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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 controllable production of ginseng and its metabolites. During the

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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 $1 \times$ 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.

sucrose, and the inoculum was 20.0 g fresh weight of excised adventitious roots, which were then incubated at 25°C in the dark. The airflow rate was 100 mL min^{-1} during cultivation. The air composition in the control was the same as atmospheric air. The roots were separated from the bioreactor and dried. Dry weight was measured after drying for 2 d at 60°C.

2.5. Analysis of ginsenosides in P. ginseng and ginseng adventitious root extracts

Five grams of dried ginseng and ginseng adventitious root powder was extracted thrice with 10 mL of methyl alcohol. The extracts were then concentrated under reduced pressure at 40°C, followed by the addition of distilled water. After defatting with Et$_2$O, the aqueous layer was further extracted with aqueous saturated n-BuOH to afford n-BuOH and aqueous fractions, respectively. The supernatant fraction was evaporated, and the solid residue was dissolved in methanol [high-performance liquid chromatography (HPLC) grade] and used for HPLC.

2.6. Optimization of fermentation conditions

To investigate the optimal conditions for the fermentation of ginseng adventitious root extracts by strain K35, the optimal medium, pH, and culture time were determined. The β-glucosidase-producing strains were cultivated in the medium. A suspension of strain K35 was incubated until it reached logarithmic phase and then mixed with the same volume of the root water extract and incubated at 30°C in a shaking incubator (150 rpm) for a few days. A substrate control was maintained without the inoculation of the microorganism, and the other culture conditions were the same as those in the biotransformation experiment. The culture was extracted with the same volume of aqueous saturated n-BuOH and then centrifuged. The supernatant was analyzed by TLC.

2.7. Standard solutions and standard curve preparation

Reference ginsenoside standards of Rg1, Rd, Rb1, and CK were accurately weighed, placed in centrifuge tubes, and dissolved in methanol to prepare a stock solution to obtain a series of concentrations for linearity validation and standard curve preparation. The different concentrations of ginsenoside CK standards were filtered using a 0.45-μm syringe filter and then analyzed by HPLC. The peak areas of the standards were calculated, and the standard curve was prepared according to the peak areas and concentrations. The data were submitted to linear regression analysis in Microsoft Excel, and the regression equation for each ginsenoside was as follows: $y = 1870.7x + 66.664$ for ginsenoside Rg1 (0.008–8.000 mg mL^{-1}, $R^2 = 0.9991$), $y = 1598.8x + 35.703$ for ginsenoside Rb1 (0.004–4.000 mg mL^{-1}, $R^2 = 0.9988$), $y = 1604.2x + 20.314$ for ginsenoside Rd (0.004–4.000 mg mL^{-1}, $R^2 = 0.9989$), $y = 2961.7x + 12.920$ for ginsenoside CK (0.002–1.500 mg mL^{-1}, $R^2 = 0.9992$), y represents peak area.

2.8. Isolation and identification of ginsenoside CK from fermented ginseng adventitious roots

Ginseng adventitious roots were fermented by strain K35 in LL medium (pH 7.0) for 9 d, and the culture was then extracted with the same volume of aqueous saturated n-BuOH. The n-BuOH fraction was evaporated to dryness under vacuum, and the residue was then dissolved in methanol (HPLC grade) and subsequently applied to the preparative HPLC system. The fractions containing the product were collected and dried. The product was then analyzed by nuclear magnetic resonance (NMR) spectroscopy, and the results were compared with those of previous reports.

2.9. Analytical methods

TLC analysis was performed using silica gel-60 plates with a mixture of CHCl$_3$:CH$_3$OH:H$_2$O (10:5:1, v/v/v) as eluent. Staining was performed by spraying 10% (v/v) H$_2$SO$_4$ in ethanol and heating at 110°C for 5 min.

HPLC analysis was performed using BDS-HYPERSIL-ODS C$_{18}$ columns (4.6 × 250 mm, ID 5 μm) connected to an HPLC system (Agilent 1100, Santa Clara, CA, USA). The column was eluted using H$_2$O (A):CH$_3$CN (B). Gradient elution started with 77% solvent A and 23% solvent B and then changed as follows: A from 77% to 54%, 0–13 min; A from 54% to 32%, 13–33 min; A from 32% to 0%, 45–55 min; and A from 0% to 77%, 60–63 min. The flow rate was 1.0 mL min^{-1}, and the samples were detected by absorption at 203 nm, with an injection volume of 10 μL.

