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Antihepatocarcinoma Activity of Lactic Acid Bacteria Fermented *Panax notoginseng*

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Panax notoginseng was used as the medium for lactic acid bacteria fermentation to manufacture product with antihepatocarcinoma activity. The fermentation broth prepared in a 250 mL Erlenmeyer flask was found to possess antiproliferation activity against hepatoma Hep3B cells. At the dosage of 500 μg/mL, the viability of hepatoma Hep3B cells was approximately 2.2%. When the fermentation was scaled up to a 6.6 L fermenter, it was found that the fermentation broth produced at 37 °C for 2 days showed the highest antihepatoma activity. Animal study revealed that when Hep3B implanted SCID mice were treated with 1000 mg/kg BW/day of the fermentation broth, tumor volume and tumor weight were reduced approximately 60% as compared to the negative control group. HPLC analyses showed that saponins in *P. notoginseng* including notoginsenoside R_1 and ginsenosides Rg_1, Rb_1, Rd, and Rh_4 decreased, but ginsenosides Rh_1 and Rg_3 increased during fermentation. LC-MS/MS revealed that the minor saponins ginsenoside F_1, protopanaxatriol, and notoginseng R_2 also exist in the fermentation product. It appears that ginsenoside Rg_3, ginsenoside Rh_1, and protopanaxatriol are possibly responsible for the enhanced antihepatocarcinoma activity of the *P. notoginseng* fermentation broth.

KEYWORDS: ***Panax notoginseng*; lactic acid bacteria; fermentation; antihepatocarcinoma activity; saponins**

INTRODUCTION

The root of *Panax notoginseng* (Burk.) F.H. Chen. (known as Sanqi in Chinese), a highly valued traditional Chinese medicine, is often used to treat cardiovascular diseases, ease inflammation, remove blood stasis, stop bleeding, relieve swelling, and alleviate pain (*1*) and has been shown to possess antitumor activity (*2*, *3*). The constituents of *P. notoginseng* include various saponins, amino acids, polysaccharides, and flavonoids (*4*, *5*), but most of the bioactivities of *P. notoginseng* are believed to be associated with saponins. The saponins of *P. notoginseng* can adjust immune responses, decrease plasma lipids, improve early postburn cardiac function, protect against ischemic brain damage, and inhibit proliferation of cancer cells (*6*−*11*). So far, 59 saponins have been identified in *P. notoginseng* (*6*). These saponins have a dammarane structure; 35 belong to the protopanaxadiols (PPD) group and 24 belong to the protopanaxatriols (PPT) group (*12*). The major saponins present in *P. notoginseng* are ginsenosides Rg_1, Rb_1, Rd, and notoginsenoside R_1 (*13*, *14*).

P. notoginseng is available in raw or steamed form. Traditionally, the raw form is widely used in Chinese medicine for its hemostatic and cardiovascular properties (*4*, *15*), whereas the steamed form has been claimed to be a tonic used to "nourish" blood (*5*). Sun et al. (*12*) examined the effect of steaming on saponin composition and anticancer activities of *P. notoginseng*. They found that steaming decreased the contents of notoginsenoside R_1, ginsenosides Rg_1, Re, Rb_1, Rc, Rb_2, Rb_3, and Rd, but increased the contents of Rh_1, Rg_2, 20*R*-Rg_2, Rg_3, and Rh_2. The extract of steamed root significantly inhibited proliferation of SW-480 human colorectal cancer cells (*12*).

In recent decades, fermentation using microorganisms including bacteria, fungi, and algae for the production of useful compounds has been extensively studied (*16*−*18*). The biotransformation with microorganisms is also an important approach in the field of applied biocatalysis. Biocatalysis is a powerful tool for the generation of new, active, and less toxic bioactive products that would be difficult to obtain from either biological systems or chemical synthesis (*19*). Fermentation is one of the most convenient techniques of biocatalytical process. During fermentation, microbial metabolism can convert raw materials to products with health-promoting properties (*20*−*22*). Bae et al. found that the ginseng saponins could be transformed to ginsenoside Rh_2 by human intestinal bacteria and that the transformants possessed anticancer activity (*23*).

Lactic acid bacteria (LAB) play essential roles in food fermentation and are often used as cell factories for the production of food and pharmaceutical products (*24*, *25*). The aims of this research were to study the antihepatocarcinoma activity of *P. notoginseng* fermented with LAB and to investigate the bioactive compounds responsible for the antihepatoma activity in the fermentation products.

