Efficient production of polysaccharide by *Chaetomium globosum* CGMCC 6882 through co-culture with host plant *Gynostemma pentaphyllum*

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Abstract

Endophytic fungus, as a new kind of microbial resources and separated from plants, has attracted increasing attention due to its ability to synthesize the same or similar bioactive secondary metabolites as the host plants. Nevertheless, the effects of the symbiotic relationship between microorganisms and elicitors existed in host plant on metabolite production are not adequately understood. In the present work, the impacts of elicitors (ginseng saponin and puerarin) and symbiotic microorganisms on endophytic fungus *Chaetomium globosum* CGMCC 6882 synthesizing polysaccharide were evaluated. Results show that the polysaccharide titers increased from 2.36 to 3.88 g/L and 3.67 g/L with the addition of 16 µg/L ginseng saponin and puerarin, respectively. Moreover, the maximum polysaccharide titer reached 4.55 g/L when *C. globosum* CGMCC 6882 was co-cultured with UV-irradiated *G. pentaphyllum*. This work brings a significant contribution to the research and interpretation of the relationship between endophytic fungus and its host plant.

Keywords Endophytic fungus · Host plant · Co-culture · Polysaccharide

Introduction

With the aggravation of environmental pollution, increase of life stress, increasing occurrences of drug-resistant microorganisms, new cases of life-threatening infections and constant recurrence of diseases, the need for searching novel medicines to treat human diseases has become urgent [1, 2]. Generally, there are three approaches for medical scientists to find new pharmacological compounds: first, designing drug purposely towards specific microbial target; second, chemically synthesizing a series of compounds and then screening the promising candidate using microbial tests; third, isolating bioactive compounds from natural biological

sources [3, 4]. Although rational drug design through microbial cell platform and synthetic chemistry could significantly shorten the research and development time of a new medicine, insufficient structural complex, restricted structural rigidity, numerous purification and bioactivity tests remained the bottlenecks of these two approaches. Concerning long-term safety, structural complexity and environmental friendliness, extractives from medicinal plants are considered promising new drugs with high biological activities [5].

Endophytic fungi, as a new kind of microbial resources, are normally separated from plants. They have attracted increasing attention due to their ability to synthesize the same or similar secondary metabolites as their hosts, and this offers a new approach for producing bioactive components from medicinal plants [5–7]. It has been reported that *Yew* endophytic fungus could produce an anticancer paclitaxel [8], *Dendrobium officinale* endophytic fungus could produce antitumor and antifungal 4-anilinoquinoline derivatives [9], *Passiflora incarnata* endophytic fungus could synthesize anti-fatigue and anti-aging flavone chrysin [10]. Unfortunately, relevant researches so far paid more attention to the bioactivities and biosynthetic pathway of the

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compounds produced from endophytic fungus, whereas the interacting mechanism of the host plants with endophytic fungus is hardly studied [11, 12].

Previously, an endophytic fungus *C. globosum* CGMCC 6882, which was isolated from *G. pentaphyllum*, could produce antibacterial polysaccharide [13]. In this research, *C. globosum* co-cultured with *G. pentaphyllum* and the influence of carbon and nitrogen source, fermentation time, pH values, particle size of wheat bran and *G. pentaphyllum*, the addition amount of saponin and puerarin on the synthesis of polysaccharide by *C. globosum* CGMCC 6882 were investigated. Meanwhile, the monosaccharide composition, molecular weight and functional groups of the polysaccharide produced in the co-culture process were analyzed.

Materials and methods

Microorganism and chemicals

Chaetomium globosum CGMCC 6882 was isolated from *G. pentaphyllum* herb and stored in China General Microbiological Culture Collection Center (Beijing, China). Chemicals used in this work, unless specified, were analytical grade and purchased from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China).

Seed medium

Spores of *C. globosum* CGMCC 6882 were collected by scratching the PDA agar plates (potato 200 g/L, glucose 20 g/L and agar 15 g/L) with a disposable spreader and washing with normal saline. A hemacytometer (Neubauer, Germany) was used to count the number of spores under a phase contrast microscope (Zeiss, Axio Imager 2, Jena, Germany). Then the spore suspension was diluted to a concentration of 1×10^7 spores/mL with normal saline and the inoculation volume was 1% (V/V).