^{13}C NMR spectra were observed on a Bruker Av 300 NMR spectrometer at 75 MHz with pyridine-d5 as the solvent.

3. Results

3.1. Analysis of ginsenosides in P. ginseng and ginseng adventitious root extracts

The methanol extracts of ginseng adventitious roots and *P. ginseng* were analyzed by HPLC. The method used to prepare the methanol extracts is outlined above. In this study, 12 ginsenosides (Rg1, Re, Rh1, Rb1, Rc, Rb2, Rd, F2, Rg3, CK, Rh2, and PPD) were analyzed by HPLC. The results shown in Fig. 2 indicated that the types of ginsenosides were reduced, and their contents were lower in ginseng adventitious roots than in ginseng cultivated in the field. Only small quantities of ginsenoside Rb1, Rd, and Rg1 were detected in ginseng adventitious roots. However, in field-cultivated ginseng, relatively high quantities of ginsenosides Rh1, Rb1, Rc, Rb2, and F2 were detected.

3.2. Screening of β-glucosidase-producing microorganisms

A total of 243 microbes were isolated from soil obtained from a ginseng field. Of these microbes, 147 β-glucosidase-producing isolates were screened using Esculin-R2A agar. Strain K35 showed excellent activity for the biotransformation of major ginsenosides in ginseng adventitious roots, and NCBI BLAST was used to determine the 16S rRNA gene sequence of this microorganism. Phylogenetic analysis based on 16S rDNA gene sequences indicated that the isolate belonged to the genus *Penicillium*, and it was most closely related to *Penicillium* sp. (HQ608083.1). The phylogenetic tree is shown in Fig. 3.

3.3. Fermentation of ginseng adventitious root extracts

Dried root powder (0.5 g) was immersed in 1.0 mL H$_2$O at 60°C for 2 d. After centrifugation, the supernatant was fermented by strain K35 to increase the ginsenoside contents. The optimal fermentation conditions (medium, pH, and fermentation time) are discussed below.

To identify the optimal medium for biotransformation, seven different liquid culture media were investigated. Strain K35 was inoculated into R2A, MRS, YM, LB, LL, and NA media and H$_2$O. The pH of each medium was 7.0. The TLC results, shown in Fig. 4a show the changes in ginsenoside contents after fermentation. A significant increase in the content of the rare ginsenoside CK was observed and the contents of major ginsenosides Rb1, Rd, and Rg1 decreased. The content of ginsenoside CK significantly increased when inoculated into the liquid culture medium LL and H$_2$O; thus, LL medium was chosen as it was best suited for the growth of strain K35.

