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Determination of ginsenosides by *Bacillus polymyxa* conversion and evaluation on pharmacological activities of the conversion products

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ABSTRACT

Bacillus polymyxa was used to transform ginseng for the first time. Furthermore, the pharmacological activities of the conversion products were evaluated. Twelve kinds of ginsenosides of conversion products were detected by high performance liquid chromatography (HPLC). The results showed that the contents of ginsenosides Rb3, Re, Rd, CK, protopanaxadiol were significantly increased. Moreover, the conversion products had anti-fatigue, immune enhancement, and antitumor activities by the certification of pharmacodynamics experiments. The study provides a theoretical basis and reference for future research on microbial transformation of ginseng.

1. Introduction

Ginseng (*Panax ginseng* C.A. Meyer), an *Araliaceae perennial* herb, which has main active ingredient ginsenosides, has anti-aging, immunity, fatigue, antitumor and other pharmacological activities [1–4]. Currently, the conversion methods of ginsenosides include acid hydrolysis, alkaline hydrolysis, the degradation of the enzyme, microbial conversion, and semisynthetic methods [5–10]. The microbial conversion is widely used because of its low costs and less by-products. A more systematic gut microbial conversion from ginsenosides of Rbl, Rb2 and Rc to ginsenoside CK was speculated [11]. The ginsenoside β-glucosidase which was successfully isolated from *Paecilomyces Beini Ye* (*Paecilomyces Bainier*) sp.229 with the microcrystalline cellulose as filler column could convert ginsenoside Rb1 into ginsenoside Rg3 [12].

In recent years, ginseng endophytes were used for the transformation of ginsenosides. Studies found that 51 bacteria were isolated from the Korean Ginseng, which at different ages could promote the growth of the ginseng [13,14]. The ginseng endophytic fungi was isolated and identified as well [15,16]. The endophytes called *Bacillus polymyxa* which was isolated from the fresh ginseng has been screened by our laboratory and this strain could significantly improve the ginsenosides concentration [17].

Bacillus polymyxa is a class of Gram-positive bacteria of the genus *Bacillus* [18]. It is a ubiquitous bacteria existing in soil, plant epicuticular, roots, and stems [19]. It is also an important plant biocontrol bacteria and plant growth-promoting rhizobacteria, which has been widely applied in the field of agriculture [20,21]. In addition, this species is classified by the Environmental Protection Agency as an organism that has commercial applications as a biocontrol agent [22]. The flocculants produced by *Paenibacillus polymyxa* play an important role in sewage treatment [23]. But there are few reports about conversing ginseng with *Bacillus polymyxa* and pharmacological activities of conversion products.

This is the first study to use ginseng endophytes-*Bacillus polymyxa* to transform ginseng and to research the pharmacological activities of conversion products, which could provide a theoretical basis and reference for the further studies of microbial transformation of ginseng.

2. Materials and methods

2.1. Materials and sample preparation

The four-year-old ginseng was collected from the Medicinal Plant Garden of Jilin Agricultural University. *Bacillus polymyxa* was from our laboratory. The 250 ml flask was filled with 100 ml water and 3 g ginseng powder (20–60 mesh), sterilized at 121 °C for 20 min and cooled. 2 ml of seed liquid (OD_{600} = 0.5) was inoculated to the flask by pipettes under sterile conditions. Kill bacteria by boiling water after shaking culture at 25 °C for 12 d. Then 1% of CMC-Na was added to triturate for preparing 39 mg/ml (native dose) of fermentation broth for pharmacological experiments.

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Kits for liver glycogen, muscle glycogen, blood lactic acid (LD), and blood urea nitrogen (BUN) were purchased from Nanjing Jiancheng Bioengineering Institute, kits for IL-2, TNF-α, IgG1, IgE, and IgM were purchased from U.S.R & D company, mice antibody with fluorescein-labeled of CD4-PE, CD8-FITC were from U.S. Pharmingen, Inc and the injection of cyclophosphamide was purchased from Shanghai Hualian Pharmaceutical Company (batch number: 120809). The levamisole was purchased from Ren Tang Pharmaceutical Co., Ltd. Shandong.