MATERIALS AND METHODS

Bacterial Strains and Inocula Preparation. *Streptococcus salivarius* subsp. *thermophlius* BCRC 12268, *Lactobacillus helveticus* BCRC 14092, *Lactobacillus rhamnosus* GG. ATCC 53103 BCRC 16000, *Lactobacillus*

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acidophilus BCRC 10695, *Bifidobacterium longum* BCRC 14602, *Bifidobacterium catenulatum* BCRC 14667, *Bifidobacterium breve* BCRC 11846, and *Bifidobacterium bifidum* BCRC 14615 were obtained from the Bioresource Collection and Research Centre, Food Industry Research and Development Institute (Hsinchu, Taiwan). Aliquots of 0.5 mL solutions of each strain were stored at −80 °C in MRS broth (Difco, Detroit, MI) plus 0.5 mL of glycerol. The cultures were propagated twice in MRS (Merck, Darmstadt, Germany) at 37 °C before use. The activated culture was diluted with saline solution to obtain a preparation containing 8 log cfu/mL, which served as the inoculum.

Fermentation and Sample Preparation. The raw *P. notoginseng* was obtained from the Microbio Co. (Taipei, Taiwan). The fermentation medium contained 10 g of raw *P. notoginseng* powder and 90 mL of water (10% w/v). For preliminary fermentation study, 100 mL of *P. notoginseng* medium or MRS broth was placed in a 250 mL Erlenmeyer flask with screw cap and 1 mL of each inoculum of the lactic acid bacteria was inoculated together. The initial population of each microorganism in the *P. notoginseng* medium was 6 log cfu/mL. Inoculated *P. notoginseng* medium was cultured statically at 37 °C for various time periods. After fermentation, the fermentation broth was filtered through a 0.22 μm membrane and evaluated for soluble solid content by oven-drying at 105 °C. In the subsequent experiments, the fermentation broth was directly used to treat cells or tumor-implanted mice, and the dosage was based on the soluble solid content.

For the experiments using a stirred tank fermenter, a volume of 5 L of *P. notoginseng* medium was charged into a 6.6 L fermentation jar. The jar was then sterilized at 121 °C for 30 min. Aliquots of 5 mL of each inoculum (0.1% v/v) were together introduced into the fermenter, which was then operated at various temperatures for different time periods.

Cell Cultivation and Viability Assay. Human hepatocellular carcinoma Hep3B cell line was purchased from the Bioresource Collection and Research Centre (Food Industry Research and Development Institute, Hsinchu, Taiwan) and cultured in DMEM medium (Gibco, Grand Island, NY). The culture medium was supplemented with 10% heat-inactivated fetal calf serum (Hyclone, Logan, UT), incubated at 37 °C in a humidified 5% CO_2 incubator, passaged every 2−3 days with trypsin−EDTA−glucose (TEG) solution (0.25% trypsin, 0.1% EDTA, and 0.05% glucose in Hanks' balanced salt solution), and maintained in exponential growth.

The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) colorimetric method was used to determine cell viability. In brief, tumor cells were cultured in a 24-well microplate (5×10^4 cells per well in 1 mL of medium) for 24 h and then incubated with various concentrations of samples for 72 h. At the end of incubation, tetrazolium dye was added as an indicator to convert tetrazolium salts to a colored product, formazan. The formazan concentration was measured by spectrophotometer at 570 nm.

The primary rat hepatocytes were obtained from rats anesthetized with sodium pentobarbital. Cell viability was confirmed to be >90% via trypan blue exclusion. After washing, the isolated hepatocytes were resuspended in L-15 cell culture medium (pH 7.6) and then supplemented with 18 mM HEPES, 2.5% FBS, 5 mg/L of insulin, 5 mg/L of transferrin, 28 mM galactose, 1 M dexamethasone, 100,000 IU/L penicillin, and 100 mg/L streptomycin at a density of 5×10^8 cells/L. Cells (2.5×10^6) were plated on 60 mm collagen precoated plastic tissue culture dish and incubated at 37 °C in a humidified incubator. After 4 h, the medium was replaced by new medium that contained the same components but replaced FBS with 2.0 g/L BSA. After 16 h, medium was renewed and testing sample was added. After 24 h, the medium was removed to stop the reaction, and cells were washed with cold phosphate-buffered saline (PBS). Then cells were removed with a cell scraper and analyzed by MTT method (*26*).