To identify the optimal pH for the growth of strain K35, six different pH values (3.0–8.0) were investigated in LL medium, and the TLC results are shown in Fig. 4b. The pH of the LL medium was regulated using hydrochloric acid and sodium hydroxide. A significant increase in the content of the rare ginsenoside CK was observed by TLC analysis when
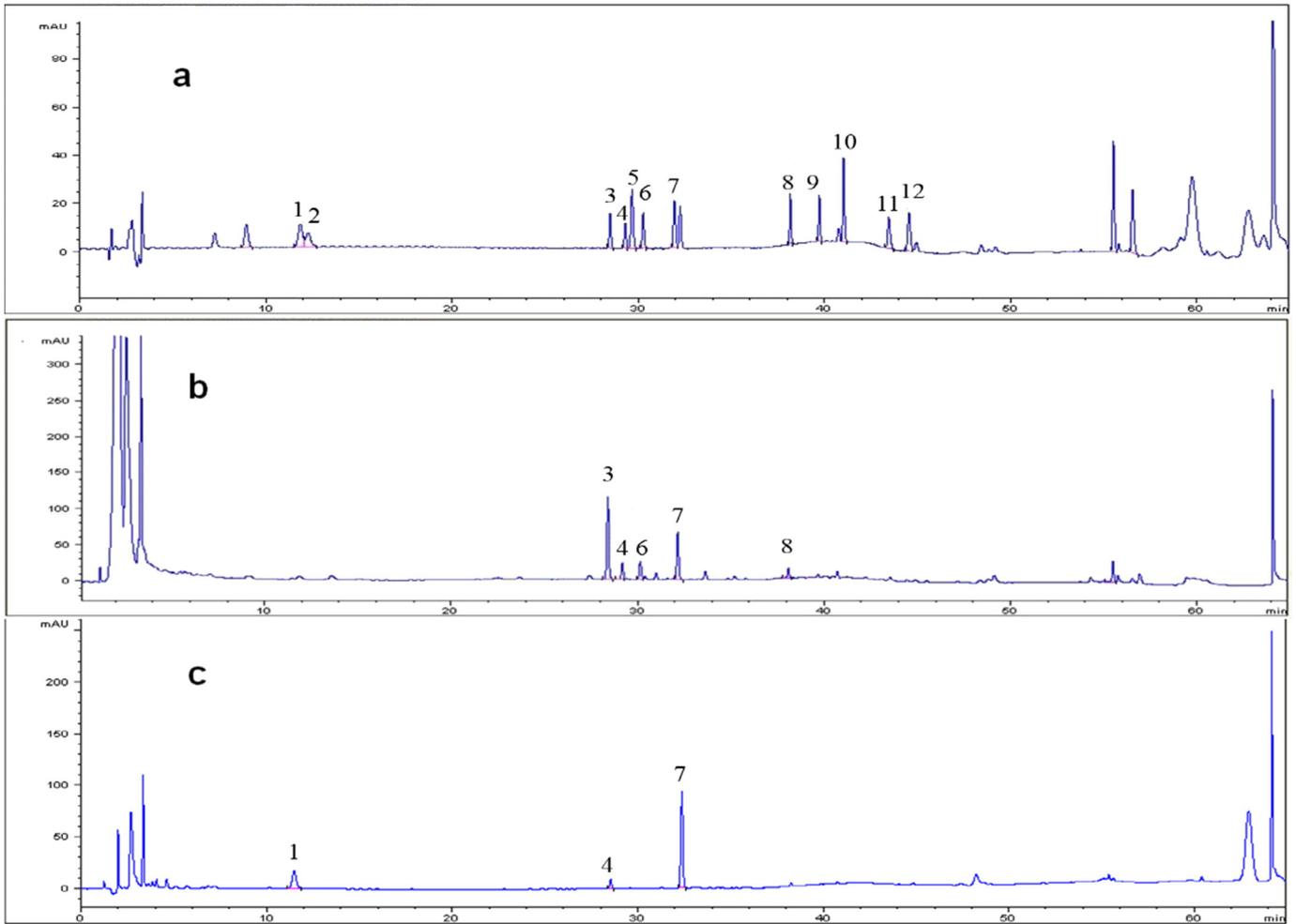
Fig. 2. The HPLC profile of ginsenoside standards (a), *P. ginseng* (b) and adventitious roots of ginseng (c). Peaks 1-12 correspond to ginsenoside Rg1, Re, Rh1, Rb1, Rc, Rb2, Rd, F2, Rg3, CK, Rh2, and PPD, respectively.
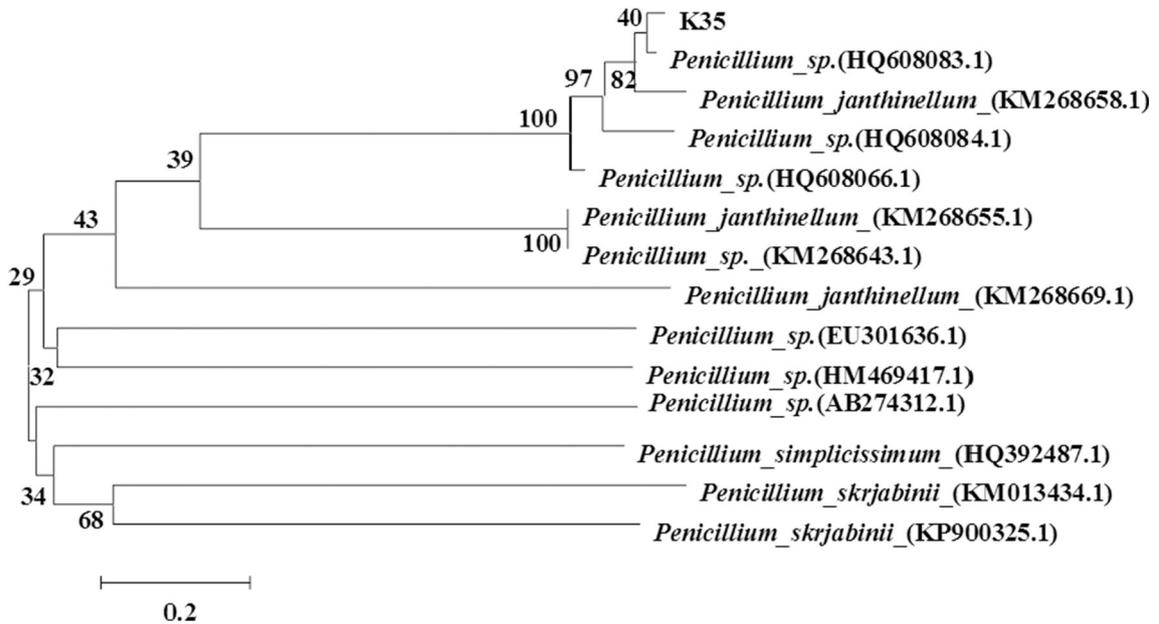

