



Optimization of fermentation of *Fomes fomentarius* extracellular polysaccharide and antioxidation of derivatized polysaccharides

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Abstract: Fermentation is a metabolic process that converts sugars into acids, gases, or alcohol. This process occurs in yeasts and bacteria, as well as in muscle cells when faced with a lack of oxygen. In this paper, isolation, culture, purification and extracellular polysaccharides of strain *Fomes fomentarius* were studied. Extraction of polysaccharides from a culture based on *F. fomentarius* extracellular polysaccharides, extracellular polysaccharides fermentation experiments was optimized and compared, the optimal fermentation method was obtained; extracellular polysaccharides were sulfated, phosphorylated experiments, selenium acidified, discussed the preparation of derivative polysaccharides and microscopic detection, and finally studied extracellular polysaccharides on DPPH, The scavenging ability, superoxide anion radical and hydroxyl radical scavenging ability of the derived polysaccharides were compared. The results showed that the extracellular polysaccharide and derivatized polysaccharide of *F. fomentarius* had certain antioxidant activity.

Key words: *Fomes fomentarius*; Response surface optimization; Antioxidation; Derivatized polysaccharide.

Introduction

Fomes fomentarius is a kind of medical fungus. It is a fruiting body of *F. fomentarius*. It grows on trees and rotten wood such as birch and poplar. The surface is a thick, horny husk, gray, light brown or black color, smooth and always with obvious concentric rings (1). Studies have shown that his chemical constituents include organic acids, sugars, polysaccharides and their glycosides, lactones, coumarins and their glycosides, phytosterols, terpenoids, anthraquinones and their glycosides. It is responsible for food, esophageal cancer, stomach cancer, and uterine cancer. Therefore, people want to get more medicinal value from it. *F. fomentarius* have many pharmacological effects, including anti-fatigue, anti-inflammatory, enhancing immune and hypoglycemic activities (2). Polysaccharides can protect neurons from cytotoxicity induced by free radicals (3). Studies have shown that fungal mycelial polysaccharides have potential antioxidant properties (4). These natural antioxidants not only protect food lipids from oxidation but also prevent the damage of free radicals to the human body. A large number of studies have shown that polysaccharides are an important active ingredient of *F. fomentarius*. Therefore, the research of this kind of medicinal fungi has broad application prospects (5).

In this study, the isolation, culture, purification of *F. fomentarius* and the extraction of extracellular polysaccharides from *F. fomentarius* were studied. The fermentation of extracellular polysaccharides was optimized. The preparation of derivative polysaccharides and the observation and test of derivatization were carried out.

Finally, the comparative study of the antioxidant activity of derivative polysaccharides was carried out. The purpose of this study is to further develop and utilize the polysaccharides secreted by *F. fomentarius*.

Materials and Methods

Materials, reagents and instruments

Strain BHT: TBHQ; homemade analytically pure; DPPH: Sigma company. 752 ultraviolet spectrophotometer: Shanghai Precision Scientific Instrument Co., Ltd; Rotary Evaporator: Shanghai Yarong Co. Shanghai Jiuyi Chemical Reagent Co. Ltd. for weak acid, alkali anion cation exchange resin, standard monosaccharide, 1,3-dihydroxy naphthalene and other reagents. SHODEXSB804 chromatographic column, detector differential, gel column (XL=8.0 x 300.00mm), protective column SB-G (XL=6.0 x 50mm), flat membrane machine Saipu membrane technology development Co., Ltd; LNG-T98 freeze concentration centrifugal dryer Taicang Huamei Biochemical Instrument Factory; DHL-A computer current balance pump, DBS-100 automatic part collector Shanghai Huxi analytical instrument Factory; 122PC Spectrophotometer Shanghai Prism Technology Co., Ltd; JY92-2D Ultrasonic Cell Crusher Ningbo New Chicken Institute; DGG-9420A Electric Heating Blower Drying Box Shanghai Senxin Experimental Instrument Co., Ltd; YP-2 Tablet Press Shanghai Mountain Science Instrument Co., Ltd; NI-COLET 5700 Infrared Spectrometer Zhengzhou Medium Spectrum Instrument Equipment Limited company; SephadexG-75 column Shanghai Long billion

Chemical Reagent Co., Ltd.

Detection method

Polysaccharide content determination: phenol-sulfuric acid method (6), Its principle is summarized as follows: Phenol-sulfuric acid method is the use of polysaccharides in the role of sulfuric acid hydrolysis into monosaccharides and quickly dehydrated to produce glucuronic derivatives, and then with phenol to form orange compounds, and then colorimetric determination. The absorbance was measured by spectrophotometer.

Experimental process

Mycelium obtained by fermentation → Cell breakage → Gauze filtration → Concentrate Alcohol precipitation → Freeze-drying → called quality → Deproteinized → Two centrifugation → Decolorization of ethanol → Dried polysaccharides → Determination of Polysaccharides → Purification → Dried polysaccharides → Analysis of polysaccharide components → Derivatization of polysaccharides and evaluation of their antioxidant activities → Observations and conclusions.

Experimental content

Deep fermentation of *F. fomentarius*

Configure the appropriate medium to facilitate the cultivation of *F. fomentarius*, the configuration should be convenient, efficient and green. Preparation of 5% phenol Accurately weigh 5 g of phenol, put it into a test tube, melt it in a water bath at 60 °C until it is completely melted, pour it into a beaker and add water to 100 mL (7). Preparation of anhydrous glucose Take appropriate amount of analytical glucose, pour into the weighing bottle, place in the oven, open the cap, dry at 105 °C for 1 h, take out the weighing until constant weight.

The fungus species of the fungus deposited at 4 °C were transferred to fresh PDA slant medium and then placed in a 25 °C incubator. The hyphae were substantially covered with a slanted surface for use. Under sterile conditions, pick 1 cm² of the shredded mycelium of the sclerotium from the PDA slope and insert it into a 250 mL flask (containing 100 mL of liquid seed medium) and place it. The culture was shaken by shaking at a constant temperature shaker (180 r/min) at 30 °C, and a seed culture solution was obtained after 7 days. According to the inoculation amount of 6%, the first-grade seed liquid was connected to a 250 mL flask (containing 100 mL of liquid seed medium) and then placed on a shaker at 30 °C to shake and culture. After 7 days, the second-stage seed solution was used.

