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Proteomic Analysis Revealed the Fruiting-Body Protein Profile of *Auricularia polytricha*

**Dinghong Jia[1,2] · Bo Wang[2] · Xiaolin Li[2] · Weihong Peng[2] · Jie Zhou[2] ·
Hao Tan[2] · Jie Tang[2] · Zhongqian Huang[2] · Wei Tan[2] · Bingcheng Gan[2] ·
Zhirong Yang[2] · Jian Zhao[1]**

Abstract *Auricularia polytricha* is one of the most widely cultivated edible mushrooms in China. Many advances have been made to *A. polytricha*, but there is still no proteomic information of this species. Our current understanding was based upon the translated information of its transcriptome or other relative species. This study presented the proteomic information of fruiting-body proteins by shotgun liquid chromatography and tandem mass spectrometry (LC–MS/MS), which identified 15,508 peptides corresponding to 1850 high-confidence proteins. Of these, 1383 were annotated across the GO subcategories with 829 (44.81%) involved in biological process, 908 (49.08%) in molecular function, and 406 (21.95%) in cellular components. Among these high-confidence proteins, 132 proteins were annotated as carbohydrate-active enzymes, of which 51 were secreted enzymes. Moreover, a number of commercially important enzymes were detected, functioning as auxiliary activity (AA) family 5 glyoxal oxidase, AA5 galactose oxidase, glycoside hydrolase (GH) family 20 hexosaminidase, and GH47 alpha-mannosidase. To the best of our knowledge, this is the first study to characterize *A. polytricha* proteome, and also fills the gap of our knowledge on the under-developed mushroom species.

✉ Jian Zhao
zj804@163.com

1   Key Laboratory of Biological Resource and Ecological Environment of the Ministry of Education, College of Life Sciences, Sichuan University, Chengdu 610064, China

2   Soil and Fertilizer Institute, Sichuan Academy of Agricultural Sciences, Chengdu 610066, China

family 20 hexosaminidase, and GH47 alpha-mannosidase. To the best of our knowledge, this is the first study to characterize *A. polytricha* proteome, and also fills the gap of our knowledge on the under-developed mushroom species.

Introduction

A. polytricha (Mont.) Sacc is known as hairy wood ear in China [30], and the fourth most important cultivated black fungus used by humans worldwide [6]. Many published studies have examined the benefits of *A. polytricha* [6]. Its polysaccharide was shown to be potential agents against dementia, hypercholesterolemia, mutagenicity, tumor, oxidation, and DNA damage [3, 7, 38, 46, 51, 53]. As a dietary supplement, it contributed a beneficial effect on postprandial levels of blood sugar [41]. As an alternative biosorbent, its fruiting bodies or powdered mycelia could be employed to aid detoxification of effluents contaminated by Cr, Cd, Cu, Pb, Zn, or emulsified oil [13, 16, 29, 42, 52]. It has drawn great attention due to its various biological activities [6].

An enormous effort has been undertaken to develop methods for cultivation [1], molecular markers [48], pathogen identification [30], and transcriptomic exploration [54]. Despite these advances, no studies were made to characterize the proteome of *A. polytricha*. Our current understanding was dependent upon translated information from its transcriptome or other closely relative species. There is an urgent need to broaden our knowledge of its proteome.

To date, proteomic technique has become an effective tool to characterize protein profiles. Among current

proteomic methods, the shotgun proteomic approach possesses the virtues of high efficiency, and time and labor savings. It is suitable for use as a high throughput technology for the identification of proteins samples in comparison with the two-dimensional electrophoresis (2-DE) combined with mass spectrometry (MS) [35]. The proteomic analyses have been performed on mushroom species such as *Ganoderma lucidum* [45], *Armillaria mellea* [8], *Grifola frondosa* [14], *Sparassis crispa* [15], *Antrodia cinnamomea* [21], *Termitomyces heimii* [31], *Lignosus rhinocerotis* [43], and *Agaricus bisporus* [26]. These may contribute to understanding the molecular mechanism of mycelial growth, fruiting-body formation, spore germination, and so on.

