J. Food Sci. Nutr.
Vol. 6, No. 3, p. 192~196 (2001)

Growth-Promoting Effects of Vegetable Extracts on Selected Human Lactic Acid Bacteria

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Abstract

Ethanol extracts from 36 vegetable samples were assayed for their growth-promoting effects on *Bifidobacterium bifidum, B. longum,* and *Lactobacillus casei.* The growth-promoting effects varied according to bacterial strain and vegetable species. In modified György broth, extracts of *Lactuca sativa, Lycopersicon esculentum* and *L. esculentum* var. *cerasiforme* exhibited strong growth-promoting responses toward *B. longum*, and significant and strong growth-promoting response toward *B. bifidum* was observed in extracts of *Actinidia arguta, Allium cepa, A. sativum, Brassica campestris subsp. napus* var. *pekinensis, Capsicum frutescens, Daucus carota* var. *sativa, L. sativa, L. esculentum* and *L. esculentum* var. *cerasiforme, Nelumbo nucifera, Cucurbita moschata, Lactuca sativa* var. *capitata*, and *Rubus coreanus.* For *L. casei*, extracts of *A. fistulosum, A. tuberosum, Cichorium intybus, Cucurbita moschata, Ipomoea batatas, L. sativa* var. *capitata, L. esculentum, P. brachycarpa, Raphanus sativus, R. coreanus*, and *S. melongena* strongly enhanced the growth of this bacteria. In modified György broth, the promoting effect was most pronounced with *B. bifidum* and *L. casei* among lactic acid bacteria used. In MRS broth, *A. arguta, A. cepa, A. sativum, B. campestris subsp. napus* var. *pekinensis, C. frutescens*, and *D. carota* var. *sativa, L. sativa* var. *capitata*, and *R. coreanus* strongly enhanced the growth of *B. bifidum*. Growth of *B. longum* was strongly affected by the addition of extracts from *L. sativa* var. *capitata.* For *L. casei*, moderate growth-promoting responses were observed in 9 vegetable extracts. The promoting effect in MRS broth was most pronounced with *B. bifidum* among lactic acid bacteria used.

Key words: intestinal bacteria, growth-promoting activity, vegetable extract

INTRODUCTION

Various microorganisms are resident in the human intestinal tract as a highly complex ecosystem with considerable species diversity. The intestinal microbiota in healthy subjects remains relatively constant but is known to be greatly influenced by physical, biological, chemical, environmental or host factors (1-3). They participate in normal physiological functions, and contribute significantly to the genesis of various disease states by biotransforming a variety of ingested or endogenously formed compounds to useful or harmful derivatives. These biotransformations may influence drug efficacy, toxicity, carcinogenesis and aging (1-4).

Previous investigations have demonstrated that there were some differences in intestinal bacteria between patients and healthy control subjects (5,6), and between younger and elderly subjects (2,7,8). Normal gastrointestinal microbiota is found to be predominantly composed of lactic acid bacteria which seem to play an important role in metabolism, host defense against infection, aging and immunopotentiation (1,2,4). On the other hand, the microbiota of cancer patients is composed of a high concentration of clostridia and eubacteria with few lactic acid bacteria. It has also been reported that elderly subjects harbour fewer bifidobacteria but larger numbers of clostridia than younger subjects. Accordingly, any disturbance of the microbiota may cause a variety of diseases of abnormal physiological states.

In relation to human health, currently much concern has focused on plant-derived bifidus factors which promote the growth of beneficial bacteria and plant-derived growth inhibitors against harmful bacteria such as *C. perfringens* and *E. coli* because plants constitute a rich source of bioactive chemicals (9). Recently, much interest has been focused on various vegetables in relation to human health since they are largely free from harmful adverse effects and have excellent nutritional, industrial and pharmacological significance (10-13). However, relatively little work has been reported recently on the growth-promoting effects of vegetables on intestinal microorganisms. In the laboratory study described herein, we assessed growth-promoting effects of various vegetable extracts on selected lactic acid bacteria originating from humans.

MATERIALS AND METHODS

Plant materials and sample preparation

The sample plants were chosen anecdotally and collected randomly (Table 1). They were dried in an oven at 60°C for

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3 days and finely powdered using a blender (Model: RM 100, F. Kurt Retsch GmbH & Co. KG, Germany). Each sample was extracted twice with 500 mL of 70% ethanol at room temperature and filtered (Toyo filter paper No. 2, Toyo Roshi, Japan). The combined filtrate was concentrated *in vacuo* at 35°C using a rotary vacuum evaporator (N-3NW, Eyela Co., Japan). The sample list and yields are shown in Table 1.

