

Research Article

Proteomic Analysis Reveals Growth Inhibition of *Coriolus versicolor* by Methanol Extracts of *Cinnamomum camphora* Xylem

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The extracts of decay-resistant tree species are important research objects for the future development of wood preservatives. To understand the antifungal mechanisms of *Coriolus versicolor* inhibition with methanol extracts of *C. camphora* xylem, the protein profiles of *C. versicolor* were analyzed using 2-DE followed by MALDI-TOF/MS and bioinformatic analyses. The results showed that 41 protein spots were obviously changed among the 366-385 protein spots of *C. versicolor* treated with methanol extracts of *C. camphora* xylem. Twenty-one protein spots were upregulated, and 20 protein spots were downregulated. Cellular localization was performed to identify these differential proteins, and biological process and functional analysis found that 9 of these proteins were in the cytoplasm, 6 were intracellular, and 5 were in the mitochondrion. A total of 18.8% were mapped to small-molecule metabolic processes, 12.5% to cellular amino acid metabolic processes, and 10.9% to cellular nitrogen compound metabolic processes. Twenty-five percent of the differential proteins were associated with ion bonding, 15% with oxidoreductase activity, and 15% with ATPase activity and transmembrane transport activity. Downregulated expression of aspartate aminotransferase, ATP synthase alpha chain, DEAD/DEAH-box helicase, and phosphoglycerate kinase showed that the methanol extracts of *C. camphora* xylem disrupted functional aspects such as nitrogen and carbon metabolism, energy metabolism, hormone signal response, and glucose metabolism, eventually leading to *C. versicolor* inhibition.

1. Introduction

Wood is an economical and environmentally friendly material and which is used for many end uses, such as furniture, wood-based panels, and wooden structures. However, some wood is vulnerable to damage or destruction by external factors, reducing its commercial value. In particular, biological decay, including wood rot fungus and sapstain fungus, is the most serious and harmful threat to wood [1–3]. Therefore, there is an urgent demand to develop the technology of wood preservation [4]. Wood preservation can extend the usable life of wood and impart relatively good outdoor weather durability to the wood. Many types of wood have

natural decay resistance properties. In addition to the different biological structures of wood, the academic community has agreed that there are differences in the chemical composition and contents of wood. These differences in chemical compositions are one of the important factors leading to variation in natural decay resistance performance [5]. These studies usually focused on antibacterial, decay resistance, and antioxidant compounds and their application [6, 7]. Recently, an increasing number of scholars have focused on plant extractives that can be used as wood preservatives [8]. The application of botanical extracts in wood preservative impregnation is one new technique in the wood science field.

The current study suggested that tree species with different decay resistance properties have different antifungal chemical compositions that can be obtained by different extraction methods and solvents. Generally, the extracts of decay-resistant wood could inhibit wood rot fungus, and nonresistant wood impregnated with decay-resistant heartwood extracts could become decay resistant [9]. Many scholars have investigated some of the local decay-resistant hardwood species and found that the related decay-resistant wood extractives often contain many phenols, terpenoids, alkaloids, and other chemical components [10]. In addition to those from wood, extracts from other parts of some trees also have certain antibacterial activity. Some studies have shown that the ethanolic extracts of *Lantana camara* roots, stems, and leaves could protect against white and brown rot fungi [11, 12]. Through analysis, it was found that the extracts contained a large amount of phenols, alkaloids, terpenoid compounds, and other substances with antifungal activity. To verify the antifungal performance of plant extracts, many scholars have studied the effects of a large number of plant extracts on wood rot fungus [13]. These researchers found that heartwood extracts of decay-resistant tree species had strong inhibitory activities against wood rot fungus. By contrasting the relationship between the chemical structure of the different parts of tree extractives and antifungal activity, heartwood usually has the best decay resistance performance. We have seen that research on natural wood preservatives from plant extracts has become a hot spot in recent years. *Cinnamomum camphora* (*C. camphora*), a species exhibiting decay-resistant wood, has received considerable attention from scholars. The antifungal and antibacterial extractives from this species have been applied in the field, but the mechanism of their decay resistance remains unknown [14–16].