In this study, proteomic analysis of fruiting-body proteins (FBPs) in *A. polytricha* was performed using the shotgun approach based on liquid chromatography and tandem mass spectrometry (LC–MS/MS). As an initial foray into *A. polytricha* proteome, it should facilitate future analysis of the localization, structure, and function of interested proteins in some biological context.

Materials and Methods

A. Polytricha Strains and Culture Conditions

Strain huanger 10 of *A. polytricha* was preserved in Soil and Fertilizer Institute, Sichuan Academy of Agricultural Sciences, China. The mycelia of strain huanger 10 were grown on potato dextrose agar (PDA) medium for one week at room temperature [40], and cultivated on packs of growth medium consisting of 33% sawdust, 30% corncob, 20% rice bran, 10% cottonseed hulls, 2% corn flour, 4% lime, and 1% plaster powder in the dark at 25 °C. The fruiting bodies were cultivated as reported by Zhou [54], and sampled at stage of initial maturity from Shifang City in China. GN fluid medium contained glucose 10 g/L, $(NH_4)_2SO_4$ 6 g/L, NaCl 1 g/L, K_2HPO_4 1 g/L, $MgSO_4$ 0.5 g/L [24]. Cellulose medium was GN medium supplemented with 10 g/L microcrystalline cellulose, and used to investigate whether tested proteins could be induced by cellulose.

Protein Extraction

The fruiting-body specimen of strain huanger 10 (1.0 g) was finely ground. The powders were precipitated in a 10% (w/v) trichloroacetic acid/acetone solution containing 65 mM dithiothreitol (DTT) at −20 °C for 1 h. The solution was centrifuged at $10,000 \times g$ for 45 min. The

supernatant was discarded, and the precipitate was vacuum-dried and solubilized in 1/10 volumes of SDT buffer (4% SDS, 100 mM DTT, and 150 mM Tris-HCl, pH 8.0). The suspensions were incubated in boiling water for 5 min followed by ultrasonication (80 w, 10 s ultrasonic at a time, every 15 s, and 10 times) [12] and incubation at 100 °C for 5 min. The crude extract was clarified by centrifugation at $13,000 \times g$ at 25 °C for 10 min [39]. The protein concentration was determined by the BCA Protein Assay Reagent (Promega, USA). The supernatants were stored at −80 °C before use.

In-Solution Digestion

Protein digestion was performed following the FASP procedure described by Wisniewski and colleagues [39]. 200 μg of proteins was mixed with 200 μL of UA buffer (8 M Urea, 150 mM Tris-HCl pH 8.0), loaded into the filter devices, and centrifuged at $14,000 \times g$ for 15 min. The concentrates were diluted in the device with 100 μL of UA buffer and centrifuged again. Then this substance was mixed with 100 μL of 50 mM IAA in UA buffer (600 rpm, 1 min) and incubated in darkness at room temperature for 30 min followed by centrifugation at $14,000 \times g$ for 10 min, after which the concentrate was diluted with 100 μL of UA buffer and concentrated, and this step repeated twice. Next, this concentrate was diluted with 100 μL of 25 mM NH_4HCO_3 and concentrated again. This step was repeated twice. The resulting concentrate was diluted to 40 μL of 25 mM NH_4HCO_3 containing 2 μg of trypsin (600 rpm, 1 min), and incubated at 37 °C for 16–18 h. Lastly, peptides were collected by centrifugation of the filter units at $14,000 \times g$ for 10 min, and their quantities were determined by OD_{280}.

Off-Line High pH Reversed-Phase Fractionation

By high pH reversed-phase chromatography, the digested peptides were fractionated with AKTA Purifier system (GE Healthcare). When reconstituted in buffer A ($NH_3 \cdot H_2O$, pH 9.0), the peptide was loaded onto an XBridge Peptide BEH 4.6 mm × 150 mm C18 Column (300 Å, 5 μm, Waters) and eluted at a flow rate of 0.8 mL/min with a gradient of 0–4% buffer B ($NH_3 \cdot H_2O$ in ACN, pH 9.0) during 0–15 min, 4–40% buffer B during 15–42 min, 40–90% buffer B during 42–47 min, 90% buffer B during 47–52 min, and buffer B was reset to 90% after 52 min. When monitored by absorbance at 214 nm [25], the elution was collected every 1 min. The collected fractions were dried by vacuum centrifugation.

