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RESEARCH NOTE

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Evaluation of Potato Varieties (*Solanum tuberosum* L.) on Fecal Microflora of Human Volunteers

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Abstract Effects of Dasom Valley and Bora Valley on fecal microflora, fecal moisture, and fecal pH of twelve healthy human volunteers were investigated. Numbers of *Bifidobacterium, Clostridium perfringens, Escherichia coli*, and *Lactobacillus* of control group were 9.24±0.63, 4.44±1.21, 7.75±0.38, and 6.98±0.81 (Log CFU/g wet feces), respectively. During administration of Dasom Valley, numbers of *Bifidobacterium* and *Lactobacillus* were 10.70±0.44 and 8.84±0.77, whereas those of *C. perfringens* and *E. coli* were 2.96±1.50 and 6.69±0.29, respectively. Administration of Dasom Valley significantly increased growth responses of beneficial bacteria, *Bifidobacterium* and *Lactobacillus*, whereas those of harmful bacteria, *C. perfringens* and *E. coli*, significantly decreased. Moisture content of feces increased and fecal pH decreased with intake of Dasom Valley. Intake of Bora Valley slightly increased numbers of *Bifidbacterium* and *Lactobacillus* and slightly decreased those of *C. perfringens* and *E. coli*. Results indicate Dasom Valley has greater intestinal-modulating effect than Bora Valley and Atlantic. Daily intake of Dasom Valley may normalize disturbed physiological functions, resulting in improvement of growth and composition of microbial community within intestinal tract.

Keywords: Atlantic, Bora Valley, Dasom Valley, fecal microflora, growth response

Introduction

Potato (*Solanum tuberosum*) is an important plant from the nutritional, agricultural, and toxicological points of view (1). Its nutritional value and protein quality are superior to those of cereals and is the second in total protein production after soybean (2). The medicinal properties of potatoes have long been recognized worldwide to include antimicrobial (3), antifungal (4), and anticarcinogenic (5) effects, as well as antihypertensive effects (6) and dietary effects on patients with peptic ulcer (7). Potatoes contain various compounds such as flavonoids, catecholamines (dopamine, norepinephrine, normetanephrine), carotenoids (violaxanthin, antheraxanthin, lutein, zeaxanthin, β-cryptoxanthin, β-carotin), and toxic glycoalkalods (3, 8, 9).

Even though potatoes have various medicinal properties, relatively little work has been done on their effects on the growth of human intestinal microorganisms. Colonic bacteria have been implicated as major determinants of health and disease in humans (10). With the elucidation of relationships between microbial community structure and the health of the host, interest in the manipulation of gut bacterial populations for improved human health has increased (10, 11). Therefore, in a previous study, we investigated the effects of ethanol-water extracts of 29 potato varieties and breeding clones on human intestinal bacteria *in vitro*, and found that all varieties and breeding clones showed no inhibitory effects against harmful bacteria such as *Clostridium perfringens* and *Escherichia coli* (the causative agents of a wide variety of human diseases), whereas some varieties and breeding clones enhanced the growths of some lactic acid-forming beneficial bacteria such as *Bifidobacterium bifium, B. breve, B. longum, Lactobacillus acidophilus*, and *L. casei*. Among the 29 potato varieties and breeding clones, Dasom Valley and Bora Valley significantly promoted the growths of Bifidobacteria and *Lactobacillus* (12, 13). Therefore, based on the results of these *in vitro* studies, we performed the present human study on the effects of Dasom Valley and Bora Valley juices on fecal microflora, pH, and moisture content of human intestines.

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Materials and Methods

Chemicals BL medium and eosinmethylenblue agar were provided by EIKEN chemical (Tokyo, Japan) and Difco (Sparks, MD, USA), respectively. MRS medium and paromomycin sulfate were supplied by Merck (Frankfuter Str., Germany) and Sigma Chemical (St. Louis, USA), respectively. Egg yolk emulsion, lithium chloride, neomycin, perfringens agar base, sodium propionate, and TSC supplement were supplied by OXOID (Basingstoke, Hampshire, UK). All other chemicals were of the reagent grade. Egg yolk emulsion, lithium chloride, neomycin, perfringens agar base, and sodium propionate were supplied by OXOID (Basingstoke, Hampshire, UK). All other chemicals were of reagent grade.

Study designs Twelve healthy human volunteers (six males and six females between 24~28 years old) were given the normal Korean diet. They were free of antibiotics and other medical therapies before and during the experiment. Dasom Valley juice (100 g) was administered to volunteers every day for 4 weeks, followed by 1 week of non-intake period. Changes in fecal microflora, fecal moisture, and fecal pH were observed before during, and after the Dasom Valley juice intake. Each assay was replicated three times. Four months after Dasom Valley intake, Bora Valley juice was also administered to volunteers under the same conditions as above. Commercially available potato (Dae-seo, *Solanum tubersum* L. cv. Atlantic) which was used as a reference was administered to volunteers simultaneously with the Bora Valley.