Proteins are made up of special amino acids that are an essential component of organisms and participate in every process within cells [17]. As a manifestation and executor of gene function, proteins have diverse biological functions, such as catalysis, signal transduction, immune protection, metabolic regulation, growth, and differentiation, which are closely related to the biological functions of life forms and physiological activities [18, 19]. Proteomics is the study of an organism's entire protein content and profile, which are then mapped to biological pathways [20]. Proteins play a key role in fungal and bacterial functions and could be helpful to reveal the genes related to the growth of wood rot fungus, especially when studying dynamic changes in proteins. It is necessary to study the proteins of wood rot fungus at the overall, dynamic, and network levels, as this will help us to obtain a comprehensive and in-depth understanding of the complicated biological activity of wood rot fungus.

In summary, researchers have explored the antifungal and decay resistance performance of many types of botanical extracts and reported a large number of applications in many areas, such as antifungal (white-rot fungi and brown-rot fungi) and insecticidal activities [21, 22]. However, the study of botanical extracts has focused on the reporting of phenomena, and there are few antifungal mechanism studies, which leads to the lack of a theoretical basis in

the development and utilization of botanical extracts. *C. camphora* is a strong decay-resistant tree species, and using its extractives as wood preservative provides a new research direction not only for the promotion of wood protection science and technology but also for the realization of low-toxicity wood protection and conservation of lumber resources [23–25]. Implementing clean production of wood preservatives has important significance [26]. Two-dimensional gel electrophoresis (2-DE) and matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF/MS) were used to analyze the differentially expressed proteins in *C. versicolor*, which were inhibited by the methanol extracts of *C. camphora* xylem. The changes in *C. versicolor* protein expression levels were revealed, which can provide a theoretical basis to explore the antifungal mechanism of methanol extracts of *C. camphora* xylem against *C. versicolor*.

2. Materials and Methods

2.1. Material and Reagents. *C. versicolor* was obtained from the Plant Protection College, Fujian Agriculture and Forestry University (Fuzhou, China). Methanol was purchased from Shanghai Chemical Reagent Factory (Shanghai, China).

pH standards (pH 3–10, $L = 24$ cm), TCA, β -mercaptoethanol (BME), ampholyte pharmalyte (pH 3–10), acrylamide, sodium dodecylsulfate (SDS), ammonium persulfate, methylene diacrylamide, urea, thiourea, DTT, CHAPS, and iodoacetamide were obtained.

A pH gradient isoelectric focusing (IPG) Ettan™-IPGphor™ III IEF system vertical gel slab electrophoresis apparatus was used. An Ettan™ Dalt II vertical system, an ImageScanner III scanning densitometer (GE Healthcare) image analysis system, a 5800 MALDI-TOF/TOF-MS instrument, a THY-111B constant shaking incubator, and a preparative ultracentrifuge (BECKMANCOULTER OPTIMAL) were used in this study.

2.2. Extract Preparation. The experimental tree (*C. camphora*) was 45 years old and grew in the Shangjie area in Fuzhou. The xylem of *C. camphora* was pulverized and had a mesh size within the range of 40 to 60. The *C. camphora* powder (100 g) was subjected to heat reflux extraction. Methanol was used as the solvent. The mixture was heated to the boiling point temperature of 64°C. The raw materials were extracted twice, first heated for 5 h with a ratio of *C. camphora* powder to solvent of 1:10 ($\text{g}\cdot\text{mL}^{-1}$), then heated for 3 h with a ratio of *C. camphora* powder to solvent of 1:10 ($\text{g}\cdot\text{mL}^{-1}$). Then, the extractives were combined and filtered to obtain the extract liquid. The solvent was removed by reduced pressure distillation, and the *C. camphora* extracts were obtained. Finally, extracts were prepared at a concentration of $80 \text{ mg}\cdot\text{mL}^{-1}$ with water.

2.3. Fungal Strains. A Petri dish containing 25 mL of 4% (w/v) malt agar (the formula for making Malt agar solid medium is 25 g malt extract, 15 g agar, and 1000 mL distilled water) solid medium was inoculated with *C. versicolor*, and

the fungus was cultured at $28 \pm 2^\circ\text{C}$ with a relative humidity of 75% to 85% for 7 days. Then, the hyphae of the *C. versicolor* strain (7 mm) were transferred to a 250 mL sterilized culture flask containing 100 mL of malt agar medium and 1 mL of the *C. camphora* xylem methanol extract (the concentration was $80\text{ g}\cdot\text{L}^{-1}$). Thereafter, two of the above-sterilized culture flasks and a controlled sample were cultured in an oscillation box at 28°C with a rotation speed of $80\text{ r}\cdot\text{min}^{-1}$ for 14 days. Finally, the hyphae were collected by vacuum filtration.