Animal Study. The C.B17/Icr-SCID male mice (5 weeks old) were obtained from the National Laboratory Animal Center (Taipei, Taiwan) and housed in a rodent facility at 23 ± 1 °C with a 12 h light−dark cycle. All experiments were performed in accordance with the regulations of the NIH Guide for the Care and Use of Laboratory Animals (DHHS publication NIH 85-23, revised 1996). 5-Fluoracil (5-FU) was purchased from Sigma-Aldrich Co. The animals were divided into six groups, including normal control group, negative control group, experimental groups (200, 400, and 1000 mg/kg of BW/day (low dose, medium dose, and high dose, respectively)), and the positive control (5-FU) group. Each group had six mice. Except the normal control group, other mice were implanted with Hep3B cells (1×10^7 cells/0.2 mL of DMEM) by subcutaneous injection. The negative control and normal control groups received PBS (8 g of NaCl, 0.2 g of KCl, 1.44 g of Na_2HPO_4, and 0.24 g of KH_2PO_4 dissolved in 1000 mL of distilled water, pH 7.4) orally with a feeding needle every day. The experimental groups received daily oral feeding of sample with 200, 400, or 1000 mg/kg of BW/day. The positive control group received 12 mg/kg of BW/day of 5-FU by intraperitoneal injection every day right after implantation.

The body weight of each mouse and size of implanted tumors were determined by a single observer. Calipers were used to measure the largest (a) and smallest (b) diameter, and the tumor volume was estimated according to the formula $0.52ab^2$. Animals were sacrificed 36 days after implantation, and then the weights of tumor and visceral organs were measured.

HPLC Analysis. A volume of 10 mL of fermentation broth was mixed with 21 mL of methanol for 3 h at room temperature for saponins extraction. The extract was centrifuged at 4800*g* for 20 min, and the supernatant was dried via rotary evaporator. The residue was then dissolved with 6 mL of 70% methanol and analyzed by HPLC according to the method of Lau et al. (*28*). The analyses were performed using an Agilent 1100 liquid chromatograph (Palo Alto, CA) equipped with a quaternary gradient pump, an autosampler, a photodiode array detection (DAD) system, and a ZORBAX ODS C_{18} column (4.6 mm × 250 mm, 5 μm particle size). The binary gradient elution system consisted of water (A) and acetonitrile (B), and separation was achieved using the following gradient: 0−30 min, 20% B; 30−60 min, 20−45% B; 60−78 min, 45−75% B; 78−80 min, 75−100% B. The column temperature was kept constant at 35 °C. The flow rate was 1 mL/min, and the injection volume was 5 mL. The UV detection wavelength was set at 203 nm, and diode array scanning was from 190 to 400 nm (*27*). Individual saponins were identified by comparing the retention times with the published data in the literature (*28*) and the standards of ginsenosides Rg_1, Rb_1, and Rd and notoginsenoside R_1 provided by the Microbio Co. Ltd. (Taipei, Taiwan).

LC-MS/MS Analysis. Bruker Daltonics ion-trap mass spectrometer (Bruker Daltonik, GmbH) Esquire 3000 plus with online nano-ESI coupled with a nano-LC system (Waters) was used for investigating the minor saponins, which could not be identified by HPLC. Separation was carried out using a PSTi-Proteomics RP chromatographic nanocolumn (10 cm × 365 μm × 75 μm). The binary gradient elution system consisted of 0.2% acetic acid in water (A) and 0.2% acetic acid in acetonitrile (B). The following gradient was used: 25% B to 25% B in 2 min, 25% B to 80% B in 80 min, maintain 80% B for 10 min, 80% B to 50% B in 1 min, 50% B to 25% B in 18 min. The ESI conditions were as follows: ion polarity, negative; dry gas, 5 L/min; dry temperature, 200 °C; HV capillary, +1500 V; capillary exit, −213.6 V; skimmer, −40 V; trap drive, 140.8; full scan range, m/z 50−3000; end plate offset, −500 V. In Auto MS2Mode, the fragmentation parameters were as follows: number of precursor ions, 2; threshold Abs, 105; excluded after 5 spectra; release after 0.6 min; collision energy in the m/z range, about 0.8 V.

Statistical Analysis. All values are means of at least three replicates ± SD. Statistical analysis was performed using ANOVA and Duncan's multiple-range test (SAS Institute Inc., Cary, NC) to determine significant differences among means ($P < 0.05$).