Fig. 3. Phylogenetic tree based on 16S rDNA gene sequences showing the phylogenetic relationships between strain K35 and related species.
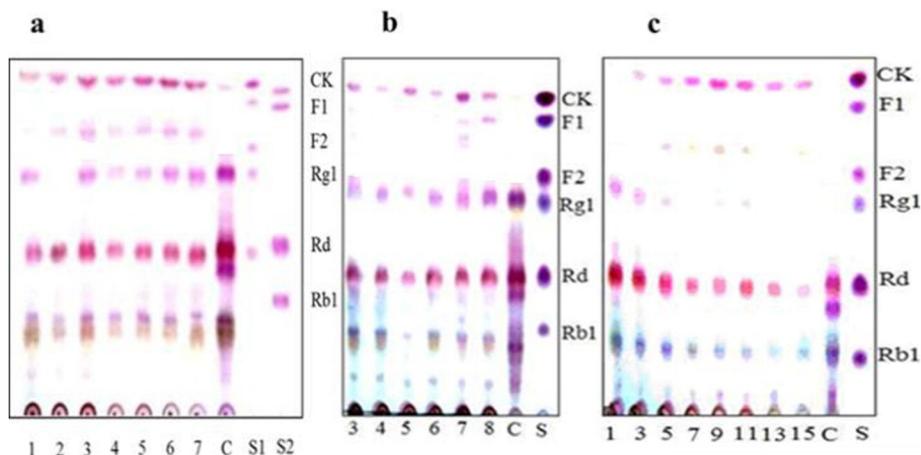
Fig. 4. (a) TLC chromatograms showing the effect of different media on the biotransformation of ginsenosides in adventitious roots of ginseng. Lanes 1–7 correspond to the media MRS, R2A, YM, NA, H$_2$O, LL, and LB, respectively. (b) Effect of different pH values on the biotransformation of ginsenosides in the roots. Lanes 3–8 correspond to the pH of LL medium: 3.0, 4.0, 5.0, 6.0, 7.0, and 8.0, respectively. (c) Effect of fermentation time on the biotransformation of ginsenosides in the roots. Lanes 1–15 correspond to the fermentation times 1, 3, 5, 7, 9, 11, 13, and 15 d, respectively, by strain K35. C: the root extracts without fermentation, S: ginsenoside standards.

the pH was 7.0. Therefore, a neutral environment (pH 7.0) is more suitable for biotransformation by strain K35 as it results in a higher content of the rare ginsenoside.

To determine the optimal fermentation time of strain K35, eight different fermentation times (1, 3, 5, 7, 9, 11, 13, and 15 d) were investigated, and the TLC results are shown in Fig. 4c. When strain K35 was inoculated into LL medium at pH 7.0 for less than 9 d, the contents of the major ginsenosides Rb1, Rd, and Rg1 gradually reduced over time, but the content of the rare ginsenoside CK gradually increased. When the fermentation time was longer than 9 d, the contents of ginsenosides Rb1, Rd, Rg1, and CK gradually decreased.

Thus, a fermentation time of 9 d was suitable to obtain the rare ginsenoside CK.