2.4. Antifungal Assay. The antifungal activity of *C. camphora* extracts was tested against *C. versicolor* by growth rate of poison medium culture method [27]. Malt agar solid medium then mixed to *C. camphora* extracts obtain the final concentrations were $0.5\text{ g}\cdot\text{L}^{-1}$, $1\text{ g}\cdot\text{L}^{-1}$, $2\text{ g}\cdot\text{L}^{-1}$, $4\text{ g}\cdot\text{L}^{-1}$, and $8\text{ g}\cdot\text{L}^{-1}$. After transferring the hypha of *C. versicolor*, cultures of each of the fungi were maintained at the center of a Petri dish and incubated for several days and incubated medium at $28 \pm 2^\circ\text{C}$ for several days to mycelia get to Petri dishes edge. The EC_{50} (concentration for 50% of maximal effect) was calculated. The mycelium growth inhibition rate was calculated using

$$\text{Inhibition rate(\%)} = \frac{X - Y}{X} \times 100\%, \quad (1)$$

where X is control colony growth diameter, and Y is treat growth diameter.

2.5. Mycelial Protein Extracted by the TCA-Acetone Method. The hyphae were ground with liquid nitrogen, mixed with 20 mL of TCA/acetone, and homogenized. The mixture was then precipitated for 12 h at -20°C . After precipitation, the sample was centrifuged for 15 min at 4°C and 11000 rpm. The supernatant was removed and then 20 mL of acetone, which was precooled at -20°C and contained 0.07% β -mercaptoethanol, and was added to the sample. After precipitating for 2 h, the sample was centrifuged for 15 min at 4°C and 11000 rpm, and this step was repeated until the precipitate turned white. After vacuum filtration, a radioimmunoprecipitation assay (RIPA) was conducted according to a standard concentration of $20\text{ }\mu\text{L}\cdot\text{mg}^{-1}$. Ultrasonic dissolution was performed for 2 h at $25\text{--}30^\circ\text{C}$. Finally, after centrifugation at 4°C and 11000 rpm, the supernatant containing proteins were obtained. The protein sample (at a concentration of $5\text{--}10\text{ }\mu\text{g}\cdot\mu\text{L}^{-1}$) was kept in a -20°C freezer.

2.6. Two-Dimensional Electrophoresis. Isoelectric focusing was carried out using the Ettan™-IPGphor™ III IEF system with a ceramic tray using the following protocol: 13 h at 30 V, 1.0 h at 500 V, 6 h at 1000 V, 8 h at 8000 V (gradient), 4 h at 8000 V (step), and 30 h at 1000 V. The strips were then equilibrated for 15 min in equilibration buffer I (10 mL of equilibrium liquid with 100 mg of DTT) and subsequently for 15 min in equilibration buffer II (10 mL of equilibrium liquid with 250 mg of iodoacetamide). Thereafter, the filter paper was used to absorb redundant equilibration buffer on the strips. The strips were rinsed and then put on a 10% polyacrylamide gel plate, and relative quality standard

proteins were added. Finally, a second vertical electrophoresis experiment was conducted [28].

2.7. Two-Dimensional Electrophoresis and Staining. The balanced IPG strips were fixed to a glass gel plate, and 1 mL of agarose sealing glue was added. Then, the glass gel plate was placed in a buffer tank of the Ettan™ DALT II vertical system. Electrophoresis was performed under 16°C water cycling conditions and a constant power of $4\text{ W}\cdot\text{glue}^{-1}$. Electrophoresis was stopped when the tracer bromophenol blue reached the bottom gel edge, and the gel was removed and stained.

2.8. Gel Image Analysis. An image scanner was applied to conduct image scanning. Image Master™ 2D Platinum 7.0 was used to achieve 2-DE fingerprint analysis [29]. Image Master 7.0 software was used to compare the two-dimensional SDS-PAGE electrophoretograms of *C. versicolor* treatment with methanol extracts from the xylem of *C. camphora* and the control.