2.2. Experimental animals

ICR mice (male) weighed 20 ± 2 g were purchased from the Changchun Biological Products. The mice were housed at a room temperature (23 ± 1 °C), with 12 h light and 12 h dark cycle (lights were kept on from 6:00 am to 6:00 pm). Drinking water was available *ad libitum*.

2.3. Determination of ginsenosides of the conversion products

2.3.1. Sample preparation

1 g of ginseng powder by microbial conversion was wrapped with filter paper and then was extracted within a soxhlet extractor for 4 h at 80 °C. After removing the petroleum ether, the methanol was added into the soxhlet extractor to extract continuously for 10 h at 80 °C. The extracted solution was collected after evaporating methanol and dissolved in methanol for HPLC to a 10 ml volumetric flask. The samples were filtrated through a 0.45 μm membrane filter prior to HPLC analysis. Ginsenosides of conversion products and the control group which was ginseng without *Bacillus polymyxa* conversion were prepared at the above conditions. There were three replicates for each group.

2.3.2. Chromatographic conditions

The HPLC system was a Shimadzu LC-2010A instrument, with liquid chromatography pump (LC-2010A), automatic injector and chromatography workstation (CLASS-vP). The separation was carried out on C18 column (150 mm × 4.6 mm, 5 μm); mobile phase was of acetonitrile–water. For HPLC analysis, 20 μl sample was injected into the column and eluted at room temperature with a constant flow rate of 1.0 ml/min. Water (solvent A) and acetonitrile (solvent B) were used. Gradient elution started with 82% solvent A and 18% solvent B, changed to 21% B for 40 min, then changed to 26% B for 2 min, then changed to 32% B for 4 min, changed to 33.5% B for 20 min, changed to 38% B for 5 min, changed to 65% B for 15 min and held for 5 min, changed to 85% B for 5 min and held for 7 min. Finally, it was changed to 18% B for 2 min and held for 1 min to rebalance the separated column. The detection wavelength was set to 203 nm [24].

2.3.3. Calibration curves of ginsenoside standards

A stock solution of standards containing 16 ginsenosides (Rg1, Re, Rf, Rb1, Rg2, Rc, Rb2, Rb3, F1, Rd, F2, Rg3, protopanaxatriol, compound K, Rh2, and protopanaxadiol were purchased from chemistry laboratory of natural medicine in Jilin University and all standards were of biochemical-reagent grade and at least 98% pure.) (Fig. 1) in methanol was prepared and the 2, 4, 6, 10, 16, and 20 μl of the mixed-standard solutions were injected in triplicate, and then the calibration curves were constructed by plotting the peak areas *versus* the amounts of each analyte. The concentrations of 16 ginsenosides in these samples were calculated according to the standard curves of 16 ginsenosides.

2.4. Antifatigue experiments

The mice were fed for 1 week and randomly divided into three groups ($n = 12$) as follows: saline control group (0.4 ml/d), ginseng group (15.6 mg/0.4 ml/d) and microbial conversion of ginseng group (15.6 mg/0.4 ml/d). 30 min after the administration, the mice were placed in plastic cylinder (height 50 cm, 10 cm in diameter), which was filled with water to a depth of 30 cm and maintained at 30 ± 1 °C, then were forced to swim for 30 min every 2 days, dried with a towel and returned to their original cages. Animals in each group were intragastrically administered for 28 d.

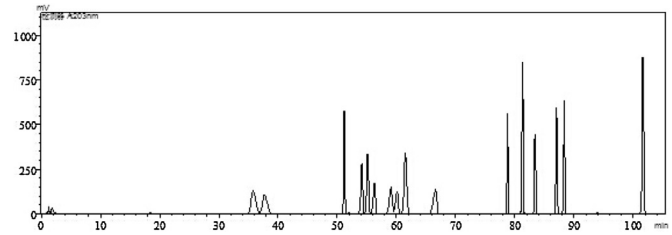

Fig. 1. HPLC chromatogram of mixed subject, the ginsenosides peak from the left to right order is Rg1, Re, Rf, Rb1, Rg2, Rc, Rb2, Rb3, F1, Rd, F2, Rg3, protopanaxatriol, CK, Rh2, and protopanaxadiol.