Liquid Chromatography and Tandem Mass Spectrometry

Experiments were performed on a Q Exactive mass spectrometer coupled to Easy nLC (Thermo Fisher Scientific). Samples were loaded onto a the C18 reversed-phase column (75 μm i.d., 150 mm length) packed in-house with RP-C18 5 μm resin in mobile phase A (0.1% Formic acid in HPLC-grade water) and separated with a linear gradient of 4–100% mobile phase B (0.1% Formic acid in 84% acetonitrile) at a flow rate of 250 nL/min over 60 min [9, 22]. With a data-dependent top 10 method, MS data were acquired dynamically choosing the most abundant precursor ions from the survey scan (300–1800 m/z) for HCD fragmentation. The target value is determined based on predictive Automatic Gain Control (pAGC), with the following dynamic exclusion duration was 20 s. Survey scans were acquired at a resolution of 70,000 at m/z 200 and resolution for HCD spectra was set to 17,500 at m/z 200. Normalized collision energy was 27 eV and the underfill ratio was defined as 0.1%. The instrument was run with peptide recognition mode enabled.

Bioinformatic Analysis

The acquired MS/MS spectra were searched using MASCOT engine (Matrix Science, London, UK; version 2.2) [10] against combined protein database including the sequences of NCBI Auriculariales proteins (14 October 2015, 47,231 sequences) and putative proteins translated from *A. polytricha* transcriptome (33,573 sequences) (Supplemental dataset). For protein identification, the options were as follows: Peptide mass tolerance = 20 ppm, MS/MS tolerance = 0.1 Da, Enzyme = Trypsin, Missed cleavage = 2 [50], Fixed modification: Carbamidomethyl (C), Variable modification: Oxidation (M), False discovery rate (FDR) <0.01 at peptide and protein level.

With unique peptides ≥2, high-confidence proteins were chosen for further analysis [35]. Using the NCBI BLAST+ client software (ncbi-blast-2.2.28+-win32.exe), these sequences were locally searched against SwissProt database to find homologue sequences from which the functional annotation can be transferred to the studied sequences.

Using *E*-value less than $1e^{-3}$ for each query sequence, the top 10 blast hits were retrieved and loaded into Blast2GO1 (Version 2.7.2) for GO2 mapping and annotation. Annotation configurations with *E*-value filter of 1e-6, default gradual EC weights, Gene ontology (GO) weight of 5, and annotation cutoff of 55 were chosen. With more

permissive parameters, un-annotated sequences were then re-annotated. The sequences without BLAST hits and un-annotated sequences were then selected to go through an InterProScan3 against EBI databases to retrieve functional annotations of protein motifs and merge the InterProScan GO terms to the annotation set [10]. Subsequently, the studied proteins were blasted against the Kyoto Encyclopedia of Genes and Genomes (KEGG) GENES (fungi, database updated: October 28, 2015) to retrieve their KOs and mapped to pathways in KEGG4.

The identified proteins were annotated using dbCAN for carbohydrate-active enzyme (CAZyme) families (based on CAZyDB 07/15/2016) [2, 44]. And secreted proteins were predicted by the SignalP 4.1 server [32, 33].

Quantitative Real-Time PCR (qRT-PCR) Analysis

In order to validate the proteins identified by this study, qRT-PCR assay was performed. Strain huanger 10 was cultured in cellulose medium and GN fluid medium (as control) [24]. GH37 trehalase (protein ID: comp21191_c0_seq1, intracellular proteins, Treh), GH3 beta-glucosidase (protein ID: comp15213_c0_seq2, intracellular proteins, beta-Glu(N)), and GH3 beta-glucosidase (protein ID: comp21080_c0_seq1, extracellular proteins, beta-Glu(Y)) were chosen as tested proteins, and glyceraldehyde phosphate dehydrogenase (*Gapdh*) gene as the endogenous control. The reverse transcriptions were performed using an HiScript 1st Strand cDNA Synthesis Kit from Vazyme Biotech Co., Ltd. Their reverse transcripts from each biological replicate were used as templates. Gene-specific primers based on selected proteins are listed in Table 1. PCR protocol: 95 °C 40 s, 1 cycle; 95 °C 20 s, 60 °C 20 s, plate read, 45 cycles. The linear amount of the target gene expression to the calibrator was calculated by $2^{-\Delta\Delta C_T}$ [49].