2.9. In-Gel Proteolytic Digestion and Identification. Protein spots of interest were manually excised from the CCB-stained gels and subjected to in-gel trypsin digestion. After digestion, peptides were then extracted twice using 0.1% trifluoroacetic acid (TFA) in 50% ACN. The extracts were pooled and dried completely by a SpeedVac apparatus. Peptide mixtures were redissolved in 0.1% TFA, and $0.8\text{ }\mu\text{L}$ of peptide solution was mixed with $0.4\text{ }\mu\text{L}$ of matrix (CHCA in 30% ACN, 0.1% TFA) before spotting on the target plate.

Protein identification was performed on an AB SCIEX MALDI-TOF/TOF-MS™ 5800 analyzer (AB SCIEX, Foster City, CA) equipped with a neodymium:yttrium-aluminum-garnet laser (laser wavelength: 349 nm). The TOF/TOF calibration mixtures (AB SCIEX) were used to calibrate the mass spectrometer to a mass tolerance within 100 ppm. For MS mode, peptide mass maps were acquired in positive reflection mode, and a $700\text{--}3600\text{ m}\cdot\text{z}^{-1}$ mass range was used with 1000 laser shots per spectrum. A maximum of 50 precursors per spot with a minimum signal/noise ratio of 25 were selected for MS/MS analysis using ambient air as the collision gas with a medium pressure of 10^{-6} Torr. The contaminant m/z peaks originating from human keratin, trypsin autodigestion, or the matrix were excluded for MS/MS analysis. An energy of 2 KV was used for collision-induced dissociation (CID), and 2500 acquisitions were accumulated for each MS/MS spectrum. The raw MS and MS/MS spectra were processed using GPS Explorer™ TM software with the following criteria: fungi (1757520 sequences; 762750636 residues), release date (2016.03.11), trypsin as the digestion enzyme, one missed cleavage, a 100 ppm precursor tolerance, an MS/MS ion tolerance of 0.6 Da, and methionine oxidation as a variable modification. Proteins with protein score confidence intervals (C.Is.) above 95% (protein score > 75) were considered confident identifications.

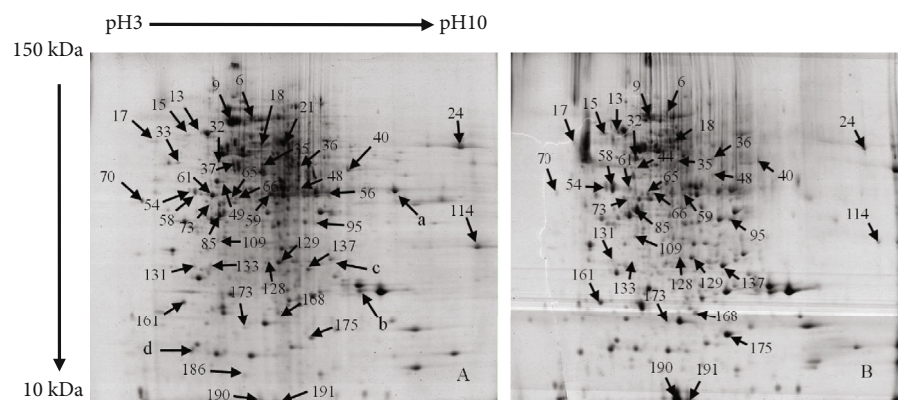


FIGURE 1: The two-dimensional electrophoresis gel diagram of *Coriolus versicolor* treated with methanol extracts of *Cinnamomum camphora* xylem.

TABLE 1: Differential expressed spots of *Coriolus versicolor* treated with methanol extracts identified by mass spectrometry.

