Supplementary material

1. Materials and Methods

1.1 Strains

Aspergillus fumigatus (*A. fumigatus*) G-13, which could degrade lignin used in this work was isolated from the samples collected from soil near the sewage draining exit of a paper mill in Harbin.

1.2 Instruments

Shimadzu LC-20AB liquid chromatograph, Shimadzu LC-20AD nanoliter liquid chromatograph, ESC tandem mass spectrometer: Triple TOF 5600 (SCIEX, Framingham, MA, USA), Ion source is Nanospray III source (SCIEX, Framingham, MA, USA), Emitter is a needle drawn from quartz material (New Objectives, Woburn, MA, USA).

1.3 Preparation of fungus suspension

The oblique surface of *A. fumigatus* G-13 PDA medium stored at 4 °C was taken out, and the fungal spores were washed with sterile water to prepare a spore suspension of 10^6 cells/mL (measured by blood cell counting method). Keep it in a 4 °C refrigerator.

1.4 Fermentation culture

The lignin model compounds are ferulic acid, sinapic acid and p-coumaric, glucose as an auxiliary carbon source, and the cellulose co-substrate is carboxymethyl cellulose. Using different dilution factors, the concentration of the model compound in the inorganic salt medium is 0.1, 0.5, 1, 2 mol/L. The medium with the same other components without adding lignin model compound substrate was used as a control

group. Subsequently, the spore suspension was connected according to the volume ratio of fungus suspension to culture solution at 1:25, and cultured on a shaker at 160 r/min at 30 ℃, and its OD_{600} was measured every 3 hours to select the optimal lignin model compound concentration.

Using a liquid culture method, added 100 mL of a large amount of elemental nutrient solution and 0.1 mL of a trace element nutrient solution to a 250 mL Erlenmeyer flask, wherein the concentration of lignin monomer model compound was 0.1 mmol/L, cellulose co-substrate and glucose concentration were both 10 g/L, autoclave at 120 °C for 20 min. Then, 4 mL of the spore suspension was added and cultured at a temperature of 30 °C. When using shake flask culture, the rotation speed was 160 r/min. Samples were taken on the 3rd, 6th, 9th, 12th, and 15th day of culture, and measured the enzyme activity. Wherein each set of three replicates. The two groups with the largest difference in enzyme activity were used as samples for subsequent proteomic analysis.

1.5 Label-free analysis of protein samples

1.5.1 Sample concentrate

Took fermentation broth to a 30K ultrafiltration tube and centrifuged at 6000 r/min for 15 min. Repeated this step several times until the culture medium was separated from mycelium, and the substance intercepted by ultrafiltration membrane was a concentrated protein sample.

1.5.2 Extraction of protein

TCA/acetone precipitation + SDT cracking method: take an appropriate amount

of sample and grind it into a fine powder in a mortar in liquid nitrogen, add 5 times the volume of TCA/acetone (1: 9), vortex to mix, and place at -20 ℃ precipitation over 4 h. Centrifuge at 6000 g for 4 min at 4 ℃, discard the supernatant. Add pre-chilled acetone and wash 3 times. Dry the pellet in a fume hood. Weigh 20-30 mg of the dried powder, add 30 times the volume (m / v) of SDT lysate, Votex resuspend the precipitate, and boil in a water bath for 5 min. Ultrasonic disruption, boiling water bath for 15 min. After centrifugation at 14000 g for 15 min, the supernatant was filtered through a 22 μm filter. The protein was quantified by the BCA method, and the samples were stored at -80 ℃ after aliquoting.

