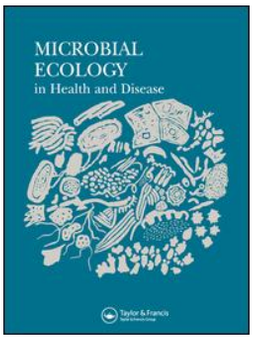

Microbial Ecology in Health and Disease

ISSN: (Print) 1651-2235 (Online) Journal homepage: informahealthcare.com/journals/zmeh20

Selective Growth Responses of Human Intestinal Bacteria to *Araliaceae* Extracts

Y-J. Ahn, M-J. Kim, T. Yamamoto, T. Fujisawa & T. Mitsuoka

To cite this article: Y-J. Ahn, M-J. Kim, T. Yamamoto, T. Fujisawa & T. Mitsuoka (1990) Selective Growth Responses of Human Intestinal Bacteria to *Araliaceae* Extracts, Microbial Ecology in Health and Disease, 3:4, 223-229, DOI: 10.3109/08910609009141546

To link to this article: https://doi.org/10.3109/08910609009141546

MICROBIAL ECOLOGY IN HEALTH AND DISEASE VOL. 3: 223–229 (1990)

Selective Growth Responses of Human Intestinal Bacteria to *Araliaceae* Extracts

Y-J. AHN*†§, M-J. KIM†, T. YAMAMOTO‡, T. FUJISAWA§ and T. MITSUOKA§‖

†*Central Research Institutes, Taiyo Kagaku Co., Yokkaichi, Mie 510,* ‡*Department of Biotechnology, Fukuyama University, Fukuyama City, 729-02,* §*Frontier Research Program, Laboratory for Intestinal Flora, RIKEN, Wako, Saitama 351-01 and* ‖*Faculty of Agriculture, The University of Tokyo, Tokyo 113 and The Institute of Physical & Chemical Research, Wako, Saitama 351-01, Japan.*

Received 28 April 1989; revised 26 March 1990

The growth responses of a variety of human intestinal bacteria to extracts of *Panax ginseng* and five other oriental medicinal *Araliaceae* were evaluated *in vitro*. The extracts enhanced the growth of *Bifidobacterium breve* and *B. longum* in media with or without carbon sources, suggesting that bifidus factor(s) might be involved in the phenomenon. This effect was most pronounced with water extract of *P. ginseng*, the growth of 27 bifidobacteria strains belonging to *B. adolescentis*, *B. longum*, *B. breve* and *B. infantis* being greatly stimulated, whereas seven *B. bifidum* strains and other bacteria such as clostridia and *Escherichia coli* had little or no ability to utilise it for growth. Methanol extracts of *P. ginseng* were found to selectively inhibit growth of various clostridia including *C. perfringens* and *C. paraputrificum*, but this effect was not observed on other bacteria including bifidobacteria. These results may be an indication of at least one of the pharmacological actions of *P. ginseng* as an adaptogen.

KEY WORDS—Araliaceae plant; *Panax ginseng*; Intestinal bacteria; Bifidus factor(s); Growth inhibition.

INTRODUCTION

Various kinds of microorganisms are resident in the human intestinal tract as a highly complex ecosystem with considerable species diversity. It is well known that 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 to useful or harmful derivatives. Accordingly, this biotransformation may influence drug efficacy, toxicity, carcinogenesis and ageing.

The term 'adaptogen' is defined as a substance that increases non-specific resistance of the organisms to environmental stress and disease. Adaptogenic activity was first reported for *Eleutherococcus senticosus*.[5] Evidence has been accumulating that *Panax ginseng* also contains adaptogens.[5,15,17] These two plants belong to the family *Araliaceae*. However, the effect of extracts from *Araliaceae* plants on the human intestinal microorganisms remains unknown, although much current concern is focused on these organisms in relation to human health. We have therefore examined the effect of various *Araliaceae* extracts on a variety of intestinal bacteria.

*Author to whom correspondence should be addressed. Present address: Department of Agrobiology, College of Agriculture, Seoul National University, Suweon 441-744, Korea.