2.4.1. Loaded swimming test

After 28 days, the mice were taken from each group for the test. A total of 60 min after the last administration, the mice were weighed. After that, a lead sinker (10% of the body weight) was attached to the tail root of each mouse. When the mice were underwater within 5 s, the swimming time was recorded immediately. Then the mice were removed from the water and dried with a towel.

2.4.2. Determination of LD, BUN, liver glycogen, and muscle glycogen in mice

After four weeks, some biochemical indexes were analyzed. A total of 60 min after the last administration, the mice were forced to swim for 30 min and gently touched to keep the state of swimming. After the mice rested for half an hour, blood was collected through their eyeballs and serum was prepared by centrifugation at 4000 rpm at 4 °C for 15 min. The livers and quadriceps of the mice were immediately collected and homogenized to 10% solution with normal saline at 4 °C. The levels of LD, SUN, liver glycogen, and muscle glycogen were determined according to the kits.

2.5. Immunization test

The mice were fed for one week and were randomly divided into five groups ($n = 12$) as follows: saline control group, model group, positive control group administered levamisole tablets powder (15.6 mg/0.4 ml/d), microbial conversion group (15.6 mg/0.4 ml/d) and ginseng group (15.6 mg/0.4 ml/d). In addition to saline control group, the mice of other groups were injected subcutaneously with cyclophosphamide (20 mg/kg) for preparation of immunodeficient model in the back of mice neck. After 3 consecutive days, saline control group and modeling group were randomly selected to determine organ index and lymphocyte counts. The model was made successfully if there is a significant difference ($P < 0.05$) between the groups. Then the animals in each group were intragastrically administered 28 d, 60 min after the last administration, the experiment was carried out.

2.5.1. Immune organ index

After the last administration, the mice were fasted for 12 h, weighed, and sacrificed. The spleen and thymus were weighed in sterile condition and calculated the immune organ index of the spleen and the thymus according to the following formula:

$$\text{Spleen index} = \text{spleen weight (mg)/body weight of mice (g)}$$
$$\text{Thymus index} = \text{weight (mg)/mouse body weight (g)}$$

2.5.2. Determination of CD4⁺, CD8⁺ of spleen lymphocyte subsets

The spleen was added into 10 ml of precooled PBS solution under sterile conditions and the spleen was taken in a sterile 200 mesh aperture of the web with grinding by using a syringe needle core and filtered and washed twice with adding cold PBS into single cell suspension to make cell count reach the final concentration of 1×10^6 cells/ml at least. The 100 μl of spleen cell suspension was drawn into centrifuge tube then added 1 μl of $CD4^+$-FITC and 2 μl of $CD8^+$-PE, after mixing, incubated in the dark for 30 min, washed with PBS and filtered to add into the dedicated pipe of FACS and finally detected the positive expression rate of $CD4^+$ and $CD8^+$ in T cells by flow cytometry.

2.5.3. Assay of IL-2, TNF-α, IgG1, IgE, and IgM in mice

The IL-2, TNF-α, IgG1, IgE, and IgM in mice were determined by kits.

2.6. Antitumor test in vitro

The logarithmic growth phase of breast cancer tumor cell MCF-7 were seeded in 96-well plates at 1×10^4 cells/well and each well was added 100 μl and then cultured for 24 h to make the final concentrations of 100, 50, 25, 12.5, 6.25, and 3.125 μg/ml, respectively, according to the principle of increasing concentrations of reagents. Each concentration was set at six parallel holes. The blank control group was the cell culture medium without cells. The negative control group was the cell culture medium containing cells but no drug. They were incubated in an atmosphere of 5% CO_2 in air at 37 °C in a standard CO_2 incubator for 2 d. After that, each hole was added 20 μl of 5 mg/ml MTT and continued to foster for 4 h, carefully aspirating culture medium and then added 150 μl of DMSO to each well. The OD value was read in microplate reader (BIO-RAD) at 490 nm and calculated the rate of cell proliferation inhibition (CI) and half inhibitory concentration (IC50).

