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Original Article

Enhanced active extracellular polysaccharide production from *Ganoderma formosanum* using computational modeling

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ARTICLE INFO

Article history:
Received 23 July 2016
Received in revised form
30 November 2016
Accepted 7 December 2016
Available online 16 February 2017

Keywords:
β-glucan
extracellular polysaccharide
Ganoderma formosanum
medium composition
response surface methodology

ABSTRACT

Extracellular polysaccharide (EPS) is one of the major bioactive ingredients contributing to the health benefits of *Ganoderma* spp. In this study, response surface methodology was applied to determine the optimal culture conditions for EPS production of *Ganoderma formosanum*. The optimum medium composition was found to be at initial pH 5.3, 49.2 g/L of glucose, and 4.9 g/L of yeast extract by implementing a three-factor–three-level Box–Behnken design. Under this condition, the predicted yield of EPS was up to 830.2 mg/L, which was 1.4-fold higher than the one from basic medium (604.5 mg/L). Furthermore, validating the experimental value of EPS production depicted a high correlation (100.4%) with the computational prediction response model. In addition, the percentage of β-glucan, a well-recognized bioactive polysaccharide, in EPS was 53 ± 5.5%, which was higher than that from *Ganoderma lucidum* in a previous study. Moreover, results of monosaccharide composition analysis indicated that glucose was the major component of *G. formosanum* EPS, supporting a high β-glucan percentage in EPS. Taken together, this is the first study to investigate the influence of medium composition for *G. formosanum* EPS production as well as its β-glucan composition.

1. Introduction

Lingzhi (*Ganoderma* spp.), generally recognized as a safe medical mushroom, has been used for centuries as a nutraceutical to promote health and longevity [1]. Nowadays, the global *Ganoderma* market has an estimated size of US$2.5 billion [2]. Polysaccharide is one of the major components contributing to the health benefits of *Ganoderma* spp., including immune-modulation, anti-inflammation, and

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http://dx.doi.org/10.1016/j.jfda.2016.12.006

obesity management [3]. For commercialization of dietary supplement, however, it takes several months to cultivate the fruit bodies of *Ganoderma*, and it is difficult to control their quality when they come from different batches [4]. As a result, submerged fermentation has received considerable attention by dint of its time-saving and economic properties for industrial production.

Ganoderma formosanum is an endemic species of *Ganoderma* in Taiwan [5]. Currently, owing to its novelty, few studies have demonstrated its pharmacological potential, among which the investigation of bioactive extracellular polysaccharide (EPS) is the most understood [6–9]. PS-F2, an EPS fraction from the submerged cultivation of *G. formosanum*, was reported to stimulate macrophage activation in mice via multiple pattern-recognition receptors including TLR4, CR3, and Dectin-1 [7,8]. Moreover, PS-F2 exerted antitumor effects toward melanoma, adenocarcinoma, and sarcoma in mice without adverse effects [9]. Nevertheless, optimization for the production of *G. formosanum* EPS has not yet been explored.

Optimizing medium composition is a key step to ameliorate the production of bioactive components. The effects of initial pH, carbon source, nitrogen source, inoculation density, and temperature have been investigated to ameliorate polysaccharide and biomass production in *Ganoderma* spp. [10–13]. A previous study indicated that a maximum level in the biomass of *Ganoderma lucidum* was obtained at high initial pH (6.5), whereas low initial pH (3.5) was favorable for EPS production [10]. Another study reported that an increase in glucose concentration (from 20 g/L to 50 g/L) gradually led to a higher biomass and endopolysaccharide production of *G. lucidum*. However, the production of polysaccharide was inhibited at a higher concentration of glucose (65 g/L) because of the high osmotic pressure [14]. Therefore, it was noteworthy that the fermentation response was not in proportion to the cultivation parameter. In view of this, a more reliable statistical strategy is needed to optimize fermentation variables.

Traditionally, one-factor-at-a-time (OFAT) approach has been carried out by analyzing the effect of a single factor on experimental response during fermentation [15]. Although this technique provides a simple way to monitor the influence of the variables studied, some drawbacks still exist, including time consumption, laboriousness, and diseconomy [16]. Furthermore, the major downside of OFAT is that it does not depict the interaction between different factors. Therefore, a collection of statistical and mathematical approaches is needed to investigate the multiple variables and their interaction during cultivation. Response surface methodology (RSM) is a statistical approach based on the fit of a polynomial regression model, which can be applied to validate the value of independent variables considering the interaction among them.

Briefly, RSM is composed of three steps: (1) executing a set of designed experiment; (2) evaluating the coefficients of polynomial model; and (3) predicting the response model and obtaining the optimum value. Box–Behnken design (BBD) and central composite design (CCD) are the most popular RSM alternatives; however, BBD is preferable to CCD when three factors are used as it decreases the number of experiments [17,18].

To date, there is still no consensus about the optimal cultivation conditions for EPS production of *Ganoderma* spp. Therefore, the aim of this study was to investigate the optimal initial pH, glucose, and yeast extract concentration of the medium for *G. formosanum* ESP production using the RSM technique, while revealing the relationship between morphology and EPS production. The percentage of β-glucan in EPS was also studied. The insights gleaned from this study enable us to increase EPS production for further application.

2. Materials and methods

2.1. Fungal strain and fermentation

G. formosanum (ATCC76537) was obtained from the American Type Culture Collection (Rockville, MD, USA). The mycelia were cultured on potato dextrose agar (PDA) plates at 25°C for 10 days. For preparation of seed inoculums, an 8-cm^2 mycelium from dish culture was inoculated to an Erlenmeyer flask (250 mL) containing 100 mL potato dextrose broth and incubated at 25°C on a rotary shaker (120 rpm) for 7 days. For the fermentation stage, a 10% (v/v) inoculum was poured to the basic medium that was obtained from a previous study with modification [19], including 35 g glucose, 7.5 g yeast extract, 0.88 g KH_2PO_4, 0.5 g $MgSO_4 \cdot 7H_2O$, and 0.05 g vitamin B_1 per liter of deionized water, with 120 rpm shaking at 25°C for 9 days. To optimize the culture condition for EPS, the medium was supplemented with various levels of glucose, yeast extract, and initial pH in a total of 13 different conditions (Tables 1 and 2).

2.2. Determination of biomass and exopolysaccharide (EPS)

To determine the mycelial biomass of *G. formosanum*, mycelia were separated from culture broth with mesh filter and washed with sterile water, then dried to a constant weight in the lyophilizer (T10; HCS, New Taipei City, Taiwan). For EPS preparation, the culture broth without mycelia was added with 95% ethanol by four volume times to precipitate EPS at 4°C overnight. After isolation of EPS by centrifugation at 7,200 × *g* for 15 minutes, EPS was resuspended with 95% ethanol and centrifuged again. The insoluble component was dissolved with 1N NaOH at 60°C for 1 hour, and the amount of EPS was measured using the phenol–sulfuric acid method [19].

2.3. Effects of carbon, nitrogen, and pH levels (experimental design)

A three-level–three-factor Box–Behnken design response surface methodology (BBD-RSM) was applied to optimize

Table 1 – Levels of factors chosen for the Box–Behnken design.

Factors	Symbols	Coded levels		
		−1	0	1
Initial pH	X_1	3.5	5	6.5
Glucose (g/L)	X_2	25	45	65
Yeast extract (g/L)	X_3	0	3.75	7.5