0891–060X/90/040223–07 $05.00

MATERIALS AND METHODS

Bacteria and culture conditions

The bacterial strains used in this study were as follows; 27 bifidobacteria, 19 bacteroides, 15 clostridia, 15 eubacteria, eight lactobacilli, five *E. coli*, five peptostreptococci, four mitsuokellae, three fusobacteria, two propionibacteria, and one each of *Streptococcus*, *Megasphaera*, *Rikenella* and *Megamonas* species. They were, except as noted, from the RIKEN culture collection. Stock cultures of all strains were routinely stored on EGLF agar at −80°C and when required were subcultured on BL and EG agar (Eiken Chemical Co., Ltd, Tokyo, Japan) with 5 per cent horse blood for bifidobacteria and other organisms, respectively. All plates were incubated for 2 d at 37°C in an atmosphere of 100 per cent CO_2. On the following day, bifidobacteria were grown in Briggs liver broth (pH 6·8) in an atmosphere of 100 per cent CO_2,

Table 1. Effect of extracts from several *Araliaceae* plants on the growth of *B. longum* and *B. breve*

Test plant	*B. longum* E194b		*B. breve* S1	
	György*	PYF	György*	PYF
Panax ginseng				
M	—	—	—	++
W	++	++	++	++
P. japonicum				
M	—	—	—	—
W	++	+	++	++
P. notoginseng				
M	—	—	++	++
W	++	—	++	++
Acanthopanax sp.				
M	—	+	++	++
W	++	+	++	++
Aralia elata				
M	++	++	++	++
W	++	+	++	+
A. cordata				
M	—	—	—	—
W	++	(+)	++	(+)

M = methanol extract; W = water extract.
Responses were scored as described in the text.
*György broth[9] modified by Yoshioka.[26]

whereas other kinds of bacteria were grown in EGF broth (pH 7·2) in an atmosphere of 80 per cent N_2, 15 per cent CO_2 and 5 per cent H_2. All cultures were checked for contamination at the end of the growth cycle.

Plant materials and sample preparation

The medicinal *Araliaceae* plants used in this study were as follows: four-year-old *P. ginseng* (root); *P. japonicum* (root); *P. notoginseng* (root); *Acanthopanax* sp. (cortex); *Aralia elata* (cortex); *A. cordata* (root). These samples were finely powdered using a blender, extracted three times with methanol at 25°C and filtered (Toyo filter No. 2). The combined filtrate was concentrated *in vacuo* at 35°C. The residue was extracted with water at 80°C for 1 h and filtered. The filtrate volume was reduced with a rotary evaporator and freeze dried *in vacuo*.

Microbiological assay

For growth measurements with microorganisms, the testing methods of Mitsuoka[19] were applied. In the experiments for bifidus factor(s) derived from non-carbon sources, György broth[9] (pH 6·8) as modified by Yoshioka[26] was used. In the experiments for bifidus factor(s) derived from carbon sources, PYF broth (pH 7·8) was used. Bacteria grown in Briggs liver broth or EGF broth were centrifuged at 3000 r.p.m. for 10 min, washed three times with 10 ml of sterile physiological saline (0·85 per cent NaCl, 0·1 per cent L-cysteine-HCl, and 0·1 per cent sodium thioglycolate), and suspended in 5 ml of reduced saline. Two drops of the suspension were inoculated on to the media described above. Filter-sterilised test materials and ascorbic acid solution (sterilised at 115°C for 20 min) were added to the media in a final volume of 10 ml. Solutions of the test materials were prepared using methanol or distilled water as a solvent. The methanol concentration in the solutions did not exceed 2 per cent which was found to be without adverse effect on the bacteria tested. Samples from test and control solutions were assayed by the membrane filter procedure. The media were incubated anaerobically at 37°C for 48 h, and the bacterial growth determined by change in pH value.

The growth response to the test samples was determined by comparing with the value of each control. The responses were classified as follows: the strongest response ++, pH 4·5–5·0 for modified György broth and PYF broth; moderate +, pH 5·1–5·5 for the same broths; weak (+), pH 5·6–6·0 for PYF broth; and no response, −. Each assay was repeated three or more times.