Results

Identification of FBPs of *A. Polytricha*

SDS-PAGE with silver staining indicated that proteins were successfully extracted from fruiting bodies (Fig. 1). BCA protein assay showed that its concentration was 2.3 μg/μL. The fruiting-body sample was digested with the FASP method and identified using the shotgun LC–MS/MS proteomic technique. According to the criteria of high-confidence, candidate proteins were chosen for the following GO and KEGG pathway analysis. The results demonstrated that 15,508 peptides were identified from fruiting-body samples corresponding to 1850 high-

Table 1 Primer sets for PCR amplification of mRNA encoding GH3 beta-glucosidase(N), GH37 trehalase(N), GH3 beta-glucosidase(Y), and glyceraldehyde phosphate dehydrogenase

Genes	Primer sequences	Product length (bp)
GH3 beta-glucosidase(N)	Forward: 5′-CTCATCGAACGCACGCTCACT-3′	137
	Reverse: 5′-TGCTCCTCGCCAACGCTCTT-3′	
GH37 trehalase(N)	Forward: 5′-AGGAAGACACGGATGGCGACTT-3′	135
	Reverse: 5′-GCAGCAGGTTGGAGAGCATGT-3′	
GH3 beta-glucosidase(Y)	Forward:5′-GCGGTCAACAACGGCTCCAT-3′	144
	Reverse:5′-CGGAAGTCGTCGGTGGTGATGA-3′	
Glyceraldehyde phosphate dehydrogenase	Forward:5′-GCCGTATCGGTCGGATTGTGAC-4′	168
	Reverse:5′-TGAGCTTGCCGTCCTTGGTCT-4′	
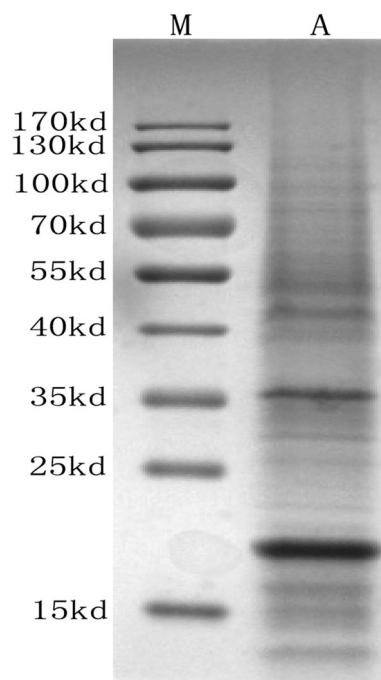
Fig. 1 Separation of fruiting-body proteins of *Auricularia polytricha* by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. *Lane M*, protein marker (15–170 kDa); *Lane A*, the extracted fruiting-body proteins of *A. polytricha*

confidence proteins (Table S1), of which 221 proteins (27.8%) are putative or uncharacterized proteins. 1383 proteins were annotated across the GO subcategories with 829 (44.81%) involved in biological process, 908 (49.08%) in molecular function, and 406 (21.95%) in cellular components (Table 2). Among these GO classifications, the rich cellular component was cell (GO: 0005623) accounting for 24.5%. The rich molecular function was catalytic activity (GO: 0003824) accounting for 45.7%. The rich biological process was metabolic process (GO: 0008152) accounting for 50.6% (Fig. 2, Table S2). So many proteins were assigned to cell (GO: 0005623), catalytic activity (GO: 0003824), and metabolic process (GO: 0008152), reflecting that *A. Polytricha* FBPs continued exuberant metabolic activities resulting in cell growth.

