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# Growth-Inhibiting Effects of Vegetable Extracts on Beneficial and Harmful Human Intestinal Bacteria

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**Ethanol extracts of 38 vegetables were subjected to an *in vitro* screening for their growth-inhibitory activities towards *Bifidobacterium bifidum*, *B. longum*, *Clostridium perfringens*, *Lactobacillus acidophilus*, *L. casei*, and *Escherichia coli* using paper disc agar diffusion methods under anaerobic conditions. The responses varied with both bacterial strain and vegetable species. In a test with 20 mg/disc, *Zingiber officinale* extracts showed significant growth-inhibitory responses against *B. bifidum*, and strong inhibitions against *L. casei* were detected in the extracts of *Chrysanthemum coronarium* var. *spatiosum* and *Lactuca sativa*. The extracts of *Allium sativum*, *Capsicum annuum*, *L. esculentum*, *L. esculentum* var. *cerasiforme*, and *Z. officinale* showed strong inhibitory activities against *C. perfringens*, while moderate growth-inhibitory responses were observed in the extracts of *C. ffutescens*, *Cucurbita moschata*, *Daucus carota* var. *sativa*, and *Rubus coreanus*. However, all vegetable extracts showed no inhibitions against *B. longum*, *L. acidophilus*, and *E. coli*. In tests with 5 mg/disc, moderate inhibitions were observed in the extracts of *C. coronarium* var. *spatiosum* and *L. sativa* against *L. casei* and *Z. officinale* against *B. bifidum*. Vegetables extracts, except for *C. coronarium* var. *spatiosum*, *L. sativa*, and *Z. officinale*, did not affect the growth of beneficial bacteria. Strong inhibitory responses against *C. perfringens* were detected in the extracts of *C. annuum* and *L. esculentum* var. *cerasiforme*. Daily intake of vegetables may be important in the prevention of human diseases caused by the intestinal bacteria.**

**Key words:** *intestinal bacteria, growth-inhibitory activity, vegetables.*

Various microorganisms are resident in the human intestinal tract as a highly complex ecosystem with considerable species diversity. The intestinal microbiota in healthy subject remains relatively constant but is known to be greatly influenced by physical, biological, chemical, environmental or host factors.[1-3] They not only participate in normal physiological functions, but also contribute significantly to the genesis of various disease states by biotransforming a variety of ingested or endogenously formed compounds into useful or harmful derivatives. These biotransformations influence drug efficacy, toxicity, carcinogenesis, and aging.[1-4]

Previous investigations have demonstrated that there were some differences in the intestinal bacteria between patients and healthy control subjects,[5,6] and also between the younger and elderly subjects.[2,7,8] The normal gastrointestinal microbiota is found to be predominantly composed of lactic acid bacteria which seem to play important roles in metabolism, host defense against infection, aging and immunopotentiation.[1,2,4] On the other hand, the microbiota of cancer patients is composed of high concentrations 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 the 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 concerns have been focused on the 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] In particular, much interests have 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 significances.[10-13] However, relatively little work has been carried out on the effects of vegetables on the growth responses of intestinal microorganisms compared to other areas of intestinal microbiology. In the laboratory study described herein, we assessed growth responses of various vegetable extracts to human lactic acid and harmful intestinal bacteria.

## Materials and Methods

**Plant materials and sample preparation.** The vegeta-

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**Abbreviations:** *B. longum*, *Bifidobacterium longum*; *C. perfringens*, *Clostridium perfringens*; *E. coli*, *Escherichia coli*.

**Table 1. Yields of 70% ethanol extracts from 38 vegetables.**

| Scientific Name | Family Name | Fresh Weight (g) | DriedWeight (g) | Yield[a] (%) |
|---|---|---|---|---|
| *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 |
| *Angelica keiskei* | Umbelliferaeceae | 620 | 295.1 | 4.3 |
| *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 |
| *Brassica oleracea* var. *acephala* | Brassicaceae | 600 | 270.0 | 5.5 |
| *Capsella bursapastoris* | Brassicaceae | 500 | 111.1 | 1.8 |
| *Capsicum annuum* | Solanaceae | 500 | 17.9 | 3.8 |
| *Capsicum ffutescens* | 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 |
| *Solanum 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 |

[a](Dried weight of 70% ethanol extract/dried weight of the sample vegetable) × 100.

bles were randomly and anecdotally collected (Table 1). They were dried in an oven at 60°C for 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 (Model: N-3NW, EYELA, Japan). The yields of 38 vegetable extractions are shown in Table 1.