Potato preparation Dasom Valley and Bora Valley potatoes were kindly provided by Dr Lim, Hak-Tae of Center for the Korea Plant Genetic Resources, Kangwon National University, and stored at 21°C in a refrigerator until used. The potatoes were mashed with grinder after washing and administered to volunteers in the morning.

Fecal preparation Fresh feces were collected and immediately stored in anaerobic pouches (Anaeropack-Anero, Mitsybishi Gas Chemical, Tokyo, Japan) at 4°C. The stool specimen (1 g/wet weight) was homogenized by vortexing for 5-10 min in saline solution (9 mL) and serially diluted 10-fold in the same solution to 10^{-8}.

Fecal microfloral analysis From each dilution, a portion (0.1 mL) was taken and inoculated in the following four selective media according to the method of Mitsuoka (2). BS agar composed of BL medium, sodium propionate, paromomycin sulfate, neomycin, and lithium chloride was used for *Bifidobacterium*, MRS agar for *Lactobacillus*, EMB agar for *Escherichia coli*, and TSC agar composed of perfringens agar base, TSC supplement, and egg yolk emulsion for *C. perfringens*. The plates were incubated at 37°C for 2 days in an atmosphere of 80% N_2, 15% CO_2, and 5% H_2 in an anaerobic chamber (Coy Lab., Grass Lake, MI, USA). After 2 days, the colonies were counted to determine the mean number of anaerobes in each of the fecal samples. Plates with 30-300 colonies, or closest to that range, were counted. The number of bacteria was expressed as a log colony forming units per g feces.

Fecal moisture and pH The fecal moisture (%) was measured every day using a vacuum-dry oven at 105°C for 24 hr. Final weight was recorded, weight loss was computed, and percent moisture as weight loss divided by sample weight was calculated. Fecal pH was measured every day using a digital pH meter (MA235 model: Mettler Toledo, Hightstown, NJ, USA).

Statistical analysis Total bacterial counts were expressed as means ± standard error (SE). Statistical comparison of data was performed using the unpaired student's *t*-test. A *p*-value of $p<0.01$ was defined as statistically different.

Results and Discussion

Total counts of fecal microflora Growth-promoting effects of Dasom Valley and Bora Valley on fecal microflora of twelve healthy human volunteers were investigated. Fecal microflora and properties of twelve adults before the intake of Dasom Valley and Bora Valley, and the effects of Dasom Valley and Bora Valley intake on the numbers of *Bifidobacterium* and *Lactobacillus* are shown in Table 1. During the control period, the mean numbers of total *Bifidobacterium* and *Lactobacillus* population were 9.24±0.63 and 6.98±0.81 (Log CFU/g wet feces), respectively. During the administration of Dasom Valley, the log fecal numbers of *Bifidobacterium* and *Lactobacillus* were significantly increased to 10.70±0.44 and 8.84±0.77, respectively, whereas those of *C. perfringens* and *E. coli* decreased significantly from 4.44 ± 1.21 to 2.96±1.50 and 7.75±0.38 to 6.69±0.29, respectively. However, after stopping the intake of Dasom Valley, the numbers of *Bifidobacterium*, *Lactobacillus*, and *E. coli* decreased for 1 week, whereas *C. perfringens* increased to the initial level. On the other hand, the intake of Bora Valley slightly increased the mean numbers of *Bifidobacterium* (from 9.46±0.64 to 9.95±1.05) and *Lactobacillus* (from 7.13±0.86 to 8.04±1.05). Growths of harmful bacteria, *C. perfringens* and *E. coli*, were slightly decreased by the intake of Bora Valley juice, but were similar to those for the control period and after feeding period. Table 1 shows the effects of commercially available potato intake (Dae-seo, *Solanum tubersum* L. cv. Atlantic) on *Bifidobacterium* and *Lactobacillus*. In the test with Atlantic intake, no differences were detected on the numbers of *Bifidobacterium* and *Lactobacillus*.

Change of fecal moisture The changes in fecal moisture content for Dasom Valley and Bora Valley intakes are shown in Table 1. The fecal moisture content of the control was 74.13±9.70%. In the test with Dasom Valley, the moisture content of the feces significantly increased from 74.13±9.70 to 84.60±3.34% during the feeding periods. On the other hand, the moisture content of the feces slightly increased from 73.85±9.93 to 77.59±7.06% for the Bora Valley intake. After stopping the intake of Dasom Valley and Bora Valley, the moisture content of the feces slightly decreased to 78.23±7.18 and 76.55±6.73, respectively.