1.5.3 FASP digestion

Take 80 μg protein solution sample, add DTT to a final concentration of 100 mol/L, and cool to room temperature after 5 min in a boiling water bath. Add 200 μL of UA buffer to mix, then add it to a 30kD ultrafiltration centrifuge tube for centrifugation, discard the filtrate after 15 minutes of centrifugation, and repeat once. Add 100 μL IAA buffer, shake at 600 r/min for 1 min, react for 30 min at room temperature in the dark, and then centrifuge at 12500 g for 15 min. Then add 100 μL of UA buffer and centrifuge at 12500 g for 15 min, repeating twice. Then, add 100 μL of 40 mol/L NH_4HCO_3 solution, centrifuge at 12500 g for 15 min, and repeat twice. Add 40 μL trypsin buffer, shake at 600 r/min for 1 min, and then place at 37 ℃ for 16 h. After replacing the collection tube, centrifuge at 12500 g for 15 min, add 20 μL of 40 mol/L NH_4HCO_3 solution, then centrifuge at 12500 g for 15 min and collect the filtrate. Finally, desalting treatment was carried out. After the peptides were

lyophilized, 40 μL of 0.1% formic acid solution was added for reconstitution, and the peptides were quantified at OD_{280}.

1.5.4 Mass spectrometry

The samples were separated using the Easy nLC system with a nanoliter flow rate, in which solution A: 0.1% formic acid in water and solution B: 0.1% formic acid in acetonitrile. A liquid equilibrated chromatographic column was loaded onto the analytical column for separation (50um×15cm, nano viper, P/N164943) with a flow rate of 300 nL/min. Use Q Exactive Plus mass spectrometer for mass spectrometry analysis for 60 min. The detection method is positive ions and the scanning range of the parent ions is 350-1800 m/z. The first-level mass spectrometer has a resolution of 70000, and the first-level Maximum IT is 50 ms. The secondary mass spectrometer has a resolution of 17500 and the secondary maximum IT of 45 ms.

1.5.5 Bioinformatics analysis

The study used a non-labeled quantitative method based on MS1 data integration. Maxquant software first recognizes the peptide feature, and then performs graphic integration to calculate the intensity value. Then use Blast2GO software to perform GO annotation on the target protein collection, including sequence alignment, GO entry extraction, GO annotation and supplementary annotation.

1.6 Non-targeted metabolomics analysis

1.6.1 Metabolite extraction

The collected samples were thawed on ice, and metabolite were extracted with 50% methanol Buffer. Briefly, 20 μL of sample was extracted with 120 μL of

precooled 50% methanol, vortexed for 1 min, and incubated at room temperature for 10 min; the extraction mixture was then stored overnight at -20°C. After centrifugation at 4,000 g for 20 min, the supernatants were transferred into new 96-well plates. The samples were stored at -80°C prior to the LC-MS analysis. In addition, pooled QC samples were also prepared by combining 10 μL of each extraction mixture.

1.6.2 LC/MS analysis

A high-resolution tandem mass spectrometer TripleTOF5600plus (SCIEX, UK) was used to detect metabolites eluted from the column. The Q-TOF was operated in both positive and negative ion modes. The curtain gas was set at 30 PSI, Ion source gas1 was set at 60 PSI, Ion source gas2 was set 60 PSI, and an interface heater temperature was 650 ℃.For positive ion mode, the Ionspray voltage floating were set at 5000 V, respectively. For negative ion mode, the Ionspray voltage floating were set at -4500 V, respectively. The mass spectrometry data were acquired in IDA mode. The TOF mass range was from 60 to 1200 Da. The survey scans were acquired in 150 ms and as many as 12 product ion scans were collected if exceeding a threshold of 100 counts per second (counts/s) and with a 1+ charge-state. Total cycle time was fixed to 0.56 s.Four time bins were summed for each scan at a pulser frequency value of 11 kHz through monitoring of the 40 GHz multichannel TDC detector with four-anode/channel detection. Dynamic exclusion was set for 4 s.During the acquisition, the mass accuracy was calibrated every 20 samples. Furthermore, in order to evaluate the stability of the LC-MS during the whole acquisition, a quality control sample

(Pool of all samples) was acquired after every 10 samples.