Extraction of crude polysaccharide

Concentrated sugar solution: First obtain the crude sugar solution, then concentrate on the appropriate volume through decompression and finally complete the preparation of a concentrated sugar solution. Ethanolysis of polysaccharides (8): The crude sugar solution after concentration was added with 4 times volume of ethanol, and the polysaccharides were precipitated after 24 hours. Freeze centrifugal drying and weighing quality of crude polysaccharides: The polysaccharides obtained by alcoholysis were transferred to a small plastic centrifugal tube and put into freeze concentration centrifugal

dryer for freeze centrifugal drying (9). The dried polysaccharides are weighed.

Sevag was used to remove protein

Trichloroacetic acid-n-butanol or n-amyl alcohol were mixed with 5:1 (V/V) and added to the sample water solution to shake. The supernatant was centrifugally removed, the gel protein was removed, and the absorbance was measured at ultraviolet wavelengths at 280nm wavelengths. The ethanol decolorization was washed and precipitated several times with 75% ethanol until the color of the precipitate no longer changed. In the oven drying (not more than 60 degrees), that is, a crude polysaccharide. Polysaccharide content determination of total sugar: phenol - sulfuric acid method.

Preparation of exo-polysaccharide

After deep culture, the fermentation broth was obtained by vacuum suction and concentration to a certain volume (10). The final concentration reached 70% by adding anhydrous ethanol. The fermentation broth was kept in the refrigerator at 4°C for the night. The polysaccharide solution was deproteinized by the trichloroacetic acid-n-butanol method, i.e. adding 1/3 volume of trichloroacetic acid-n-butanol (volume ratio 5:1) mixed solution to the crude polysaccharide solution. After shaking fully for several minutes, the polysaccharide solution was put into layers and the upper polysaccharide solution was taken. Repeat the above extracellular polysaccharide many times until the static position can be divided into 2 layers of the bright solution. After adding 1/10 volume of 30% H₂O₂ to the crude polysaccharide solution, the temperature was constant at 50°C until the solution was colorless. The pretreated polysaccharide solution was concentrated in a dialysis bag with a retention of 13 000 U. The tap water flowed for 48 hours and the crude product was obtained by vacuum freeze-drying after concentration.

Determination of mycelial biomass and exopolysaccharide

After culture, the fermentation broth under different fermentation conditions was centrifuged, mycelium was collected, washed and filtered with deionized water, repeated three times, and the mycelium was dried at 50°C to calculate mycelium biomass (g/L) = mycelium dry weight/fermentation liquid volume. The exopolysaccharide was calculated by the phenol sulfuric acid method (11).

Preparation of derivatized polysaccharide

The polysaccharide of sulfate and its preparation

The polysaccharide extracted in this study was derivatized and tested, and the derivatization included sulfation, selenation and phosphorylation (12-13). The principle of the sulfation process is that the polysaccharide dissolved in a certain solvent system reacts with the corresponding sulfating agent under a certain condition to cause certain hydroxyl groups on the polysaccharide residue to be attached to the sulfate group. Taking the chlorosulfonic acid reagent as an example, the thioindigolation reaction of the polysaccharide is a polysaccharide sulfate obtained by neutralizing -H in the polysaccharide hydroxyl group by -SO₃ in a Lewis base solu-

tion. The detection of polysaccharide sulfation includes the presence of a sulfate group on the polysaccharide molecule. The content of SO_4^{2-} can be determined by BaCl_2 -gelatin turbidimetry or the position of the sulfate group on the specific glycocalyx of the sulfate group can be checked by IR. Chemical analysis and nuclear magnetic resonance methods are generally considered to be susceptible to the leaching of sulfate groups during the methylation process. This method is not suitable for the determination of the position of the sulfate group substitution, and the chemical shift can resonate with the position of the carbon species.

Preparation of sulfate reagent

The anhydrous pyridine pre-cooled in the refrigerator was placed in a three-necked bottle with condensation tube and stirring device, cooled by ice salt bath, slowly dripped chlorosulfonic acid under intense stirring condition, pyridine and chlorosulfonic acid was added in a certain proportion, the reaction temperature was controlled at about 4°C , and the esterification reagent was obtained after 40 minutes of reaction. Esterification reaction: accurately weigh 400 mg of HBHS, and suspend it in 35 mN, N-dimethylformamide DMF, adding the prepared esterification reagent, stirring reaction at a certain temperature for 1-3 hours. When 95% ethanol was added, the concentration of ethanol was 70%. After centrifugal separation, the precipitate was dissolved in distilled water, dialyzed for 7 hours in flowing water, dialyzed for 12 hours in distilled water, and sulfated polysaccharide was obtained by vacuum concentration and freeze-drying.

The polysaccharide of selenylation and its preparation

The chemical structure of selenium polysaccharide is different from ordinary polysaccharides, forming a special selenium oxygen bond. Selenium polysaccharide has a variety of physiological activities, which can antagonize the ability of heavy metal poisoning and anti-reactive oxygen damage by increasing the activity of related enzymes (14-15). It can inhibit the DNA synthesis of cancer cells and inhibit the growth of cancer cells. The separation and purification of selenized polysaccharides are similar to the separation and purification of general polysaccharides.

Referring to other people's methods, the purified polysaccharide was dissolved in 25 mL ultra-pure water, and then dissolved in isomagnetic stirring and added 1 mL glacial acetic acid. Sodium selenite was dissolved in a small amount of ultra-pure water and slowly added to the above-mentioned polysaccharide solution and heated to 50°C for 48 h. After the reaction is completed, 3 times the volume of absolute ethanol will be added to make it precipitate completely centrifugally. After precipitation, washed with absolute alcohol for 3 times and added a small amount of ultrapure water to dissolve. 24h was purified by ultrapure water, and selenized polysaccharide was obtained by freeze-drying.