**Bacterial strains and culture conditions.** Six bacterial strains used in this study are as follows: *Bifidobacterium bifidum* ATCC 15696, *B. longum* ATCC 15707, *Clostridium perfringens* ATCC 13124, *Lactobacillus acidophilus* KCTC 4356, *L. casei* KCTC 393, and *Escherichia coli* ATCC 11775 isolated from human feces. Stock cultures of these strains were routinely stored on Eggerth-Gagnon Liver extract-Fieldes slant[14] 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 investigation of the inhibitory effect of each vegetable extract on the microorganisms,

**Table 2. Growth-inhibitory activities of ethanol extracts against beneficial and harmful intestinal bacteria.**

| Sample Name | Bacterial Strain[a] | | | | | |
|---|---|---|---|---|---|---|
| | *B. bifidum* | *B. longum* | *L. acidophilus* | *L. casei* | *C. perfringens* | *E. coli* |
| *A. arguta* | -[b] | - | - | - | - | - |
| *A. cepa* | - | - | - | - | + | - |
| *A. fistulosum* | - | - | - | - | - | - |
| *A. monanthum* | - | - | - | - | - | - |
| *A. sativum* | - | - | - | + | +++ | - |
| *A. tuberosum* | - | - | - | - | - | - |
| *A. mangostanus* | - | - | - | - | - | - |
| *A. keiskei* | - | - | - | - | - | - |
| *A. glehni* | - | - | - | - | - | - |
| *B. campestris subsp. napus* var. *pekinensis* | - | - | - | - | - | - |
| *B. campestris* var. *chinensis* | - | - | - | - | + | - |
| *B. oleracea* var. *acephala* | - | - | - | - | - | - |
| *C. bursapastoris* | - | - | - | - | - | - |
| *C. annuum* | - | - | - | - | +++ | - |
| *C. ffutescens* | - | - | - | - | ++ | - |
| *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* | +++ | - | - | - | +++ | - |

[a]Exposed to 20 mg/disc.

[b]Strong response +++, zone diameter >20 mm; moderate ++, zone diameter 16-20 mm; weak +, zone diameter 10-15 mm; no response -, zone diameter <10 mm.

one loopful of bacteria was suspended in 1 m*l* sterile physiological saline. An aliquot (0.1 m*l*) of the bacterial suspensions was seeded onto the EG agar. Ten milligrams of the extracts dissolved in ethanol were applied using a Drummond glass microcapillary to paper discs (Advantec, 8 mm, Toyo Roshi, Japan). After evaporation, the discs were placed on the EG agar surface and incubated at 37°C for 2 days in an anaerobic chamber. Control discs were applied with ethanol only. All inhibition tests were triplicated. The growth responses of test samples were determined through comparison with those of the controls. The inhibitory responses were classified as follows: strong response +++, zone diameter >20 mm; moderate ++, zone diameter 16-20 mm; weak +, zone diameter 10-15 mm; and no response -, zone diameter <10 mm.[15,16]

## Results and Discussion

The growth-inhibitory responses of 70% ethanol extracts of

**Table 3. Growth-inhibitory activities of seven extracts against beneficial and harmful intestinal bacteria.**

| Test material | Bacterial Strain[a] | Dose, mg/disc | | |
|---|---|---|---|---|
| | | 5 | 10 | 20 |
| *A. sativum* | | | | |
| | *C. perfringens* | + | ++ | +++ |
| *C. annuum* | | | | |
| | *C. perfringens* | +++ | +++ | +++ |
| *C. coronarium var. spatiosum* | | | | |
| | *L. casei* | ++ | +++ | +++ |
| *L. sativa* | | | | |
| | *L. casei* | ++ | +++ | +++ |
| *L. esculentum* | | | | |
| | *C. perfringens* | ++ | +++ | +++ |
| *L. esculentum var. cerasiforme* | | | | |
| | *C. perfringens* | +++ | +++ | +++ |
| *Z. officinale* | | | | |
| *B. bifidum* | *C. perfringens* | - | + | +++ |

[a]Strains with no activity were not shown.