1.6.3 Bioinformatics Analysis

First, use MSConvert software to convert the original mass spectrum data into mzXML format. Then, use XCMS software for peak extraction and quality control at the same time, add ion annotation to the extracted substances, and use metaX software for metabolite identification. Subsequently, annotate the candidate identification substances and explain the physicochemical properties and biological functions of the metabolites. Finally, metaX was quantified and screened using metaX software to finally obtain differential metabolites.

1.7 Joint analysis of omics data

The differential metabolites and differentially expressed proteins identified by the *A. fumigatus* G-13 fermentation lignin model compound were integrated using software tools based on the R package and mapped to the KEGG metabolic pathway for joint analysis of two omics data.

2. Results

2.1 Effects of different concentrations of model compounds on the growth of *A. fumigatus* G-13

The growth of *A. fumigatus* G-13 was investigated when the lignin model compound concentrations were 0.1 mol/L, 0.5 mol/L, 1 mol/L, 2 mol/L, and no lignin model compound was added. The results are shown in Fig. 1. With p-coumaric acid and erucic acid as substrates, the growth of *A. fumigatus* G-13 was inhibited as the concentration of lignin monomer model compounds increased. With sinapic acid and

p-coumaric as substrates, the growth of *A. fumigatus* G-13 was inhibited as the concentration of lignin monomer model compounds increased. When the substrate concentration was 0.5 mol/L and 1 mol/L, the growth of *A. fumigatus* G-13 was significantly better than 2 mol/L. Although the growth of the strain was good and roughly the same when the substrate concentration was 0.1 mol/L and no lignin model compound was added, it was still lower than that of 0.5 mol/L and 1 mol/L. The OD_{600} of 2 mol/L sinapic acid and p-coumaric substrate was significantly lower than that of the blank control group, indicated that the growth of the strain was inhibited to a certain extent. It has been reported that higher concentrations of sinapic acid do not support the growth of *Pseduomonas fluorescensde* [17, 18]. When the fermentation substrate was ferulic acid, the situation was opposite to the other two substrates. Within a certain concentration range, a higher concentration of ferulic acid obviously promoted the growth of this strain. It can be seen from Fig. 1 (c) that when the ferulic acid concentration is 2 mol/L, the strain grows better than other concentrations. Therefore, 2 mol/L ferulic acid and 1 mol/L sinapic acid and p-coumaric were selected for further study.

2.2 Enzymatic activity analysis of lignin model compound fermented by *A. fumigatus* G-13

Fig. 1 shows the curve of the enzyme activity of three lignin model compounds fermented by *A. fumigatus* G-13 with time. It can be seen from Fig. 2 that the three lignin enzymes produced by fermenting the three substrates have roughly the same activity trend. The sinapic acid substrate was the most favorable for fermentation to

produce enzymes, while fermentation has the lowest enzyme activity for p-coumaric. The activity of manganese peroxidase (Mnp) produced by the three substrates reached the highest on the 9th day. The enzyme activities from high to low were erucic acid (54.79 U/L), ferulic acid (51.37 U/L) and p-coumaric acid (48.12 U/L). Lignin peroxidase (Lip) peaked at 12 d. The enzyme activity produced by sinapic acid substrate (21.62 U/L) was 1.6 times that of p-coumaric (13.5 U/L). Ferulic acid substrate-producing enzymes were among the two, at 15.42 U/L. For laccase (Lac), the sinapic acid substrate maintained a high enzyme activity at 9-12 d, and para-coumaric acid reached a maximum of 4.65 U/L at 12 d. The Lac enzyme activity produced by *A. fumigatus* G-13 was much lower than that of Mnp and Lip, indicating that this strain mainly produced Mnp and Lip, and the Lac yield was low. Through analysis, the two groups with the largest difference in enzyme production (sinapic acid and p-coumaric) were selected for omics analysis.

2.3 Proteomics study on the model compound of *A. fumigatus* G-13 degrading lignin

2.3.1 Quantitative analysis of protein

Mass spectrometry data was obtained by merging the biological samples three times, which represents the whole proteome of the strain fermented lignin model compounds. Table 1 shows the results of protein identification and quantification. In this study, a total of 1447 peptides and 208 proteins were identified. In the analysis of the significant difference of quantitative results, at least two non-null data of the three repeated experimental data in the sample group are screened for statistical analysis.