The polysaccharide of phosphorylated and its preparation

Among the sugar derivatives, phosphate esters are a relatively important class of sugar derivatives, and phosphate esters of sugar compounds such as sulfates

are also important active substances. They have great potential for developing new drugs. Phosphate as a reflecting reagent for phosphorylation, their reactivity is extremely low, and it is difficult to obtain a product with a high degree of substitution. Compared with phosphoric acid, the advantage is that it does not generally cause degradation of polysaccharide. Commonly used as phosphorylating reagent is hydrogen phosphate. Sodium, sodium dihydrogen phosphate, sodium tripolyphosphate, sodium metaphosphate or a mixed salt thereof. Put a certain amount of phosphate in a beaker, add an appropriate amount of water, stir to completely dissolve it. Adjust with acid to the desired The pH value is added to the sugar sample while stirring, and the reaction is heated. The degree of substitution of the product obtained by the phosphorylation method is extremely low, and the degree of substitution is generally less than 0.05. In order to prepare a product with a high degree of substitution, repeated operations are often required.

Mixing 8.57 g sodium tripolyphosphate with 1.43 g sodium trimetaphosphate, dissolving with 5.0 g sodium sulfate in 100 mL double steamed water, then adding 1.00 g polysaccharide, then adjusting pH to 9.0 with NaHCO_3 , adding 5 times volume of 95% $\text{C}_2\text{H}_5\text{OH}$ after 5.0 h of constant temperature water bath at 80°C , standing at room temperature for 24 hours, centrifuging, collecting precipitation and dissolving precipitation with distilled water; Dialysis was performed on distilled water for 2 days. Finally, PFFEP-1 was obtained by filtration and freeze-drying.

Determination of antioxidant activity

Effect of DPPH on scavenging capacity

The free radical scavenging activity of extracellular polysaccharide DPPH from *F. fomentarius* xylostella was studied in Shimadaetal literature. Under room temperature, the 2 mL extracellular polysaccharide solution and 2 mL 0.2 mmol/L DPPH ethanol solution were mixed evenly. The reaction time was 30 minutes and the absorbance was measured at 517 nm. Low absorbance showed that polysaccharides had higher free radical elimination ability (16). The formula of DPPH scavenging free radicals is:

$$\text{Scavenging rate (\%)} = (1 - A_1/A_0) \times 100$$

In the form: A_0 is empty; A_1 is extracellular polysaccharide; BHT and TBHQ are positive control.

Superoxide radical scavenging test

Tris-HCl buffer of 50 mmol/L pH 8.2 and exopolysaccharide solution of 3.8 mL of different concentrations was reacted at 25°C for 5 minutes, and 200 L pyrogallol was added after 10 minutes. After 5 minutes, the solution was detected at 320 nm for 4 minutes, and the change of absorbance was measured. The rate of self-oxidation is expressed as $\Delta A/\text{min}$, and the inhibition rate of scavenging superoxide anion radical is:

$$\text{Inhibition rate of phthalic three phenol autoxidation (\%)} = (1 - A_1/A_0) \times 100$$

In the form: A_0 is a blank self-oxidation rate; A_1 is the rate of self-oxidation under the condition of test sample; BHT and TBHQ are positive control; Ascorbic acid was a positive control.

Determination of hydroxyl radical scavenging capacity

The reaction system (3.0 mL) consisted of 0.2 mmol/L EDTA-Fe chelate, 3.0 mmol/L H_2O_2 and 2.0 mmol/L salicylic acid, which were reacted in a water bath at 37 °C for 15 min, followed by the addition of 0.8 mL samples of different concentrations in a water bath at 37 °C for 60 min. The scavenging capacity of hydroxyl radical was measured at 510 nm. The formula is:

$$\text{Scavenging ability (\%)} = (1 - A_1/A_0) \times 100$$

In the form: A_0 is the absorbance of the blank sample; A_1 is the absorbance of exopolysaccharide.; Ascorbic acid was a positive control.

Results and Discussion

Effects of carbon and nitrogen sources on the yield of polysaccharides

Ten different carbon sources (30g/L): glucose, maltose, sucrose, lactose, galactose, fructose, mannitol, xylose, soluble starch and malt extract were selected. Based on the yield of extracellular polysaccharide and mycelium content, the best carbon source was selected by deep culture (18). High mycelial biomass and exopolysaccharides were obtained when the carbon sources were glucose, sucrose, galactose and mannitol. When the carbon source was glucose, the mycelial biomass was the highest (7.48 g/L), and sucrose was the carbon source, the yield of extracellular polysaccharide was the highest (0.89 g/L). However, there was no significant difference in the yield of exopolysaccharides between glucose and sucrose (19). This may be due to different carbon sources inhibiting the degradation of microbial secondary metabolism. This phenomenon has been found in many fungi in deep culture. Comparing with other carbon sources, sucrose was selected as a fermentation carbon source because of its low cost and easy to obtain. Ten nitrogen sources (5.0g/L): ammonium nitrate, ammonium chloride, sulfuric acid, phosphoric acid, urea, L-lysine, L-aspartate, peptone, tryptone, beef paste were selected. When the nitrogen source was peptone or tryptone, mycelial biomass and extracellular polysaccharide increased significantly (20-23). In the organic nitrogen source, the mycelium biomass was 8.15g/L and the yield of exopolysaccharide was 0.89g/L. Compared with organic nitrogen sources, mycelial biomass and extracellular polysaccharide content of inorganic nitrogen sources were relatively low. This is related to the fact that most fungi in the literature prefer complex organic nitrogen sources, which may require essential amino acids in deep culture, which inorganic nitrogen does not have.

The results showed that the content of *F. fomentarius* extracellular polysaccharide increased first and then decreased with the increase of sucrose when the inoculation amount was fixed and the casein peptone was set to a certain value (24). The contour plots showed that the shape tended to be elliptic, indicating that the interaction between the two factors was significant. The concentration of contour lines showed that the effect of sucrose and casein peptone on the content of extracellular polysaccharide was almost the same. Sucrose and casein peptone had the same effect on the production of *F. fomentarius* extracellular polysaccharide. The interac-

tion of the amount of surface factors is shown in Figure 1.