Bacterial strains and culture conditions

Three bacterial strains used in this study are as follows: *Bifidobacterium bifidum* ATCC 15696, *B. longum* ATCC 15707, and *Lactobacillus casei* KCTC 393 isolated from human feces. Stock cultures of these strains were routinely stored on Eggerth-Gagnon Liver extract-Fieldes slant at -80°C and, when required, were subcultured on Eggerth-Gagnon (EG) agar (Eiken Chemical Co., Ltd, Tokyo, Japan). The plates were incubated anaerobically 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).

Microbiological analysis

For growth measurements with microorganisms, the testing methods of Mitsuoka (14) and Lee and Ahn (15) were applied. In the experiments for bifidus factor (s) derived from non-carbon sources, György (16) broth (pH 6.8) as modified by Yoshioka (17) was used. In the experiments for bifidus factor(s) derived from carbon sources, MRS (15) broth (pH 7.8) was used. Lee and Ahn (15) already reported that 10~20 mg/disc of a plant extract did not cause any problems such as insolubility and difficulty in detection of its minor active components. One per cent of each seed was inoculated on to the media described above, and 10 mg of each membrane filter-sterilized test material was added to the media in a final volume of 10 mL. Solutions of the test materials were prepared using ethanol as solvent. The ethanol concentration in the solutions did not exceed 2 percent which was found to be without adverse effect on the bacteria tested. The media were incubated anaerobically at 37°C for 2 days, and the bacterial growth was measured spectrophotometically at 600 nm. Growth-promoting response was expressed as growth increase rate (GIR=A_{600} sample/A_{600} reference). The responses were determined as previously described: significant response ++++, GIR > 2.5; strong response +++, GIR > 2.0

Table 1. The yield of 70% ethanol extracts from 36 vegetables

Scientific name	Family name	Fresh weight (g)	Dried weight (g)	Yield[1)] (%)
Actinidia arguta	Actinidiaceae	500	60.4	4.8
Allium cepa	Liliaceae	500	46.6	5.5
Allium fistulosum	Liliaceae	500	17.2	4.9
Allium monanthum	Liliaceae	500	92.6	1.9
Allium sativum	Liliaceae	500	23.4	1.4
Allium tuberosum	Liliaceae	500	31.8	2.0
Amaranthus mangostanus	Amaranthaceae	500	32.2	2.5
Aster glehni	Compositae	389	59.0	14.2
Brassica campestris subsp. napus var. *pekinensis*	Brassicaceae	500	108.3	4.3
Brassica campestris var. *chinensis*	Cruciferae	500	23.8	1.3
Capsella bursapastoris	Brassicaceae	500	111.1	1.8
Capsicum annuum	Solanaceae	500	17.9	3.8
Capsicum frutescens	Solanaceae	500	50.0	4.1
Chrysanthemum coronarium var. *spatiosum*	Compositae	500	35.7	1.6
Cichorium intybus	Compositae	500	20.0	1.2
Colocasia antiquorum var. *esculenta*	Araceae	500	150.0	1.6
Cucurbita moschata	Cucurbitaceae	500	29.4	3.4
Cucumis sativus	Cucurbitaceae	500	51.3	2.6
Daucus carota var. *sativa*	Umbelliferaeceae	500	90.4	6.5
Ipomoea batatas	Convolvulaceae	500	206.0	6.7
Lactuca sativa	Compositae	500	25.0	2.0
Lactuca sativa var. *capitata*	Compositae	500	85.1	4.7
Lycopersicon esculentum	Solanaceae	500	16.9	5.5
Lycopersicon esculentum var. *cerasiforme*	Solanaceae	500	38.9	6.4
Nelumbo nucifera	Nymphaeaceae	500	157.9	2.1
Oenanthe javanica	Umbelliferaeceae	500	26.9	2.0
Perilla frutescens	Labiatae	500	66.7	1.9
Petroselinum crispum	Umbelliferaeceae	500	50.0	8.7
Pimpinella brachycarpa	Umbelliferaeceae	500	132.1	3.8
Raphanus sativus	Brassicaceae	500	68.8	5.0
Rubus coreanus	Rosaceae	268	94.3	5.0
Salanum tuberosum	Solanaceae	500	195.3	6.1
Sedum sarmentosum	Crassulaceae	500	12.5	1.9
Solanum melongena	Solanaceae	500	94.6	1.9
Spinacia oleracea	Chenopodiaceae	500	26.3	2.4
Zingiber officinale	Zingiberaceae	500	27.5	1.2

[1)](Dried weight of ethanol extract / dried weight of the sample vegetable) × 100.

; moderate ++, 2.0 > GIR > 1.6; weak, 1.5 > GIR > 1.0; and no response -, GIR < 1.0. Each assay was replicated three times (15,18).