Spot no.	Protein description	Accession no.	Peptides	MW/pI	Protein score	Sequence coverage	Organism
13	Similar to UBA/TS-N domain containing protein	Gi 312216952	2	152573/5.69	65	3%	<i>Leptosphaeria maculans</i>
15	Protein phosphatase	Gi 320591516	14	48259/4.69	182	24%	<i>Grosmannia clavigera</i> kw1407
24	ATP synthase alpha chain, mitochondrial precursor	Gi 114193514	23	59832/9.14	406	34%	<i>Aspergillus terreus</i> NIH2624
33	Hypothetical protein SNOG_12700	Gi 169619014	17	28025/8.83	60	49%	<i>Phaeosphaeria nodorum</i> SN15
36	RecName: full = phosphoglycerate kinase	Gi 400757	9	44353/6.16	182	22%	<i>Trichoderma viride</i>
37	DEAD/DEAH box helicase	Gi 310790877	27	44680/5.02	642	40%	<i>Glomerella graminicola</i> M1.001
49	ATPB_NEUCR ATP synthase beta chain, mitochondrial precursor	Gi 46116940	23	54852/5.40	393	38%	<i>Gibberella zeae</i> PH-1
56	Glyceraldehyde-3-phosphate dehydrogenase	Gi 228551660	13	36053/6.46	263	23%	<i>Cordyceps militaris</i>
58	Predicted protein	Gi 302918373	5	15925/5.84	209	24%	<i>Nectria haematococca</i> mpVI 77-13-4
59	Hypothetical protein SMAC_03111	Gi 336269525	19	44437/8.70	447	34%	<i>Sordaria macrospora</i> k-hell
114	Predicted protein	Gi 302914537	17	37541/9.48	320	30%	<i>Nectria haematococca</i> mpVI 77-13-4
168	Hypothetical protein FG00036.1	Gi 46102664	32	176621/5.75	75	19%	<i>Gibberella zeae</i> PH-1
175	Predicted protein	Gi 302918373	6	15925/5.84	188	24%	<i>Nectria haematococca</i> mpVI 77-13-4
186	Hypothetical protein SERLADRAFT_468747	Gi 336383691	15	35195/9.83	74	39%	<i>Serpula lacrymans</i> var. <i>lacrymans</i> S7.9
190	Malonyl CoA-acyl carrier protein transacylase	Gi 322698339	9	47145/9.33	68	28%	<i>Metarhizium acridum</i> CQMa 102
a	Aspartate aminotransferase	Gi 295669158	16	47022/9.25	176	31%	<i>Paracoccidioides brasiliensis</i> Pb01
b	Hypothetical protein NECHADRAFT_91822	Gi 302920406	7	21737/6.71	134	19%	<i>Nectria haematococca</i> mpVI 77-13-4
c	Vacuolar ATP synthase subunit E	Gi 322705501	7	25896/6.69	110	23%	<i>Metarhizium anisopliae</i> ARSEF 23
d	Pentafunctional AROM polypeptide, putative	Gi 218724284	19	74261/6.72	61	27%	<i>Talaromyces stipitatus</i> ATCC 10500

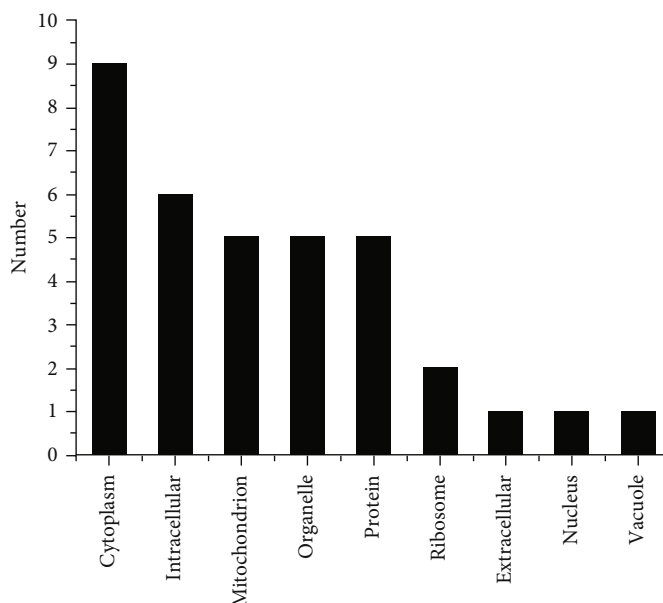


FIGURE 2: The cellular localization of difference protein of *Coriolus versicolor* in response to methanol extracts from the xylem of *Cinnamomum camphora*.