It can be seen from Fig. 2 that when sucrose is set to a certain value under the condition of fixed casein, the polysaccharide content first increases gradually with the increase of the inoculum amount, and then gradually decreases. The shape of the contour line tends to be round, indicating that the interaction between sucrose and inoculum is not significant.

It can be seen from Fig. 3 that under the condition of fixing sucrose, when the inoculum size is set to a certain value, the EPS content first increases greatly with the increase of casein, and then decreases slightly. It can be seen from the contour plot that the pattern is close to a circle, and the interaction between the two influencing factors of casein and inoculum is not significant.

Influence of the C/N ratio

Protein peptone was determined as a nitrogen source (5.0 g/L), and the amount of sucrose was changed. The C/N ratio was adjusted as follows: 1:1, 3:1, 6:1, 9:1, 12:1, 18:1, 24:1, 36:1, 48:1. The optimum C/N ratio was 6:1. The mycelial biomass was 8.18 g/L and the yield of extracellular polysaccharide was 0.92 g/L.

Trace element

The effects of mineral elements on mycelial biomass and exopolysaccharide production were studied with different mineral ions at the same concentration (0.05%). As shown in Table 1, the optimum combination is MgSO_4 and CaCl_2 , which is consistent with the preference of most basidiomycetes for Mg^{2+} and Ca^{2+} in deep culture. It has been reported that Ca^{2+} has dual roles in fungal growth: (1) it can change cell membrane permeability by controlling intracellular calcium concentration gradient and the activity of some fungal enzymes in the

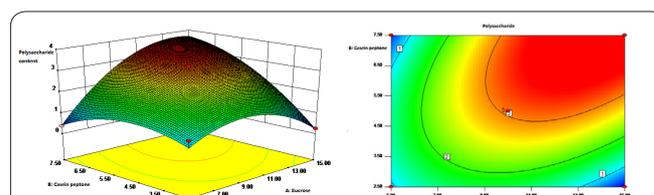


Figure 1. Interaction between sucrose and casein on the yield of *F. fomentarius* extracellular polysaccharide.

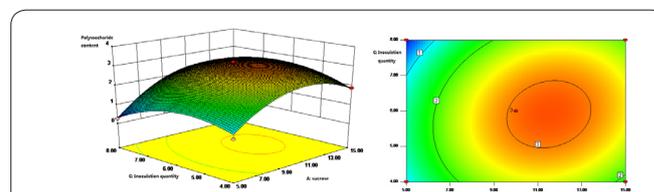


Figure 2. Interaction of sucrose and inoculum on polysaccharide yield of *F. Fomentarius*.

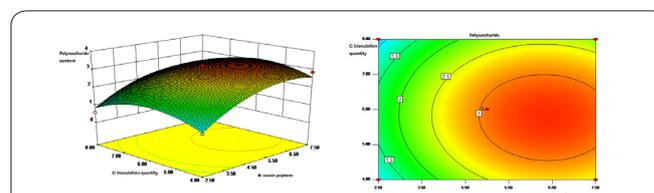


Figure 3. Interaction of casein and inoculum on polysaccharide yield of *F. Fomentarius*.

Table 1. Effects of minerals and vitamins on mycelial biomass and polysaccharides in submerged culture of phahoda.

Factor	Mycelial biomass (g/L)	P<0.01	Exopolysaccharide (g/L)	P<0.01
Mineral	MgSO ₄	7.35±0.86	0.78±0.06	*
	K ₂ HPO ₄	7.16±0.57	0.62±0.08	
	KH ₂ PO ₄	7.28±0.79	0.54±0.08	*
	CaCl ₂	7.43±0.36	0.72±0.07	*
	FeSO ₄ ·7H ₂ O	4.17±0.53	0.21±0.03	*
	MnSO ₄	3.12±0.24	0.29±0.05	**
	CaCl ₂ +MgSO ₄	7.96±0.48	0.84±0.08	
	blank	7.03±0.41	0.55±0.07	
Vitamin	V _{B1}	6.48±0.26	0.62±0.04	
	V _{B2}	8.16±0.63	0.81±0.05	*
	V _{B6}	4.86±0.41	0.59±0.05	*
	V _D	7.17±0.83	0.75±0.07	*
	inositol	5.49±0.31	0.51±0.03	*
	folic acid	7.65±0.84	0.76±0.05	*
	blank	6.63±0.59	0.56±0.08	

cell wall; (2) it can inhibit the synthesis of biopolymers through intracellular calcium ion gradient, thereby affecting the content of protein and neutral sugar. Mg²⁺ is also important for all fungi. It assists in the reaction of enzymes and the stability of the plasma membrane. It is evident that Mg²⁺ and Ca²⁺ can promote mycelial biomass and exopolysaccharide production in deeply cultured fungi. Vitamins, at very low concentrations, usually act as coenzymes or coenzyme-containing components in cells that catalyze microbial growth. In this experiment, V_{B1}, V_{B2}, V_{B6}, V_D, inositol and folic acid were selected to investigate the effects of vitamins. Based on mycelial biomass and extracellular polysaccharide production, higher mycelial biomass and extracellular polysaccharide could be obtained by deep fermentation when V_{B2} was used as a growth factor.

Effects of incubation temperature and initial pH on the yield of Polysaccharides

In this experiment, the best culture temperature was selected based on the exopolysaccharide yield of the fungus. The mycelium biomass was 7.48 g/L and the extracellular polysaccharide of *F. fomentarius* was 0.92 g/L when the culture temperature was 25, which was lower than that of other fungi when the culture temperature was 20. Initial pH of fermentation broth may affect cell morphology and structure, the function of the cell membrane, absorption of various nutrients and biosynthesis of functional components. In the experiment, when the initial pH was 6.0, the maximum amount of extracellular polysaccharide (0.92g/L) could be produced by fermentation. When the initial pH was 5.0, the optimal mycelial biomass was 7.65g/L, but there was no significant difference between the initial pH of 5.0 and 6.0.