Among them, differentially expressed proteins were screened with fold-change> 2 and p-value <0.05.

2.3.2 Protein annotation statistics

According to *A. fumigatus* G-13 fermentation of different substrates secreted protein, comprehensive evaluation of protein expression changes between two different substrates. A total of 134 proteins changed significantly. Among them, *A. fumigatus* G-13 fermented sinapic acid substrate had 73 proteins up-regulated and 61 proteins down-regulated compared to p-coumaric substrate. Compared with p-coumaric substrate, exo-β-1,3-glucanase, isoamyl alcohol oxidase, α-1,2-mannosidase, aldose 1-epimerase, catechol dioxygenase, glyceraldehyde-3-phosphate dehydrogenase, superoxide dismutase [Cu-Zn] and α,α-trehalose glucohydrolase TreA/Ath1 were significantly upregulated during the fermentation of sinapic acid substrate by *A. fumigatus* G-13. The number of differential proteins that were significantly down-regulated was small, and most of them were related to energy metabolism and nucleotide synthesis related proteins. Table 3-3 shows the significant changes in differential protein information. The proteins whose expression was up-regulated are mainly endonuclease/exonuclease/phosphatase family proteins, amino acid oxidase, carboxypeptidase, monosaccharide transporter, glycolytic protein and some uncharacterized proteins. The down-regulated proteins are mainly partial glycosyl hydrolases, adenosine deaminase family proteins, and metal ion transport proteins.

2.3.3 GO annotation and enrichment analysis

Using Blast2 GO for data analysis, the GO analysis results of the target protein collection are shown in Fig. 3. According to the functional characteristics of different proteins, they are classified, including biological process, molecular function and cellular component. Among them, 22 differential proteins participate in biological processes, mainly focusing on metabolic and cellular processes. There are 13 involved in molecular functions, mainly focusing on catalytic activity and binding. Furthermore there are 17 participating cellular components, concentrated in the extracellular region, membrane and cell parts.

The GO annotation of target protein is classified from three aspects: biological process, molecular function and cell composition, reflecting the degree of influence of biological treatment on classification in experimental design. The significantly enriched GO term mainly includes hydrolase activity, hydrolyzing O-glycosyl compounds, ribonuclease T1 activity, xyloglucan catabolic process, glucanosyltransferase activity, cellulose catabolic process, amino sugar metabolic process, glucan endo-1,3-β-glucanase activity, starch metabolic process, endoribonuclease activity, polysaccharide catabolic process. Differential proteins enriched in the secondary metabolic pathway are of great significance for the metabolic analysis of A. fumigatus G-13 degradation lignin model compounds.

2.3.4 KEGG pathway enrichment analysis

In order to further investigate the functions of these proteins, the metabolic pathways of the obtained differential proteins were analyzed using the KEGG database. Fig. 4 is a schematic diagram of pathway enrichment of differential

abundance protein. Among the annotated metabolic pathways, the identified differential proteins mainly involved metabolic pathways, two-component systems, oxidative phosphorylation, starch and sucrose metabolism, glycolysis/gluconeogenesis, ribosome, pentoses and glucuronic acid interconversion, riboflavin metabolism, degradation of aromatic compounds, protein processing in endoplasmic reticulum, butanoate metabolism, Benzoic acid metabolism, purine metabolism, pyrimidine metabolis, ubiquinone, and other terpenoid-quinone biosynthesis, etc.