3. Results and Discussion

3.1. Antifungal Activity. The methanol extracts of *C. camphora* xylem showed certain antifungal activities against *C. versicolor*. This growth inhibition ratio was calculated in the ranges of 0.5 g·L⁻¹ to 10.39%, 1 g·L⁻¹ to 13.15%, 2 g·L⁻¹ to 17.98%, 4 g·L⁻¹ to 23.01%, and 8 g·L⁻¹ to 28.02%, respectively. EC₅₀ values of methanol extract were calculated as 79.92 g·L⁻¹.

3.2. Proteomic Differential Display Analysis. The results which the 2D-GE diagram of *C. versicolor* treated with methanol extracts of *C. camphora* xylem can be seen in Figure 1, which shows that the two-dimensional electrophoretogram spectral patterns of the two proteins are basically the same, and the protein spots are mainly concentrated in the pH range of 4–7.

3.3. The Dynamic Changes in Differential Protein. Using software to match the two-dimensional electrophoretograms of each protein, it was found that 41 proteins exhibited abundance changes of more than 2 times with good reproducibility and obvious changes in expression, of which 21 protein spots were upregulated and 20 protein spots were downregulated (Figure S1).

3.4. Mass Spectrometric Identification of Differentially Expressed Proteins. Proteomics means the large-scale analysis of all the proteins encoded by the genome. It can be divided into expression proteomics and functional proteomics. The 20 protein spots of *C. camphora* xylem methanol extracts that could inhibit white-rot fungi were treated by cutting, decolorization, trypsin digestion, and desalination. Finally, the peptide mass fingerprints corresponding to MALDI-TOF/TOF-MS data were measured, and a total of 14 proteins were identified by MASCOT software in the

NCBI database. Using the ATP synthase alpha chain and mitochondrial precursor (spot 24) as an example, the tandem mass spectrogram of a protein can be seen in Figure S2. In the graph of the peptide mass fingerprint of ATP synthase alpha chain and mitochondrial precursor, b and c are the MS/MS maps of the peptides with ion scores of 82 and 78, respectively.

Proteins can be divided into the following categories through the inference of physiological function [30, 31]. Some proteins of *C. versicolor* are related to cell wall biosynthesis, a class of proteins related to small-molecule metabolic processes, cellular amino acid metabolic processes, and cellular nitrogen compound metabolic processes. Some proteins are involved in ion binding, oxidoreductase activity, ATPase activity, and transmembrane transporter activity. Proteins involved in reproducing translations are energy-related (Table 1).

3.5. Functional Analysis of Differentially Expressed Proteins. It can be found that 14 differential proteins identified by mass spectrometry were associated with cellular localization (Figure 2). Nine proteins were located in the cytoplasm, 6 existed intracellularly, 5 belonged to the mitochondrion, 5 were in the organelle, 5 were in in the protein complex, 2 were in the ribosome, 1 was in the extracellular region, 1 was in the cell nucleus, and 1 was in the vacuole.

It can be seen from Figure 3 that the proteins in *C. versicolor* that were differentially expressed after treatment with the methanol extract were involved in biological processes, namely, 18.8% were involved in small-molecule metabolic processes; 12.5% in cellular amino acid metabolic processes; 10.9% in transport processes; 10.9% in cellular nitrogen compound metabolic processes; 9.4% in transmembrane transport processes; 6.3% in translation processes; and 6.3% in the generation of precursor metabolites and energy