RESULTS AND DISCUSSION

Growth-promoting activity of extracts obtained from 36 vegetable samples on lactic acid bacteria such as *B. bifidum*, *B. longum*, and *L. casei* was investigated by spectrophotometric method. For determination of bacterial growth, two kinds of media were used : modified György broth as a carbon source-containing medium and MRS broth as a carbon source-free medium. The growth-promoting responses varied with vegetable species and bacteria strain tested.

Growth-promoting activity of the test bacteria by the extracts from vegetable samples in modified György broth is shown in Table 2. In tests with *B. longum* which are predominant in the intestines of adults, extracts of *Lactuca sativa*, *Lycopersicon esculentum* and *L. esculentum* var. *cerasiforme* exhibited strong growth-promoting responses (+++), whereas remaining samples showed weak (+) or no promoting activity. With *B. bifidum* which is dominant bacteria in the intestines of infants, significant growth-promoting response (++++) was observed in extracts of *Allium sativum*, *Brassica campestris subsp. napus* var. *pekinensis*, *Capsicum frutescens*, *Daucus carota* var. *sativa*, *L. sativa*, *L. esculentum* and *L. esculentum* var. *cerasiforme*, and *Nelumbo nucifera*, and strong growth-promoting response (+++) was obtained in extracts of *Actinidia arguta*, *A. cepa*, *Cucurbita moschata*, *Lactuca sativa* var. *capitata*, and *Rubus coreanus*. However, remaining samples except for *Cucumis sativus*, *Pimpinella brachycarpa*, and *Solanum melongena* contained moderate promoting activity (++) exhibited weak or no growth-promoting activity. For *L. casei*, extracts from *A. fistulosum*, *A. tuberosum*, *Cichorium intybus*, *Cucurbita moschata*, *Ipomoea batatas*, *P. brachycarpa*, *Raphanus sativus*, and *S. melongena* significantly enhanced the growth of this bacteria, and strong growth-promoting activity was observed in extract of *L. sativa* var.

Table 2. Effect of vegetable extracts on the growth of beneficial bacteria in modified György broth

Sample name	Strain[1)]		
	B. bifidum	*B. longum*	*L. casei*
A. arguta	+++[2)]	+	++
A. cepa	+++	+	++
A. fistulosum	-	-	++++
A. monanthum	-	-	-
A. sativum	++++	+	++
A. tuberosum	-	-	++++
A. mangostanus	-	-	-
A. glehni	-	-	-
B. campestris subsp. napus var. *pekinensis*	++++	+	++
B. campestris var. *chinensis*	-	-	-
C. bursapastoris	-	-	-
C. annuum	-	-	-
C. frutescens	++++	+	++
C. coronarium var. *spatiosum*	-	-	-
C. intybus	-	-	++++
C. antiquorum var. *esculenta*	-	-	-
C. moschata	+++	-	++++
C. sativus	++	-	-
D. carota var. *sativa*	++++	+	+
I. batatas	-	-	++++
L. sativa	++++	+++	-
L. sativa var. *capitata*	+++	+	+++
L. esculentum	++++	+++	+++
L. esculentum var. *cerasiforme*	++++	+++	-
N. nucifera	++++	-	++
O. javanica	-	-	-
P. frutescens	-	-	-
P. crispum	-	-	-
P. brachycarpa	++	-	++++
R. sativus	-	-	++++
R. coreanus	+++	+	+++
S. tuberosum	-	-	-
S. sarmentosum	-	-	-
S. melongena	++	+	++++
S. oleracea	-	-	-
Z. officinale	-	-	-

1)Exposed to 10 mg/disc.
2)Growth increase rate (GIR = A_{600} sample / A_{600} reference) : significant response ++++, GIR > 2.5; strong response +++, GIR > 2.0; moderate ++, 2.0 > GIR > 1.5; weak +, 1.5 > GIR >1.0; no response -, GIR<1.0.

capitata, *L. esculentum*, and *R. coreanus*. However, moderate growth-promoting responses were produced from 6 vegetable extracts. The promoting effect was most pronounced with *B. bifidum* and *L. casei* among lactic acid bacteria used.

Table 3 shows growth-promoting activity of lactic acid bacteria to 36 vegetable samples in MRS broth. In tests with *B. bifidum*, *A. sativum*, *C. frutescens*, and *D. carota* var. *sativa* significantly enhanced the growth of this bacteria, and strong growth-promoting activity was observed in extract of *A. arguta*, *A. cepa*, *B. campestris subsp. napus* var. *pekinensis*, *L. sativa* var. *capitata*, and *R. coreanus*. Growth of *B. longum* was strongly affected by the addition of extracts from *L. sativa* var. *capitata*. However, weak or no promoting activity against *L. casei* was obtained from extracts of remaining samples. For *L. casei*, moderate growth-promoting responses were observed in 9 vegetable extracts, whereas weak or no promoting activity was obtained from extracts of remaining samples. The promoting effect in MRS broth was most pronounced with *B. bifidum* among lactic acid bacteria used.