Carbon source and nitrogen source

Starch, maltose, sucrose, glucose, lactose and xylose were used to study the influence of different carbon sources on polysaccharide production. The fermentation medium used for *C. globosum* CGMCC 6882 culture consisted of 1.5 g/L beef extract, 1.5 g/L $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 1 g/L KH_2PO_4 , 1 g/L K_2HPO_4 , and 35 g/L of a certain kind of the carbon resources selected. Fermentation conditions are set as follows: temperature, 28 °C; cultivation time, 7 d; pH value of the culture system was maintained at 7.0 ± 0.02 with 2 mol/L NaOH and 2 mol/L HCl; agitation and aeration were set as 100 rpm and 0.8 vvm and the culture proceeded in a 7.0-L fermenter (BioFlo 115, New Brunswick, USA) with 3.5 L

working volume. Different carbon resources and different concentrations of glucose were studied to obtain the optimum glucose concentration for polysaccharide production.

Influence of different nitrogen sources on the polysaccharide production was analyzed using various nitrogen sources, i.e., NaNO_3 (A), NH_4NO_3 (B), NH_4Cl (C), $(\text{NH}_4)_2\text{SO}_4$ (D), $(\text{NH}_4)_2\text{CO}_3$ (E), ammonia liquor (F), beef extract (G), yeast extract (H), peptone (I) and soybean powder (J). Culture medium and fermentation conditions were the same as above but beef extract was replaced by a certain kind of nitrogen source. Meanwhile, the influence of initial yeast extract concentration on polysaccharide accumulation was further investigated.

Fermentation time and culture medium pH

Fermentation medium and culture conditions were kept constant, but fermentation time was set as 2, 3, 5, 7, 9, 11 and 13 days to study the influence of fermentation time on the polysaccharide production. After that, the initial pH of culture medium was regulated to 5, 6, 7, 8, 9 with 2 mol/L NaOH and 2 mol/L HCl to study the influence of medium pH on the polysaccharide production.

Particle size of wheat bran

Wheat bran was ground and divided into six meshes (12, 24, 50, 100, 150 and 200). Then 5 g/L of different particle size wheat bran was added into the original culture medium. Inoculation volume and fermentation conditions were the same as above.

Co-culture with *G. pentaphyllum* herb

Gynostemma pentaphyllum herb was ground and divided into six meshes (12, 24, 50, 100, 150 and 200). In one group, 5 g/L of *G. pentaphyllum* herb with different particle size was added into the original culture medium and sterilized at 121 °C for 20 min. Meanwhile, another group of *G. pentaphyllum* herb was irradiated by UV for 24 h to kill the bacteria on the surface of *G. pentaphyllum*. After the culture medium was sterilized at 121 °C for 20 min and cooled to room temperature, irradiated *G. pentaphyllum* was added into the fermenter through inoculation hole. Inoculation volume, culture medium and fermentation conditions were the same as above.

Addition with saponin and puerarin

The influence of two bioactive compounds in *G. pentaphyllum* herb, saponin (i.e., 1, 2, 4, 8, 16 µg/L) and puerarin (i.e., 1, 2, 4, 8, 16 µg/L), on the polysaccharide production by *C. globosum* CGMCC 6882 was investigated.

Polysaccharide titer determination

At the end of fermentation, culture broth was filtered through a Buchner funnel, the filter liquor was centrifuged at $20,000 \times g$ for 30 min. After that, supernatant (10 mL) was precipitated with alcohol (95%, 50 mL) at 4 °C overnight. Achieved precipitates, i.e., the crude polysaccharide extract, were redissolved in 10 mL deionized water and the content of polysaccharide was determined using phenol–sulfuric acid method at 490 nm.

Polysaccharide extraction and characterization

The extraction, purification and characterization of polysaccharide produced by *C. globosum* CGMCC 6882 co-culturing with *G. pentaphyllum* were conducted according to the methods described in our previous work [13].

Statistical analysis

All data were expressed as mean $\pm$ standard deviation (SD). One-way ANOVA was performed using the Origin Lab software (Origin Pro 8.5).

Results and discussion

Carbon source and nitrogen source on polysaccharide production

Both carbohydrate and nitrogen are major nutrient sources in a culture process, and may significantly affect cell growth and metabolite formation. As shown in Fig. 1a, among all the investigated carbon sources (i.e., starch, maltose, sucrose, glucose, lactose and xylose), glucose is the optimal carbon source for *C. globosum* CGMCC 6882 to synthesize polysaccharide and polysaccharide titer could reach 1.88 g/L. With the increase of glucose content from 10 to 40 g/L, polysaccharide titer increased from 1.21 to 2.03 g/L. However, with

Fig. 1 Effect of carbon source (a), glucose concentration (b), nitrogen source (c) and beef extract concentration (d) on polysaccharide production. The nitrogen sources used in this work are A: NaNO_3 , B:

NH_4NO_3 , C: NH_4Cl , D: $(\text{NH}_4)_2\text{SO}_4$, E: $(\text{NH}_4)_2\text{CO}_3$, F: ammonia liquor, G: beef extract, H: yeast extract, I: peptone and J: soybean powder

the concentration of glucose furtherly increased from 50 to 80 g/L, polysaccharide titer decreased gradually (Fig. 1b). This might be due to the inhibition of cell growth induced by high glucose concentration, and in previous work, we found that the cell growth time was prolonged when glycerol concentration was higher than 40 g/L [14]. Meanwhile, in the work reported by Yang et al. [15], glycerol concentration was controlled by fed-batch strategy to reduce the inhibition of high glycerol concentration on cell growth.