KEGG Pathway Analysis

The sequences of 1850 high-confidence proteins were searched against the KEGG reference pathway database. The result showed that these proteins were assigned to 309 pathways according to KEGG pathway taxonomy (Table S3). In general, the pathways were classified into six categories: cellular processes (255 proteins, 22 subcategories), environmental information processing (273, 30), genetic information processing (370, 22), human diseases (620, 65), metabolism (816, 99), and organismal systems (466, 71), in which protein numbers of metabolism and human diseases were more than others (Fig. 3). Among these pathways, the top 3 proteins were involved in ribosome (70), RNA transport (51), and protein processing in endoplasmic reticulum (50). All the three pathways fell into the category of genetic information processing (Table S4), suggesting the significance of genetic information processing during initial maturity of *A. polytricha*.

Analysis of CAZymes

The CAZyme database annotation of *A. polytricha* identified a total of 132 CAZymes, which were grouped into carbohydrate-binding modules (CBMs, 10 proteins), auxiliary activities (AAs, 16), glycoside hydrolases (GHs, 77), carbohydrate esterases (CEs, 9), polysaccharide lyases (PLs, 3), and glycosyltransferases (GTs, 24) (Table S5). The top proteins were assigned to GH families, in which nine were classified to GH3 family, a preponderance of proteins in GH subfamilies. Among them, eight GH3 proteins were annotated as beta-glucosidase, and one as beta-xylosidase.

Table 2 Proteins identified from the fruiting bodies of *Auricularia polytricha*

	Number
Identified peptides	15,508
Proteins[a]	1850
Proteins searching against putative protein sequence database of *Auricularia polytricha*[a]	1054
Proteins searching against NCBI Auriculariales sequence database[a]	796
Hypothetical proteins[a]	221 (27.76%)
Annotated proteins in GO categories of biological process[a]	829 (44.81%)
Annotated proteins in GO categories of molecular function[a]	908 (49.08%)
Annotated proteins in GO categories of cellular component[a]	406 (21.95%)

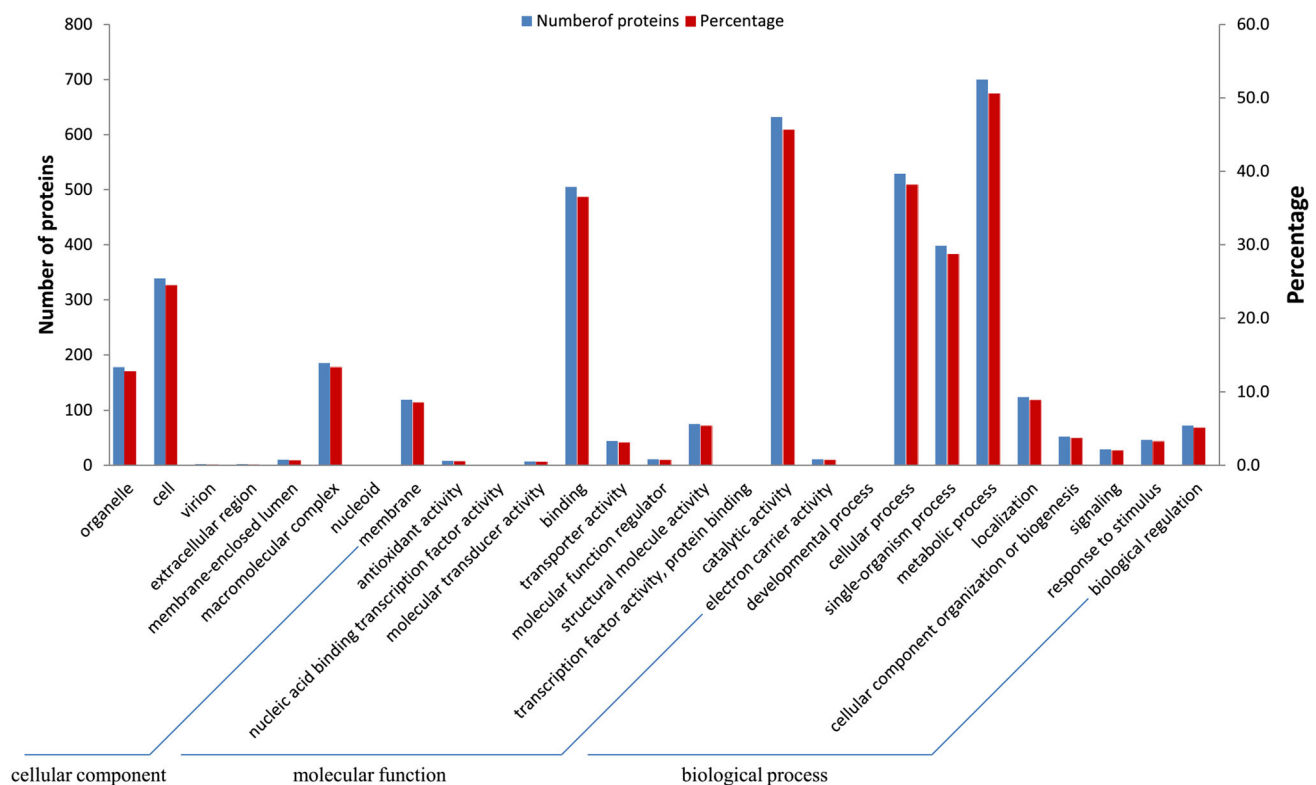[a] High-confidence proteins with two or more unique peptides

