

Antiulcerogenic and Anticancer Activities of Korean Red Ginseng Extracts Bio-transformed by *Paecilomyces tenuipes*

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Abstract In the present study, red ginseng extracts were fermented by *Paecilomyces tenuipes* and the protopanaxdiol-type ginsenosides in the extracts were bio-transformed to F2, Rg3, Rg5, Rk1, Rh2, and CK determined by a high-pressure liquid chromatography analysis. It indicates that *P. tenuipes* is a microorganism to biotransform protopanaxdiol-type ginsenosides to their less glucosidic metabolites. Other biotransformed metabolites during fermentation were also analyzed using a GC-MS and identified as 2-methyl-benzaldehyde, 4-vinyl-2-methylphenol, palmitic acid, and linoleic acid. Antiulcerogenic activity of the fermented red ginseng extract (FRGE) on gastric mucosal damage induced by 0.15 M HCl in ethanol in rats was evaluated. FRGE was shown to have a potent protective effect on gastritis with

60.5% of inhibition rate at the dose of 40 mg/kg when compared to 54.5% of the inhibition rate at the same dose for stillen, the currently used medicine for treating gastritis. Linoleic acid showed a strong inhibition on gastritis with 79.3% of inhibition rate at the dose of 40.0 mg/kg. FRGE exhibited a distinct anticancer activity including growth inhibition of the two human colon cancer cells HT29 and HCT116. HT29 cells were less susceptible to FRGE in comparison with HCT116 cells. Taken together, fungal fermentation of the red ginseng extract induced hydrolysis of some ginsenosides and FRGE exhibited potent antiulcerogenic and anticancer activities. These results refer to use FRGE as a new source for treating human diseases.

Keywords fungal fermentation · gastritis, rat · human colon cancer cells · *Paecilomyces tenuipes* · red ginseng extract

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Introduction

Korean ginseng (*Panax ginseng* C. A. Myer) has been known to possess heterogeneous pharmacological activities including prevention of obesity in high fat diet-induced obese C57BL/6J mice (Lee et al., 2012a), antidiabetic effects on type 2 diabetic rats induced by high-fat and streptozotocin (Liu et al., 2013), restoring antioxidant capacity in aged rats (Ramesh et al., 2012), antiapoptotic activity in rat pancreatic b-cells (Che et al., 2012) and anticancer activity in breast cancer cells (Kang et al., 2011).

However, there are two major problems that should be blended before use of ginseng extracts. One is safety and tolerability of ginseng extracts. The other one is bioavailability of bioactive compounds in ginseng extracts. Recently, a clinical trial report reveals that *Panax ginseng* root extract has been shown to be safe, tolerable, and free of any untoward toxic effect in Korean healthy male and females (Lee et al., 2012b). Bioavailability is serious bottleneck of intake of bioactive ginsenosides from the ginseng extract (Xu et al., 2003). The authors demonstrated that only

4.35% of Rb1 and 18.40% of Rg1 were available and absorbed through the digestive tract after oral administration in rats (Xu et al., 2003). In relation to increase bioavailability of bioactive ginsenosides, many researchers have introduced fermentation method to metabolize ginsenosides to be less glucosidic compounds using *Lactobacillus plantarum* (Kim et al., 2011), *Phellinus linteus* (Lee et al., 2009), and *Lentus edodes* and *Inonotus obliquus* (Bae et al., 2011). The biotransformation of ginsenosides by living organisms successfully increases bioavailability (Ryu et al., 2013).

Paecilomyces tenuipes originally known as entomopathogenic fungus is a mushroom showing anticancer and immune activities with other medicinal interest (Han et al., 2004; Chen et al., 2008). Even if *P. tenuipes* is a carnivorous fungus, it can survive without supply of minute insects as nutrients. Recently, *P. bainier* sp. 229 has been isolated for ginsenoside hydrolysis as it mediates the biotransformation of Rb1 and Rb1 to Rd and CK, respectively (Yan et al., 2008; Ye et al., 2010).

The purpose of this study was to enhance bioavailability of red ginseng extracts by a biotransformation process of ginsenosides using *P. tenuipes* and find some pharmacological activities. In the present study, biological activities of fermented red ginseng extract (FRGE) were determined in comparison to the non-fermented ginseng extracts (NFRGE).