3.4. Changes in ginsenosides during the fermentation of ginseng adventitious roots

Strain K35 was used to ferment an aqueous extract of ginseng adventitious roots in LL medium at pH 7.0. Following fermentation for 9 d, the fermentation products were analyzed by HPLC, and the contents of ginsenosides were calculated using the standard curve. The results are shown in Fig. 5 and Table 1. The composition of
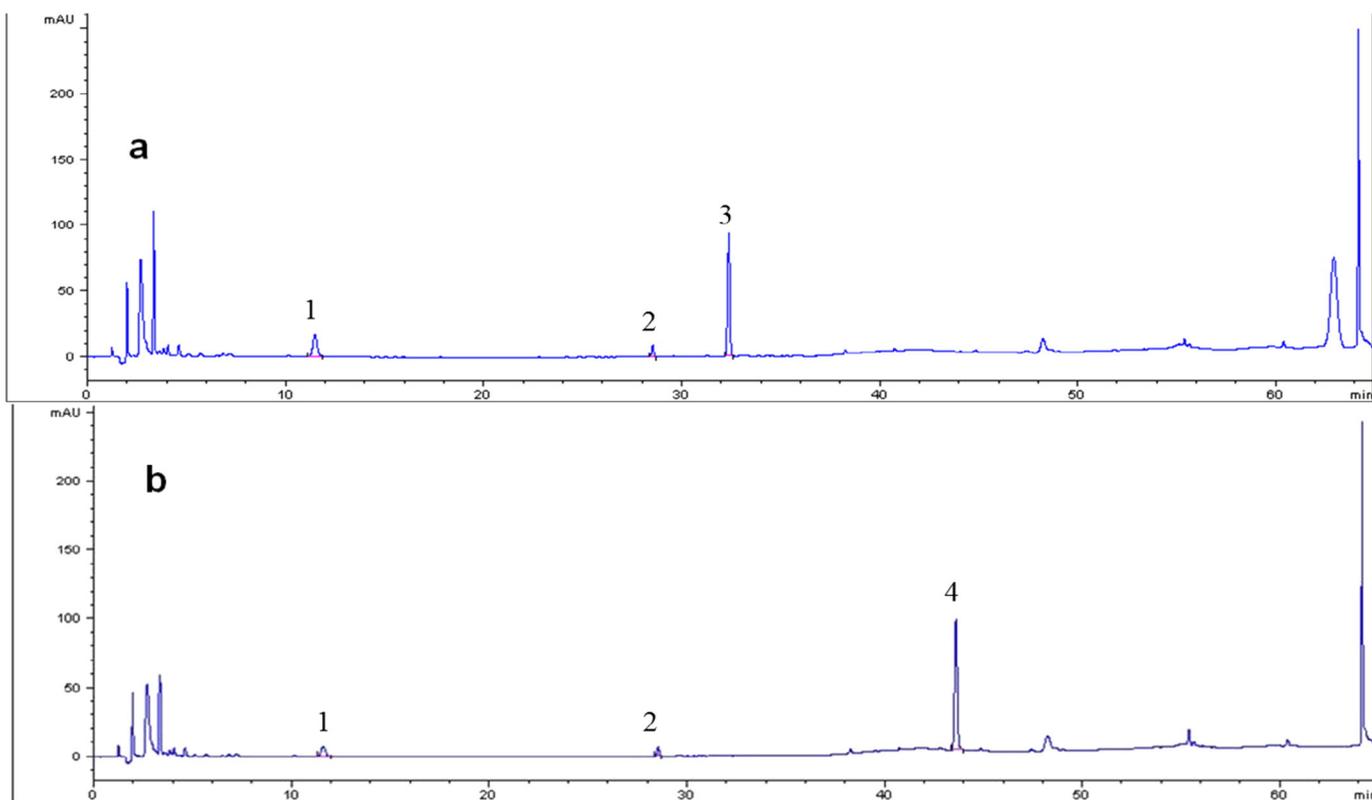

Fig. 5. HPLC analysis of ginsenosides in non-fermented and fermented adventitious roots of ginseng. (a): substrate control was run without inoculation of the microorganism, (b): fermented adventitious roots of ginseng by strain K35 in LL medium (pH 7.0) for 9 d. Peaks 1–4 correspond to ginsenoside Rg1, Rb1, Rd, and CK, respectively.

Table 1
The contents of ginsenosides in fermented and unfermented ginseng adventitious roots. A represents unfermented ginseng adventitious roots, and B represents fermented ginseng adventitious roots.

