Clinical evaluation of probiotic Lactobacillus paracasei A221 for standardizing ginseng-hydrolyzing potentials of human intestinal bacteria

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Human intestinal bacteria hydrolyze ginsenoside, triterpenoid glycoside of Panax ginseng C. A. MEYER (Araliaceae) to the active metabolites; however, metabolite-producing potentials of intestinal bacteria are shown to differ among individuals. In the present study, we evaluated the probiotic effects of L. paracasei A221, a bacterium capable of producing metabolites from ginsenoside, on 136 human volunteers. Four-week-ingestion of L. paracasei A221 improved fecal smell (p < 0.001, n = 119) and constipation (p < 0.001, n = 55). In addition, L. paracasei A221 also improved the symptoms of pruritus (67.7%, p < 0.001, n = 31), dermatopath (52.3%, p < 0.001, n = 65), pollinosis (49.0%, p < 0.001, n = 51), dry skin (46.3%, p < 0.001, n = 41), and sleep disruption (28.8%, p < 0.02, n = 52). Besides results from T-RFLP analysis confirmed the recovery of L. paracasei A221 from the feces of volunteers. These data provide evidence that L. paracasei A221 is one probiotic strain. As for metabolite-producing potentials, the treatment of volunteers with no or less metabolite-producing potentials with L. paracasei A221 was highly effective in augmenting metabolite-producing potentials. Moreover, the average score of ginseng efficacy was actually improved with an efficiency of 63.2% (p < 0.0001, n = 57) by 4-week-ingestion of L. paracasei A221. These results permit us to speculate that L. paracasei A221 is available for standardizing metabolite-producing potentials of intestinal bacteria.

Key words Panax ginseng, ginsenoside, intestinal bacteria, metabolic activation, probiotics, Lactobacillus paracasei A221.

Introduction

Ginseng (the roots of Panax ginseng C. A. MEYER, Araliaceae) has been used as one of the most valuable traditional medicines in the Orient for over 2000 years. The main ingredients of ginseng are ginsenosides, glycosides containing an aglycone (protopanaxadiol or protopanaxatriol) with a dammarane skeleton. So far, numerous researchers have contributed to the accumulation of evidence that ginsenosides are responsible for the pharmacological effects of ginseng. Ginseng is orally ingested, in general. Therefore, its ingredients must meet gastric juice, digestive and bacterial enzymes in the intestines. Orally ingested ginsenoside passes through the stomach and small intestine without decomposition by either gastric juice or liver enzymes into the large intestine, where ginsenoside is hydrolyzed (deglycosylated) by colonic bacteria followed by transit to the circulation: Colonic bacteria cleave the oligosaccharide connected to the aglycone stepwise from the terminal sugar to afford the major metabolites, 20S-protopanaxadiol 20-O-β-D-glucopyranoside (M1) and 20S-protopanaxatriol (M4). M1 is gradually hydrolyzed to the aglycone, 20S-protopanaxadiol (M12), 20S-protopanaxtriol 20-O-β-D-glucopyranoside (M11) is the intermediate metabolite of M4 (Fig. 1). Many kinds of bacteria including Bacteroides uniformis,2) Escherichia A-44,3) Bifidobacterium K506,3) Bacteroides JY6,3) and Fusobacterium K-603) seem to cooperatively metabolize ginsenoside. Accumulating evidence strongly suggests that the metabolites are the real active molecules in the body.4)

We have been so far interested in the relatedness of ginseng efficacy with metabolite-producing potentials. Intestinal microbiota is well known to be very changeable in dependence on host conditions (diet, health, and even stress). In fact, we have observed the individual differences in metabolite-producing potentials of 58 human subjects.5) Furthermore, we have found that the anti-metastatic activities of orally ingested ginsenoside are correlated with the metabolite-producing potentials of mice.6) Therefore, intestinal microbiota is suspected of affecting ginseng efficacy.

During the course of screening bacteria for metabolite-producing potentials, Lactobacillus paracasei A221 was isolated from fermented food. The genus Lactobacillus bacteria are used as starters for fermented foods including yogurt and cheese. Their safety as probiotics has been traditionally established.7) In this study, we attempted to standardize metabolite-producing potentials of human volunteers using probiotic L. paracasei A221.