RESULTS AND DISCUSSION

Effect of Fermentation Medium on Antihepatoma Activity. Both the *P. notoginseng* medium and the MRS medium were fermented with LAB at 37 °C for 24 h to investigate the effect of medium on the antihepatoma activity of the fermentation broth. The MRS broth is a common medium for LAB, but *P. notoginseng* is not. However, it was found that the LAB could also grow well in the *P. notoginseng* medium at 37 °C, indicating that this herb does not contain any antibacterial component and can provide basic nutrients for LAB growth. The total bacteria count of MRS fermentation broth was 10^9 cfu/mL, and the bacteria count of *P. notoginseng* fermentation broth was 10^8 cfu/mL. On the other hand, the antihepatoma activity of *P. notoginseng* fermentation broth was significantly higher than that of MRS fermentation broth (**Figure 1**). At the dosage of 500 μg/mL, the viability of Hep3B cells treated with *P. notoginseng* fermentation broth was only 2.2% as compared with >100% viability of the cells treated with MRS fermentation broth. In addition, at the same dosage

the viability of Hep3B cells treated with the unfermented *P. notoginseng* medium (also incubated at 37 °C for 24 h but without inoculation of LAB) was approximately 80% (data not shown). These results strongly suggested that the antihepatoma constituents were produced during fermentation of *P. notoginseng*.

For cytotoxicity study in vitro, we determined the IC_{50} of *P. notoginseng* fermentation broth to the primary rat hepatocytes. It was found that the IC_{50} was 976 μg/mL, indicating that the LAB fermentation broth of *P. notoginseng* had no strong toxic effect on normal hepatocytes, and it may be a potential remedy for hepatocelluar carcinoma treatment.

Effect of Fermentation Conditions on Antihepatoma Activity. It was observed that *P. notoginseng* fermented with LAB in a 250 mL flask exhibited antihepatoma activity. To improving efficacy, fermentation was then carried out in a 6.6 L stirred tank fermenter. Because the powder of the herb in the medium will precipitate without agitation, the fermentation was conducted with 100 rpm agitation. In addition, agitation could facilitate nutrient transfer during fermentation.

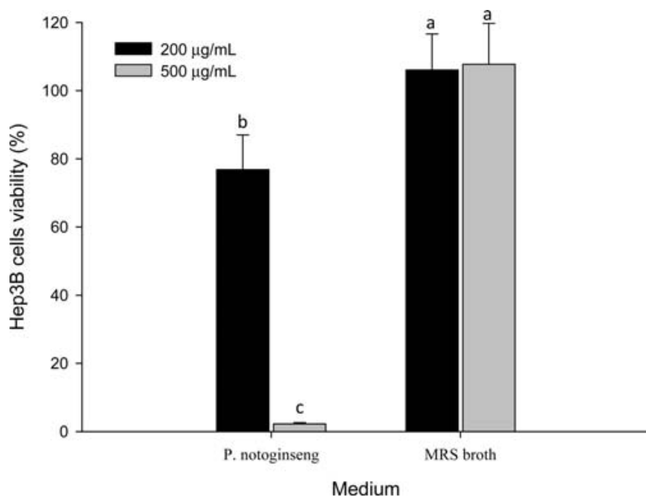


Figure 1. Viability of Hep3B hepatoma cells treated with lactic acid bacteria fermentation broths using MRS broth or *P. notoginseng* as the medium. Fermentation was carried out at 37 °C for 24 h in 250 mL flasks. Means not sharing a common alphabetic letter are significantly different ($p < 0.05$).

Figure 2 shows the effects of *P. notoginseng* fermentation broths cultured for various time periods at 32, 37, and 42 °C on the viability of Hep3B cells. The best antitumor activity of fermentation broth was obtained after fermentation for 2 days at 37 °C, indicating that 37 °C is a better temperature for LAB to utilize *P. notoginseng* to yield antihepatoma constituents. Perhaps some of the bioactive constituents in the fermentation broth might be degraded or further metabolized by the bacteria when the fermentation was carried out at a higher temperature or for a longer time. Nevertheless, results of this fermentation study proved that *P. notoginseng* could be fermented with LAB to produce antihepatoma constituents in a regular stirred tank fermenter. For the subsequent in vivo study, samples were prepared using 6.6 L fermenters, and the fermentation was carried out at 37 °C for 48 h with 100 rpm agitation.

Antihepatoma Activity in Vivo. To investigate the antihepatoma activity of *P. notoginseng* fermentation broth in vivo, we established a tumor model through transplantation of the human hepatocellular carcinoma Hep3B cells into C.B17/Icr-scid male mice and then treated the animals with test samples. As shown in **Table 1**, there was no significant difference in tumor growth among the low-dose group, the medium-dose group, and the negative control group. However, the high-dose group had a significantly smaller tumor size ($p < 0.05$) than the negative control group. In fact, the tumor volume of the high-dose group was approximately 40% of the negative control group.

When the mice were sacrificed on day 36 after implantation, the average weights of tumors of the low-dose, medium-dose, high-dose, and positive control groups were 1170, 1084, 537, and 376 mg, respectively, versus 1316 mg of the negative control group, which correspond to 11.1, 17.6, 59.1, and 71.4% inhibition (**Table 2**). This result indicated that administration of high-dose fermentation product could effectively suppress tumor growth in vivo.