2.4 Metabolomics study on the model compound of *A. fumigatus* G-13 degrading lignin

2.4.1 Metabolite identification

The TIC of the sample in this analysis is shown in Fig. 5. In POS mode and NEG mode, the obtained QC sample total ion current maps were compared for spectral overlap. Convert the original WIFF data format of the mass spectrometry, and import the R package for peak extraction to obtain metabolite-related information, including the ion peak m/z, RT, and ion area. Using metaX software, the first-level m/z of the substance detected by XCMS was matched with KEGG to obtain the first-level identification result. The study used an in-house metabolite secondary mass spectrometry library to match the secondary mass spectrometry data of the experimental sample metabolites. Table 3 is the total ion number and identification statistics. The POS and NEG models obtained 8832 and 4223 metabolites, of which 4173 and 1596 were annotated, respectively.

The metabolites identified above were assigned to the KEGG database. Fig. 6 is a classification diagram of KEGG pathway in POS / NEG mode. Under the POS model, 4122 metabolites are classified into 21 KEGG second-level entries, and under the NEG model, 1568 metabolites are classified as 19 KEGG second-level entries. Under the POS and NEG models, the substances classified as "metabolism" were 3719 and 1228 respectively. Among them, the most important for the metabolic process was the global and overview map, followed by the metabolism of terpenoids and polyketides, amino acid metabolism, xenobiotics biodegradation and metabolism, lipid metabolism and carbohydrate metabolism.

Different substances were fragmented in the mass spectrometer, and the fragment information was used to generate secondary spectra. Matched the standard secondary spectrum of the public database and scored the matching result, and finally obtained the secondary identification result of the metabolite, as shown in Fig. 7. All metabolites obtained by the secondary identification (286 in POS mode and 121 in NEG mode) were assigned to the HMDB database, matched and divided into 14 and 10 HMDB superclasses in POS and NEG, respectively. Among them, 59 metabolites are included in lipids and lipid-like molecules, 56 metabolites are included in organoheterocyclic compounds, and 52 metabolites are included in organic acids and derivatives. It represents most of all categories in the POS model. At the same time, lipids and lipid-like molecules containing 28 metabolites are the first category, followed by organic heterocyclic compounds, benzenoids, and organic acids and derivatives in the NEG model.

2.4.2 Screening and functional analysis of differential metabolites

Fig. 8 is a comparison chart of differential metabolites at various stages of a model compound for degradation of lignin by *A. fumigatus* G-13. Under POS/NEG conditions, the number of significantly different metabolites were 6 d > 9 d > 12 d > 3 d > 15 d. It can be seen that *A. fumigatus* G-13 fermented lignin model compound, the changing trend of its differential metabolites is first increasing and then decreasing. On the 6th day of fermentation, the number of significant differential metabolites produced by the strain was the largest.

$p < 0.05$ was used as the significance measure of Pathway, and KEGG Pathway enrichment analysis was performed on different metabolites at different stages. The results of KEGG Pathway enrichment at 3 d, 6 d, 9 d, 12 d and 15 d of fermentation are shown in Fig. 9 respectively. The results showed that amino acid-related metabolic pathways in samples fermented with erucic acid as a substrate changed significantly, including alanine, aspartic acid and glutamic acid metabolism, phenylalanine metabolism, biosynthesis of valine, leucine and isoleucine, lysine degradation, glycine, serine and threonine metabolism, arginine and proline metabolism and other related metabolic pathways. Among them, aminoacyl-tRNA biosynthesis, ABC transport, purine metabolism, pyrimidine metabolism, butyrate metabolism, pantothenic acid and CoA biosynthesis, ketone body synthesis and degradation, glutathione metabolism, propionate metabolism, nicotinate and niacinamide metabolism pathways also appeared significant enrichment.