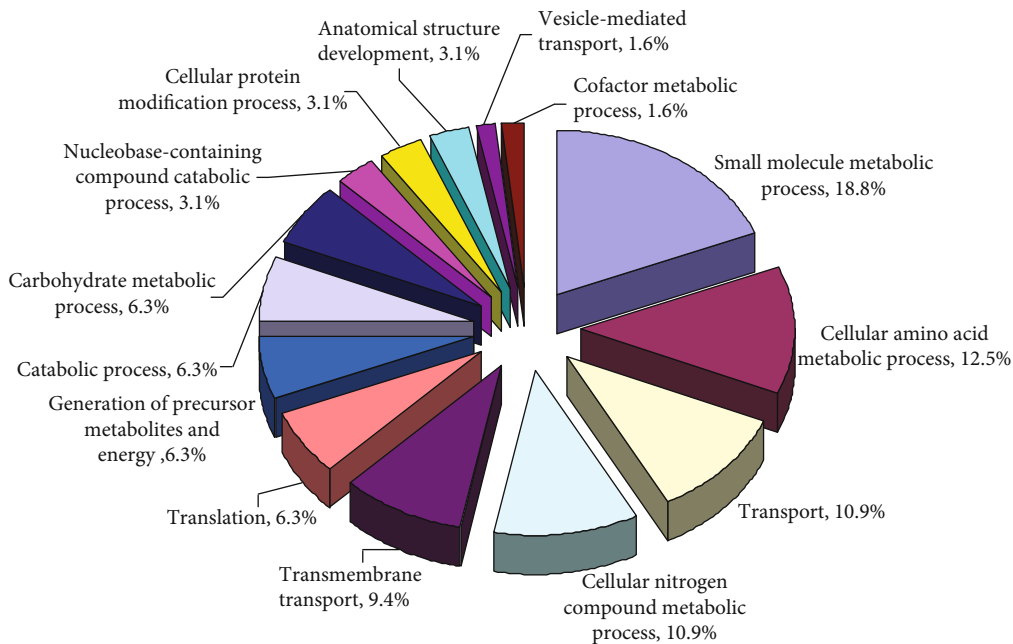


FIGURE 3: The biological processes of differences protein of *Coriolus versicolor* in response to methanol extracts from the xylem of *Cinnamomum camphora*.

generation, catabolism processes and carbohydrate metabolism, and other processes.

The percentage of protein function in each class is shown in Figure 4. Twenty-five percent of the differential proteins were related to ion binding, 15% were related to oxidoreductase activity, 15% were related to ATPase activity, 15% were associated with transmembrane transport activity, 6% were related to translation factor activity, and 3% were associated with DNA, helicase activity, peptidase activity, kinase activity, transferase activity, phosphatase activity, lyase activity, and isomerase activity.

3.6. Functional Categories of Differentially Expressed Proteins. Aspartate aminotransferase is an enzyme with high catalytic activity and is widely found in animals, plants, and microorganisms. This enzyme plays a very important role in nitrogen and carbon metabolism in cells [32]. Aspartate aminotransferase is important for metabolite exchange between mitochondria and the cytosol. This enzyme plays an important role in the biosynthesis of amino acids, and the reaction process includes the metabolic processes of cell amino acids and derivatives, biological synthesis of cells, etc. The specific functions of this enzyme are catalytic activity, aminotransferase activity, and transferase activity. The downregulation of aspartate aminotransferase here shows that the methanol extracts of *C. camphora* xylem inhibited the metabolism of nitrogen and carbon in *C. versicolor*.

ATP synthase alpha chain is an enzyme associated with energy metabolism. This enzyme produces ATP from ADP in the presence of a proton gradient across the membrane [33]. ATP synthase is widely distributed in the mitochondrial inner membrane and in the heterotrophic bacterial and photosynthetic bacterial plasma membrane. The physiological function of the ATP synthetase alpha chain may

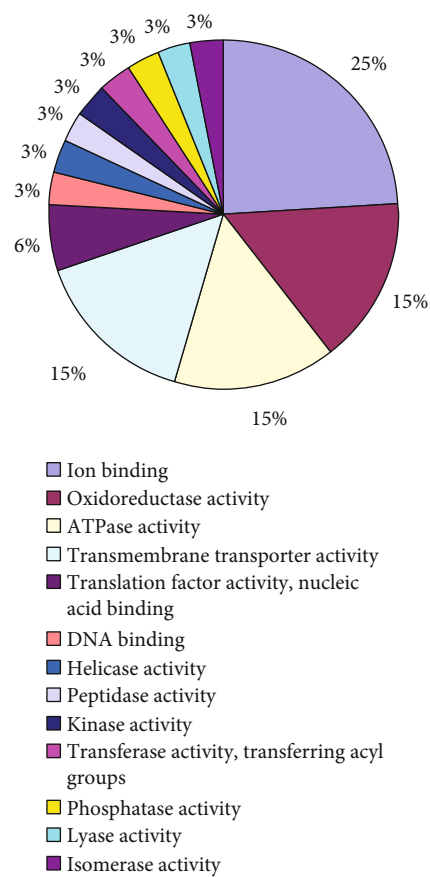


FIGURE 4: The functional groups of the proteins of *Coriolus versicolor* in response to methanol extracts from the xylem of *Cinnamomum camphora*.

involve cell proliferation and cytotoxicity. The downregulation of the ATP synthase alpha chain in *C. versicolor* after treatment with methanol extracts of *C. camphora* xylem indicates that energy metabolism in *C. versicolor* was inhibited.