At the day of sacrifice, the average body weights of all the tumor-implanted groups were significantly lower than that of the normal control ($p < 0.05$), and the negative control group was the lowest among all experimental groups (data not shown), indicating that the tumor severely retarded the growth of the animal. The weights of all vital organs of mice implanted with Hep3B cells

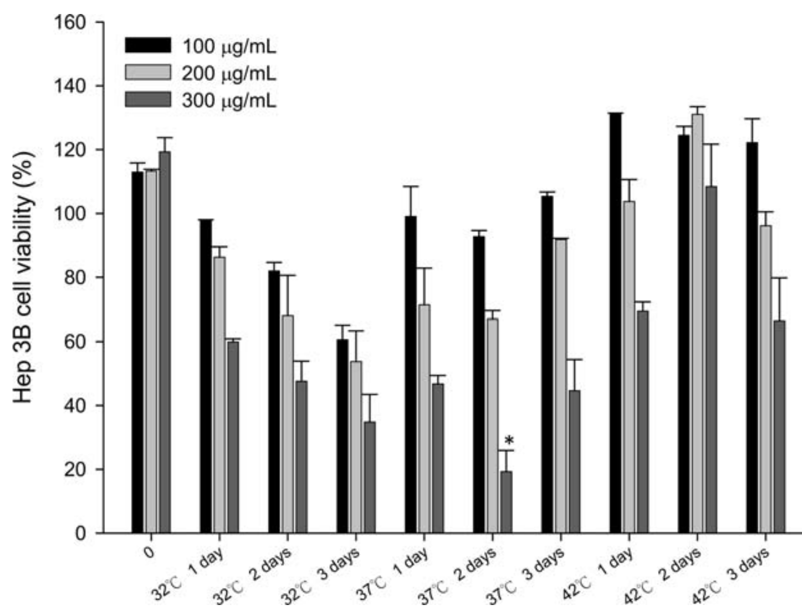


Figure 2. Viability of Hep3B hepatoma cells treated with lactic acid bacteria fermentation broths of *P. notoginseng* fermented at different temperatures for various time periods.

Table 1. Changes of Tumor Volume (mm^3) of C.B17/Icr-SCID Male Mice Fed Various Amounts of Samples during the Experimental Period[a]

group[b]	21 days	25 days	28 days	32 days	35 days
negative control	91.6 ± 18.2 ab	350.8 ± 131.8 a	693.8 ± 211.3 a	1089.6 ± 432.8 a	1533.9 ± 533.8 a
low dose	113.5 ± 71.4 a	278.0 ± 111.5 ab	603.0 ± 256.4 ab	993.0 ± 450.9 a	1534.5 ± 428.1 a
medium dose	63.3 ± 9.0 ab	205.5 ± 112.2 abc	510.6 ± 233.3 ab	682.8 ± 220.2 ab	1232.3 ± 532.7 ab
high dose	68.9 ± 22.7 ab	109.6 ± 77.7 bc	229.5 ± 145.4 bc	392.1 ± 207.2 b	621.1 ± 231.0 bc
positive control	27.2 ± 20.3 b	46.2 ± 12.4 c	102.2 ± 88.5 c	131.3 ± 88.2 b	333.8 ±140.0 c

[a] Means followed by different letters within a column are significantly different ($p < 0.05$). [b] Low dose, 200 mg/kg of BW/day; medium dose, 400 mg/kg of BW/day; high dose, 1000 mg/kg of BW/day.

Table 2. Changes of Tumor and Organ Weights (mg) in C.B17/Icr-SCID Mice Fed Various Amounts of Sample for 35 Days[a]

group[b]	heart	liver	spleen	lung	kidney	tumor
normal control	149 ± 20 a	1356 ± 123 a	100 ± 12 a	191 ± 8 ab	411 ± 13 a	
negative control	117 ± 18 b	1115 ± 143 b	59 ± 12 b	179 ± 14 b	278 ± 45 b	1316 ± 403 a
low dose	112 ± 10 b	1161 ± 131 b	60 ± 3 b	179 ± 31 b	321 ± 46 b	1170 ± 311 a
medium dose	108 ± 6 b	1212 ± 190 ab	62 ± 12 b	185 ± 14 b	325 ± 45 b	1084 ± 344 a
high dose	123 ± 12 b	1111 ± 66 b	66 ± 8 b	192 ± 20 ab	282 ± 23 b	537 ± 392 b
positive control	143 ± 15 a	1171 ± 103 b	119 ± 36 a	216 ± 23 a	328 ± 27 b	376 ± 233 b

[a] Means followed by different letters within a column are significantly different ($p < 0.05$). [b] Low dose, 200 mg/kg of BW/day; medium dose, 400 mg/kg of BW/day; high dose, 1000 mg/kg of BW/day.