In the early stage of fermentation (3rd to 6th days), *A. fumigatus* G-13 fermented two model compounds, the main differential pathways were enriched in pyruvate metabolism and glycolysis/gluconeogenesis. The study found that *A. fumigatus* G-13 will produce more precursor metabolites such as glyceraldehyde-3-phosphate, glycerin-3-phosphate, phosphoenolpyruvate during the fermentation of sinapic acid substrate. It indicates that the strain will release more energy through glycolysis in the early stage of degrading sinapic acid. Therefore, sinapic acid substrate is more beneficial to be used by *A. fumigatus* G-13 to complete the aerobic oxidation of glucose. From the 6th day of fermentation, various amino acid metabolism pathways in the sinapic acid experimental group began to appear, and a series of protein synthesis pathways involved in lignin degradation also show differences, including polycyclic aromatic hydrocarbon degradation, benzoate degradation, aromatic compound degradation, C5-branched dibasic acid metabolism, etc. Hadibarata et al. used *Pleurotus eryngii* to ferment lignin model compounds and conducted pathway enrichment analysis [19]. They were found that aminobenzoic acid metabolism, toluene metabolism, naphthalene metabolism, xylene metabolism are all related to benzoic acid metabolism. Benzoic acid metabolism is crucial in the process of lignin degradation, which supports the results of this study. On the 9th day of fermentation, the synthesis and degradation of ketone bodies and glutathione metabolism began to be significantly enriched. Glutathione, the product of glutathione metabolism, is a prosthetic group of glyceraldehyde phosphate dehydrogenase and an important coenzyme of glyoxalase and triose dehydrogenase. It participates in the tricarboxylic

acid cycle and sugar metabolism in the cell, and can activate the thiol (SH) enzyme-coenzyme to achieve the purpose of promoting the metabolism of sugar, fat and protein. Some studies have shown that glutathione can cleave aryl ethers to attack the non-phenolic structure of lignin, and glutathione reductase can effectively reduce the levels of hydrogen peroxide and oxygen free radicals in the cells, accelerate the growth of strains, and relieve the stress effect of lignin [20, 21]. This study found that the time when *A. fumigatus* G-13 fermentation started to produce a large amount of enzymes coincided with the significant enrichment time of a series of amino acid metabolism pathways (the 6th day of fermentation). It shows that various tissue proteins and metabolites synthesized by amino acid metabolism can promote the secretion of lignin-degrading enzymes by this strain. The difference of metabolic pathway may be related to the difference of substrate structure. The study shows that the syringyl structure is more conducive to being used by *A. fumigatus* G-13, which in turn promotes the metabolic activity of the strain. The syringyl propane structure of sinapic acid is more likely to undergo oxidation under the action of *A. fumigatus* G-13. That is, after the carbon-carbon bond on the side chain of the monomer is broken, the terminal carbon atom connected to the benzene ring undergoes an oxidation reaction to form an aldehyde, which is then oxidized to an acid. Or the carbon atoms in the side chain directly oxidize, and a series of reactions affect the metabolism of the strain and the production of lignin degrading enzymes.

3. Discussion

3.1 Induction of pentose phosphate pathway

Glycolysis is the main metabolic pathway for anaerobic bacteria to produce energy, but studying how *A. fumigatus* G-13 transfers glycolysis intermediate products to other pathways may be an effective way to overcome metabolic inhibition. One example is the pentose phosphate pathway (PPP), which uses glycolysis intermediates to produce pentose (C5) sugars required for biosynthesis of nucleotides and aromatic amino acids [22]. Among the 11 related enzymes detected in the pentose phosphate pathway, 5 have changed significantly over time, including glucokinase [EC: 2.7.1.12], glucose 6-phosphate-1-dehydrogenase [EC: 1.1. 1.49], 6-phosphate glucose isomerase [EC: 5.3.1.9], 6-Phosphofructose kinase 1 [EC: 2.7.1.11], deoxyribose phosphate aldolase [EC: 4.1.2.4], phosphoglucose mutant enzyme [EC: 5.4.2.2]. Among them, glucose 6-phosphate-1-dehydrogenase is a rate-limiting enzyme, and deoxyribose phosphate aldolase content will increase when entering the early stationary phase[23]. The high abundance and continued accumulation of deoxyribose phosphate aldolase indicate the production of sugar precursors for the metabolism of purines and pyrimidines. It also makes intermediate substances in the metabolic process to synthesize more deoxyribonucleotides and ribonucleotides[24]. Usually, this part of the pentose phosphate pathway is up-regulated towards the end of the culture, and the demand for growth-related nucleotides and aromatic amino acids will also decrease accordingly [25]. After comparison, it was found that during the fermentation of sinapic acid by *A. fumigatus* G-13, this part of the up-regulation time appeared earlier. Inferred from this, the conversion of sinapic acid substrate to the pentose phosphate pathway can provide an important shunt, transfer carbon/electrons