Ginsenoside content (mg mL^{-1})	Rg1	Rb1	Rd	CK
A	0.105	0.017	0.444	0
B	0.027	0.009	0	0.253

ginsenosides in ginseng adventitious roots was significantly altered following fermentation with strain K35. The content of ginsenoside Rg1 in ginseng adventitious roots was 0.105 mg mL^{-1}, Rb1 was 0.017 mg mL^{-1}, and Rd was 0.444 mg mL^{-1}. The content of ginsenoside Rg1 in fermented ginseng adventitious roots was 0.027 mg mL^{-1}, Rb1 was 0.009 mg mL^{-1}, and CK was 0.253 mg mL^{-1}; the actual mass of ginsenoside CK was 0.1765 mg. The content of ginsenosides Rb1 and Rg1 decreased slightly, and the major ginsenoside Rd was completely converted into the rare ginsenoside CK after 9 d of fermentation.

3.5. Structure of the final product ginsenoside CK by NMR

Ginseng adventitious roots were fermented by strain K35 in LL medium (pH 7.0) for 9 d, and the culture was then added to a preparative HPLC system for the isolation of ginsenoside CK. The fractions containing the product were collected and dried. The product was analyzed by NMR, and the results were compared with those of previous reports. The carbon NMR (^{13}C NMR) spectrum was obtained on a Bruker Av 300 NMR spectrometer, with CD$_3$OD as the solvent. The signals of the products and their assignments are as follows: ^{13}C NMR (75 MHz, CD$_3$OD): δ 39.33(C-1), 28.05 (C-2), 77.82 (C-3), 39.33 (C-4), 56.14 (C-5), 18.54 (C-6), 34.95 (C-7), 39.85 (C-8), 50.08 (C-9), 37.13 (C-10), 30.76 (C-11), 69.91 (C-12), 49.31 (C-13), 51.35 (C-14), 30.76 (C-15), 26.42 (C-16), 51.35 (C-17), 16.13 (C-18), 15.81 (C-19), 83.05 (C-20), 22.09 (C-21), 35.97 (C-22), 22.95 (C-23), 125.75 (C-24), 130.66 (C-25), 25.52 (C-26), 17.17 (C-27), 28.46 (C-28), 16.10 (C-29), and 17.52 (C-30); 20-O-glc: δ 98.03 (C-1), 74.90 (C-2), 79.13 (C-3), 71.49 (C-4), 78.05 (C-5), and 62.71 (C-6). These results were very similar to the signals of standard ginsenoside CK reported by Li et al. [22]. Thus, it was assumed that the product obtained was ginsenoside CK.

4. Discussion

Ginsenosides have recently attracted considerable interest because of their significant pharmacological activity. Due to the special structures of the dammarane skeleton, only special β-glucosidases can hydrolyze ginsenoside-β-glucoside linkages [10]. In the present study, we isolated 147 β-glucosidase-producing microorganisms from soil obtained from a ginseng field and identified one strain. Strain K35 belongs to the genus *Penicillium* and has the advantages of rapid growth, high fecundity, and a high yield of enzyme. It can produce β-glucosidase and has a strong ability to convert ginsenosides; thus, strain K35 was used for the fermentation of ginseng adventitious roots. Previous studies have reported that the transformation pathway of major ginsenosides is Rb1 → Rd → F2 → CK [12,23], and the results of the present study indicated that strain K35 was a potential microorganism for converting ginsenosides into rare ginsenoside CK.

To investigate the optimal conditions for the fermentation of the adventitious roots of ginseng by strain K35, the culture medium, pH, and fermentation time were investigated. The culture medium must supply carbon sources, nitrogen sources, growth factors, inorganic salts, water, and many other moieties to enable biotransformation; in addition, it must meet the demands for microbial cell growth and reproduction because if there is a large number of cells, large number of products will be generated. Thus, an appropriate

medium is essential for the adequate growth of a microbial strain. As shown in Fig. 4a, the content of ginsenoside CK notably increased when strain K35 was inoculated into the liquid medium LL and H$_2$O. These results indicated that LL medium and H$_2$O were suitable for the biotransformation of ginsenoside Rd into ginsenoside CK. The LL medium was chosen because it contained more nutrients than H$_2$O.

It is well known that pH plays a vital role in the progress of fermentation. The pH of the culture medium mainly affects the cytomembrane of the microorganism, the degree of ionization of nutrients (thereby affecting the absorption of nutrients by the microorganism), and biologically active substances such as enzymes [23]. Fig. 4b shows that a significant increase in the content of the rare ginsenoside CK was observed in fermented root extracts when the pH was 7.0. Therefore, a neutral environment (pH 7.0) is more suitable for biotransformation by strain K35 because it results in a higher content of the rare ginsenoside CK. This result may be because strain K35 is more suitable for growing in a neutral environment or catalysis of β-glucosidase in a neutral environment is more efficient.