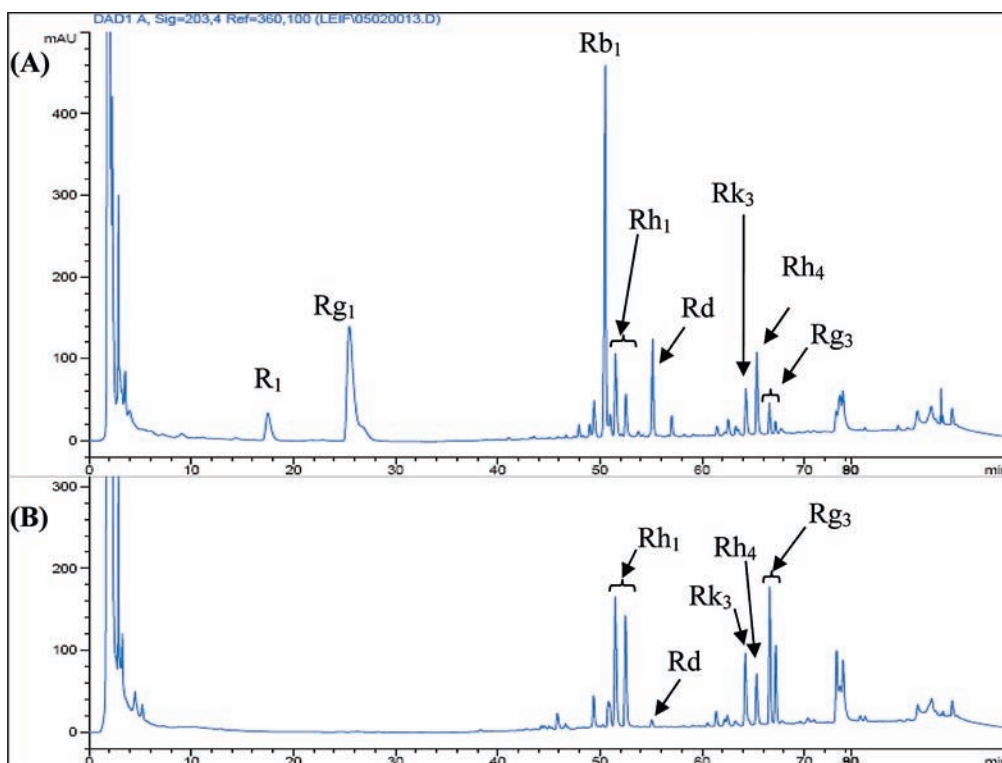


Figure 3. HPLC profiles of *P. notoginseng* saponins without fermentation (**A**) and with lactic acid bacteria fermentation at 37 °C for 48 h (**B**).

were also lower than those of the normal control group (**Table 2**). However, there was no significant difference in body and vital organ weights among the negative control and treatment groups. These results indicated that the LAB fermentation broth of *P. notoginseng* possesses antihepatoma activity without retarding the growth of mice with hepatoma.

Saponins Analysis. It is generally believed that the major bioactive compounds of *P. notoginseng* are saponins. Therefore, changes of saponins profile of *P. notoginseng* after LAB fermentation were investigated. **Figure 3** shows the HPLC profiles of saponins before and after LAB fermentation. The HPLC profile of saponins of *P. notoginseng* medium before fermentation was similar to the saponins profile of steamed *P. notoginseng* (*28*). Because the *P. notoginseng* medium was sterilized before fermentation, this result indicates that sterilization has the same thermal effect on the saponins of *P. notoginseng* as steaming. According to the retention times of the standards and the published data (*28*), it was found that notoginsenoside R_1 and ginsenosides Rg_1, Rb_1, and Rd are the major saponins, and ginsenosides Rh_1, Rk_3, Rh_4, and Rg_3 exist in minor amounts in the thermally processed and unfermented *P. notoginseng*. However, notoginsenoside R_1 and ginsenosides Rg_1, Rb_1, Rd, and Rh_4 disappeared or decreased significantly after fermentation. On the other hand, ginsenosides Rh_1 and Rg_3 increased as the result of LAB fermentation. This finding indicated that biotransformation occurred during LAB fermentation. Besides, to investigate the minor saponins that

could not be identified by HPLC, liquid chromatography−electrospray ionization−mass spectrometry and collision-induced dissociation experiments were used to identify the backbone and glycosidic linkage sites of these saponins in the fermentation product. We found three main ions at *m*/*z* 475, 697, and 769, and their collision-induced dissociation fragmentation patterns were