As shown in Fig. 1c, the polysaccharide titer produced from *C. globosum* CGMCC 6882 was significantly influenced by the type and concentration of nitrogen source in the fermentation medium. Among all the studied nitrogen sources (i.e., NaNO_3 , NH_4NO_3 , NH_4Cl , $(\text{NH}_4)_2\text{SO}_4$, $(\text{NH}_4)_2\text{CO}_3$, ammonia liquor, beef extract, yeast extract, peptone and soybean powder), the maximum polysaccharide titer (2.08 g/L) was obtained with yeast extract. To obtain a higher polysaccharide yield, the initial yeast extract concentration in the culture medium was further optimized, and the polysaccharide titer reached 2.17 g/L when 3 g/L yeast extract was added (Fig. 1d). This result is in accordance with previous studies by others. Liu et al. [16] reported that yeast extract was the most favorable nitrogen source for cell growth of *Tuber sinense*, and the dry cell weight reached 19.53 ± 0.13 g/L with an initial yeast extract concentration at 25 g/L.

Fermentation time and culture medium pH

Except for the influence of carbohydrate and nitrogen sources, the culture conditions are also crucial for target compound production [17, 18]. The influence of fermentation time is shown in Fig. 2a, the polysaccharide titer increased from 0.01 to 2.25 g/L from day 2 to day 7, and decreased gradually to 1.7 g/L at the 13th day. This might be brought by the polysaccharide decomposition for

maintaining cell growth when the carbon source was deficient in culture medium [19, 20]. Likewise, Skriptsova et al. [21] also found that the production of water-soluble polysaccharides reached a plateau at around day 7.

The influence of culture medium pH is shown in Fig. 2b, from which it could be seen that the maximum polysaccharide titer (2.36 g/L) was obtained when the original culture medium pH was 7.0. Many researchers have also reported the influences of fermentation pH on target compound production. Jiang et al. [22] reported that alkaline pH shock could increase Validamycin A production by 27.43%. Wu et al. [23] adopted a two-stage pH-shift control strategy to improve the (2S)-pinocembrin production from D-glucose by engineered *Escherichia coli*. Some studies revealed some of the mechanisms of the pH influence. For instance, Zou et al. [24] found that nitrifying aerobic granular sludge (N-AGS) alkaline fermentation facilitated the carbon source recovery, and N-AGS acidic fermentation benefited the phosphorus recovery.

Co-culture with wheat bran and *G. pentaphyllum* herb

Given that fungus forms a pellet during the submerged fermentation process and then affects bacterial growth and target compound synthesis [25, 26], wheat bran and *G. pentaphyllum* herb were used as upholder to investigate the fungal morphology on polysaccharide production. As shown in Fig. 3a, polysaccharide titer was 3.18 g/L when the particle size of wheat bran was 12 mesh, and the polysaccharide titer decreased with the reduction of wheat bran particle size. Although *G. pentaphyllum* herb as upholder obtained the similar tendency of polysaccharide production to wheat bran (Fig. 3b), the maximum polysaccharide titers of *G. pentaphyllum* herb with UV irradiation and sterilization were 4.55 g/L and 4.25 g/L, respectively. Because of the

Fig. 2 Fermentation time (a) and culture medium pH (b) on polysaccharide production

Fig. 3 Particle size of wheat bran (a) and host plant *G. pentaphyllum* herb (b) on polysaccharide production

low content of bioactive ingredients (including polysaccharide) in plants [5, 6], we assume that the polysaccharide in fermentation medium was mainly produced by *C. globosum* CGMCC 6882. At the same time, polysaccharide titers of UV irradiation cases were all higher than those of sterilization. This is possibly due to that *G. pentaphyllum* herb not only provided upholder for *C. globosum* CGMCC 6882 growth, but also offered symbiotic environment for polysaccharide synthesis, such as the interacting microorganisms and substances [27, 28]. Therefore, polysaccharide titer of *G. pentaphyllum* herb group was higher than that of wheat bran, and UV irradiation case was higher than that of the sterilization case.

