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RESEARCH NOTE

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Clinical Effects of Intake of Juice Valley and Gogu Valley toward Fecal Microflora of Healthy Human Volunteers

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Abstract Juice Valley and Gogu Valley were administered to twelve healthy young volunteers for 4 weeks to study their clinical effects on human intestinal microflora. Changes in fecal microflora, fecal moisture, and fecal pH were observed for Juice Valley intake. Administration of Juice Valley significantly increased numbers of *Bifidobacterium* and *Lactobacillus* from 8.69 and 7.02 to 10.89 and 9.02 (Log CFU/g wet feces), respectively, whereas those of *Clostridium perfringens* and *Escherichia coli* decreased. Moisture content of feces increased, and fecal pH decreased after 4 weeks of Juice Valley intake, intake of Gogu Valley slightly increased growth responses of *Bifidobacterium* and *Lactobacillus* and decreased growth responses of *C. perfringens* and *E. coli*. Su-mi potato, as a reference, had no effect on *Bifidobacterium* and *Lactobacillus* numbers. This study confirmed Juice Valley has better effects than Gogu Valley and Su-mi, and has important role on growth promotion and inhibition of human intestinal bacteria.

Keywords: fecal microflora, Gogu Valley, growth response, intestinal bacteria, Juice Valley

Introduction

The human gut microflora is extremely complex, comprising more than 500 species of bacteria with total numbers reaching up to 10^{12} CFU/g contents in the colon (1, 2). Based on the impact of the gut microflora on human health and disease (3), they are generally divided into potentially deleterious and health-promoting species. For example, some *Clostridium* spp., proteolytic *Bacteriodes* spp., and enterobacteria are considered potentially harmful due to their association with certain acute and chronic gastrointestinal complaints, whereas *Bifidobacterium* spp. and *Lactobacillus* spp. are considered to play important roles in promoting a healthy gut ecosystem through their antagonistic activities towards potential pathogens, immunomodulatory activities, production of short chain fatty acids, and reduction of microflora-associated enzyme activities involved in the production of carcinogens and genotoxins (4, 5). Recently, there has been much interest in supplementing the health-promoting moieties of the human gut microflora, with increased numbers of *Bifidobacterium* spp. and *Lactobacillus* spp. considered to be desirable. However, the species diversity of the human gut microflora gives rise to a high degree of self-regulation and homeostasis (6). Thus, augmentation of the gut microflora through deliberate consumption of health-promoting bacteria may only have limited and short term success.

Potatoes, commonly cultivated for human consumption worldwide, have been thoroughly examined through the nutritional, agricultural, and toxicological points of view (7). Furthermore, many researches have been reported in relation to antihypertensive (8), antimicrobial (9), antifungal (10), and anticarcinogenic effects of potatoes (11) and dietary therapy for patient with peptic ulcer of potato (12). Its nutritional value and protein quality are superior to those of cereals and is considered as the second crop in total protein production after soybean (13). In addition, the medicinal properties of potatoes have long been recognized worldwide. However, relatively little work has been carried out on the effect of potato on growth of human intestinal microorganisms (14). Thus, this study examined the growth effects of potato varieties on the human intestinal bacteria.

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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 reagent grade.

Study designs Twelve healthy human volunteers (six males and six females between 24~28 years old) were given the normal Korean diet (Table 1). They were free of antibiotics, other medical therapies, and some foods such as dairy products and liquor, which might alter the intestinal bacterial ecosystem, before and during the experimental period. Juice Valley juice (100 g) was administered to volunteers once a day before meals at 7:00 in the morning 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 sample intake. Each assay was replicated three times. Gogu Valley juice was also administered to volunteers

Table 1. General articles of twelve healthy human volunteers

Articles	Subjects											
	1	2	3	4	5	6	7	8	9	10	11	12
Sex	male	male	male	male	male	male	female	female	female	female	female	female
Age	24	27	28	26	28	28	24	24	25	27	25	28
Weight (kg)	60	65	70	68	73	75	45	50	55	49	53	52
Height (cm)	165	170	175	173	175	178	156	168	162	161	165	162

Potato (100 g) was administered to volunteers once a day before meals at 7:00 in the morning for 4 weeks, followed by 1 week of non-intake period.

under the same conditions 4 months later. Commercially available potato (Su-mi), used as a reference, was administered to volunteers simultaneously with the Juice Valley.