Table 3. LC-MS and Corresponding Collision-Induced Dissociation Data in Negative Ion Mode (*m*/*z* Value)

identification	$[M - H]^-$ (*m*/*z*)	MS/MS fragment ions (*m*/*z*)
protopanaxatriol	475	
ginsenoside F_1	637	475 $[M - H - Glc]^-$
notoginsenoside R_2	769	637 $[M - H - Xyl]^-$; 475 Agl

also checked at the same time (**Table 3**). Referring to the information of the chemical compositions of *P. notoginseng* and its congeneric and conspecific plants (*29*, *30*), we believe that these ions represent protopanaxatriol, ginsenoside F_1, and notoginsenoside R_2 (**Figure 4**).

Several investigations have indicated that ginsenoside Rg_1 would be metabolized to ginsengoside Rh_1 or ginsenoside F_1 in the human digestive tract, and both of them could be further metabolized to protopanaxatriol (*31*−*33*). Dan et al. found that ginsenoside F_1 is a metabolite produced during *P. notoginseng* fermentation (*30*). Due to the natural absence of protopanaxatriol, the protopanaxatriol is often prepared by microbial conversion (*34*). Additionally, ginsenoside Rh_1 was found to be the intermediate product of protopanaxatriol (*33*, *34*). Therefore,

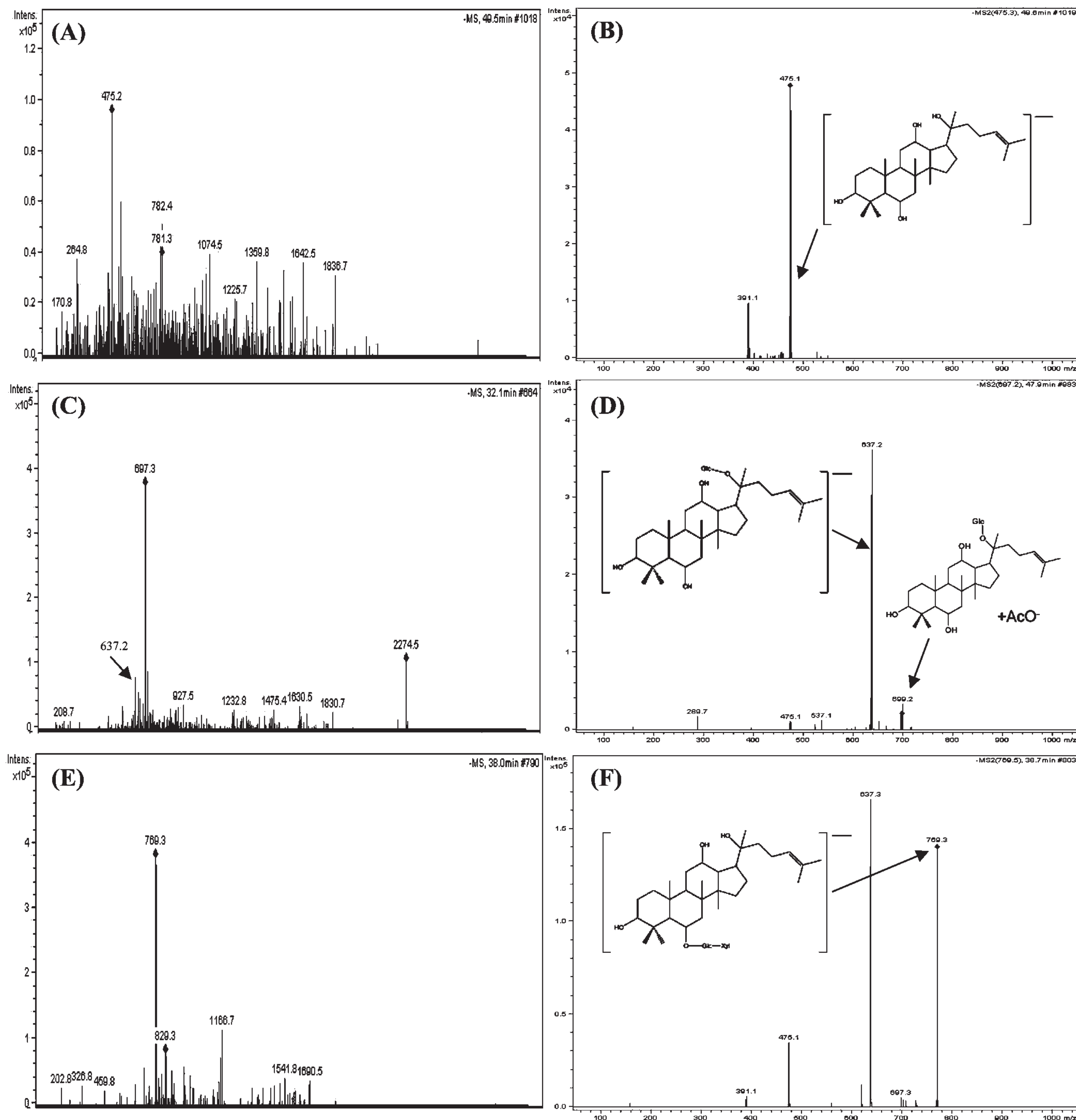
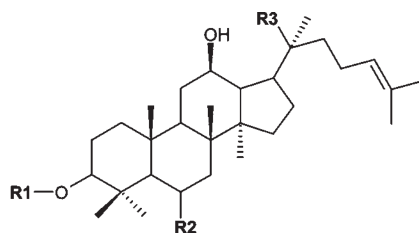
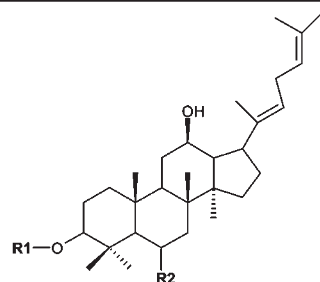