Effect of medium capacity and speed of shaking table

In the deep culture of higher fungi, the capacity of the medium is closely related to the supply of oxygen. In the deep culture of medicinal fungi, oxygen supply can obviously affect the formation and accumulation of bioactive substances. Therefore, medium-capacity plays an important role in the production of mycelium

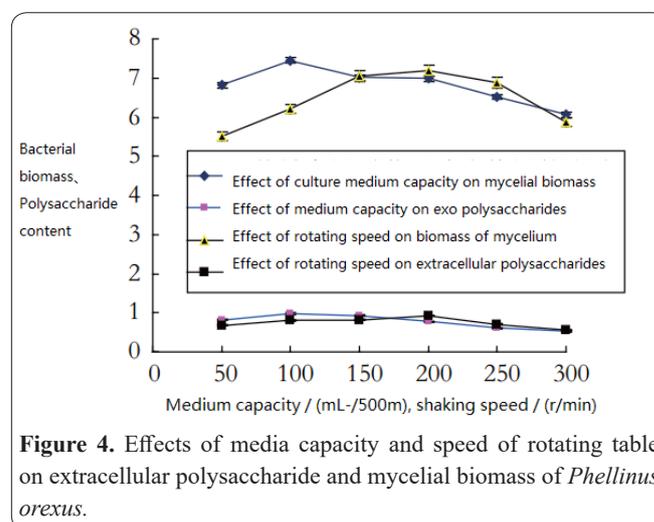


Figure 4. Effects of media capacity and speed of rotating table on extracellular polysaccharide and mycelial biomass of *Phellinus orexus*.

and extracellular polysaccharide. In this experiment, *F. fomentarius* was cultured in a 500 mL triangular flask, changing the medium capacity, and other conditions remained unchanged. It was found that the mycelial biomass and extracellular polysaccharide production did not change significantly when the medium capacity increased from 50 mL to 200 mL. The mycelial biomass and extracellular polysaccharide production decreased rapidly over 200 mL. Considering the economic benefits, the medium-capacity increased from 100 mL to 150 mL, which was the best for mycelial biomass and extracellular polysaccharide yield. In addition, when the shaker speed increased from 150 r/min to 200 r/min, mycelial biomass and extracellular polysaccharide yield did not change significantly, and the output decreased rapidly as the rotational speed continued to increase as the Figure 4 shows.

Optimization of extracellular polysaccharides

The effects of carbon source, nitrogen source, mineral element, culture temperature, initial pH, medium capacity, rotating speed of shaker and culture time on polysaccharide yield were investigated by a single factor experiment. According to the results of the single-factor experiment, an orthogonal experiment was carried out to obtain the best medium formula (17).

Based on the mycelial biomass and extracellular polysaccharide content, the influential factors were screened out: sucrose, peptone, vitamin and mineral elements, and further optimized by orthogonal L16. The fermentation temperature, initial pH, medium capacity, rotating speed of shaker, age of bacteria, inoculation quantity and culture time were controlled at 25 C, 6.0, 100 mL, 150 r/min, 8 d, 6% and 7d, respectively. The orthogonal design of the factor level is shown in Table 2. The results of the orthogonal test are shown in Table 3, and the analysis is shown in Table 4.

According to Table 1, four groups of data with different levels of factors were used. The first group was multiplied by sucrose, peptone and trace elements in the second group; the third group was multiplied by CaCl₂, while the fourth group was multiplied by each factor. In addition, adding 0.5 times the concentration of CaCl₂ solution constitutes a factor level orthogonal design experiment.

The results of Table 4 showed that the order of influence of each factor on mycelial biomass was sucrose > peptone > CaCl₂ > MgSO₄ > VB₂, and the order of influence on extracellular polysaccharide yield was sucrose > peptone > CaCl₂ > VB₂ > MgSO₄. The selected factors had significant effects on mycelial biomass and exopolysaccharide production during fermentation. As shown in Table 1, the formula is: sucrose 10.0 g/L, peptone 5.0 g/L, CaCl₂ 1.0 g/L, MgSO₄ 0.25 g/L, V_{B2} 0.50 mg/L. Under these conditions, the biomass of mycelium was 21.06 g/L and the yield of exopolysaccharide was 3.52 g/L, which was significantly higher than that of the orthogonal experiment group. Compared with K value, A₄B₂C₃D₁E₂ was the optimum condition for mycelium biomass, that is, the optimum formula for a submerged culture of *F. fomentarius* xylophilus was: sucrose 10.0 g/L, peptone 5.0 g/L, CaCl₂ 1.0 g/L, MgSO₄ 0.25 g/L, V_{B2} 0.50 mg/L. Under these conditions, the biomass of mycelium was 21.06 g/L and the yield of exopolysac-

Table 2. four groups of data with different levels of factors.

Level	Factor				
	Sucrose (g/L)	Peptone (g/L)	CaCl2/(g/L)	MgSO4/(g/L)	VB2/(mg/L)
	A	B	C	D	E
1	3	3	0.25	0.25	0.25
2	5	5	0.5	0.5	0.5
3	8	8	1.0	0.75	0.75
4	10	10	1.5	1.00	1.00

Table 3. Orthogonality of mycelial biomass and extracellular polysaccharides in submerged culture of foramen PHAE Optimization results.

Number	Factor					Biomass (g/L)	Extracellular polysaccharide(g/L)
	A	B	C	D	E		
1	1	1	1	1	1	8.51±0.64	0.9440.08
2	1	2	2	2	2	9.35±0.36	1.35±0.07
3	1	3	3	3	3	7.56±0.35	1.04±0.09
4	1	4	4	4	4	6.26±0.47	0.96±0.07
5	2	1	2	3	4	11.30±0.30	1.25±0.06
6	2	2	1	4	3	9.84±0.56	1.81±0.05
7	2	3	4	1	2	13.27±0.67	1.77±0.11
8	2	4	3	2	1	10.35±0.55	1.26±0.08
9	3	1	3	4	2	16.26±0.65	2.01±0.13
10	3	2	4	3	1	14.35±0.46	2.71±0.08
11	3	3	1	2	4	9.46±0.28	3.51±0.09
12	3	4	2	1	3	10.40±0.42	3.51±0.09
13	4	1	4	2	3	16.09±1.86	1.92±0.07
14	4	2	3	1	4	20.05±0.95	1.18±0.06
15	4	3	2	4	1	13.25±0.92	2.71±0.08
16	4	4	1	3	2	8.51±0.64	0.9440.08

Note: A, B, C, D and E represent sucrose, peptone, VB₂, MgSO₄ and CaCl₂ respectively; each group is parallel three times, and the result is expressed as average (±) standard deviation.

