Optimization of extraction method for extracellular polymeric substances of *Phanerochaete chrysosporium*

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**Abstract.** There are many methods for extracting extracellular polymeric substances of white rot fungi. In this experiment, we studied the efficiency of NaOH method, EDTA-2Na method and high-speed centrifugation method. Without destroying the cell structure and the activity of extracellular polymeric substances, we maximized the effect of extracellular polymeric substances. The results showed that in the ultrasonic centrifugation method, the polysaccharide concentration in the extracted EPS solution gradually increased with the increase of the ultrasonic action time. The concentration of polysaccharide in the EPS solution extracted under the ultrasonic action for 10 min and 12000 rpm was the highest (613.638 mg/L); on the whole, the ultrasonic treatment time has a greater impact on the extraction efficiency of polysaccharides in the extracellular polymer of *Phanerochaete chrysosporium*, and the polysaccharide concentration changes greatly. This study can provide useful information for the follow-up experiments related to extracellular polymeric substances of white rot fungi.

1. **Introduction**

White rot fungi are a collection of filamentous fungi that cause white rot in wood, so they are called white rot fungi. Deterioration of trees or wood, usually under natural conditions [1]. The surface of white rot fungi mycelium has a large amount of filamentous structure, which increases the specific surface area, and is rich in extracellular polysaccharides[2], which can tolerate and adsorb heavy metals in the environment, so that white rot fungi are affected by microbial remediation of heavy metal pollution[3]. Extracellular polymers are polymeric compounds that are secreted by microorganisms under specific conditions and attached to or around the cell surface, and their components are generally dominated by polysaccharides, proteins, and humus [4]. Extracellular polymers are rich in functional groups (such as hydroxyl, carboxyl, amino, etc.), which play an important role in the active defense and removal of heavy metal ions by microorganisms [5].

EPS is combined on the surface of the cells. To study the characteristics of EPS production and its role in the process of heavy metal removal, it is necessary to select an appropriate method to extract EPS. There are many methods for extracting EPS, such as ultrasonic method, cation exchange resin (CER) method, NaOH method, EDTA-2Na method, high-speed centrifugation method, etc. [6].

The purpose of this experiment is to study the extraction method of various extracellular polymers of *Phanerochaete chrysosporium*, and find a suitable method for the extracellular polymer of
Phanerochaete chrysosporium, without damaging the cell structure and the activity of extracellular polymer. Maximize the extraction amount of extracellular polymer, and finally determine the extraction method used in the subsequent experiments, and provide reference for subsequent experiments.

2. Materials and Methods

2.1 Acquisition of Phanerochaete Chrysosporium
In this paper, Phanerochaete chrysosporium ME-446, a model strain of white rot fungus, was used. The strain was purchased from China's typical culture preservation center of Wuhan University. After activation, the strain was subcultured on glucose potato AGAR medium and cultured in microelement liquid medium for experiments.

2.2 EPS extraction method
In this paper, based on the published report on the extraction method of microbial EPS, combined with the nature of Phanerochaete chrysosporium and the needs of subsequent experiments, consider using EDTA-2Na method, ultrasonic method and high-speed centrifugation to extract EPS [7].

2.3 Three methods of measuring indicators and their measurement
In order to more accurately express the extraction effect of high-speed centrifugation, EDTA-2Na method and ultrasonic centrifugation on the extracellular polymer of Phanerochaete chrysosporium, the concentration of protein, polysaccharide and EPS extracted by three methods can be compared. Suitable extracellular polymer extraction methods are employed with reference to subsequent experimental purposes.

3. Results and Discussion

![Figure 1. the protein concentration in the EPS solution extracted by three methods](image)

As can be seen from Figure 1, the protein concentration in the EPS solution extracted by high-speed centrifugation, EDTA-2Na and ultrasonic centrifugation is different. High-speed centrifugation is used as a reference, and the protein concentration and high-speed centrifugation of EPS extracted by ultrasonic centrifugation are used. The extraction method was almost the same, and the protein concentration in EPS extracted by EDTA-2Na method was significantly higher than that extracted by high-speed centrifugation. Among the three methods, the EDTA-2Na method has the highest protein extraction efficiency. If the concentration of protein in EPS under heavy metal stress and the role of protein in heavy metal removal by EPS are studied in subsequent experiments, the extracellular polymer is considered to be extracted by EDTA-2Na method.
Figure 2. the concentration of polysaccharides in EPS solutions extracted by three methods

Figure 2 shows that there are significant differences in the concentration of polysaccharides in EPS solutions extracted by high-speed centrifugation, EDTA-2Na and ultrasonic centrifugation, using high-speed centrifugation as a reference. The concentration of polysaccharides in EPS extracted by EDTA-2Na is generally lower than that of polysaccharides extracted by high-speed centrifugation, and the concentration of polysaccharides in EPS extracted by ultrasonic centrifugation is generally higher than that of polysaccharides extracted by high-speed centrifugation[9]. The extraction efficiency of polysaccharides in the three methods was ultrasonic centrifugation, high-speed centrifugation and EDTA-2Na. If we focus on the concentration of polysaccharides in EPS under heavy metal stress and the role of polysaccharides in the removal of heavy metals in EPS, we consider the use of ultrasonic centrifugation to extract extracellular polymers.

Figure 3. EPS concentrations extracted by three methods

From Figure 3, it can be seen that there are significant differences in EPS concentrations extracted by high-speed centrifugation, EDTA-2Na and ultrasonic centrifugation, using high-speed centrifugation as a reference. The EPS concentration extracted by EDTA-2Na method is generally lower than the EPS concentration extracted by high-speed centrifugation, and the EPS concentration extracted by ultrasonic centrifugation is generally higher than the EPS concentration extracted by high-speed centrifugation. The EPS extraction efficiency of the three methods was ultrasound centrifugation, high-speed centrifugation and EDTA-2Na method. If the concentration of EPS under heavy metal stress and the removal of heavy metals by EPS are focused in subsequent experiments, the use of ultrasonic centrifugation to extract extracellular polymers is considered for further study.

4. Conclusion
(1) In the EDTA-2Na method, as the concentration of EDTA-2Na solution increases, the protein
concentration in the extracted EPS generally increases. However, EPS was extracted by EDTA-2Na method, and EDTA-2Na with chelation was added to EPS, which caused some interference to the subsequent experiments.

(2) In the ultrasonic centrifugation method, the concentration of polysaccharide in the extracted EPS solution gradually increased with the increase of the ultrasonic action time. The concentration of polysaccharide in the EPS solution extracted under the conditions of 10 min and 12000 rpm was the largest. It reached 613.638 mg / L; on the whole, the ultrasonic treatment time had a great influence on the extraction efficiency of polysaccharides in the extracellular polymer of *Phanerochaete chrysosporium*, and the polysaccharide concentration changed greatly.

(3) The polysaccharide in the extracellular polymer is the most important component, and the polysaccharide concentration is significantly higher than the protein concentration. If the concentration of EPS under heavy metal stress and the removal of heavy metals by EPS are mainly studied in subsequent experiments, the extracellular polymer is extracted by ultrasonic centrifugation for further study.

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