Potato preparation Juice Valley, Gogu Valley, and Su-mi 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 About 5 g of fresh feces was collected three times a week. Samples were immediately stored in anaerobic pouches (Anaeropack-Anero, Mitsybishi Gas Chemical, Tokyo, Japan) at 4°C before analysis. For the analyses, the inner part (1 g/wet weight) of a fecal sample was thoroughly homogenized by vortexing for 5-10 min in an anaerobic solution (4.5 g KH_2PO_4, 6.0 g Na_2HPO_4, 0.5 g L-cysteineHCl·H_2O, 1 g Tween 20, 2 g Galtin, 1 L distilled water; 9 mL) and serially diluted 10-fold in the same solution to 10^{-8}.

Fecal microflora analysis From each dilution, a portion (0.1 mL) was inoculated in the following four selective media according to the method of Mitsuoka (13). BS agar (composed of BL medium, sodium propionate, paromomycin sulfate, neomycin, and lithium chloride), MRS agar, EMB agar, and TSC agar (composed of perfringens agar base, TSC supplement, and egg yolk emulsion) were used for *Bifidobacterium*, *Lactobacillus*, *Escherichia coli*, and *C. perfringens*, respectively. 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 fecal samples. Plates with 30-300 colonies, or closest to that range, were counted. The number of bacteria was expressed as log colony-forming units per g feces.

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

Statistical analysis The percent mortality was determined and transformed into arcsine square-root values for analysis of variance (ANOVA). Treatment means were compared and separated by Duncan test at $\alpha = 0.05$ level (15).

Results and Discussion

Table 2 shows changes in fecal microflora, fecal moisture, and fecal pH before, during, and after intake of Gogu Valley and Juice Valley, and effects of Juice Valley and Gogu Valley intakes on the growth of *Bifidobacterium* and *Lactobacillus*. During the control period of Juice Valley intake, the mean total *Bifidobacterium* and *Lactobacillus* populations were 8.69 and 7.02 (Log CFU/g wet feces), respectively. During the administration of Juice Valley, the log fecal numbers of *Bifidobacterium* and *Lactobacillus* significantly increased to 10.89 and 9.02, whereas those of *C. perfringens* and *E. coli* decreased from 4.80 to 3.21 and 7.32 to 5.61, respectively (Table 2). However, after stopping the intake of Juice Valley, the log fecal numbers of *Bifidobacterium* and *Lactobacillus* slightly decreased to 9.76 and 8.75, respectively, whereas those of *C. perfringens* (from 3.21 to 4.16) and *E. coli* (from 5.61 to 6.23) increased for 1 week. On the other hand, intake of Gogu Valley slightly increased the numbers of *Bifidobacterium* (from 9.36 to 9.60) and *Lactobacillus* (from 7.10 to 7.42, and slightly reduced the growth responses of *C. perfringens* and *E. coli*. However, after stopping the intake of Gogu Valley, the numbers of fecal bacteria returned to levels similar to those of the control period. On the other hand, intake of commercially available potato (Su-mi, *Solanum tubersum* L.) had no effect on *Bifidobacterium* and *Lactobacillus*.

The changes in the fecal moisture content of control, and after intake of Juice Valley and Gogu Valley are shown in Table 2. The moisture content of the control was 72.34%. During the administration of Juice Valley, the moisture content of the feces successively increased from 72.34 to 83.33%. After stopping the intake of Juice Valley, the moisture content of the feces slightly decreased to 81.06%. On the other hand, Gogu Valley had no effect on the number of moisture content. Effects of Juice Valley and Gogu Valley intakes on fecal pH are shown in Table 2. Intake of Juice Valley significant decreased fecal pH from 6.78 to 6.43. Fecal pH after stopping intake of Juice Valley was 6.65. However, intake of Gogu Valley had no marked effects on fecal pH.

Intake of Juice Valley and Gogu Valley promoted the growth of *Bifidobacterium* and *Lactobacillus*, inhibited the growth of *C. perfringens* and *E. coil*, increased fecal moisture content, and decreased fecal pH in human volunteers. In particular, Juice Valley showed more excellent effects than Gogu Valley and Su-mi.