Table 4. Orthogonal test analysis of mycelial biomass and exopolysaccharides in submerged culture of foramen PHAE.

Index	Biomass (mg/L)					Extracellular polysaccharide (mg/L)				
	A	B	C	D	E	A	B	C	D	E
K ₁	94.90	155.22	114.70	156.42	139.36	13.42	15.23	20.64	22.32	22.31
K ₂	133.86	160.62	132.56	135.43	149.86	17.39	17.40	25.53	23.39	19.04
K ₃	151.41	130.57	162.58	132.65	131.64	28.56	25.29	16.04	18.14	23.46
K ₄	181.53	114.27	149.92	136.92	140.93	23.24	24.74	20.36	18.78	17.84
K ₁	7.92	13.05	9.71	13.04	11.62	1.12	1.28	1.75	1.86	1.86
K ₂	11.14	13.37	11.02	11.26	12.49	1.35	1.46	2.13	1.96	1.58
K ₃	12.62	10.86	13.54	11.05	11.02	2.38	2.11	1.34	1.52	1.94
K ₄	15.12	9.52	12.88	11.41	11.73	1.92	2.05	1.70	1.58	1.49

Note: the biomass of K_i= mycelia or the average value of exopolysaccharide/3.

charide was 3.52 g/L, which was significantly higher than that of the orthogonal experiment group.

Analysis of exosporous polysaccharides and exopolysaccharide derivatives

IR spectra of polysaccharide

Fig.5 shows the IR spectra of polysaccharide. The absorption peaks at 4000-2500 cm^{-1} are X-H stretching vibrations, X can be O, N, C, S atoms, but they have their ranges. There is a strong absorption peak at 3457.4 cm^{-1} in the spectrum, so the group is O-H; there is an absorption peak at 2926.3 cm^{-1} , and the group is C-H.

There are two-bond stretching vibrations at 200-1500 C m^{-1} , including C=C, C=O, C=N, -NO₂ and shear vibrations of -NH₂. The absorption peaks at 1658.8 and 1554.7 cm^{-1} in the atlas contain C=O and sugar esters should contain ester amine. In addition, there are other absorption peaks at 2000 ~ 1500 cm^{-1} , indicating that there are other double bond groups. 1500-600 cm^{-1} is a single bond vibration and fingerprint region, mainly including C-O, C-N, C-X (halogen), N-O and C-C, C-O related skeleton vibration. The absorption peaks in this region are more complicated. Pyranoside has three absorption peaks at 1100-1010 cm^{-1} , and the polysaccharide contains pyranoside. Near 840 cm^{-1} indicated the alpha glycosidic bond.

Infrared spectroscopy analysis of derivatized polysaccharides

Infrared spectroscopy was used to analyze the contents of extracellular polysaccharide sulfated, selenized and acidified, purified water eluted and phosphorylated. The analysis curve was shown in Fig. 6 (1-4).

Electron microscopic determination of derivatized polysaccharides

Electron microscopic determination of the content of the derived polysaccharides is shown in the following figure. Fig. 7 (1) shows that the polysaccharides are closely arranged and the overall surface has an irregular convex and concave shape, and the overall surface of the polysaccharides is rough. Fig.17 (2-4) is the result of polysaccharide measurement under different parts and magnification.

Then we sulfated the polysaccharides and observed the sulfated polysaccharides by electron microscopy. The results are shown in Figure 7. Figure 8 (1-4) shows

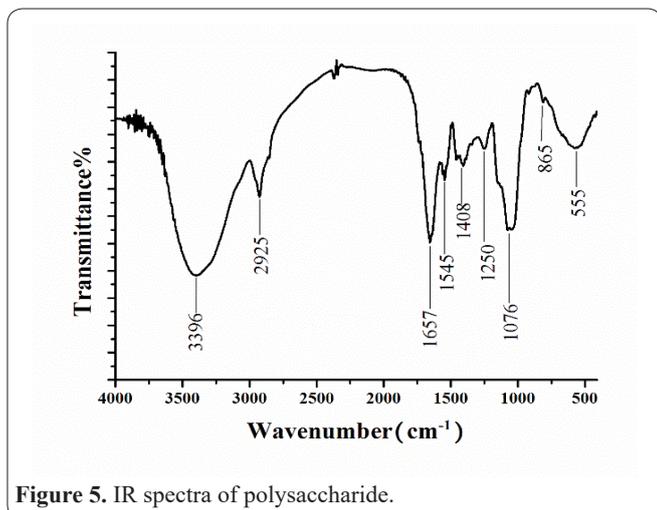


Figure 5. IR spectra of polysaccharide.

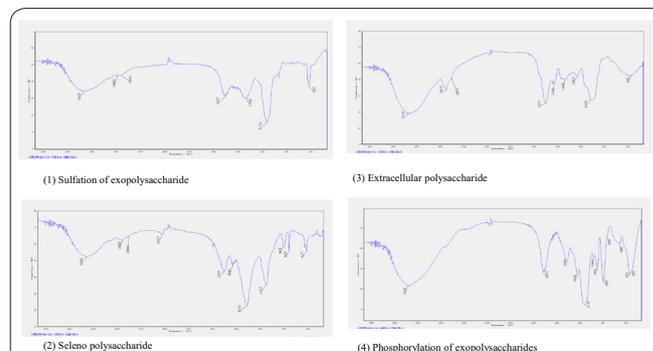


Figure 6. Infrared transmittance of polysaccharides in different treatment processes.