CI (%) = (the value of the negative control group − the value of treatment group)/the value of negative control group × 100%

2.7. Statistical analysis

Data were subjected to a one-way analysis of variance (ANOVA) with the program SPSS 13.0. The treatments were determined according to Duncan's multiple range test at $P < 0.05$ in order to identify significant correlates between treatments. Correlations between selected parameters were tested with the Pearson correlation coefficient. Tables were made with Microsoft excel.

3. Results

3.1. HPLC analysis on the conversion products

Microbial conversion group was compared with the control group (Table 1), ginsenosides Rb3, Re, Rd, CK, and protopanaxadiol were significantly increased. In the HPLC chromatogram of transformation products (Figs. 1–3), the ratio of some ginsenosides peak area in the control group was less than that of the conversion group, especially the concentrations of protopanaxadiol and ginsenoside Rd.

3.2. Antifatigue experiments

3.2.1. Loaded swimming test

The ginseng group and microbial conversion group were compared with the control group (Table 2), they could significantly prolong the swimming time of mice ($P < 0.05$), in which the swimming time of the microbial conversion group was significantly longer than the ginseng group.

Table 1
Transformation product and the reference of the monomer saponin concentration (%) ($N = 3$).

Monomer saponin	Content of conversion products (%)	Content of the control group (%)
Rg1	0.2087 ± 0.0285	0.2437 ± 0.0092
Re	0.1830 ± 0.2520	0.1541 ± 0.0050
Rf	0.0322 ± 0.0090	0.0383 ± 0.0059
Rb1	0.1970 ± 0.0209	0.2119 ± 0.0057
Rg2	0.0028 ± 0.0020	0.0056 ± 0.0002
Rc	0.1018 ± 0.0098	0.1840 ± 0.0023
Rb2	0.0759 ± 0.0160	0.0900 ± 0.0053
Rb3	0.0142 ± 0.0110	0.0027 ± 0.0106
Rd	0.1732 ± 0.0183	0.1151 ± 0.0256
F2	0.0235 ± 0.0047	0.0247 ± 0.0010
CK	0.0357 ± 0.0038	0.0150 ± 0.0057
Protopanaxadiol	0.2422 ± 0.0486	0.0276 ± 0.0223

This value represents the saponin concentration in each monomer with mean ± standard deviation.

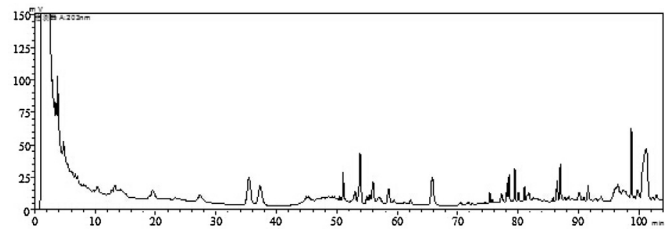

Fig. 2. HPLC chromatogram of conversion products, the ginsenosides peak from the left to right order is Rg1, Re, Rf, Rb1, Rg2, Rc, Rb2, Rb3, Rd, F2, CK, and protopanaxadiol.

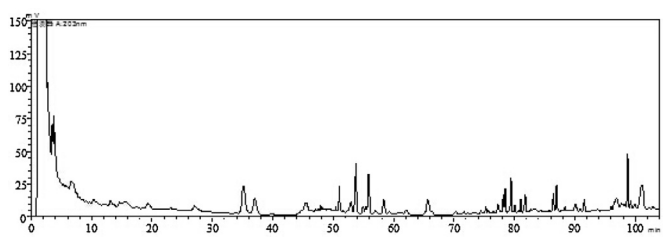

Fig. 3. HPLC chromatogram of the control group, the ginsenosides peak from the left to right order is Rg1, Re, Rf, Rb1, Rg2, Rc, Rb2, Rb3, Rd, F2, CK, and protopanaxadiol.

3.2.2. Determination of LD, BUN, liver glycogen, and muscle glycogen in mice

The ginseng group and microbial conversion group compared with the control group (Tables 3 and 4), the levels of LD, BUN in plasma were significantly lower ($P < 0.05$), in which the levels of LD, BUN of the microbial conversion group were significantly lower than ginseng group. The contents of muscle glycogen and liver glycogen were significantly increased ($P < 0.05$), in which the content of muscle glycogen and liver glycogen of the microbial conversion group was significantly higher than ginseng group.