Change of fecal pH Effects of Dasom Valley and Bora Valley on fecal pH are shown in Table 1. Intake of Dasom Valley significantly decreased the fecal pH from 6.99±0.05 to 6.61±0.11. The fecal pH after termination of Dasom Valley intake was 6.74±0.07, whereas no marked effects were observed with Bora Valley intake.

The objective of the present study was to investigate the effects of Dasom Valley and Bora Valley intakes on fecal microflora and properties. As expected, the intake of Dasom Valley and Bora Valley promoted the growth of *Bifidobacterium* and *Lactobacillus*, inhibited the growth of *C. perfringens* and *E. coli*, increased the fecal moisture content, and decreased the intestinal pH in human volunteers. Furthermore, Dasom Valley showed much higher intestinal-modulating effect than Bora Valley and Atlantic. Results of the test with Dasom Valley suggest that the ingredients of Dasom Valley could be used by *Bifidobacterium* and *Lactobacillus* as bifidus factors for lowering the intestinal pH. Furthermore, the low intestinal pH produces a more favorable environment for growth of beneficial bacteria, thereby resulting in high counts of beneficial bacteria, which, in turn, inhibit the proliferation of pathogenic bacteria such as *C. perfringens* and *E. coli* (14). In addition, the inhibition of *C. perfringens*, which causes constipation, may promote the fecal moisture

Table 1. Changes of fecal microflora and properties by the administration of Dasom Valley, Bora Valley, and Atlantic

		control	intake 1	intake 2	intake 3	intake 4	after 1
pH	Dasom Valley	6.99±0.05	6.93±0.05	6.82±0.11	6.80±0.11	6.61±0.11	6.74±0.07
	Bora Valley	6.76±0.06	6.70±0.04	6.71±0.06	6.72±0.04	6.79±0.02	6.74±0.04
Moisture (%)	Dasom Valley	74.13±9.70	82.69±0.98	84.60±3.34	83.06±4.46	85.58±2.10	78.23±7.18
	Bora Valley	73.85±9.93	77.59±7.06	77.42±6.01	77.33±6.01	77.19±5.68	76.55±6.73
Bifidobacterium	Dasom Valley	9.24±0.63	9.92±0.58	98.61±0.58	9.58±0.59	10.70±0.44	9.16±0.43
	Bora Valley	9.46±0.64	9.47±0.40	9.09±0.48	9.95±1.05	9.25±0.40	9.06±0.42
	Atlantic	9.06±0.21	8.99±0.84	9.16±0.64	9.14±0.21	9.27±0.47	8.99±0.55
Lactobacillus	Dasom Valley	6.98±0.81	7.68±0.70	7.99±0.70	8.60±0.69	8.84±0.77	7.54±0.63
	Bora Valley	7.13±0.86	7.25±0.89	7.78±0.85	8.04±1.05	7.89±1.40	8.14±1.60
	Atlantic	7.02±1.20	7.16±1.01	7.01±0.81	7.13±0.56	7.50±0.45	7.29±0.91
C. perfringens	Dasom Valley	4.44±1.21	4.43±1.14	3.60±1.33	3.33±1.49	2.96±1.50	4.33±0.64
	Bora Valley	4.61±1.23	4.94±0.90	4.13±1.39	4.21±1.02	4.32±0.41	4.48±0.67
E. coli	Dasom Valley	7.75±0.38	7.96±0.80	7.69±0.23	6.94±0.63	6.69±0.29	6.18±0.58
	Bora Valley	6.32±0.72	6.22±0.43	6.28±0.53	6.06±0.55	5.55±0.96	6.11±0.78

Control: before Dasom Valley and Bora Valley intake, intake 1: 1st week during Dasom Valley and Bora Valley intake, intake 2: 2nd week during Dasom Valley and Bora Valley intake, intake 3: 3rd week during Dasom Valley and Bora Valley intake, intake 4: 4th week during Dasom Valley and Bora Valley intake, after: 1 week after Dasom Valley and Bora Valley intake. Mean standard deviation of $\log_{10}$ count per gram of wet feces.

contents (15). Recent *in vivo* investigations using human volunteers have shown that intake of ginseng extract (16) and green tea-derived polyphenols (17) favorably affected fecal microflora and biochemical aspects of feces. Some researches demonstrated that potato starch-isoflavone diet increased the fecal *Bifidobacterium* in adult mice (18). Furthermore, the addition of resistant potato starch to the rat diet resulted in an increase in the fecal total short chain fatty acid concentration and a decrease in pH (19).

In conclusion, our limited data and some earlier findings indicate that daily intake of Dasom Valley may normalize the disturbed physiological functions, which, in turn, alters the growth and composition of the microbial community and modulates the genesis of potentially harmful products within the intestinal tract, thus preventing diseases caused by pathogens and maintaining optimal human health. Further work is necessary to establish whether Dasom Valley plays significant roles such as prevention against various pathogens in the human large intestine, and to identify the biologically active compounds of Dasom Valley.

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