For assay of the inhibitory effect of *P. gingseng* on the organisms, one loopful of bacteria was suspended in 1 ml of sterile physiological saline. An aliquot (0·1 ml) of the bacterial suspensions was seeded on Brucella agar (Difco) supplemented with 5 per cent horse blood. A sample (10 mg) in methanol or water solution (100 µl) was applied by Drummond glass microcapillary to a paper disc (ADVANTEC φ 8 mm Toyo Roshi, Japan). After evaporation of solvents, the paper discs were placed on the agar surface. All plates were incubated for 2 d at 37°C in an atmosphere of 80 per cent N_2, 15 per cent CO_2 and 5 per cent H_2. Control discs received methanol or water only. All tests of inhibition were performed at least in duplicate, and a mean inhibition zone of 10 mm or greater was considered positive (+).

RESULTS

The effects of extracts from six *Araliaceae* plants on the growth of bifidobacteria are given in Table 1.

Table 2. Bifidobacteria growth-promoting activity at various concentrations of *P. ginseng**

	Gyögy†						PYF					
	PWM			PME			PWE			PME		
Strain	0·01	0·1	1	0·01	0·1	1	0·01	0·1	1	0·01	0·1	1
B. adolescentis E194a	—	+	++	—	—	+	—	—	++	—	—	++
B. longum E194b	—	++	++	—	—	—	—	—	++	—	—	—
B. breve S1	—	+	++	—	—	—	—	(+)	++	—	—	++
B. infantis S12	—	+	++	—	—	—	—	—	++	—	—	—
B. bifidum Ti	—	—	—	—	—	—	—	—	—	—	—	—

PWE = water extract (w/v, %) of *P. ginseng*; PME = methanol extract (w/v, %) of *P. ginseng*.
*Four-year-old ginseng root was used.
†György broth[9] modified by Yoshioka.[26]

Table 3. Growth responses of many strains of bifidobacteria to PWE*

	Growth response	
Strain	György†	PYF
B. adolescentis E-298b	+	(+)
E-319a, M-101-4, M-602 and U-601	++	++
M-601, S-601 and S-602	++	—
B. longum Kd-5-6, M-101-2 and S-601	+	—
M-601 and S-3	++	—
B. breve I-53-8w and S-46	++	++
B. infantis I-10-5	++	—
B. bifidum A-234-4, E-319, M-601, S-28a, S-601 and S-602	—	—

Responses were scored as described in the text.
*Water extract of *P. ginseng*.
†György broth[9] modified by Yoshioka.[26]

For determination of bacterial growth, two kinds of media were used: modified György broth as a carbon source-containing medium and PYF broth as a carbon source-free medium. *Bifidobacterium longum* and *B. breve* were used as representatives of the organisms dominant in the intestines of adults and infants, respectively. In modified György broth, all water extracts from the plants tested showed either strong or moderate growth-promoting activity for both *B. longum* and *B. breve*. Methanol extracts from all except *A. elata* showed little or no growth stimulation on *B. longum*. However, the methanol extracts from *P. notoginseng*, *Acanthopanax* sp. and *A. elata* stimulated growth of *B. breve*.

Results obtained in the test on PYF broth were similar although the quantitative response was slightly different (Table 1).

Dose-growth responses for five bifidobacteria strains were studied using extract from 4 y-old *P. ginseng* root (Table 2). Water extract of *P. ginseng* (PWE) at 1 per cent (w/v) strongly enhanced the growth of *B. adolescentis*, *B. longum*, *B. breve* and *B. infantis* in both procedures. Moderate responses were obtained at 0·1 per cent (w/v) PWE on György broth, but this concentration produced no growth

Table 4. Growth responses of various strains of intestinal bacteria except bifidobacteria to PWE*