Fig. 2 Gene ontology (GO) categories of the identified fruiting-body proteins of *Auricularia polytricha*. *Y*-axis (*left*) represents the protein number, and *Y*-axis (*right*) represents percentages of proteins identified. The proteins were categorized based on GO annotation, and the number of each category is displayed based on biological process, cellular components, and molecular functions (Color figure online)

Lignin Oxidases and Hemicellulases

Degradation of cellulose is restricted by lignin and hemicelluloses, which are heterogeneous polysaccharides formed by various types of sugar units, mainly including mannans and xylans [18]. This study identified lignin oxidases such as AA2 peroxidase, AA5 galactose oxidase, AA5 glyoxal oxidase, and also detected hemicellulases including CE1 Acetyl xylan esterase, GH27 alpha-galactosidase, GH2 alpha-L-arabinofuranosidase, GH29 alpha-L-fucosidase, GH47 alpha-mannosidase, GH31 alpha-xylosidase, GH35 beta-galactosidase, GH3 beta-xylosidase, GH52 beta-xylosidase, CE1 carboxylesterase, and GH5 endo-1,4-beta-mannanase.
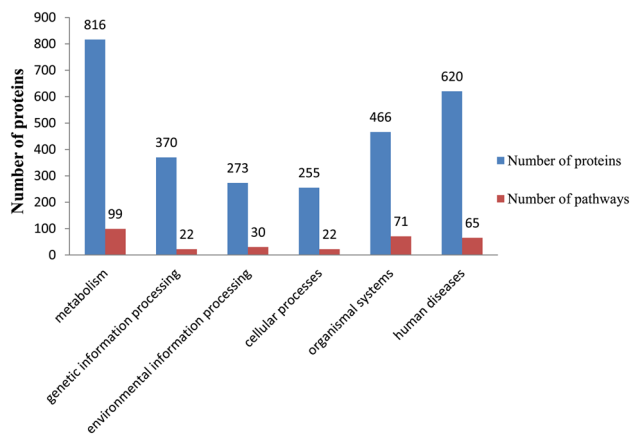
Fig. 3 Number of annotated proteins and their pathways, in which the top 3 proteins were involved in metabolism (816), human diseases (620), and organismal systems (466) (Color figure online)

Cellulases

Nineteen cellulose-degrading proteins were detected in *A. polytricha* proteome. These proteins included GH3 beta-glucosidase, GH94 cellobiose phosphorylase, GH16 endo-1,3(4)-beta-glucanase, GH5 endo-beta-1,4-glucanase, GH17 glucan 1,3-beta-glucosidase, and GH74 oligoxyloglucan reducing end-specific cellobiohydrolase.