Materials and Methods

Probiotic bacteria. The origin of L. paracasei A221 (FERM BP-10123) was a traditional fermented food. Its 16S
rRNA sequence was deposited in the GenBank database under accession number AB126872. *L. paracasei* A221 hydrolyzed plant glycosides including ginsenoside, glycyrrhizin (Glycyrrhizae Radix), and soy isoflavone glycosides (Glycine max). As for ginsenoside, *L. paracasei* A221 hydrolyzed ginsenosides Rb1, Rb2, Re, and Rd (protopanaxadiol-type), and also ginsenosides Rg1 and Re (protopanaxatriol-type). Bacteria were cultured in 10% skim milk broth at 35°C for 48 h, chilled at -35°C overnight, followed by freeze-drying to give powders with bacteria of approximate 5 x 10^10 cfu/g.

**Human volunteers.** After informed consent, a total 136 volunteers participated in all aspects of the study. Their age distribution is shown in Table 1.

**Clinical study.** The clinical study was conducted from February 2003 to March 2003 according to ethical principles of the Declaration of Helsinki. The clinical protocol was as follows: Volunteers ingested freeze-dried powders of *L. paracasei* A221 at a dose of 200 mg (1 x 10^{10} cfu)/day for 4 weeks. Volunteers interviewed them weekly about 22 items of lifestyle and symptoms as well as conditions of feces and number of defecation, and scored corresponding items. Scored symptoms are listed in Table 2. Each score consisted of deterioration (D) (1 point), slight deterioration (SD) (2 points), no change (NC) (3 points), improvement (I) (4 points), and remarkable improvement (RI) (5 points). Average score was calculated using the following formula:

Average score (point, mean ± SEM) = (number of volunteers who complained of symptom) x 100.

![Fig. 1 Metabolic pathway of ginsenoside by intestinal bacteria](image)

<table>
<thead>
<tr>
<th>Table 1 Age distribution of volunteers</th>
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| Table 2 Changes in score of symptom after ingestion of *L. paracasei* A221 |
|------------------|------------------|------------------|------------------|
| Symptom          | (n)              | D                | SD               | NC               | IM               | RI               | Average score (point) | ρ                   | Efficacy (%) |
| Smell of feces   | 118              | 1                | 2                | 56               | 56               | 4                | 3.5 ± 0.06          | < 0.001             | 50.4        |
| Dermopathy       | 65               | 0                | 4                | 27               | 26               | 8                | 3.7 ± 0.10          | < 0.001             | 52.3        |
| Sleep disruption  | 52               | 0                | 4                | 33               | 15               | 0                | 3.2 ± 0.05          | < 0.02              | 28.8        |
| Pollinosis       | 51               | 0                | 6                | 18               | 21               | 4                | 3.5 ± 0.12          | < 0.001             | 49.0        |
| Dry skin         | 41               | 0                | 1                | 21               | 15               | 4                | 3.5 ± 0.11          | < 0.001             | 46.3        |
| Pruritus          | 30               | 0                | 1                | 9                | 18               | 3                | 3.8 ± 0.13          | < 0.001             | 67.7        |

\(a\) Number of volunteers who complained of symptom. \(b\) D, deterioration; SD, slight deterioration; NC, no change; IM, improvement; RI, remarkable improvement. \(c\) Average score (point, mean ± SEM) = (number of volunteers who scored D x 1 + number of volunteers who scored SD x 2 + number of volunteers who scored NC x 3 + number of volunteers who scored IM x 4 + number of volunteers who scored RI x 5) / (total number of volunteers who complained of symptom). \(d\) Statistical significance of scores between pre-ingestion and post-ingestion, evaluated by paired two-tailed Student's t-test. \(e\) Efficacy (%) = (number of volunteers who scored I or RI) / (total number of volunteers who complained of symptom) x 100.
teers who scored D x 1 + number of volunteers who scored SD x 2 + number of volunteers who scored NC x 3 + number of volunteers who scored IM x 4 + number of volunteers who scored RI x 5 / (total number of volunteers who complained of symptom). Improvement ratio was calculated using the following formula: efficacy (%) = (number of volunteers who scored I or RI) / (total number of volunteers who complained of symptom) x 100. One hundred sixteen volunteers complained of symptoms suggestive of pruritus, dermopathy, pollinosis, dry skin, and sleep disruption. Fifty-seven volunteers routinely ingesting ginseng scored ginseng efficacy without change of daily dosage during the period of study. The scores were analyzed between pre-ingestion and post-ingestion.

