation of results, draft manuscript preparation: Wei Q, Song J, Wang L, Li M, Ji H, He C; supervision: Liu S, Ju H; manuscript proofreading and editing: Liu S. All authors reviewed the results and approved the final version of the manuscript.

Data availability

The data that support the findings of this study are available in the NCBI with accession number GSE302790 (ECSQ/ECCK). This data can be found here: www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE302790.

Acknowledgments

This work was partly supported by the National Natural Science Foundation of China (No. 32572068, 32171801), the New Functional

Materials Design and Green Synthesis Innovation Team, Jilin Engineering Normal University (20250601084RC), and the Jilin Province Enterprise Science and Innovation Commissioner Program (2023-2-300). We thank Kanehisa Laboratories for providing the copyright permission of KEGG pathway maps.

Conflict of interest

The authors declare that they have no competing interests.

Supplementary information accompanies this paper online at: https://doi.org/mpb-0026-0012.

Dates

Received 21 October 2025; Revised 25 May 2026; Accepted 28 May 2026; Published online 14 July 2026

References

[1] Bai X, Fu R, Duan Z, Liu Y, Zhu C, et al. 2021. Ginsenoside Rh4 alleviates antibiotic-induced intestinal inflammation by regulating the TLR4-MyD88-MAPK pathway and gut microbiota composition. *Food & Function* 12(7):2874−2885

[2] Zhang K, Sun C, Hu Y, Yang J, Wu C. 2021. Network pharmacology reveals pharmacological effect and mechanism of *Panax notoginseng* (Burk.) F. H. Chen on reproductive and genetic toxicity in male mice. *Journal of Ethnopharmacology* 270:113792

[3] Feng W, Lu A, Huang D, Jiang C, Chen B, et al. 2022. Network pharmacology reveals the targets and mechanism for *Panax notoginseng* (Burk.) F. H. Chen against venous thromboembolism. *Pharmacological Research – Modern Chinese Medicine* 2:100035

[4] Dou L, Lu Y, Shen T, Huang X, Man Y, Wang S, et al. 2012. *Panax notoginseng* saponins suppress RAGE/MAPK signaling and NF-kappaB activation in apolipoprotein-E-deficient atherosclerosis-prone mice. *Cellular Physiology and Biochemistry* 29(5−6):875−882

[5] Wang LD, Xu ZM, Liang X, Qiu WR, Liu SJ, et al. 2021. Systematic review and meta-analysis on randomized controlled trials on efficacy and safety of *Panax notoginseng* saponins in treatment of acute ischemic stroke. *Evidence-Based Complementary and Alternative Medicine* 2021:4694076

[6] Shen S, Zhou C, Zeng Y, Zhang H, Hossen MA, et al. 2022. Structures, physicochemical and bioactive properties of polysaccharides extracted from *Panax notoginseng* using ultrasonic/microwave-assisted extraction. *LWT* 154:112446

[7] Kim DH. 2012. Chemical diversity of *Panax ginseng, Panax quinquifolium*, and *Panax notoginseng*. *Journal of Ginseng Research* 36(1):1−15

[8] Hou M, Wang R, Zhao S, Wang Z. 2021. Ginsenosides in *Panax* genus and their biosynthesis. *Acta Pharmaceutica Sinica B* 11(7):1813−1834

[9] Liu H, Lu X, Hu Y, Fan X. 2020. Chemical constituents of *Panax ginseng* and *Panax notoginseng* explain why they differ in therapeutic efficacy. *Pharmacological Research* 161:105263

[10] Yang C, Qu L, Wang R, Wang F, Yang Z, et al. 2024. Multi-layered effects of *Panax notoginseng* on immune system. *Pharmacological Research* 204:107203

[11] Li YF, Liang YZ, Cui XM, Shao LJ, Lou DJ, et al. 2022. Production of minor ginsenosides from *Panax notoginseng* flowers by *Cladosporium xylophilum*. *Molecules* 27(19):6615

[12] He Y, Hu Z, Li A, Zhu Z, Yang N, et al. 2019. Recent advances in biotransformation of saponins. *Molecules* 24(13):2365

[13] Jiang Y, Li W, Fan D. 2021. Biotransformation of ginsenoside Rb1 to ginsenoside CK by strain XD101: a safe bioconversion strategy. *Applied Biochemistry and Biotechnology* 193(7):2110−2127