Saccharide-Degrading Proteins

By searching against the CAZyme database, saccharide-degrading proteins were annotated. These enzymes included GH33 sialidase, GH37 trehalase, GH38 alpha-mannosidase, GH63 alpha-glucosidase, GH76 alpha-1,6-mannanase, GH88 D-4,5-unsaturated beta-glucuronyl hydrolase, GH92 alpha-1,2-mannosidase, GH13/CBM48 alpha-amylase, GH109 alpha-N-acetylgalactosaminidase, GH15/CBM20 glucoamylase, GH31 alpha-1,4-glucosidase, GH72/CBM43 beta-1,3-glucanosyltransglycosylase, GH81 endo-beta-1,3-glucanase, PL14 alginate lyase, and PL8 chondroitin ABC lyase.

CAZymes Secreted by *A. Polytricha*

Among *A. polytricha* CAZymes, 51 secreted proteins were predicted by the SignalP 4.1 server [33, 34] accounting for 38.6% (51/132) (Table S5). Enzymes secreted by *A. polytricha* can be classified into AA, GH, CE, PL, CBM, and GT families. These secretomic proteins included two lignin oxidases, 12 hemicellulases, six cellulases, 14 saccharide-degrading proteins, three pectin-degrading proteins (GH43 endo-1,5-alpha-L-arabinanase, PL3 pectate lyase, GH79 beta-glucuronidase), and four chitin-degrading proteins (CE4 chitin deacetylase, GH18/CBM5 chitinase, GH20

beta-hexosaminidase). These secreted enzymes would contribute to the degradation of lignocellulosic substrate.

Proteins Involved in TCA Cycle

The citrate cycle (TCA cycle) is an important aerobic pathway involved in the conversion of carbohydrates, fats, and proteins to form energy [4]. The TCA cycle plays a central role in all living cells because energy production is essential [47]. In this study, 21 proteins are assigned to the citrate cycle (Table S4), including citrate synthase, ATP citrate (pro-S)-lyase, isocitrate dehydrogenase, 2-oxoglutarate dehydrogenase, pyruvate carboxylase, and so on (Table S3). So many proteins were involved in TCA cycle, suggesting the significance of TCA cycle in stage of initial maturity.

In summary, 1850 high-confidence proteins identified by this study confirmed the existence of many putative proteins translated from *A. Polytricha* transcriptome. This greatly enriched *A. Polytricha* proteomic database.

Validation of Selected Proteins by qRT-PCR

The qRT-PCR results showed that three tested genes were all detected both in GN fluid medium and cellulose medium, and were upregulated 1.10- to 1.59-fold when cultivated in cellulose medium compared with GN fluid medium. GH3 beta-glucosidase (protein ID: comp21080_c0_seq1) was annotated as secreted enzyme. Its expression level increased up to 1.59-fold over control. GH37 trehalase (protein ID: comp21191_c0_seq1) and GH3 beta-glucosidase (protein ID: comp15213_c0_seq2) were predicted as intracellular proteins, and had 1.10- and 1.30-fold increase (Fig. 4, Table S6). It shows that secreted GH3 beta-glucosidase is more prone to be induced by cellulose and high expression.

Discussion

15508 peptides were identified in the tested sample corresponding to 1850 high-confidence proteins. With FDR <0.01 at peptide and two or more unique peptides, the high-confidence proteins were chosen for further analysis, which made the proteins identified more reliable [36]. And qRT-PCR assay provided further evidence to validate these proteomics data.

A. polytricha is saprophytic and cultivated on an extensive range of wood substrates [5] which includes cellulose, hemicellulose, and lignin (lignocellulosic complex) [19]. It is indicated that numerous lignocellulases are essential to *A. polytricha*. This study annotated 105 lignocellulases which are more than 61 ones identified in
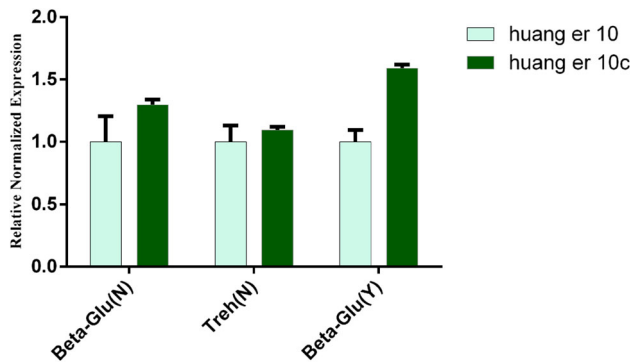
Fig. 4 qPCR validation of selected proteins from fruiting bodies of *Auricularia polytricha* at the mRNA level. Samples were normalized with the reference gene *Gapdh*. Vertical lines represent ± S.D. Specimens of strain huanger 10 and huanger 10c harvested from GN fluid medium and cellulose medium, respectively. Treh(N) represents GH37 trehalase (protein ID: comp21191_c0_seq1, intracellular proteins). Beta-Glu(N) represents GH3 beta-glucosidase (protein ID: comp15213_c0_seq2, intracellular proteins). Beta-Glu(Y) represents GH3 beta-glucosidase (protein ID: comp21080_c0_seq1, extracellular proteins) (Color figure online)

