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Research article

Microbial transformation of ginsenosides extracted from *Panax ginseng* adventitious roots in an airlift bioreactor

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ABSTRACT

Background: Ginsenoside is the most important secondary metabolite in ginseng. Natural sources of wild ginseng have been overexploited. Although root culture can reduce the length of the growth cycle of ginseng, the number of species of ginsenosides is reduced and their contents are lower in the adventitious roots of ginseng than in the roots of ginseng cultivated in the field.
Results: In this study, 147 strains of β-glucosidase-producing microorganisms were isolated from soil. Of these, strain K35 showed excellent activity for converting major ginsenosides into rare ginsenosides, and a NCBI BLAST of its 16S rDNA gene sequence showed that it was most closely related to *Penicillium* sp. (HQ608083.1). Strain K35 was used to ferment the adventitious root extract, and the fermentation products were analyzed by high-performance liquid chromatography. The results showed that the content of the rare ginsenoside CK was 0.253 mg mL^{-1} under the optimal converting conditions of 9 d of fermentation at pH 7.0 in LL medium, which was significantly higher than that in the adventitious roots of ginseng.
Conclusion: These findings may not only solve the problem of low productivity of metabolite in ginseng root culture but may also result in the development of a new valuable method of manufacturing ginsenoside CK.

1. Introduction

Panax ginseng C.A. Meyer can promote health and treat diseases and has been used as a traditional Chinese medicine for thousands of years in Asian countries. A number of ginseng species including *P. ginseng* (Asian ginseng), *Panax notoginseng* (Sanqi in Chinese), and *Panax quinquefolius* (American ginseng) are commonly consumed and have been investigated [1]. The use of ginseng to treat diabetes and cardiovascular disease and prevent cancer has been well documented [2]. These beneficial effects are mainly attributed to ginsenosides, which are composed of a dammarane backbone with several side chains, including glucose, arabinose, xylose, and rhamnose side chains [3]. Over 150 types of ginsenosides have been isolated, of which six types of major ginsenosides (Rb1, Rb2, Rc, Rd, Re, and Rg1) constitute the main portion of *P. ginseng* ginsenosides [4,5]. Recently, it was reported that rare ginsenosides have significant pharmaceutical activity and can be easily absorbed by the human body [6], of which the rare ginsenoside CK has recently attracted considerable interest because of its significant pharmacological activity including antiallergic, antidiabetic, anticarcinogenic, anti-inflammatory, antiaging, and hepatoprotective effects. With a high level of safety and diverse biological functions, ginsenoside CK may be a potential therapeutic agent for many diseases [7,8,9,10]. The rare ginsenoside CK is a non-natural diol-type saponin, but it can be obtained from major ginsenosides by various methods such as heating, hydrolysis by acid or alkali, and enzymatic and microbial transformation [10]. Chemical transformations induce side reactions and environmental pollution. Enzymatic transformation has disadvantages such as difficulty in purification and characterization of the enzyme as the enzyme can easily lose its activity. Of these methods, microbial transformation is the most promising because of its low cost, eco-friendliness, and ability to be scaled up for industrial-scale production [10,11,12].

In recent years, ginseng has been processed into many types of commercial health products, including ginseng soups, drinks, capsules, and cosmetics [13]. However, natural sources of wild ginseng have been overexploited. Ginseng requires 5–7 years for field cultivation, during which time labor is needed for plant growth as ginseng is very sensitive to many environmental factors including shade, soil, climate, pathogens, and pests [14,15]. The current sources of ginseng are mainly field cultivation, which requires time and labor [16]. As a result, tissue and organ culture have been developed as an alternative as this emerging biotechnological method results in more efficient and

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controllable production of ginseng and its metabolites. During the cultivation of plant cells and organs, the bioreactor culture system has a number of merits as the culture conditions in the bioreactor such as temperature and pH and the concentrations of oxygen, carbon dioxide, and nutrients in the medium can be controlled [17].

Although the root culture method can reduce the length of the growth cycle of ginseng, the number of species of ginsenosides is reduced and their contents are lower in cultivated roots than in ginseng cultivated in the field [18]. Thus, a lot of effort has been made to improve the content of ginsenosides in ginseng adventitious roots. A previously published study has reported that ginsenoside content in an adventitious root culture of *P. ginseng* increased significantly following the addition of 10 mg L^{-1} jasmonic acid [18]. Some researchers have shown that organic germanium can be used as an elicitor to enhance biomass accumulation and ginsenoside production in ginseng adventitious roots [19]. Although these methods can increase the content of ginsenosides in ginseng adventitious roots, the content of rare ginsenosides was not increased. There are currently many reports on the biotransformation of major ginsenosides into the rare ginsenoside CK, but the methods used are expensive and time-consuming; thus, the industrial preparation of ginsenoside CK using these methods is unprofitable.