**Table 4. Growth-inhibitory responses of four vegetable extracts fractions against *B. bifidum*, *L. casei*, and *C. perfringens*.**

| Plant Species | Fraction[b] | Bacterial Strain[a] | | |
|---|---|---|---|---|
| | | *B. bifidum* | *L. casei* | *C. perfringens* |
| *C. annuum* | | | | |
| | *Chloroform* | - | - | +++ |
| | *Ethyl-acetate* | - | - | ++ |
| *L. sativa* | | | | |
| | *Ethyl-acetate* | - | +++ | - |
| *L. esculentum var. cerasiforme* | | | | |
| | *Chloroform* | - | - | +++ |
| | *Water* | - | - | ++ |
| *Z. officinale* | | | | |
| | *Hexane* | +++ | - | - |

[a]Exposed to 5 mg/disc.
[b]Fractions (hexane, chloroform, ethyl-acetate, butanol, water) without activity were not shown.

various vegetables against *B. bifidum*, *B. longum*, *C. perfringens*, *L. acidophilus*, *L. casei*, and *E. coli* were investigated *in vitro*. Because plants constitute a rich source of bioactive organic chemicals with various pharmacological actions[8,9,10,11] and native herbal practices have been replaced by modern medical practices,[17] in recent years much interest has been focused on the vegetable materials as potentially useful products or lead compounds for synthetic compounds in various medicinal fields.[10-12] It has been acknowledged that vegetables have various pharmacological actions.[10-12] The details along with economic importance of majority of vegetable are provided by Billson *et al.*[12] and Kipopoulou et al.[13]

Growth-inhibitory activities of the beneficial intestinal bacteria such as *B. bifidum*, *B. longum*, *L. acidophilus*, and *L. casei* against the extracts of various vegetables are shown in Table 2. The growth-inhibitory responses to the beneficial intestinal bacteria tested varied between plant species and bacterial strains. In tests with *B. bifidum* which is predominant in the intestines of infants, *Zingiber officinale* extracts showed significant growth-inhibitory responses (+++) at a concentration of 20 mg/disc, whereas remaining samples (37 vegetables) showed no inhibitory responses. With *B. longum* which is dominant in the intestines of adults, no inhibitory responses were obtained in all vegetable extracts tested. Furthermore, no inhibitory effects against *L. acidophilus* were obtained from all vegetable extracts. Significant growth-inhibitory responses (+++) to *L. casei* were determined in the extracts of *Chrysanthemum coronarium* var. *spatiosum* and *Lactuca sativa*, whereas weak activity (+) was obtained from the extracts of *Allium sativum*. The remaining samples (33 vegetables) showed no inhibitory responses.

Among the beneficial bacteria, bifidobacteria are the most closely related to the human health. Their acknowledged physiological effects pertain to the improvement of intestinal microbiota by preventing colonization of pathogens, amelioration of diarrhea or constipation,[18] nutrition production such as vitamins and essential amino acids, improvement of lactose tolerance of milk products,[4] decrease in serum cholesterol levels, immunity activation, and antitumorigenic activity.[19,20] Bifidobacteria growth-promoting factors, known as bifidus factors, have therefore been extensively studied since Gyrögy *et al.*[21] reported of their existence in the human milk.[4] Bifidus factors are classified into lacteal secretions, fructooligosaccharides, derivatives of lactose, and xylooligosaccharides.[18] Current studies revealed the extracts of various medicinal plants, legume seeds, and grains to have selective growth-promoting activities for lactic acid bacteria only.[15,16,22] In our study, even though 36 vegetable extracts showed no inhibitory effects on the beneficial bacteria, daily intake of vegetables, except for *C. coronarium* var. *spatiosum*, *L. sativa*, and *Z. officinale*, may affect the faecal microbiota and biochemical aspects of faeces, an indication of at least one of the phamacological actions of vegetables.[10-12] However, daily intake of *C. coronarium* var. *spatiosum*, *L. sativa*, and *Z. officinale* should be caused for beneficial bacteria because they inhibited *B. bifidum* and *L. casei*.