Materials And Methods

Materials. Human colorectal cancer cell lines HCT-116 and HT-29 cells were purchased from Korean Cell Line Bank (Korea). *Paecilomyces tenuipes* mycelia were kindly presented by Dr. Shin (Bion Co., Korea). Red ginseng fermented with *P. tenuipes* (FRGE) was manufactured by Nanotoxtech Inc. (Korea). Red ginseng extract was purchased from Nonghyub Company (Korea). RPMI1640 and DMEM culture media were obtained by Invitrogen Co. (USA). Standard ginsenosides Rg1, Re, Rf, Rh1, Rg2(s), Rg2(r), Rb1, Rc, Rb2, Rd, F2, Rg3(s), Rg3(r), CK, Rg5, Rk1, Rh2(s), and Rh2(r) were supplied by Prof. Moon (Hankyong National University, Korea). Other reagents and antibiotics were purchased from Sigma Chemical Co. (USA) and were used as received.

Biological materials. Male Sprague-Dawley rats (180–210 g) were used in the experiments. All animals were housed in a temperature-controlled room with a 12 h light period. They were fed commercial solid food (Samyang Yuji Co. Ltd., Korea) and tap water *ad libitum*. The test materials were suspended in 2% carboxymethylcellulose solution and given in a volume of 0.2 mL/100 g body weight. The doses of the test materials were chosen based on the yields obtained from the original extract or fractions. The room temperature was maintained at 25°C.

Microorganisms and fermentation. The strain of *P. tenuipes* maintained on potato dextrose agar slant was produced as previously described by fermentation of *P. tenuipes* mycelia with the following modification (Lee et al., 2009). A potato dextrose

broth or potato dextrose agar was used as a medium which seed cultivations were carried out for 10 days at 24.5 and 145 rpm. Ten percent red ginseng extract (2 L) was prepared as a media for the fermentation and two hundreds milliliters of the seed culture broth were then added to initiate the fermentation. After 10 days of cultivation, mycelia and culture media were harvested by filtration of culture broths and were filtered for further high-pressure liquid chromatography (HPLC) analysis. A filtered sample was ready to be analyzed by HPLC.

Determination of ginsenosides using HPLC. The levels of major 11 ginsenosides were determined by using a method previously reported (Ryu et al., 2013). A HPLC system as an Agilent 1100 (Agilent Technologies, USA) was equipped with a quaternary solvent delivery system and a column of Imtakt Cadenza CD-C18 (4.6×75 mm; Imtakt Co., Japan). Gradient elution was employed, using 10% acetonitrile and 90% acetonitrile at 35°C. The flow rate was at 1.0 mL/min, and the volume of the injected sample was 2 µL. A typical HPLC chromatogram of 18 ginsenoside standards is shown in Fig. 1.

Determination of fatty acids and polyacetylenes using GC-MS. After a 10-day fermentation, the culture medium was filtered through glass wool and centrifuged (6,000×g, 10 min). The supernatant was extracted with ethyl acetate (3×500 mL). The extracts were combined and some metabolites were analyzed on a gas chromatograph-mass spectrometer (GC-MS). GC-MS analyses were performed using a GC-MS-QP 2010 spectrometer (Shimadzu, Japan), equipped with a 30 mm×0.25 mm (i.d.), 0.25 µm DM-5 capillary GC column (Dikma Technologies, China). The carrier gas was helium at a rate of 2 mL/min. The column temperature started from 120°C (2 min), increased to 280°C at a rate of 2°C min⁻¹, and was held at 280°C for 10 min. Injector and analyzer temperatures were 270 and 280°C, respectively. The mass spectrometer was operated in EI mode. Initially, detection of the two compounds was performed using the full-scan mode in the range *m/z* 40–380. Selected ion monitoring (SIM) GC-MS analysis was applied to identify polyacetylenes in ginseng and SIM mode with scanning at 159 and *m/z* 121 for the detection of panaxynol and panaxydol, respectively, was carried out for the identification of the polyacetylenes.

Cell culture and cell viability assay. HCT-116 and HT-29 cells were kept in McCoy's 5A medium supplemented with 10% fetal bovine serum and penicillin-streptomycin (50 units/mL). Cells were grown and maintained in a tissue culture dish (100 mm i.d.) and kept in a humidified incubator (5% CO₂ in air at 37°C) with a medium change every 2–3 days. Cell viability was measured by a method using 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) (Duan et al., 2005). In a reaction mixture MTT at a concentration of 0.5 mg/mL was added to every well and incubated for 4 h, after which the media was removed and replaced with dimethyl sulphoxide (DMSO). Level of optical density was measured by Spectra MAX 190 Reader (MDS Inc., USA) at 570 nm after reduced MTT was dissolved in DMSO for 30 min.

Ethanol/HCl-induced gastric lesion in rats. The gastroprotective