**Metabolite-producing potential assay.** Metabolite-producing potential was determined by the following TLC method. Fecal specimens from 17 healthy volunteers 29 to 53 years of age were taken into 3 ml GAM semisolid without out dextrose "Nissui" (Nisui Pharmaceutical Co. Ltd., Japan) with 0.5% ginsenoside fraction, and anaerobically incubated at 37°C for 48 h. After incubation, a part (0.3 ml) of cultures was extracted with water-saturated n-ButOH (0.2 ml) and centrifuged (15,000 rpm, 3 min). Aliquots (2 μl) of the n-ButOH layer were analyzed by TLC: plates, silicagel 70 F254; developing solvents, CHCl3-MeOH-H2O (65 : 35 : 10 v/v/v, lower phase) and CHCl3-EtOH (8 : 1); detection of spots, spraying 8% vanillin in MeOH-72% H2SO4 (1 : 5 v/v) followed by heating (140°C, 3-4 min). Positive reaction of metabolite-producing potentials means that spots of the same Rf values as reference metabolites appear on TLC.

**T-RFLP analysis.** T-RFLP analysis of fecal specimens was done according to the method described previously. The analytical study was performed under the approval of RIKEN ethics committees.

**Statistical analysis.** The statistical significance of differences between the groups was determined by applying two-tailed paired Student's t-test.

### Results

**Individual differences in metabolite-producing potentials.** Seventeen randomly selected volunteers were assayed for metabolite-producing potentials. As shown in Fig. 2, remarkable individual differences in metabolite-producing potentials were observed: Volunteers C and J showed M11-, M1-,-, and M12-producing potentials. Volunteer 1 showed M11-, M1-, and M12-producing potentials. Volunteer L showed M11-, M1-, and M4-producing potentials. Volunteer M showed M11- and M1-producing potentials. Volunteer O showed M11- and M12-producing potentials. Volunteers E, F, and Q showed just M4-producing potential. Volunteers A, B, D, G, H, K, N, and P showed no or less metabolite-producing potentials. The latest 8 volunteers were selected as non-metabolite-producers.

**Effect of L. paracasei A221 on metabolite-producing potentials.** With a view toward standardizing metabolite-producing potentials, we examined the effect of *L. paracasei* A221 on metabolite-producing potentials. Freeze-dried powders of *L. paracasei* A221 were given to 8 non-metabolite-producers (volunteers A, B, D, G, H, K, N, and P) for 3 weeks. Figure 3 demonstrates that the ingestion of *L. paracasei* A221 resulted in marked augmentation of metabolite-producing potentials. T-RFLP analysis indicated that the *Mps I*-derived T-RF specific to *L. paracasei* A221 was detected in feces of volunteers after 3-week-ingestion (Fig. 4).

**Effect of *L. paracasei* A221 on symptoms.** As for total number of scored items, 3.0±0.24 items on average (n = 136) were significantly (p < 0.001) reduced after 4-week-ingestion of *L. paracasei* A221 (post-ingestion: 6.1±0.27 items vs. pre-ingestion: 9.0±0.29 items). The score of feces smell was significantly (p < 0.001, n = 119) increased by 0.5 ± 0.06 points on average (50.4% efficacy, Table 2), indicating that *L. paracasei* A221 cleaned the intestinal environment. The changes in score of symptom after ingestion of *L. paracasei* A221 are summarized in Table 2. Four-week-ingestion of *L. paracasei* A221 resulted in statistically significant improvement in pruritus (67.7%, p < 0.001), dermopathy (52.3%, p < 0.001), pollinosis (49.0%, p < 0.001), dry skin (46.3%, p < 0.01), and sleep disruption (28.8%, p < 0.02) compared with pre-ingestion. As for the volunteers complaining of constipation (n = 55), the defecation number (3.91±0.22 times per week on average) before ingestion was significantly (p < 0.001) increased up to 3.33 ± 0.35 times per week after ingestion.