Table 2
Effect of different groups on weight-loading swimming time in mice.

Experimental group	Number of animals	Swimming time (s)
The control group	12	461.83 ± 44.08^{c}
Ginseng group	12	740.83 ± 50.62^{b}
Microbial conversion group	12	781.60 ± 34.74^{a}

This value represents mean ± standard deviation, [abc] indicates significant differences ($P < 0.05$), the same letters are not significant but different letters have significant differences.

Table 3
Effect of different groups on levels of LD, BUN in mice.

Experimental group	Number of animals	LD (mmol/l)	BUN (mmol/l)
The control group	12	8.90 ± 0.71[a]	10.57 ± 0.97[a]
Ginseng group	12	3.91 ± 0.17[b]	7.49 ± 0.75[b]
Microbial conversion group	12	2.01 ± 0.12[c]	6.21 ± 0.51[c]

This value represents mean ± standard deviation, [abc] indicates significant differences ($P < 0.05$), the same letters are not significant but different letters have significant differences.

Table 4
Effect of different groups on contents of glycogen and muscle glycogen in mice.

Experimental group	Number of animals	Muscle glycogen (mg/g)	Liver glycogen (mg/g)
The control group	12	1.51 ± 0.09[c]	3.89 ± 0.32[c]
Ginseng group	12	2.03 ± 0.19[b]	4.85 ± 0.35[b]
Microbial conversion group	12	2.37 ± 0.21[a]	6.67 ± 0.29[a]

This value represents mean ± standard deviation, [abc] indicates significant differences ($P < 0.05$), the same letters are not significant but different letters have significant differences.

Table 5
Effect of different groups on organ indexes in mice.

Experimental group	Numbers of animals	Thymus (mg/g)	Spleen (mg/g)
The control group	12	2.71 ± 0.12[a]	5.87 ± 0.17[a]
model group	12	1.59 ± 0.07[c]	3.20 ± 0.13[c]
Positive control group	12	2.27 ± 0.10[b]	4.13 ± 0.32[b]
Ginseng group	12	2.04 ± 0.19 [b]	4.29 ± 0.34[b]
Microbial conversion group	12	2.76 ± 0.01[a]	5.98 ± 0.02[a]

This value represents mean ± standard deviation, [abc] indicates significant differences ($P < 0.05$), the same letters are not significant but different letters have significant differences.

Table 6
Effect of different groups on subgroup ratio of spleen cells in mice (%).

Experimental group	Numbers of animals	$CD4^+$	$CD8^+$	$CD4^+/CD8^+$
Control group	12	6.26 ± 0.06[d]	3.10 ± 0.35[b]	2.06 ± 0.05[e]
Model group	12	11.87 ± 0.78[a]	3.40 ± 0.26[a]	3.51 ± 0.90[a]
Positive control group	12	9.40 ± 2.34[b]	2.77 ± 0.67[c]	3.39 ± 0.05[b]
Ginseng group	12	7.50 ± 3.61[c]	2.50 ± 0.98[c]	2.94 ± 0.25[c]
Microbial conversion group	12	7.27 ± 2.44[c]	3.00 ± 1.14[b]	2.42 ± 0.31[d]

This value represents mean ± standard deviation, [abcd] indicates significant differences ($P < 0.05$), the same letters are not significant but different letters have significant differences.

3.3. Immunization experiments

3.3.1. Immune organ index

After modeling, the organ index of model group was significantly reduced compared with the control group ($P < 0.05$) (Table 5), the organ index of positive control group, ginseng group, and microbial conversion group was significantly increased compared with the model group ($P < 0.05$), where microbial transformation group significantly improved than the organ index of ginseng group and positive control group ($P < 0.05$). It was also showed that, after the treatment of levamisole, ginseng group and microbial transformation, the thymus and spleen index of immunocompromised mice could significantly improve.