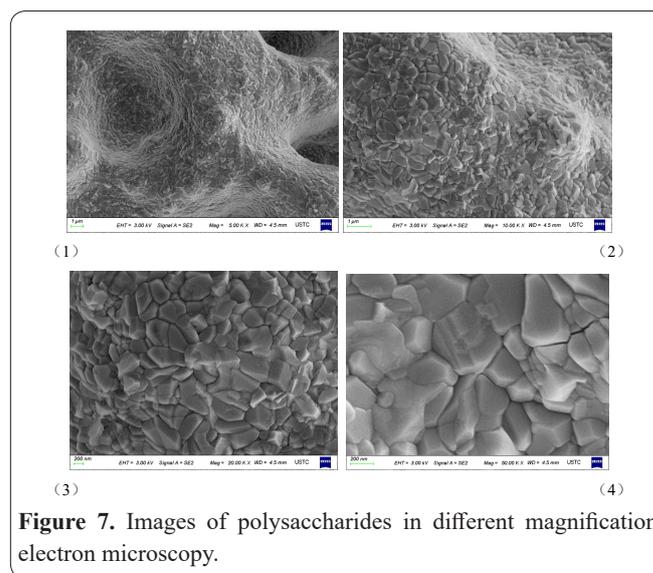


Figure 7. Images of polysaccharides in different magnification electron microscopy.

that the original arrangement of the polysaccharides changed from tight to sparse after the sulfation of the polysaccharides.

Then we carried on the phosphorylation experiment to the polysaccharide, still observed the polysaccharide after the phosphorylation experiment by the electron microscope, the result is as shown in Figure 9, in which Figure 9 (1-4) are a different magnification of the phosphorylated polysaccharide electron microscope photos, from the graph, after the phosphorylation experiment of polysaccharide, the original arrangement of sparse sulfated polysaccharide structure. Stratification is more clear, and polysaccharides are relatively isolated.

The electron microscopic results of the polysaccharide after selenation are shown in Fig. 10. Selenic acid can bind negatively with neutral sugar. The selenium atom in selenic acid has an empty orbital that can hold electrons and act as a Lewis acid. In an aqueous solution, a selenium atom can form a coordination compound with OH and make the solution acidic. The coordination of polyhydroxy carbohydrates with selenic acid makes the solution weakly acidic, which can improve separation and increase sample loading. The concentration of the selenic acid solution used was 0.03 mol/L. When forming a carbohydrate complex with selenic acid, their optical rotation can be increased. The addition of selenic acid to the carbohydrate increases its optical rotation, which facilitates the identification of the coordination reaction between the carbohydrate and selenic acid.

The results of electron microscopy showed that the extracellular polysaccharides were successfully sulfa-

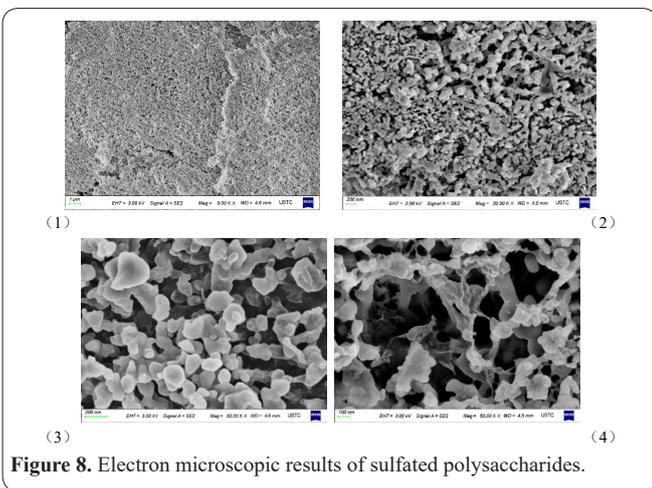


Figure 8. Electron microscopic results of sulfated polysaccharides.

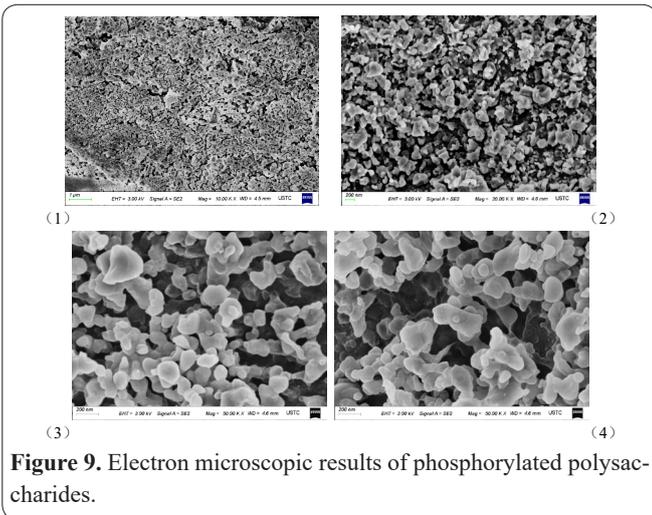


Figure 9. Electron microscopic results of phosphorylated polysaccharides.

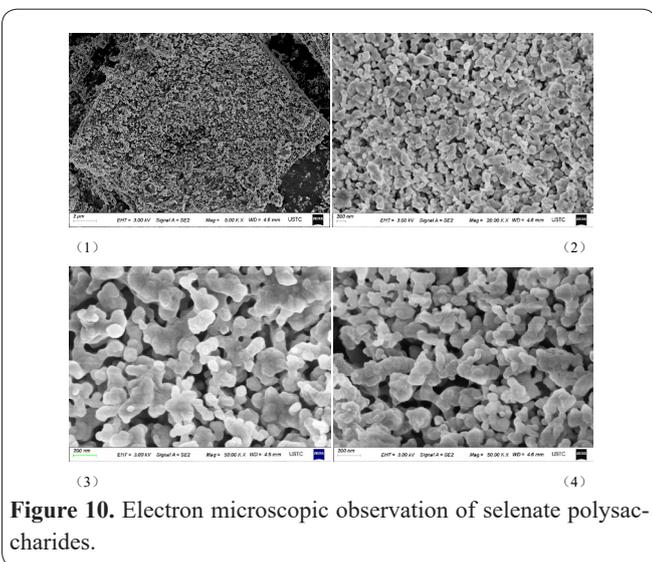


Figure 10. Electron microscopic observation of selenate polysaccharides.

ted, selenium acidified and phosphorylated. The molecular structure of the polysaccharides changed significantly, which proved that the experiment was successful.