**Effect of *L. paracasei* A221 on ginseng efficacy.** We also evaluated the effect of *L. paracasei* A221 for ginseng
efficacy. When \textit{L. paracasei} A221 was administered for 4 weeks to 57 volunteers who routinely took ginseng, improvement in ginseng efficacy was observed in 36 volunteers (63.2\% efficiency) versus 19 volunteers of no change and 2 volunteers of deterioration. The average score of ginseng efficacy before ingestion (n = 57) was significantly ($p < 0.0001$) increased by 0.6±0.07 points after 4-week ingestion.

**Discussion**

We evaluated the probiotic effects of \textit{L. paracasei} A221. Salminen \textit{et al.} have defined that probiotics are foods which contain live bacteria which are beneficial to health.\textsuperscript{9} Certain species of the genus \textit{Bifidobacterium}, \textit{Lactobacillus}, \textit{Enterococcus}, and \textit{Saccharomyces} have been already shown to be probiotic bacteria.\textsuperscript{10} As for \textit{L. paracasei} A221, its ingestion improved the smell of farts and feces. In addition, \textit{L. paracasei} A221 also improved the symptoms including pruritus, dermatopathy, pollinosis, dry skin, and sleep disruption (Table 2). Results from T-RFLP analysis also confirmed the recovery of \textit{L. paracasei} A221 from the feces of volunteers (Fig. 4). These data provide evidence that \textit{L. paracasei} A221 is one probiotic strain. Although the underlying mechanism remains unclear, we speculate that the improvement of symptoms is possibly related to activation of metabolism and regulation of immunity by \textit{L. paracasei} A221.

We observed individual differences in metabolite-producing potentials of 17 volunteers (Fig. 2). The results are consistent with those found in the previous study.\textsuperscript{5} We evaluated the effect of \textit{L. paracasei} A221 for standardizing metabolite-producing potentials. The treatment of volunteers with no or less metabolite-producing potentials with \textit{L. paracasei} A221 was highly effective in augmenting metabolite-producing potentials (Fig. 3). Moreover, the average score of ginseng efficacy was actually improved with an efficiency of 63.2\% ($p < 0.0001$) by ingestion of \textit{L. paracasei} A221. Figure 3 demonstrates that orally ingested \textit{L. paracasei} A221 was excreted as feces and that the bacteria grew in the medium utilizing ginsenosides (Fig. 3). \textit{L. paracasei} A221 was detected in feces just after the ingestion (Fig. 4), suggesting that \textit{L. paracasei} A221 is not a general species of intestinal bacteria. Therefore, acquisition of metabolite-producing potentials by \textit{L. paracasei} A221 is possibly considered as the result that ingested \textit{L. paracasei} A221 hydrolyzes ginsenosides in the intestines. These results permit us to speculate that the augmentation of metabolite-producing potentials by \textit{L. paracasei} A221 resulted in improvement of ginseng efficacy. Collectively, we
conclude that L. paracasei A221 is available for standardizing metabolite-producing potentials of intestinal bacteria.

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References


Japanese abstract

漢方処方を構成する生薬には様々な配糖体が有効成分とし
て含まれている。ところがβ結合をもつ配糖体は、それ自身
ヒト消化酵素によって分解されるため、生体利用率が著し
く低い課題がある。しかし、配糖体は腸内細菌による代謝
（加水分解）を受けることで親油性が高まることによっては
じめて吸収され、薬効を発揮するようになる。しかし、腸内
細菌系の構成は個人差が大きくため、その加水分解率も一定
せず、薬効における個人差を引き起こす一因になることが懸
念される。そこで著者らは、配糖体を加水分解する能力があ
るプロバイオティクス（Lactobacillus paracasei A221）の
摂取で、配糖体を加水分解する能力が標準化されるかどうか
を検討した。試験は、まず人間配糖体を加水分解する能力
がほとんど認められなかった8名の被験者にプロバイオティ
クスを3週間服用させた。その結果、被験者全員にその能力
の改善が認められた。さらに、このプロバイオティクスを症
状を呈する136名の被験者に摂取させた結果、痒み（68%），
皮膚疹（52%）、便臭（50%）、花粉症（49%）、乾燥肌（46%），
不眠（29%）に有意な改善が認められた。さらに、プロバイ
オティクス効果とは別に、日常的な人間消化系においても57
名中36名に人間の効果に関するスコアの改善が認められた。
プロバイオティクスの漢方領域への応用が期待される成果と
いえる。

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