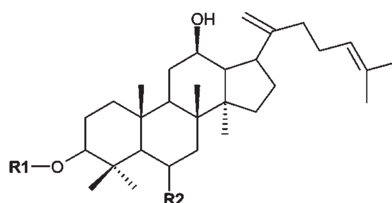
Figure 4. MS spectrum (**A**) and MS/MS spectrum (**B**) of *m*/*z* 475; MS spectrum (**C**) and MS/MS spectrum (**D**) of *m*/*z* 697; and MS spectrum (**E**) and MS/MS spectrum (**F**) of *m*/*z* 769.



Saponins	R1	R2	R3
Ginsenoside-Rg_1	-H	-O-Glc	-O-Glc
Ginsenoside-Rh_1	-H	-O-Glc	-OH
Ginsenoside-F_1	-H	-OH	-O-Glc
Protopanaxatriol	-H	-OH	-OH
Notoginsenoside-R_1	-H	-O-Glc-Xyl	-O-Glc
Notoginsenoside-R_2	-H	-O-Glc-Xyl	-OH
Ginsenoside-Rb_1	-Glc-Glc	-H	-O-Glc-Glc
Ginsenoside-Rd	-Glc-Glc	-H	-O-Glc
Ginsenoside-Rg_3	-Glc-Glc	-H	-OH



Saponins	R1	R2
Ginsenoside-Rh_4	-H	-O-Glc



Saponins	R1	R2
Ginsenoside-RK_3	-H	-O-Glc

Figure 5. Chemical structures of some saponins in the lactic acid bacteria fermented *P. notoginseng*: ginsenoside Rh_1 including 20*S*-Rh_1 and 20*R*-Rh_1; ginsenoside Rg_3 including 20*S*-Rg_3 and 20*R*-Rg_3. Abbreviations: Glc, glucose; Xyl, xylose.

it is believed that ginsenoside Rg_1 loses one molecule of glucose to convert to ginsenoside Rh_1 or ginsenoside F_1 during fermentation. When Rg_1 loses both of its glucose molecules, the aglycone protopanaxatriol is formed (**Figure 5**). Ginsenoside R_2 was possibly derived from the metabolism of ginsenoside R_1 by eliminating its glucose molecule (*35*). In addition, ginsenoside Rb_1 loses one molecule of glucose to convert to ginsenoside Rd, and ginsenoside Rd further loses one molecule of glucose to become ginsenoside Rg_3 (**Figure 5**). It is worth mentioning that the ginsenoside Rg_3, ginsenoside Rh_1, and protopanaxatriol formed during LAB fermentation of *P. notoginseng* have been proven to possess antitumor activities. Ginsenoside Rg_3 can inhibit proliferation of SW-480 cells by 33.7% at a concentration of 78.5 μg/mL (*12*). Ginsenoside Rh_1 and protopanaxatriol could inhibit proliferation of human leukemia cells (THP-1) with IC_{50} of 19 and 210 μg/mL, respectively (*36*). Results of this study have further demonstrated that fermentation of *P. notoginseng* with LAB can enhance its antihepatoma activity, and the major anticancer compounds formed during fermentation are ginsenosides Rg_3 and Rh_1 and protopanaxatriol. Thus, the LAB fermentation broth of *P. notoginseng* may be a potential remedy for treating liver cancer.

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