Table 2. Changes of fecal microflora and properties by the administration of Juice Valley, Gogu Valley, and Su-mi

	Variety	Mean	Control	Intake 1	Intake 2	Intake 3	Intake 4	After 1
pH	Juice Valley	6.62a*	6.78±0.02a	6.66±0.03a	6.62±0.02a	6.59±0.04a	6.43±0.03a	6.65±0.03a
	Gogu Valley	6.73a	6.73±0.03a	6.74±0.01a	6.74±0.01a	6.73±0.03a	6.72±0.04a	6.73±0.02a
Moisture (%)	Juice Valley	76.88a	72.34±1.78a	78.63±2.41ab	81.10±2.55ab	82.21±2.14b	83.33±1.06b	81.06±3.23ab
	Gogu Valley	79.78a	75.07±2.16a	76.94±1.66ab	77.59±2.79ab	77.60±2.29b	77.63±2.54b	76.43±2.53ab
Bifidobacterium	Juice Valley	9.79a	8.69±0.21a	9.41±0.38ab	9.72±0.53ab	10.29±0.38b	10.89±0.31b	9.76±0.55ab
	Gogu Valley	9.88a	9.36±0.31a	9.75±0.29ab	10.00±0.31ab	10.18±0.19b	10.38±0.24b	9.60±0.34ab
	Su-mi	8.78b	8.72±0.18a	8.83±0.25ab	8.76±0.33ab	8.78±0.26b	8.79±0.19b	8.77±0.19ab
Lactobacillus	Juice Valley	8.14a	7.02±0.21a	7.70±0.22ab	7.96±0.19ab	8.38±0.31ab	9.02±0.29b	8.75±0.18ab
	Gogu Valley	7.50b	7.10±0.29a	7.47±0.23ab	7.52±0.19ab	7.68±0.21ab	7.81±0.19b	7.42±0.23ab
	Su-mi	8.39a	8.34±0.16a	8.38±0.18ab	8.40±0.20ab	8.41±0.19ab	8.44±0.17b	8.35±0.21ab
C. perfringens	Juice Valley	4.06a	4.80±0.18a	4.32±0.21ab	4.26±0.20abc	3.58±0.31cd	3.21±0.19d	4.16±0.24bc
	Gogu Valley	4.21a	4.64±0.26a	4.44±0.32ab	4.26±0.24abc	4.01±0.19cd	3.76±0.25d	4.12±0.23bc
E. coli	Juice Valley	6.25a	7.32±0.25a	6.34±0.31ab	6.09±0.25ab	5.91±0.23b	5.61±0.24b	6.23±0.32ab
	Gogu Valley	7.46b	7.73±0.34a	7.57±0.23ab	7.44±0.14ab	7.31±0.24b	7.15±0.16b	7.58±0.24ab

*Significant difference between Juice Valley and Gogu Valley at α=0.05 level.
Control: before Juice Valley and Gogu Valley intake, intake 1: 1st week during Juice Valley and Gogu Valley intake, intake 2: 2nd week during Juice Valley and Gogu Valley intake, intake 3: 3rd week during Juice Valley and Gogu Valley intake, intake 4: 4th week during Juice Valley and Gogu Valley intake, after: after Juice Valley and Gogu Valley intake. Mean standard deviation of $\log_{10}$ count per gram of wet feces.

Furthermore, low intestinal pH produced a more favorable environment for beneficial bacteria, allowing higher counts of beneficial bacteria to inhibit the proliferation of pathogenic bacteria such as *C. perfringens* and *E. coli* (16). Additionally, inhibition of *C. perfringens*, which causes constipation, increased the fecal moisture content (17). These results indicate that Juice Valley plays an important role in the growth promotion and inhibition of human intestinal bacteria.

In conclusion, the excellent effects of Juice Valley confirmed its superiority and usefulness as a growth modulator against intestinal bacteria. Moreover, administration of Juice Valley (100 g) for 4 weeks resulted in no undesirable effects to the volunteers. Therefore, Juice Valley and Gogu Valley are safe for human consumption. Based on our present data and those of the earlier findings, daily intake of Vally potatoes could help correct our intestinal conditions and protect us from various diseases.

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