3.3.2. Determination of $CD4^+$, $CD8^+$ of spleen lymphocyte subsets

After modeling, the levels of $CD4^+$, $CD8^+$, and $CD4^+/CD8^+$ in the model group significantly increased compared with the control group ($P < 0.05$) (Table 6). The levels of $CD4^+$, $CD8^+$, and $CD4^+/CD8^+$ of the positive control group, ginseng group and the microbial transformation group were significantly lower ($P < 0.05$). Where CD4+, $CD8^+$, and $CD4^+/CD8^+$ of microbial conversion group were significantly lower compared with ginseng group and the positive control group ($P < 0.05$). It was also showed after the treatment of levamisole, ginseng group and microbial conversion group could significantly affect the levels of the $CD4^+$, $CD8^+$ of spleen lymphocyte subsets and improved the immunity in mice, where the effect of microbial conversion group was better.

Table 7
Effect of different groups on contents of IL-2, TNF-α, IgG1, IgE, IgM in mice.

Experimental group	IL-2	TNF-α	IgG1	IgE	IgM
Control group	1111.23 ± 27.95[c]	373.26 ± 18.60[a]	118.22 ± 11.45[a]	608.63 ± 51.41[d]	144.64 ± 5.94[a]
Model group	146.58 ± 9.60[d]	117.50 ± 11.53[c]	77.31 ± 1.19[c]	2911.24 ± 97.38[a]	29.25 ± 2.13[c]
Positive control group	1154.33 ± 32.26[c]	291.44 ± 13.86[b]	98.14 ± 4.52[b]	2249.47 ± 54.74[b]	111.61 ± 8.22[b]
Ginseng group	1225.54 ± 34.37[b]	289.09 ± 33.47[b]	91.89 ± 10.45[b]	2227.91 ± 93.79[b]	107.69 ± 11.38[b]
Microbial conversion group	1284.21 ± 22.02[a]	381.44 ± 10.16[a]	109.14 ± 3.79[a]	1301.02 ± 21.98[c]	139.99 ± 7.69[a]

This value represents mean ± standard deviation, the letters indicate significant differences ($P < 0.05$), the same letters are not significant but different letters have significant differences.

3.3.3. Determination of IL-2, TNF-α, IgG1, IgE, and IgM in mice

The contents of IL-2, TNF-α of the model group were significantly lower ($P < 0.05$) compared with the control group (Table 7). The positive control group, ginseng group and microbial conversion group were significantly increased compared with the model group and the content of microbial transformation group was the highest. For IgG1, the contents of IgG1 of model group were significantly lower ($P < 0.05$) compared with the control group. The positive