The growth inhibition to harmful bacteria also varied with plant species and bacterial strains at a concentration of 20 mg/disc. Extracts of *A. sativum*, *Capsicum annuum*, *Lycopersicon esculentum*, *L. esculentum* var. *cerasiforme*, and *Z. officinale* showed strong inhibitory activities against *C. perfringens*, while moderate growth-inhibitory responses (++) were observed from the extracts of *Capsicum ffutescens*, *Cucurbita moschata*, *Daucus carota* var. *sativa*, and *Rubus coreanus*. However, the remaining samples (19 vegetables) showed weak or no inhibitory responses. In tests with *E. coli*, extracts of all vegetables revealed no growth-inhibitory activities. Clostridia exert such harmful effects on the human health as

sudden death, toxicity, mutagenesis, carcinogenesis, and aging.[2,4] They act by biotransforming a variety of ingested or endogenously formed compounds into harmful products including *N*-nitroso compounds or aromatic steroids within the gastrointestine.[23,24] Because *C. perfringens* produces a variety of toxic enzymes such as phospholipase and collagenase, which disintegrate the plasma membrane of the host and toxins, resulting in an increase in the permeability of the intestinal blood vessels, which, in turn, causes enteritis and colitis and stimulates aging and tumorigenesis in human,[25] much interest has been focused on selective plant-derived growth inhibitors against *C. perfringens* in the intestines, based upon the fact that plant-derived materials were found to be nontoxic to human.

Seven vegetable extracts were selected and evaluated through microbial assay at concentrations of 5, 10, and 20 mg/disc to determine the changes of growth-inhibitory responses and detection of minor active components (Table 3). In tests with beneficial bacteria, *C. coronarium* var. *spatiosum* and *L. sativa* against *L. casei*, and *Z. officinale* against *B. bifidum* exhibited moderate inhibitions at a low concentration (5 mg/disc), while strong inhibitions at a concentration of 10 mg/disc. In tests with *C. perfringens* at 5 mg/disc, strong and moderate inhibitory responses were detected in the extracts of *C. annuum*, *L. esculentum* var. *cerasiforme*, and *L. esculentum*, whereas *A. sativa*, at 5 and 10 mg/disc, exhibited weak and moderate inhibitions, respectively. These results indicate that the above extracts strongly inhibited the growth of *C. perfringens* without affecting the growth of beneficial bacteria. It would be more desirable to not only inhibit the growth of any potential pathogens but also to increase the number of bifidobacteria in the human gut. Selective growth promoters for bifidobacteria or inhibitors for harmful bacteria are especially important to the human health because intake of these materials may normalize the disturbed physiological functions and prevent diseases caused by pathogens in the gastrointestinal tract. Previous *in vivo* investigations[26,27] using human volunteers have shown that intake of ginseng or green tea extract favourably affected the faecal microbiota and biochemical aspects of faeces, an indication of at least one of the phamacological actions of ginseng and green tea.[26,27] Daily intake of *A. sativum*, *C. annuum*, *L. esculentum*, and *L. esculentum* var. *cerasiforme* may alter the growth and composition of the microbial community and modulate the genesis of potentially harmful products such as carcinogenic *N*-nitroso compounds or aromatic steroids within the intestinal tract, thus providing protection from a variety of diseases and helping to maintain optimal human health.

Due to their potent inhibitory responses against the intestine bacteria, fractions of *C. annuum*, *L. sativa*, *L. esculentum* var. *cerasiforme*, and *Z. officinale* were evaluated at a low concentration of 5 mg/disc (Table 4). Strong inhibitory effects against *C. perfringens* were observed in the chloroform fractions of *C. annuum* and *L. esculentum* var. *cerasiforme*. Ethyl acetate fraction of *C. annuum* and water fraction of *L. esculentum* var. cerasiforme exhibited moderate inhibitions. In tests with *B. bifidum* and *L. casei*, strong inhibitions were observed in ethyl acetate fraction of *L. sativa* against *L. casei* and hexane fraction of *Z. officinale*.

In conclusion, vegetables showing strong activities confirm their superiority and usefulness as growth modulators, which were found to be more effective than the synthetic growth modulators against the intestinal bacteria. Further research to identify the biologically active substances in the chloroform fractions of *C. annuum* and *L. esculentum* var. *cerasiforme*, which showed the most potent growth-inhibitory activity, is in progress.

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