Study on antioxidant activity of *F. fomentarius* polysaccharides

In this study, the DPPH method was used to determine the scavenging capacity of free radicals, superoxide radicals and hydroxyl radicals. Compared with other methods, DPPH scavenging free radicals can be used to evaluate the antioxidant activity of active ingredients in a relatively short time. As shown in Fig.11,

compared with TBHQ and BHT, the scavenging rate of extracellular polysaccharides of *F. xylophilus* was lower, but the scavenging activity of free radicals was obvious at the tested concentrations. The scavenging ability of exopolysaccharides from *Phellinus* is positively correlated with its concentration. The clearance rate of DPPH by 800 mg/mL exopolysaccharide can reach 80%.

Superoxide is a relatively weak oxidant in different oxygen active components. However, it can decompose and form strong oxygen active components, such as hydroxyl radicals, which can participate in lipid peroxidation and damage cell membrane in vivo. In order to evaluate the ability of polysaccharides to eliminate superoxide, pyrogallol was used to inhibit the autoxidation of polysaccharides. The color change showed the content of superoxide anion radical and the antioxidant ability of polysaccharides. The inhibitory effect of exopolysaccharides at different concentrations on pyrogallol was shown in Figure 12. With the increase of concentration, the inhibitory ability was also enhanced, that is, the scavenging ability of exopolysaccharides from *F. fomentarius xylostella* was positively correlated with its concentration. When the concentration reaches 500 mg/mL, the inhibition rate can reach 90%.

As shown in Fig. 13, the scavenging ability of extracellular polysaccharides from *F. fomentarius xylostella* to hydroxyl radicals showed a dose-effect relationship, and the scavenging ability increased with the increase of polysaccharide concentration. From Fig. 11, we can see that extracellular polysaccharide has weak antioxidant activity compared with ascorbic acid, but still has strong antioxidant activity. The extracellular polysaccharides of *F. fomentarius xylostella* have potential antioxidant activity. Polysaccharides from different solvents have

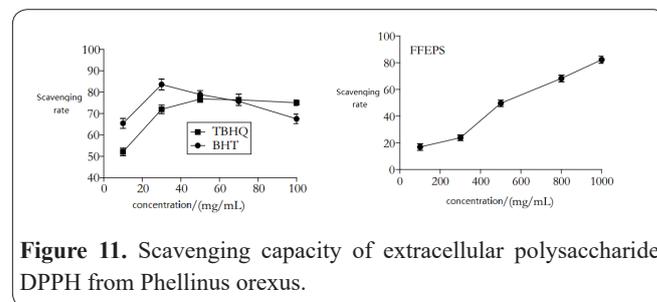


Figure 11. Scavenging capacity of extracellular polysaccharide DPPH from *Phellinus orexus*.

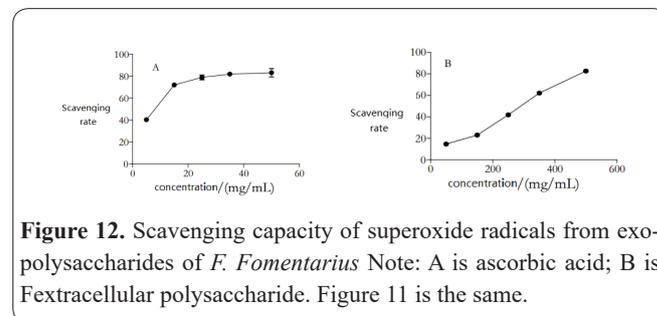


Figure 12. Scavenging capacity of superoxide radicals from exopolysaccharides of *F. Fomentarius*. Note: A is ascorbic acid; B is Fextracellular polysaccharide. Figure 11 is the same.

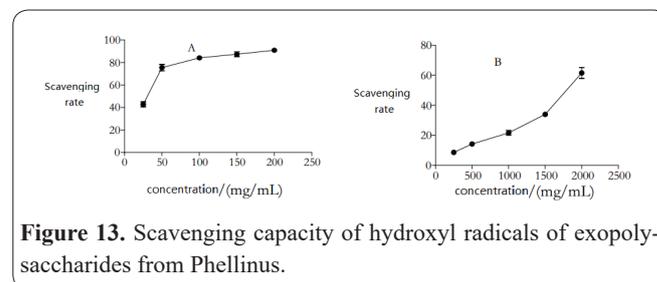


Figure 13. Scavenging capacity of hydroxyl radicals of exopolysaccharides from *Phellinus*.

certain differences in scavenging activity. Although the results are lower than those of the positive control group, they can still be used as an effective fungal metabolite for scavenging free radicals.

The antioxidant activity of derivatized polysaccharides was also studied. The results showed that the antioxidant activity of derivatized polysaccharides changed significantly, showing that the antioxidant activity increased significantly.

Fermentation is the incomplete decomposition of some metabolites (organic compounds) into simpler compounds with energy by fermentation. Fermentation of sugars with yeast, the oldest chemical process used by humans, is still very important for the preparation of ethanol and some other alcohols (25-26).

In this paper, the culture of *F. fomentarius* and the preparation and detection of extracellular polysaccharides of *F. fomentarius* were described. The technology of the deep culture of *F. fomentarius* was optimized. The results showed that the extracellular polysaccharides of *F. fomentarius* could be used for large-scale preparation. Sevag method was used to deproteinize polysaccharides. The polysaccharides were purified by ion-exchange chromatography and gel chromatography and changed from light brown to yellowish crystals. Gel chromatography showed that polysaccharides from Polypores of the *F. fomentarius* were composed of polysaccharides. The polysaccharide of *F. fomentarius* *amurensis* was polymerized by galactose. In this paper, the extracted polysaccharides were subjected to sulfation, selenation and phosphorylation tests. The experimental results show that the polysaccharide derivative has been successfully prepared. Infrared spectrum analysis showed that the polysaccharide of *P. xylophilus* was a kind of pyranose, which was linked by a-glycoside bond and contained ester amine and other groups. Polysaccharides and their derivatives are important biologically active substances. The antioxidant activity of the derivatives polysaccharides increased significantly. Although they have not been widely used so far, they have shown attractive prospects.

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