Ginsenoside Rb1 is Transformed into Rd and Rh2 by *Microbacterium trichothecenolyticum*

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Ginseng is one of the most popular medicinal plants, especially in Korea, Japan, China, and other Asian countries. Ginseng is used as a traditional medicine for boosting immunity, providing nutrition, and recovering from fatigue. Ginseng saponins (ginsenosides) are the principle component of ginseng, and these saponins have various pharmacological and biological activities such as anti-aging, anti-inflammation, antidiabetic [10], and antitumor activities [9]. Ginsenosides can be classified into three groups: protopanaxadiol (PPD), protopanaxatriol (PPT), and oleanolic acid, based on their chemical compositions. To date, over 40 ginsenosides have been isolated and identified. The known ginsenosides include Rb1, Rb2, Rd, Rc, Re, and Rg1, and these ginsenosides constitute more than 90% of total known ginsenosides [5]. Ginsenoside Rd has exhibited encouraging neuroprotective efficacy in both laboratory and clinical studies. Based on the pathophysiological mechanisms of acute ischemic stroke, Rd can be applied to impede the ischemic cascade and can protect the ischemic brain [12]. In particular, Rb1 is the most abundant ginsenoside (23%), and it can be easily converted into more pharmaceutically potent minor ginsenosides by hydrolysis of its sugar moieties [5]. Recently, many studies have focused on ginsenoside Rh2, because of its antidiabetic, anti-inflammatory, and anticancer effects. Rh2 belongs to the PPD family, and Rh2 has been shown to increase insulin secretion, reduce ischemic brain injury in rats, and inhibit the proliferation of human lung adenocarcinoma A549 cells [7,8]. In addition, Rh2 inhibits adipocyte differentiation [4] and protects against t-butylhydroperoxide cytotoxicity in hepatocellular carcinoma HepG2 cells [6]. However, the amount of Rh2 is very low in both red ginseng and wild ginseng. Several conversion methods have been previously described, including acid treatment, base treatment, heating, enzymatic conversion [11], and microbial conversion [1,2]. Chemical methods were not found to be effective at converting Rh2, and thus many recent studies have been focused on the microbial conversion method.

Members of the genus *Microbacterium* can be isolated from a wide range of habitats, including soil, insects, human clinical specimens, and marine environments. It has previously been reported that *Microbacterium* spp. isolated from ginseng fields are capable of biotransformation of
In this study, we screened for Microbacterium spp. that can transform ginsenosides, and M. trichothecenolyticum KCTC 19343 was selected as a target microorganism. M. trichothecenolyticum KCTC 19343 was purchased from the Korean Collection for Type Cultures (Daejeon, Korea). Ginsenosides Rb1, Rd, Rg3, and Rh2 were purchased from BTGin (Chungnam, Korea). Silica gel 60 F$_{254}$ plates were purchased from Merck (Darmstadt, Germany).

M. trichothecenolyticum KCTC 19343 was seeded in Luria-Bertani broth (LB), tryptic soy broth (TSB), or nutrient broth (NB) and then cultured for 24 h at 30°C. Cell growth was monitored by reading the absorbance at 600 nm. Microbial suspension (100 µl) and cell-free extracts (cells were eliminated by centrifugation at 13,000 rpm for 10 min) were

**Fig. 1.** Growth curves of Microbacterium trichothecenolyticum KCTC 19343 cultured in LB broth, TSB, or NB.

**Fig. 2.** TLC analysis of Rb1 or Rd reaction mixtures with Microbacterium trichothecenolyticum KCTC 19343 or its culture supernatants.

(A) Rb1 mixture with TSB culture; (B) Rd mixture with TSB culture; (C) Rb1 mixture with NB culture; (D) Rd mixture with NB culture.
mixed with 100 µl of 1 mM Rb1 and Rd. The reaction mixtures were then incubated on a rotary shaker at 30°C for 48 h. Each reaction mixture was extracted with 200 µl of water-saturated butanol; and 10 µl of the extracts were loaded on silica gel 60 F254 plates. The plates were developed in a chamber with chloroform/methanol/water (65:35:10, by volume) and detected by spraying with 20% H2SO4 in methanol, followed by heating. HPLC analysis was carried out using a Hitachi L-6200 pump coupled with a Sedex 75 ESLD (Sedere, Virty-sur-Seine, France) and a SIL-9A auto injector (Shimadzu, Japan) [3]. Five micrograms of each reaction mixture was injected for HPLC analysis, and peaks were assigned by comparing their retention times with that of reference compounds.

The growth of bacteria in the three different broth types was checked by measuring optical densities. The growth curves showed that the stationary phase was reached at 24 h for each broth type. The absorbance values of samples from LB broth, TSB, and NB at 24 h were 2.41, 2.08, and 1.13, respectively (Fig. 1). Next, we mixed cell suspensions or supernatants of the culture broths with Rb1, Rd, and Rg3, and examined the conversion of ginsenosides by TLC analysis. TLC analysis revealed that Rb1 was converted into Rd and then into Rh2 by bacteria from the TSB culture. Cells cultured for 12 h completely transformed Rb1 into Rd. However, the conversion of Rd into Rh2 was relatively weak. In addition, the conversions conducted with supernatants were very weak. The bacteria grown in NB converted Rd into Rh2. On the other hand, Rg3 was not converted by bacteria grown in NB (data not shown). M. trichothecenolyticum was shown to transform Rb1 into Rd and further into Rh2. The highest levels of conversion were observed when bacteria were cultured in TSB, and conversion was very weak when bacteria were grown in NB. Cell-free supernatants did not show any conversional activities (Fig. 2). Next, the 24 h reaction mixture was analyzed by HPLC. Rb1 was mainly converted into Rd and small amount of Rb2. HPLC analysis of the Rd mixture revealed that Rd was transformed to Rh2. Therefore, the results from HPLC analysis of each reaction mixture are consistent with those of the TLC analysis (Fig. 3).

In this study, we used M. trichothecenolyticum KCTC 19343 to convert Rb1 into Rd and, subsequently, into Rh2. Bacteria grown in TSB showed the highest levels of conversion activity. In addition, the transforming activity was detected with cell suspensions, suggesting cell surface proteins are responsible for transformation of Rb1 into Rd and further into Rh2.

**Fig. 3.** HPLC analysis of Rb1 or Rd reaction mixtures with bacteria suspensions from TSB. (A) Rb1 mixture and (B) Rd mixture.

**References**


