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Anti-inflammatory activity of polysaccharide from *Schizophyllum commune* as affected by ultrasonication

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ABSTRACT

Ultrasound treatment was applied to modify the physicochemical properties of an exopolysaccharide from mycelial culture of *Schizophyllum commune*. Molecular weight (MW) degradation, viscosity and anti-inflammatory property of ultrasonic treated polysaccharide were optimized with response surface methodology. The best ultrasonic parameters were obtained with a three-variable-three-level Box-Behnken design. The optimized conditions for efficient anti-inflammatory activity are initial concentration at 0.4%, ultrasonic power at 600 W, and duration of ultrasonic irradiation for 9 min. Under these conditions, the nitric oxide inhibition rate was 95 ± 0.03% which agreed closely with the predicted value (96%). Average MW of polysaccharide decreased after ultrasonic treatments. The viscosity of degraded polysaccharide dropped compared with native polysaccharide. The anti-inflammatory activity was improved by ultrasound treatment. The results suggested that ultrasound treatment is an effective approach to decrease the MW of polysaccharide with high anti-inflammatory activity. Ultrasonic treatment is a viable modification technology for high MW polymer materials.

1. Introduction

Schizophyllum commune (Fr.) is a species of basidiomycetes belonging to *Schizophyllaceae* of *Agaricales* [1]. It is one of the most widely distributed fleshy fungi, and can be isolated on all continents except for Antarctica [2]. *S. commune* is a filamentous growing fungus that produces exopolysaccharides (EPS). The most promising biological properties of these polysaccharides are anti-inflammatory and anti-tumor activities. Many studies have been attempted to correlate biological activity and molecular weight of polysaccharides. Chen et al. [3] investigated that low MW polysaccharide showed stronger inhibitory effects towards the proliferation of colorectal cancer. Moreover, recent research showed that low molecular weight chitosans with average molecular weights in the range of 5–10 kDa exhibited strong bactericidal and superior biological activities compared to chitosan with high molecular weight [4]. The application of polysaccharide from *S. commune* in pharmaceuticals and cosmetics industries have been limited for its high MW and viscosity [5]. It is necessary to set up a kind of fast, efficient, and convenient method of degrading the native polysaccharides in order to achieve the desired MW [6]. Ultrasonic technology, which currently attracts considerable attention, plays key roles in food technology, such as in processing, preservation, and extraction [7–9]. Ultrasonic degradation is a great physical method for producing homologous series of lower MW [10–13]. It appears that cellulose and guar gum derivatives exhibit a significant MW reduction upon the ultrasonic treatment, a simple, effective, and without additive method [14]. Ultrasonic degradation occurs mainly by cleavage of glycosidic bonds of the main chain of polysaccharide. The physicochemical properties of ultrasound-treated polysaccharides have been investigated in recent years [15–17]. Solution viscosity and biological activity of polysaccharides depend on their MW. Ultrasonication could decrease the viscosity of polymers and increase in the solubility, and was proved to be an effective and favorable tool to improve the bioactivities of polysaccharides [18–22].

Abbreviations: ANOVA, analysis of variance; BBD, Box-Behnken design; CV, coefficient of the variation; DMEM, Dulbecco's Modified Eagle's Medium; EPS, exopolysaccharide; FBS, Fetal bovine serum; FT-IR, Fourier transform infrared; LPS, lipopolysaccharide; MTT, 3-(4,5-Dimethylthiazol-2-yl-2, 5- diphenyltetrazolium bromide; MW, molecular weight; NO, nitric oxide; RSM, response surface methodology.

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Response surface methodology (RSM) is an effective statistical technique to evaluate the effects of influential factors on one or more response variables for optimizing complex processing technology [23,24]. The Box-Behnken design (BBD) is a RSM model that examines the relationships between several explanatory variables and one or more response variables [25] and has been applied successfully to improve food and pharmaceutical research [26–29].

However, little information is available on the effect of bio-degradation of EPS from *S. commune* on anti-inflammatory property. The objective of this study was to investigate the effect of different factors on the efficacy of ultrasonic depolymerization of polysaccharide and to develop the optimum conditions for ultrasonic degradation that would yield EPS fragments with reduced MW but with increased anti-inflammatory activity.

2. Material and methods

2.1. Experimental materials and chemicals

Dulbecco's Modified Eagle's Medium (DMEM) was purchased from Gibco-BRL (Gaithersburg, MD, U.S.A.). Fetal bovine serum (FBS), penicillin, streptomycin, 3-(4,5-dimethylthiazol-2-yl-2, 5-diphenyltetrazolium bromide (MTT), and Griess reagent were bought from Sigma-Aldrich Company. All the other chemicals used were of analytical grade.