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is a highly conserved protein that plays a role in central carbon metabolism. GAPDH is one of the most basic enzymes to provide energy activities to support life [34]. GAPDH is a key enzyme for the decomposition of sugar in all prokaryotic and eukaryotic organisms [35]. Tokunaga et al. [36] disclosed a molecular basis of glyceraldehyde-3-phosphate dehydrogenase and revealed an important role of the energy-creating reaction in cancer cell growth [36]. In addition, this enzyme is also involved in the control of posttranscriptional gene expression, acting on the processes of tRNA transport, DNA replication and repair, etc. The related protein spot (GAPDH) disappeared, which likely impacted cellular carbon metabolism. This result indicated that the enzyme was completely inhibited, resulting in the loss of energy metabolism and subsequent death of *C. versicolor*.

ATPB_NEUCR ATP synthase beta chain, a mitochondrial precursor, is an enzyme related to energy metabolism. No relevant literature reporting on details of this enzyme has been published.

The function of vacuolar ATP synthase subunit E is mainly ATP hydrolysis of proton-exchange membrane coupling. ATPase is also known to participate in proton transport and rotation mechanisms [37].

Helicases widely exist in almost all prokaryotes and eukaryotes and belong to the class of enzymes that cleave hydrogen bonds. The functions of these enzymes mainly include nucleic acid binding, helicase activity, and ATP binding [38]. A large number of studies have shown that DEAD/DEAH box helicase is involved in almost all biological processes from bacteria to eukaryotic cells, including hormonal responses, RNA metabolism, nucleocytoplasmic transport, gene expression regulation, and other important biological activities. In addition, DEAD/DEAH box helicase can also play an important role in RNA folding and RNA-protein complex processing. The downregulated expression of DEAD-box helicase indicates that hormone signal response, RNA metabolism, etc., were inhibited in *C. versicolor*.

Phosphoglycerate kinase (PGK) is a key enzyme in glycolysis that exists in almost all organisms and is an important enzyme that organisms depend on [39]. A deficiency in this enzyme can lead to dysfunctions such as functional metabolism. PGK is essential in most living cells for both ATP generation in the glycolytic pathway of aerobes and fermentation in anaerobes [40]. PGK is distributed in different organisms, and its biological functions are different in different organisms. The downregulated expression of this enzyme indicates the inhibition of glucose metabolism, which may lead to glucose metabolism dysfunction in *C. versicolor*.

4. Conclusions

The research reveals the antifungal mechanism of the secondary metabolites of *C. camphora* xylem in methanolic

extracts through 2-DE and MALDI-TOF/TOF-MS. The observed differential proteins can be divided into the classes of cell wall biosynthesis, small-molecule metabolism, cellular amino acid and nitrogen compound metabolism, and transport. The downregulated expression of aspartate aminotransferase, ATP synthase alpha chain, and DEAD/DEAH box helicase showed that amino acid metabolism, energy metabolism, nucleocytoplasmic transport, and glucose metabolism were inhibited. We concluded that the inhibiting mechanisms of these extracts for *C. versicolor* may offer a chance to develop new wood preservatives that disrupt the metabolic pathways of wood rot fungus.

Data Availability

No data was used in the study.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

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Supplementary Materials

The following are available online. Figure S1: expression levels of differentially proteins compared with those of controls (a) protein spot numbers 6-40; (b) protein spot numbers 44-114; (c) protein spot numbers 128-192. Figure S2: the mass spectrum of protein spot number 24 (ATP synthase alpha chain, mitochondrial precursor) ((a) the peptide mass fingerprint of ATP synthase alpha chain, mitochondrial precursor; (b) MS/MS map of the 1332 peptide with ion score of 82; (c) MS/MS map of the 1553 peptide with ion score of 78). (*Supplementary Materials*)

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