Fermentation time has considerable influence on the fermentation products as the strain requires enough time to produce enzymes for fermentation. If the fermentation time is too short, the strain may not produce enough enzymes, resulting in incomplete fermentation. If the fermentation time is too long, metabolism by the strain will be unstable because of insufficient nutrients and may inhibit fermentation. As shown in Fig. 4c, when strain K35 was inoculated into LL medium at pH 7.0 for less than 9 d, the contents of the major ginsenosides Rb1, Rd, and Rg1 gradually decreased, but the content of the rare ginsenoside CK gradually increased. This is because the growing strain produced more β-glucosidase to convert major ginsenosides into rare ginsenoside CK. When the fermentation time was 9–15 d, the contents of ginsenosides Rb1, Rd, Rg1, and CK gradually reduced. This may be due to lack of nutrients in the medium because of the overgrowth of strain K35, and as a result, strain K35 may use ginsenosides Rb1, Rd, Rg1, and CK to maintain normal activities.

Ginsenosides have remarkable pharmaceutical activities and intestinal absorption. Therefore, many studies have focused on the biotransformation of major ginsenosides into rare ginsenoside CK. In this study, strain K35 was used to ferment an aqueous extract of ginseng adventitious roots in LL medium at pH 7.0 to obtain ginsenoside CK. The fermentation was performed with and without the microbial strain. The changes in ginsenoside composition were evaluated by HPLC, and the contents of ginsenosides were calculated using the standard curve. Following fermentation by strain K35 for 9 d, the content of ginsenoside Rb1 and Rg1 decreased slightly, and all of the major ginsenoside Rd, with a content of 0.444 mg mL^{-1}, and some of ginsenoside Rb1 were converted into the rare ginsenoside CK after 9 d of fermentation. The content of ginsenoside CK was 0.253 mg mL^{-1}, and the actual mass of ginsenoside CK was 0.1765 mg, which was higher than that obtained in Cui's study [23]. Cui et al. [23] obtained 0.03323 mg ginsenoside CK following the fermentation of 1.5 g total ginseng saponins by *Platycodon grandiflorum* endophytes. In the present study, strain K35 directly converted ginsenoside Rd into ginsenoside CK without the formation of other products, thus making the isolation of ginsenoside CK easy. The materials in this study have the advantages of easy preparation and low cost, thus making this research very innovative and valuable. These findings have great potential for application in the pharmaceutical industry for the preparation of the rare ginsenoside CK.

Conflicts of interest

All contributing authors declare no conflicts of interest.

References

[1] Qi LW, Wang CZ, Yuan CS. Ginsenosides from American ginseng: Chemical and pharmacological diversity. Phytochemistry 2011;72:689–99. http://dx.doi.org/10.1016/j.phytochem.2011.02.012.

[2] Jia L, Zhao Y, Liang XJ. Current evaluation of the Millennium phytomedicine-ginseng (II): Collected chemical entities, modern pharmacology, and clinical applications emanated from traditional Chinese medicine. Curr Med Chem 2009;16:2924–42. http://dx.doi.org/10.2174/092986709788803204.

[3] Shibata S, Ando T, Tanaka O. Chemical studies on the oriental plant drugs. XVII. The prosapogenin of the ginseng saponins: Ginsenosides-Rb1, -Rb2, and -Rc. Chem Pharm Bull 1966;14:1157–61. http://dx.doi.org/10.1248/cpb.14.1157.

[4] Christensen LP. Chapter 1 Ginsenosides: Chemistry, biosynthesis, analysis, and potential health effects. Adv Food Nutr Res 2008;55:1–99. http://dx.doi.org/10.1016/S1043-4526(08)00401-4.

[5] Sun BS, Gu LJ, Fang ZM, Wang CY, Wang Z, Lee MR, et al. Simultaneous quantification of 19 ginsenosides in black ginseng developed from Panax ginseng by HPLC-ELSD. J Pharm Biomed Anal 2009;50:15–22. http://dx.doi.org/10.1016/j.jpba.2009.03.025.

[6] Paek IB, Moon Y, Kim J, Ji HY, Kim SA, Sohn DH, et al. Pharmacokinetics of a ginseng saponin metabolite compound K in rats. Biopharm Drug Dispos 2006;27:39–45. http://dx.doi.org/10.1002/bdd.481.