2.2. Preparation of EPS from S. commune

Strain (CFCC83289) of *S. commune* Fr. was purchased from Beijing Beina Chuanglian Biotechnology Research Institute (Beijing, China). The mycelium of *S. commune* was cultivated on potato dextrose agar slants. A 1 cm^2 of mycelia along with agar from such slants were inoculated to 50 mL of sterile seed culture medium in 250 mL conical flasks, which were incubated at 28 ± 2 °C, 180 rpm for 7 days on an orbit shaker [30]. Biomass concentration was determined by the dry mass method involving filtration of broth samples through pre-weighed filter discs (Whatman Ltd., Maidstone, UK). The filtrate was collected and stored at −20 °C for the isolation of crude exopolysaccharides. The total sugar content of polysaccharide was 89.0 ± 3.41%.

2.3. Ultrasonic treatment of polysaccharide

Ultrasonic treatment was performed with a Scientz-IID ultrasonic cell disrupter (Ningbo Scientz Biotechnology Company, Ltd., Ningbo, Zhejiang, China) of 25 kHz frequency and 950 W maximum output power. The output power of the probe is proportional to input power and controlled by the amplitude. In this work, a horn probe with a 6 mm-diameter tip was used for all experiments and the output power was set at three levels, 200 W, 500 W and 800 W.

2.4. Determination of MW

The average MW of polysaccharide was determined with Agilent High Performance Liquid Chromatography (HPLC), refractive index detector using a chromatographic column (PL aquageloh MIXED-H (8 μm, 300 × 7.5 mm)) eluted with 0.1 M $NaNO_3$ at a flow rate of 0.8 mL min^{-1}.

2.5. Measurements of viscosity

Apparent viscosity of polysaccharide solutions was measured with a LV DV-II + Pro viscometer (Brookfield Company, Stoughton, MA, U.S.A.). The concentration of original native polysaccharide samples were adjusted in deionized water at 10 g/L and the measurement was performed at 25 °C.

Table 1
The independent variables and their levels used for BBD.

Independent variables	Levels of factor		
	−1	0	+1
Initial concentration of polysaccharide (%)	0.4	0.5	0.6
Ultrasonic power (W)	200	500	800
Duration of ultrasonic irradiation (min)	5	10	15

2.6. Measurement of infrared (IR) spectrum of EPS

The IR spectrum of the EPS was determined using a TENSOR 27 Fourier transform infrared (FT-IR) spectrophotometer (Bruker Corporation, Karlsruhe, Germany). The sample was ground with spectroscopic grade KBr powder and then pressed into 1 mm pellets for FT-IR determination in the frequency range of 4000–400 cm^{-1}.

2.7. Experimental design

In this study, the most suitable variables are the following parameters: Initial concentration of polysaccharide, ultrasonic power and duration of ultrasonic irradiation. Influence of initial concentration of polysaccharide (*A*) was investigated in the range from 0.4% to 0.6%; ultrasonic power (*B*), in the range from 200 W to 800 W; and duration of ultrasonic irradiation (*C*) in the range from 5 to 15 min. Details of analysis are presented in Table 1.

A three-variable-three-level BBD [25], one of response surface methodology (Design-Expert 7.1.3 Trial version, State-Ease Inc., Minneapolis, MN, U.S.A.) was applied to optimize the effect of ultrasonic treatment on the MW degradation, viscosity and anti-inflammatory property of polysaccharide in order to obtain the polysaccharide with high anti-inflammatory activity of. A total of 17 experiments were designed according to BBD. Each experiment was performed in triplicate and the average NO inhibition of polysaccharide was taken as the response, *Y*.

Regression analysis was performed for the experimental data and was fitted into the empirical second order polynomial model, as shown in Eq. (1):

$$Y = A_0 + \sum_{i=1}^{3} AiX_i + \sum_{i=1}^{3} AiiX_i^2 + \sum_{i=1}^{2}\sum_{j=i+1}^{3} AijXij \quad (1)$$

where *Y* was the dependent variable, A_0 was constant, and A_i, A_{ii}, and A_{ij} were coefficients estimated by the model. X_i and X_j were levels of the independent variables.

2.8. Anti-inflammatory activity of polysaccharide

2.8.1. Cell culture

RAW 264.7 murine macrophages were obtained from American Type Culture Collection (ATCC, Rockville, MD, U.S.A.). The cells were cultured at 37 °C under 5% CO_2-humidified air in DMEM supplemented with 10% FBS, 100 U mL^{-1} penicillin, and 100 μg mL^{-1} streptomycin.

2.8.2. Determination of NO production

The nitrite concentration in the medium was measured by Griess reagent as an indicator of NO production as previous report [31]. Briefly, RAW 264.7 cells (1.0×10^5 cells/well in a 24-well plate with 500 μL of culture medium) were pretreated with EPS samples for 1 h and incubated with lipopolysaccharide (LPS) (100 ng mL^{-1}) for 24 h. After incubation, the nitrite concentration of the supernatants (100 μL/well) was measured by adding 100 μL of Griess reagent.