out of glycolysis in an attempt to balance metabolic flux. This effectively inhibited the metabolic inhibitory effect, which resulted in the enzyme activity of sinapic acid fermented by *A. fumigatus* G-13 being higher than that of p-coumaric acid substrate.

3.2 The ortho-cleavage pathway of dihydroxylated aromatic rings

Shi'ss research shows that the dimer phenyl coumarone produces monocyclic aromatic compound ferulic acid under the action of lyase. Subsequently, ferulic acid is converted into vanillin under the combined action of acyl-CoA synthetase (HcaA) and acyl-CoA hydratase (HcaB), and then vanillin generates vanillic acid under the action of dehydrogenase (HcaC). Vanillic acid produces protocatechuic acid under the action of demethylase oxidoreductase (VanA), and then enters the β-ketoadipate pathway under the oxidation of dioxygenase[29]. The p-coumarol fragment in the lignin structure is cleaved to form the monocyclic aromatic compound p-coumaric acid. Subsequently, p-coumaric acid is converted into p-hydroxybenzaldehyde under the combined action of acyl-CoA synthetase (HcaA) and acyl-CoA hydratase (HcaB). Then, under the action of dehydrogenase (HcaC), p-hydroxybenzoic acid is produced, and under the action of decarboxylase, phenol is produced. Finally, catechol is produced under the action of hydroxylase through the phenol metabolic pathway, and a ring-opening reaction occurs under the oxidation of catechol 1,2-dioxygenase to enter the β-ketoadipate pathway [30].

During the proteomic detection of two model compounds fermented by *A. fumigatus* G-13, a large amount of catechol dioxygenase [EC: 1.13.11.1] was found to be up-regulated. Among them, the experimental group using sinapic acid as the

substrate was more up-regulated, but no protocatechol dioxygenase was detected. Inferred from this, during the degradation of lignin model compounds by *A. fumigatus* G-13, the intermediate metabolite protocatechuic acid is produced under the action of enzymes with similar functions as protocatechuate dioxygenase. Then the benzene ring cleavage is completed and enters the β-ketoadipate pathway. The benzoic acid metabolic pathway also plays an auxiliary role in the production of the intermediate metabolite protocatechuic acid. Proteomics and metabolomics studies have found that *A. fumigatus* G-13 can produce peroxidase and aromatic ring cracking enzyme systems to break the Cα-Cβ chemical bond in the lignin structure and oxidize the side chain and catalyze the cleavage of the benzene ring. Then lignin will be further depolymerized to form oligomers such as diarylpropane and aryl ether. Such oligomers will be further oxidized under the action of peroxidase, dehydrogenase, and oxidoreductase to form monocyclic aromatic compounds with simple structures such as syringaldehyde, syringic acid, vanillin, and vanillic acid (In the secondary identification metabolites of the two model compound substrates, a large number of monocyclic aromatic compounds were detected in sinapic acid substrate compared to p-coumaric acid substrate). Under the action of dehydrogenase and dioxygenase, this type of compound undergoes a ring-opening reaction through the protocatechuic acid or catechol pathway to produce β-ketohexanedioic acid intermediate metabolites. It then combines with CoA, decomposes into acetyl-CoA and succinic acid through the β-ketoadipate pathway, and finally enters the tricarboxylic acid cycle. During the degradation of lignin model compounds by *A. fumigatus* G-13, peroxidase can also act

on the ester bond and ether bond between lignin structural units, so that the lignin is decomposed into simple structural units, which are further broken under the action of the aromatic ring cracking enzyme system, thereby aggravating the complete degradation of lignin.