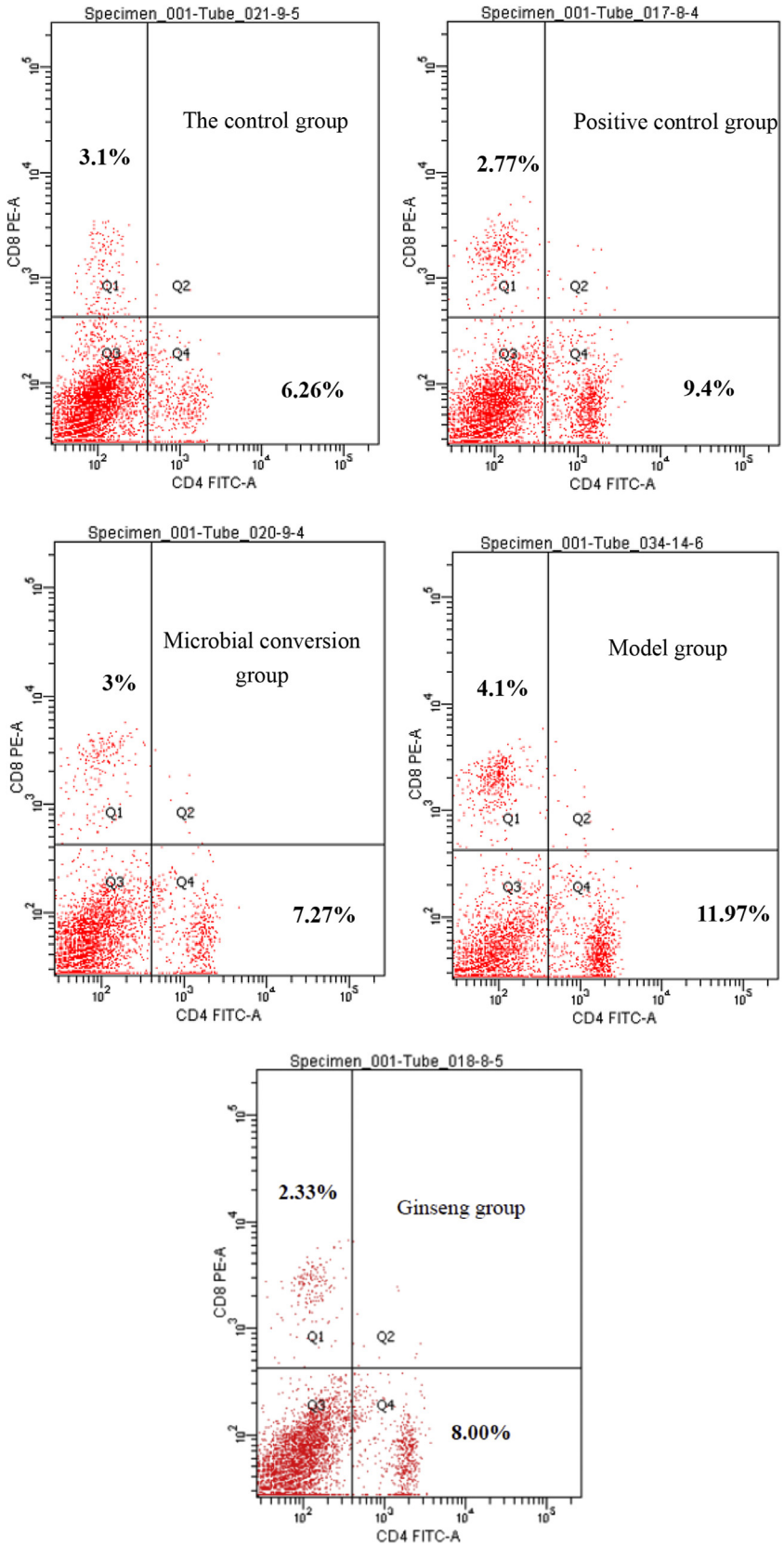

Fig. 4. The effects of different groups on spleen lymphocyte subsets of mice.

Table 8
the effect of transforming products and ginseng group on breast cancer tumor cells.

Drug concentration (μg/ml)	Microbial conversion group CI (%)	Ginseng group CI (%)
100	47.80	36.68
50	47.37	36.14
25	45.45	35.98
12.5	41.91	34.38
6.26	39.56	31.10
3.125	37.05	26.17
IC_{50} (μg/ml)	102.81	259.49

This value is expressed as mean. The CI and IC50 were calculated.

control group, ginseng group and microbial transformation group were both significantly increased compared with the contents of IgG1 of the model group, of which the highest contents was microbial transformation group. For IgE, a significant increase in levels of IgE was the model group compared with the control group ($P < 0.05$). The positive control group, ginseng group and microbial transformation group were significantly decreased compared with the levels of IgE of the model group, of which the lowest levels were microbial transformation group. For IgM, the positive control group, ginseng group and microbial conversion were significantly increased compared with the levels of IgM of the model group. The highest levels were microbial transformation group. After the treatment of levamisole, ginseng group and microbial transformation, which could significantly affect the levels of IL-2, TNF-α, IgG1, IgE, and IgM in immunocompromised mice and improved immunity in mice and the effect of microbial transformation group was the better.

3.4. *Antitumor experiments* in vitro

With the increase of drug concentration (Table 8), the inhibition rate of microbial groups showed a gradual upward trend compared with the ginseng group. The IC50 value of microbial groups was significantly lower, indicating that ginseng products by *Bacillus polymyxa* conversion had better antitumor activity compared with ginseng group.

4. Discussions

In the present study, HPLC method is the common determination method of ginsenosides, which can express peak area of ginsenosides and determine its content. If the HPLC combined with UV, the structure and concentration of ginsenosides were known [25]. In our study, the contents of ginsenosides in conversion products were analyzed by HPLC (Table 1). The HPLC chromatogram of conversion products was illustrated (Figs. 1–3). Which enzyme of *Bacillus polymyxa* can convert ginsenosides is an area that needs further study.