In tests with two types of media, growth-promoting effects of *C. moschata*, *L. sativa*, *L. esculentum*, *L. esculentum* var. *cerasiforme*, and *N. nucifera* on *B. bifidum* were enhanced more in modified György broth than in MRS broth. For *B. longum*, similar results were observed in extracts of *L. esculentum* and *L. esculentum* var. *cerasiforme*. Furthermore, toward *L. casei*, growth-promoting responses of *A. tuberosum*, *C. intybus*, *Ipomoea batatas*, *L. esculentum*, *P. brachycarpa*, and *R. sativus* were enhanced more in modified György broth than in MRS broth. In this study, these results indicate that growth promoters of vegetables above should be a carbon source.

Among the various human intestinal microorganisms, bifidobacteria are often taken as useful indicators of human health under most environmental conditions. This is based upon the facts that they play important roles in metabolism, e.g. nutrition production such as vitamin and essential amino acid, aid defense against infection, are associated longevity, pathogen inhibition, immunity activation, improvement of lactose

Table 3. Effect of vegetable extracts on the growth of beneficial bacteria in MRS broth

Sample name	Strain[1]		
	B. bifidum	*B. longum*	*L. casei*
A. arguta	+++[2]	+	++
A. cepa	+++	+	++
A. fistulosum	-	-	++
A. monanthum	-	-	-
A. sativum	++++	+	++
A. tuberosum	-	-	-
A. mangostanus	-	-	-
A. glehni	-	-	-
B. campestris subsp. napus var. *pekinensis*	+++	-	++
B. campestris var. *chinensis*	-	-	-
C. bursapastoris	-	-	-
C. annuum	-	-	-
C. frutescens	++++	-	++
C. coronarium var. *spatiosum*	-	-	-
C. intybus	-	-	-
C. antiquorum var. *esculenta*	-	-	-
C. moschata	-	-	++
C. sativus	-	-	-
D. carota var. *sativa*	++++	+	+
I. batatas	-	-	+
L. sativa	-	+++	-
L. sativa var. *capitata*	+++	-	+
L. esculentum	-	-	-
L. esculentum var. *cerasiforme*	-	-	-
N. nucifera	-	-	-
O. javanica	-	-	-
P. frutescens	-	-	-
P. crispum	-	-	-
P. brachycarpa	-	-	-
R. sativus	-	-	-
R. coreanus	+++	+	++
S. tuberosum	-	-	-
S. sarmentosum	-	-	-
S. melongena	+	-	++
S. oleracea	-	-	-
Z. officinale	-	-	-

[1]Exposed to 10 mg/disc.
[2]Growth increase rate (GIR = A_{600} sample / A_{600} reference) : significant response ++++, GIR > 2.5; strong response +++, GIR > 2.0; moderate ++, 2.0 > GIR > 1.5; weak +, 1.5 > GIR > 1.0; no response -, GIR < 1.0.

tolerance of milk products, and antitumorigenic activity (1, 3,19,20). Bifidobacteria growth-promoting factors, usually called bifidus factor, have therefore been extensively studied since György et al. (16) suggested their existence in human milk. Bifidus factors are classified into lacteal secretions, fructooligosaccharides, derivatives of lactose, and xylooligosaccharides (21).

In our microbial study, extracts from 20 vegetables enhanced the growth of lactic acid bacteria in media with or without carbon sources, suggesting that bifidus factor(s) might be involved in the phenomenon. It would be most desirable to both inhibit the growth of potential pathogens and/or increase the numbers of bifidobacteria in the human gut. Selective growth promoters for bifidobacteria or inhibitors for harmful bacteria are especially important for human health, because intake of these materials may normalize disturbed physiological functions which result in the prevention of diseases caused by pathogens in the gastrointestinal tract. Similar *in vitro* results were also reported in extracts from grain, legume seeds, and medicinal plants (15,18,22-24). Recent *in vivo* investigations using human volunteers have shown that intake of ginseng extract (24) and green tea-derived polyphenols (25) favorably affected faecal microbiota and biochemical aspects of faeces, suggesting an indication of at least one of their pharmacological actions. Accordingly, the daily intake of vegetable-derived materials might be expected to alter the growth and composition of the microbial community and modulate the genesis of potentially harmful products such as carcinogenic *N*-nitroso compounds of aromatic steroids within the intestinal tract, thus protecting from a variety of diseases and maintaining optimal human health.

ACKNOWLEDGMENTS

This work was supported by Agricultural R & D Promotion Center through Chonbuk National University to Moo-Key Kim.

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(Received June 23, 2001)