Strain	Growth response	
	György†	PYF
Lactobacillus casei ATCC-7469	++	—
IFO-3425	—	—
L. acidophilus ATCC-4356 and Omf1	—	++
L. gasseri F-164	—	++
M-601	++	++
L. salivarius ATCC-11741 and ATCC-11742	—	++
Bacteroides distasonis B-26, M-602, M-603, S-601 and U-604	—	+
B. fragilis 3676 and M-601	—	+
B. melaninogenicus NCTC-9337	—	—
B. thetaiotaomicron AS-126	—	+
B. uniformis M-601	—	+
B. vulgatus B-19 and B-24	—	++
S-601, 602, 603, 604, 606 and F-92	—	+
S-605	—	—
Clostridium bifermentans B-1 and B-4	—	—
C. butyricum ATCC-14823	—	++
S-601	—	—
C. coccoides B-2	—	(+)
C. difficile ATCC-9689	—	—
C. innocuum M-601	—	—
C. paraputrificum B-3-4, B-78 and VPI-6372	—	—
C. perfringens ATCC-13124, C-01 and B-165-16	—	—
C. ramosum ATCC-25582 and C-00	+	++
Eubacterium aerofaciens M-608, M-609, M-610, S-601, S-602, S-603, S-604, S-605 and S-606	—	—
E. lentum M-601	—	—
E. limosum ATCC-8486 and E-1	—	—
VPI-1939	—	+
E. nitritogenes ATCC-25547	+	+
E. tortuosum ATCC-25548	—	+
Escherichia coli E-605	—	+
M-602 and O-601	—	—
Mitsuokella multiacida F1-376	+	++
NCTC-10934, NCTC-10935 and P-208-58	++	++
Peptostreptococcus anaerobius X-36	—	—
P. asaccharolyticus VPI-5045A	—	—
P. parvulus 1612	—	—
P. prevotii ATCC-9321	—	—
P. productus ATCC-27340	—	+
Streptococcus faecalis IFO-3971	—	++
Propionibacterium acnes ATCC-6919 and ATCC-11829	—	—
Fusobacterium biacutus PAS-4476	+	(+)
F. necrophorum W-12, 2013	—	—
F. varium P103-112	—	—
Megasphaera eisdenii F1-375	—	—
Rikenella microfusus NCTC-11190	—	(+)

Responses were scored as described in the text.
*Water extract of *P. ginseng*.
†György broth[9] modified by Yoshioka.[26]

Table 5. Inhibitory effects of *P. ginseng* on various intestinal bacteria

Strain	Growth inhibition*	
	PME	PWE
Bifidobacterium adolescentis E-194a	—	—
B. longum E194b	—	—
B. breve S1	—	—
B. infantis S12	—	—
B. bifidum Ti	—	—
Bacteroides distasonis M-602 and S-601	—	—
B. fragilis 3676 and M-601	—	—
B. thetaiotaomicrom AS-126	—	—
B. vulgatus F-92 and S-601	—	—
Clostridium bifermentans B1	—	—
C. butyricum ATCC-14823 and S-601	+	—
C. coccoides B-2	+	—
C. difficile ATCC-9689	—	—
C. innocuum M-601	+	—
C. paraputrificum B-78 and VPI-6372	+	—
C. perfringens ATCC-13124, C-01 and B-165-16	— +	— —
C. ramosum ATCC-25582 and C-00	+	—
Eubacterium aerofaciens S-601 and S-605	—	—
S-605	+	—
Escherichia coli E-605, F-604, M-602, O-601 and V-603	—	—

PME = methanol extract of 4 y-old *P. ginseng* root (10 mg/disc); PWE = water extract of 4 y-old *P. ginseng* root (10 mg/disc).
*Inhibition zone of 10 mm or greater was considered positive (+).

response on PYF broth. No effect on bacterial growth in either broth was seen with 0·01 per cent PWE. The methanol extract of *P. ginseng* (PME) showed little or no growth-promoting effect on the five bifidobacteria tested.

The bifidus factors so far reported are known to be effective on only some strains of bifidobacteria. Therefore, the effect of PWE on a variety of bifidobacteria was investigated (Table 3). The result obtained by using 1 per cent PWE showed that growth response was indeed strain dependent. PWE enhanced the growth of many strains of *B. adolescentis*, *B. longum*, *B. breve* and *B. infantis* strongly or moderately in the test on both media described above, whereas none was observed with six *B. bifidum* strains.

Table 4 shows the effect of 1 per cent PWE on various kinds of other microorganisms. The effect was also strain dependent. PWE showed strong growth-promoting activity for lactobacilli on either György or PYF broth. However, other microorganisms including eubacteria, clostridia and *E. coli* showed little or no growth in the two media with 1 per cent PWE.

Inhibitory effects of PME and PWE on the bacteria mentioned above were also examined by paper-disc method (Table 5). At a concentration of 10 mg/disc, PME inhibited various strains of clostridia including *C. perfringens* and *C. paraputrificum*. PWE showed no inhibitory effect on these bacteria by this method.