Ganoderma lucidum [45]. Proteins assigned to GHs (77) were the most numerous in *A. polytricha*. This result is in accordance with data described by Ospina-Giraldo et al., who found out that most homologs belonged to the GH families by scanning the genomes of *Phytophthora infestans*, *P. sojae*, and *P. ramorum* [28], and also in accordance with the data from *Ganoderma lucidum* proteome [45], reflecting GHs are a widespread group of enzymes. Additionally, so many identified lignocellulases may also enrich our knowledge of such proteins.

In comparison with *A. polytricha* transcriptome [54], GO terms of the rich cellular component, molecular function, and biological process are in agreement, except that the rich cellular component is also cell part in *A. polytricha* transcriptome. The general agreement on GO terms validates the two studies. In descending order of the top 3 proteins involved in metabolism, the KEGG pathways were carbohydrate metabolism (263 proteins), amino acid metabolism (157 proteins), and energy metabolism (90 proteins) in *A. polytricha* proteome (Table S7), while amino acid metabolism (265 ESTs), carbohydrate metabolism (248 ESTs), and energy metabolism (193 ESTs) in *A. polytricha* transcriptome. The different order of pathways may be resulted from the discrepancy between transcriptome and proteome, or resulted from different developmental stages, in which mature fruiting bodies were used as experimental materials in transcriptomic analysis [54], and initial maturity fruiting bodies were used in this study.

51 secreted CAZymes were predicted by the SignalP 4.1 server, such as GH3 beta-glucosidase, AA11 lytic polysaccharide monooxygenase, GH18 chitinase, GH3 beta-xylosidase, AA3 cellobiose dehydrogenase, GH37 trehalase, PL3 pectate lyase, GH81 endo-beta-1,3-glucanase, CE4 chitin deacetylase. These secreted proteins fall into AA, GH, CE, PL, CBM, and GT families. Numerous secreted CAZymes were detected, and may be able to provide an explanation of phenomenon that *A. polytricha* could be cultivated on an extensive range of wood substrates.

Enzymes of higher fungi have a potential for use in the industry [11]. Alginate lyases have been expected to become potential enzymes in the bioenergy generation from alginate [27]. Alpha-Glucosidases are involved in diverse physiological processes including carbohydrate assimilation [17]. AA5 glyoxal oxidase and AA5 galactose oxidase belong to the copper radical oxidases family [20], and are involved in lignin degradation. GH2 alpha-L-arabinofuranosidase and xylan degrading enzymes have been used in the food, feed, and pulp industries as well as conversion of hemicellulosic biomass to biofuels and chemicals [34]. GH2 alpha-L-arabinofuranosidase is more stable at higher temperatures, and has great potential to be used in industrial processes [34]. GH20 hexosaminidases are found in crustaceans, insects, and fungi, participating in the degradation of chitin [37]. GH88 D-4,5-unsaturated beta-glycosidases have been implicated in the degradation of unsaturated oligosaccharides. GH47 alpha-mannosidases are thought to participate in post-translational modification of secreted proteins [23]. Our assay detected the above functional proteins. This information may help shed some light on those who are prepared to apply biologically active proteins for industry.

Direct proteomic information of *A. polytricha* was unavailable previously, thus we got this information depending on the translated sequences of transcriptome of *A. polytricha* or other related species. This study firstly presented the proteomic information for researchers based on LC–MS/MS, and would give helpful information for the further development of such species.

Compliance with Ethical Standards

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