Two points should be mentioned here regarding the polysaccharide produced by *C. globosum* CGMCC 6882 in this work. First, glycosides are the major bioactive ingredients and chemical constituents in *G. pentaphyllum* herb [29], researchers pay more attention to glycosides, but less

attention to polysaccharides. Second, *C. globosum* is mainly studied by researchers for chaetoglobosin A synthesis [30, 31], there are few researches for polysaccharide production by *C. globosum*. Therefore, the current polysaccharide titer by *C. globosum* CGMCC 6882 is hardly compared, but we believe that this titer should not be the highest and will continue to increase in the future through genomic, transcriptomic, and metabonomic investigations.

Elicitors ginseng saponin and puerarin

Endophytic fungi may produce many bioactive compounds including saponin, flavonoid, polysaccharide, alkaloid and growth regulating agent, to promote host plant growth and help the host plant to keep healthy [32]. Nevertheless, there are few researches on the effects of host plant bioactive compounds as elicitors for endophytic fungus synthesizing bioactive metabolites. Figure 4a shows that the increase of

Fig. 4 Elicitors of ginseng saponin (a) and puerarin (b) on polysaccharide production

polysaccharide titer in fermentation medium was consistent with the addition of ginseng saponin, and the polysaccharide titer could reach 3.88 g/L when the ginseng saponin concentration was 16 µg/L. Meanwhile, the addition of puerarin acted in the same influencing tendency as ginseng saponin, and the polysaccharide titer was 3.67 g/L with the addition of 16 µg/L puerarin (Fig. 4b). This might be that the ginseng saponin and puerarin used in this work enhanced the metabolic process of polysaccharide synthesis in *C. globosum* CGMCC 6882 [32, 33], and these will be studied in our future work. Meanwhile, many researchers obtained results similar to this work, for instance, Tian et al. [33] found that flavonoids produced by host plant *Cephalotaxus harringtonia* displayed significant beneficial effects on the hyphal growth of endophytic fungus *Paraconiothyrium variabile*. Conversely, Shweta et al. [34] demonstrated that camptothecin produced by host plant *Nothapodytes nimmoniana* (Graham) Mabb. (Icacinaeae) inhibited the fungal endophyte growth.

Characterization of polysaccharide produced from *C. globosum* CGMCC 6882 co-culturing with host plant *G. pentaphyllum* herb

Bioactivities of polysaccharide were affected by its chemical structure including repeating unit, monosaccharide types, glycosidic bond of main chain, chain length, branch degree of chain, flexibility and configuration of chains [35]. In this work, the elution profiles (Fig. 5a) and FT-IR spectroscopy results (Fig. 5b) of polysaccharide produced from *C. globosum* CGMCC 6882 co-culturing with host plant *G. pentaphyllum* perfectly matched with the previously published results for antibacterial polysaccharide GCP [13]. Meanwhile, Table 1 shows that the monosaccharide composition, weight-average molecular weight (Mw), number-average

Table 1 Characteristics of polysaccharide produced by *C. globosum* CGMCC 6882 from co-culture with *G. pentaphyllum* herb

Items	GCP	Polysaccharide
Arabinose (µmol/L)	0.64	0.58
Galactose (µmol/L)	2.58	2.89
Glucose (µmol/L)	23.53	21.03
Xylose (µmol/L)	0.90	0.67
Mannose (µmol/L)	2.47	1.95
Glucuronic acid (µmol/L)	0.27	1.24
Weight-average molecular weight (Mw, Da)	8.093×10^4	7.904×10^4
Number-average molecular weight (Mn, Da)	7.982×10^4	7.695×10^4
Polydispersity (Mw/Mn)	1.014	1.027

molecular weight (Mn) and polydispersity (Mw/Mn) all corresponded with GCP, which further suggested that polysaccharide produced by *C. globosum* CGMCC 6882 from co-culturing with host plant *G. pentaphyllum* was the same polysaccharide as GCP in our previous work [13].

Conclusions

This work demonstrated that the production of polysaccharide by an endophytic fungus, *C. globosum* CGMCC 6882, was not only affected by fermentation conditions, including carbon source, nitrogen source, fermentation time, culture medium pH and fungal morphology, but also influenced by other symbiotic endophytic fungi in host plant and the compounds produced by host plant, i.e., saponin and puerarin. Our findings bring a significant contribution to the research and interpretation of the relationship between endophytic fungus and its host plant. Moreover, this work provided

Fig. 5 Elution profiles (a) and FT-IR spectroscopy (b) of polysaccharide produced by *C. globosum* CGMCC 6882 from co-culture with *G. pentaphyllum* herb

useful approaches for enhancing production through co-culture with host plant or addition of elicitors.

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Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest.

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