DISCUSSION

The intestinal microflora in healthy man remains relatively constant but is known to be greatly influenced by physical, biological, chemical, environmental or host factors.[20] Alterations to the flora may cause abnormal physical conditions or diseases. In the present paper, growth responses to the intestinal microorganisms *in vitro* were investigated for the extracts from *P. ginseng* and five other oriental medicinal *Araliaceae* plants.

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 such as amino-acid production,[18,20] aid defense against infection,[13] are associated with longevity,[12,21] pathogen inhibition[6,7] and immunopotentiation.[2,20] Bifidobacteria growth-promoting factors, usually called bifidus factors, have therefore been extensively studied since György *et al.*[11] suggested their existence in human milk. Many oligosaccharides in human milk and N-acetylglucosamine derivatives are growth factors for the organism and also called bifidus factors.[10] Lactulose, oligosaccharides, peptide or peptide-like and vitamine-like substances have been identified as bifidus factors in human milk,[1,3,4,8,10,14,23] carrot,[24-26] and soybean.[16]

In our microbial assay, addition of extracts from six *Araliaceae* plants to carbon source-free or carbon source-containing media enhanced the growth of *B. breve* and *B. longum*, indicating the presence of bifidus factor(s) in the extracts.

However, the bifidus factors mentioned above were effective on only some strains of bifidobacteria. There are large variations in the bifid flora in individuals.[12,21,22] It would be desirable to both inhibit the growth of potential pathogens and/or increase the numbers of many kinds of bifidobacteria in the human gut. Therefore, the growth response of various strains of bacteria to *P. ginseng* was investigated.

The present work revealed that PWE showed strong or moderate growth-promoting activities for many strains of *B. adolescentis*, *B. longum*, *B. breve* and *B. infantis*, although it showed no effect on the growth of all strains of *B. bifidum* tested. Also, it was ineffective for the growth of other bacteria including clostridia and *E. coli*. On the other hand, PME selectively inhibited the growth of certain strains of clostridia.

Pharmacological or clinical efficacy of *P. ginseng* to a variety of diseases has been reported.[15,17] It is also well known that *P. ginseng* is an excellent adaptogen. Brekhman and Dardymov[5] established the concept of the tonic effect of *P. ginseng*, suggesting that it normalises disturbed physiological functions rather than treats a specific disease. The pharmacological actions of panax may be explained at least partly by the effect on intestinal microflora. Based upon our data, the intake of *P. ginseng* would be expected to alter the growth and composition of the intestinal microbial community and to modulate the generation of potentially harmful agents within the intestinal tract. This pharmacological effect may also affect toxicity, carcinogenesis, ageing and other processes in which intestinal microorganisms participate.

Further work to identify the biologically active substance of *P. ginseng* is in progress.

REFERENCES

1. Azuma N, Yamauchi K, Mitsuoka T. (1984). Bifidus growth-promoting activity of a glycomacropeptide derived from human *K*-casein. *Agricultural and Biological Chemistry* **48,** 2159–2162.
2. Berg RD. (1983). Host immune response to antigens of the indigenous intestinal flora. In: Hentges DJ (ed) *Human Intestinal Microflora in Health and Disease*. Academic, New York, pp. 101–126.
3. Bezkorovainy A, Nichols JH. (1976). Glycoproteins from mature human milk whey. *Pediatric Research* **10,** 1–5.
4. Bezkorovainy A, Grohlich D, Nichols JH. (1979). Isolation of a glycopolypeptide fraction with *Lactobacillus bifidus* subspecies *pennsylvanicus* growth-promoting activity from whole human milk casein. *American Journal of Clinical Nutrition* **32,** 1428–1432.
5. Brekhman II, Dardymov IV. (1969). New substances of plant origin which increase nonspecific resistance. *Annual Review of Pharmacology* **9,** 419–430.
6. Bullen CL, Willis AT. (1971). Resistance of the breast-fed infant to gastroenteritis. *British Medical Journal* **3,** 338–343.
7. Bullen JJ, Rogers HJ, Leigh L. (1972). Iron-binding proteins in milk and resistance to *Escherichia coli* infection in infants. *British Medical Journal* **1,** 69–75.
8. Dehnert J. (1957). Untersuchung üder die Gram-positive stuhlflora des Brustmilchkindes. *Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene, I Originale* **169,** 66–83.
9. György P. (1953). A hitherto unrecognized biochemical difference between human milk and cow's milk. *Pediatrics* **11,** 98–107.
10. György P, Rose CS. (1955). Microbiological studies on growth factor for *L. bifidus* var. *pennsylvanicus*. *Proceedings of the Society for Experimental Biology and Medicine* **90,** 219–223.
11. György P, Norris RF, Rose CS. (1954). Bifidus factor. I. A variant of *Lactobacillus bifidus* requiring a special growth factor. *Archives of Biochemistry and Biophysics* **48,** 193–201.
12. Haenel H. (1963). Über die Mikroökologie alter Menschen. *Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene, Originale* **188,** 219–230.
13. Hentges DJ. (1983). Role of the intestinal microflora in host defense against infection. In: Hentges DJ (ed) *Human Intestinal Microflora in Health and Disease*. Academic, New York, pp. 311–331.