In the present study, to obtain the rare ginsenoside CK, the ginsenosides in ginseng adventitious roots were fermented with β-glucosidase produced by microorganisms isolated from a ginseng field. The optimal fermentation conditions with *Penicillium* sp. (HQ608083.1) were determined, and the changes in ginsenosides were analyzed under different fermentation conditions. These findings could result in the development of a new method for manufacturing the rare ginsenoside CK.

2. Materials and methods

2.1. Chemicals

Standard ginsenosides including Rb1, Rb2, Rd, F1, F2, and CK were purchased from the Shanghai Winherb Medical Technology Co., Ltd., China. Silica gel-60 for thin-layer chromatography (TLC) was obtained from Merck KGA (Darmstadt, Germany). R2A agar was purchased from Difco (Detroit, MI, USA). Esculin (6,7-dihydroxycoumarin 6-glucoside) was purchased from Sigma (St. Louis, MO, USA). The CoreOne bacterial DNA extraction kit, PCR purification kit, and API ZYM kit were purchased from Coretech and Bioneer (Daejeon, Korea). All other chemicals were of analytical reagent grade.

2.2. Isolation of microorganisms

A soil sample was collected from a ginseng field in Changbai Mountain, Jilin Province, China, at a depth of 20–30 cm. The 0.1-g sample was diluted with 99.9-mL sterile distilled water, and 0.2 mL of the suspension was inoculated onto R2A agar plates and incubated at 30°C for 1–3 d. The morphological appearance of the inoculated plates was observed, and distinct colonies were subcultured to obtain pure isolates.

2.3. Screening of microorganisms producing β-glucosidase

The pure strains were transferred onto the Esculin-R2A agar plates (yeast extract 0.5 g, dextrose 0.5 g, casamino acids 0.5 g, proteose peptone 0.5 g, soluble starch 0.5 g, sodium pyruvate 0.3 g, dipotassium phosphate 0.3 g, magnesium sulfate 0.05 g, and agar 15.0 g containing 1.0 g of esculin and 0.5 g of ferric citrate per liter). This method allows esculin to be used as a substrate for a convenient zymogram technique to locate β-glucosidase in polyacrylamide gels. The rationale behind this technique is that the natural β-glucoside esculin is split into esculetin (6,7-dihydroxycoumarin) and glucose by the action of β-glucosidase, and then esculetin reacts with ferric ions to form a black precipitate [20]. Microorganisms were isolated from the soil obtained from the ginseng field by direct plating onto Esculin-R2A agar. The microorganisms producing β-glucosidase appeared as colonies surrounded by a brown to dark brown zone and were then incubated for 3 d at 30°C.

Total genomic DNA was extracted using the CoreOne bacterial DNA extraction kit. DNA quality was assessed by 1× TAE agarose gel electrophoresis stained with ethidium bromide nucleic acid gel stain. The bacterial 16S rDNA was amplified using the universal primers 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-TACGGYTACCTT GTTACGACTT-3′). Following PCR, the PCR product was sequenced by Shanghai Invitrogen Biotechnology Co. Ltd., China. The sequence was compared to the GenBank databases using the BLAST algorithm. A phylogenetic tree was constructed by the neighbor-joining method and the MEGA 6.0 program with bootstrap values based on 1000 replicates.

2.4. Cultivation of ginseng adventitious roots using an airlift bioreactor

Roots of *P. ginseng* obtained from a local market in the city of Yanji, Jilin, China, were washed in water and blotted dry. After spraying the roots with 70% ethanol, the internal tissue of the roots was cut into 10-mm sections and inoculated into Murashige and Skoog (MS) media [21] containing 1.0 mg L^{-1} 2,4-dichlorophenoxyacetic acid (Sigma, USA), 30.0 g L^{-1} sucrose, and 6.5 g L^{-1} agar. A callus was obtained following incubation at 25°C in the dark for 30 d.

For the induction of adventitious roots from the callus, the proliferated callus was inoculated into modified MS medium supplemented with 5 mg L^{-1} IBA and 30.0 g L^{-1} sucrose (pH 5.8) and cultured at 25°C in the dark for 30 d. Adventitious roots were induced following this incubation period (Fig. 1) and were proliferated in the same medium by subculturing at 30-d intervals.

Further culturing was performed in a 5-L bioreactor containing 4 L of modified MS medium supplemented with 5 mg L^{-1} IBA and 30.0 g L^{-1}

Fig. 1. Adventitious roots of *P. ginseng* cultured in a 5-L airlift bioreactor for 30 d.

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