[14] Liu CY, Zhou RX, Sun CK, Jin YH, Yu HS, et al. 2015. Preparation of minor ginsenosides C-Mc, C-Y, F2, and C-K from American ginseng PPD-ginsenoside using special ginsenosidase type-I from *Aspergillus niger* g. 848. *Journal of Ginseng Research* 39(3):221−229

[15] Chen H, Zhang S, He S, Runa A, Wang M, et al. 2022. The necrotroph *Botrytis cinerea* promotes disease development in *Panax ginseng* by manipulating plant defense signals and antifungal metabolites degradation. *Journal of Ginseng Research* 46(6):790−800

[16] Ge Y, Wang Y, Liu Y, Tan Y, Ren X, et al. 2016. Comparative genomic and transcriptomic analyses of the Fuzhuan brick tea-fermentation fungus *Aspergillus cristatus*. *BMC Genomics* 17:428

[17] Kanehisa M, Goto S. 2000. KEGG: kyoto encyclopedia of genes and genomes. *Nucleic Acids Research* 28:27−30

[18] Wang J, Feng D, Xiang Y, Guo J, Huang N, et al. 2023. Synthesis and inhibitory activity of euparin derivatives as potential dual inhibitors against α-glucosidase and protein tyrosine phosphatase 1B (PTP1B). *Fitoterapia* 169:105596

[19] Lee SB, Lee S, Park JY, Lee SY, Kim HS. 2020. Induction of p53-dependent apoptosis by prostaglandin A2. *Biomolecules* 10(3):492

[20] Mahour R, Klapproth J, Rexer TFT, Schildbach A, Klamt S, et al. 2018. Establishment of a five-enzyme cell-free cascade for the synthesis of uridine diphosphate N-acetylglucosamine. *Journal of Biotechnology* 283:120−129

[21] Liu T, Zhu L, Wang L. 2022. A narrative review of the pharmacology of ginsenoside compound K. *Annals of Translational Medicine* 10(4):234

[22] Bogojevic O, Zhang Y, Wolff CD, Nygaard JV, Wiking L, et al. 2023. A sustainable and regioselective synthesis of Hemi-bis(monoacylglycero)phosphates and bis(diacylglycero)phosphates. *iScience* 26(7):107075

[23] Lee JY, Jeong KW, Kim Y. 2009. Flavonoids can be potent inhibitors of human phenylethanolamine N-Methyltransferase (hPNMT). *Bulletin of the Korean Chemical Society* 30(8):1835−1838

[24] Song X, Wu H, Piao X, Yin Z, Yin C. 2017. Microbial transformation of ginsenosides extracted from *Panax ginseng* adventitious roots in an airlift bioreactor. *Electronic Journal of Biotechnology* 26:20−26

[25] Ge F, Huang Z, Yu H, Wang Y, Liu D. 2016. Transformation of *Panax notoginseng* saponins by steaming and *Trichoderma longibrachiatum*. *Biotechnology & Biotechnological Equipment* 30(1):165−172

[26] Kim H, Kim JH, Lee PY, Bae KH, Cho S, et al. 2013. Ginsenoside Rb1 is transformed into Rd and Rh2 by *Microbacterium trichothecenolyticum*. *Journal of Microbiology and Biotechnology* 23(12):1802−1805

[27] Lee S, Reddy CK, Ryu JJ, Kyung S, Lim Y, et al. 2021. Solid-state fermentation with *Aspergillus cristatus* enhances the protopanaxadiol- and protopanaxatriol-associated skin anti-aging activity of *Panax notoginseng*. *Frontiers in Microbiology* 12:602135

[28] Chen H, He S, Zhang S, Runa A, Li W, et al. 2022. The necrotroph *Botrytis cinerea* BcSpd1 plays a key role in modulating both fungal pathogenic factors and plant disease development. *Frontiers in Plant Science* 13:820767

[29] Bains RK, Nasseri SA, Wardman JF, Withers SG. 2024. Advances in the understanding and exploitation of carbohydrate-active enzymes. *Current Opinion in Chemical Biology* 80:102457

[30] Hu Z, He J, Chen K, Wang Z, Liu J, et al. 2019. Molecular cloning and biochemical characterization of a new flavonoid glycosyltransferase from the aquatic plant lotus. *Biochemical and Biophysical Research Communications* 510(2):315−321

[31] Chen D, Song Z, Han J, Liu J, Liu H, et al. 2024. Targeted discovery of glycosylated natural products by tailoring enzyme-guided genome mining and MS-based metabolome analysis. *Journal of the American Chemical Society* 146(14):9614−9622