14. Hirano S, Hayashi H, Terabayashi T, Onodera K, Iseki S, Kochibe N, Nagai Y, Yagi N, Nakagaki T, Imagawa T. (1968). Biologically active glycopeptides in human colostrum. *The Journal of Biochemistry* **64,** 563–565.
15. Hong SS. (1978). The clinical effects of *Panax ginseng*. In: Bae HW (ed) *Korean Ginseng*. Korea Ginseng Research Institute, Seoul, pp. 163–189.
16. Kobayashi Y, Echizen R, Mada M, Mutai M. (1984). Effect of hydrolysates of *Konjac* mannan and soybean oligosaccharides on intestinal flora in man and rats. In: Mitsuoka T (ed) *Intestinal Flora and Dietary Factors*. Japan Scientific Societies Press, Tokyo, pp. 69–90.
17. Kim ND. (1978). Pharmacological properties of ginseng. In: Bae HW (ed) *Korean Ginseng*. Korea Ginseng Research Institute, Seoul, pp. 120–125.
18. Matteuzzi D, Crociani F, Emaldi O. (1978). Amino acids produced by bifidobacteria and some clostridia. *Annales de Microbiologie (Paris)* **129B,** 175–181.
19. Mitsuoka T. (1969). Vergleichende Untersuchungen über die Bifidobakterien aus dem Verdauungstrakt von Menschen und Tieren (Zugleich die Beschreibung von *B. thermophilum* nov. spec. und *B. pseudolongum* nov. spec.). *Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene, I Originale* **210,** 52–64.
20. Mitsuoka T. (1984). *A Color Atlas of Anaerobic Bacteria*. Shobunsha, Tokyo.
21. Mitsuoka T, Hayakawa K. (1973). Die Faekalflora bei Menschen. I. Mitteilung: Die Zusammensetzung der Faekalflora der verschiedenen altersgruppen. *Zentralblatt für Bakteriologie Mikrobiologie und Hygiene. Reihe A* **223,** 333–342.
22. Mitsuoka T, Ohno K. (1977). Die Faecklflora bei Menschen. V. Mitteilung: Die Schwankungen in der Zusammensetzung der Faekalflora gesunder Erwachsener. *Zentralblatt für Bakteriologie Mikrobiologie und Hygiene. Reihe A* **238,** 228–237.
23. Petuely F. (1957). Bifidusflora bei Flaschenkindern durch bifidogene Substanzen (Bifidus factor). *Zeitschrift für Kinderheilkunde* **79,** 174–179.
24. Yoshioka M, Tamura Z. (1971). Bifidus factors in carrot. II. The structure of the factor in fraction IV. *Chemical & Pharmaceutical Bulletin* **19,** 178–185.
25. Yoshioka M, Tamura Z. (1971). Bifidus factors in carrot. II. The structure of the factor in fraction V. *Chemical and Pharmaceutical Bulletin* **19,** 186–189.
26. Yoshioka M, Yoshioka S, Tamura Z, Ohta K. (1968). Growth responses of *Bifidobacterium bifidum* to coenzyme A, its precursors and carrot extract. *Japanese Journal of Microbiology* **12,** 395–402.