The loaded swimming test was to evaluate the index of the effect of antifatigue and the length of the swimming time to exhaustion indicated the degree of fatigue [26,27]. The ginseng products by the conversion of *Bacillus polymyxa* could significantly increase the swimming time of mice and enhance vitality (Table 2). The body will go on an anaerobic movement when it runs to a certain extent, then it will generate a lot of lactic acid from sugar decomposition and results in fatigue symptoms [28]. The ginseng products of *Bacillus polymyxa* conversion could significantly reduce the formation of lactic acid in mice and reduced fatigue. When a certain amount of sugar is broken down and then it began to consume protein to provide energy with producing urea nitrogen [29]. Studies have shown that the levels of blood urea nitrogen increased with the increase of movement load. The worse the body's resilience was, the more obvious serum urea nitrogen increased, which were the products of movement load beyond a certain amount [30]. However, the conversion products could significantly reduce the generation of serum urea nitrogen in mice (Table 3). The contents of glycogen directly reflected the state of the body's metabolism of glycogen, in theory, increasing the reserves of liver glycogen and muscle glycogen reserves could provide energy for the body during exercise and delay reduced fatigue [31]. The ginseng products by *Bacillus polymyxa* conversion could reduce the consumption of the body's liver glycogen and muscle glycogen (Table 4). Besides, the comparison of microbial conversion group with the control group, could significantly increase the swimming time of the mice and reduce blood lactate, blood urea nitrogen, muscle glycogen, and liver glycogen in mice. They also significantly improved the resistance to fatigue compared with ginseng group.

T lymphocyte cell is an important population in immune system. Under normal circumstances, the $CD4^+$ T cells and $CD8^+$ T cells of body maintain homeostasis, particularly the ratio of $CD4^+/CD8^+$ in the immune cell reflected the ability to regulate the immune response [32]. T cells also secrete cytokines, such as IL-2, TNF-α and it also promotes the generation of some immunoglobulins, such as IgG1, IgM to enhance immunity. The study was to prepare immunocompromised model by injecting cyclophosphamide in mice and measure the immune organ index to affirm successful modeling. By measuring the immune organ index (Table 5), the ginseng products by *Bacillus polymyxa* conversion could significantly increase the levels of the spleen and thymus index compared with ginseng group. And the ginseng products by *Bacillus polymyxa* conversion could significantly affect the percent of spleen lymphocyte subsets $CD4^+$, $CD8^+$ and the ratio of $CD4^+/CD8^+$ and improve immunity of mice (Table 6) (Fig. 4). The ginseng products by *Bacillus polymyxa* conversion could significantly affect the levels of the IL-2, TNF-α, IgG1, IgE, and IgM levels in immunocompromised mice and the ginseng products by *Bacillus polymyxa* conversion could effectively enhance the immune capacity compared with ginseng group (Table 7).

The anti-tumor experiments *in vitro* have been widely used as a model system for evaluating the antitumor of compounds [33]. As a result, the ginseng products by *Bacillus polymyxa* conversion had a certain antitumor activity compared with ginseng group. With the increase of drug concentration of the conversion products, the inhibition rate of the conversion products increased (Table 8). However, the mechanism of antitumor of conversion products needed further study.

5. Conclusions

This is the first time to use ginseng endophyte-*Bacillus polymyxa* to transform ginseng. The conversion products by *Bacillus polymyxa* could prolong the swimming times, reduce the amount of LD and SUN in mice after movement, reduce the consumption of glycogen and muscle glycogen in mice after exercise. The products significantly improve the ability of antifatigue in mice. They could also improve the index of spleen, thymus in mice, affect the ratio of $CD4^+/CD8^+$ of spleen lymphocyte subsets in mice and the IL-2, TNF-α, IgG1, IgE, and IgM in mice have affected in varying degrees, which showed the ginseng products by *Bacillus polymyxa* conversion had the strong immunity. Meanwhile, the conversion products had a certain antitumor activity.

Acknowledgments

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