[7] Zhou W, Yan Q, Li JY, Zhang XC, Zhou P. Biotransformation of Panax notoginseng saponins into ginsenoside compound K production by Paecilomyces bainier sp. 229. J Appl Microbiol 2008;104:699–706. http://dx.doi.org/10.1111/j.1365-2672.2007.03586.x.

[8] Shin YW, Kim DH. Antipruritic effect of ginsenoside Rb1 and compound K in scratching behavior mouse models. J Pharmacol Sci 2005;99:83–8. http://dx.doi.org/10.1254/jphs.fp0050260.

[9] Zhang ZY, Du GJ, Wang CZ, Wen XD, Calway T, Li ZJ, et al. Compound K, a ginsenoside metabolite, inhibits colon cancer growth via multiple pathways including p53–p21 interactions. Int J Mol Sci 2012;14:2980–95. http://dx.doi.org/10.3390/ijms14022980.

[10] Yang XD, Yang YY, Ouyang DS, Yang GP. A review of biotransformation and pharmacology of ginsenoside compound K. Fitoterapia 2015;100:208–20. http://dx.doi.org/10.1016/j.fitote.2014.11.019.

[11] Zhao X, Gao L, Wang J, Bi H, Gao J, Du X, et al. A novel ginsenoside Rb1-hydrolyzing β-D-glucosidase from Cladosporium fulvum. Process Biochem 2009;44:612–8. http://dx.doi.org/10.1016/j.procbio.2009.01.016.

[12] Fu Y, Yin Z, Wu L, Yin C. Diversity of cultivable β-glycosidase-producing microorganisms isolated from the soil of a ginseng field and their ginsenosides-hydrolysing activity. Lett Appl Microbiol 2014;58:138–44. http://dx.doi.org/10.1111/lam.12166.

[13] Paek KY, Chakrabarty D, Hahn EJ. Application of bioreactor systems for large scale production of horticultural and medicinal plants. Plant Cell Tiss Org Cult 2005;81:287–300. http://dx.doi.org/10.1007/s11240-004-6648-z.

[14] Kanigel R. Getting to the root of ginseng. Health 2000;28:26–32.

[15] Wu JY, Wong K, Ho KP, Zhou LG. Enhancement of saponin production in Panax ginseng cell culture by osmotic stress and nutrient feeding. Enzyme Microb Technol 2005;36:133–8. http://dx.doi.org/10.1016/j.enzmictec.2004.07.010.

[16] Gao WY, Jia W, Duan HQ, Xiao PG. Industrialization of medicinal plant tissue culture. China J Chin Mater Med 2003;28:385–90 [In Chinese].

[17] Sivakumar G, Yu KW, Paek KY. Production of biomass and ginsenosides from adventitious roots of Panax ginseng in bioreactor cultures. Eng Life Sci 2005;5:333–42. http://dx.doi.org/10.1002/elsc.200520085.

[18] Yu KW, Gao W, Hahn EJ, Paek KY. Jasmonic acid improves ginsenoside accumulation in adventitious root culture of Panax ginseng C.A. Meyer. Biochem Eng J 2002;11:211–5. http://dx.doi.org/10.1016/s1369-703x(02)00029-3.

[19] Yu KW, Murthy HN, Jeong CS, Hahn EJ, Paek KY. Organic germanium stimulates the growth of ginseng adventitious roots and ginsenoside production. Process Biochem 2005;40:2959–61. http://dx.doi.org/10.1016/j.procbio.2005.01.015.

[20] Kwon KS, Lee J, Kang HG, Hah YC. Detection of β-glucosidase activity in polyacrylamide gels with esculin as substrate. Appl Environ Microbiol 1994;60:4584–6.

[21] Murashige T, Skoog F. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol Plant 1962;15:473–97. http://dx.doi.org/10.1111/j.1399-3054.1962.tb08052.x.

[22] Li PY, Liu JP, Lu D. Standard NMR spectrum of ginsenosides. 1st ed. Beijing: Chemical Industry Press; 2012.

[23] Cui L, Wu SQ, Zhao CA, Yin CR. Microbial conversion of major ginsenosides in ginseng total saponins by Platycodon grandiflorum endophytes. J Ginseng Res 2016;40:366–74. http://dx.doi.org/10.1016/j.jgr.2015.11.004.