Effect of Diet and Aging on Human Fecal Microflora

Yoshimi BENNO¹ and Tomotari MITSUOKA^{2,3}¹*Japan Collection of Microorganisms, ²Frontier Research Program,
RIKEN, Wako, Saitama 351-01 and*³*Nippon Veterinary and Animal Science University,
Musashino, Tokyo 180*

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The incidence of colon cancer varies widely throughout the world (8, 9). It has been demonstrated that the disease is much rarer in Africa, Asia, and South America than in Western Europe or North America (37). The reason for this variation is unclear but epidemiological studies, focusing on genetic, cultural, environmental and economic factors suggest that the diet, in particular an increased intake of fat and animal protein, is strongly correlated with the incidence of colon cancer (19, 36).

Epidemiologic studies (7, 31, 36) also suggest that decreases in dietary fiber intake actually increase the incidence of several colonic disorders including: diverticular disease, cancer and constipation. Since some of the predominating bacteria in human fecal contents ferment various types of hemicelluloses (34), it would be of interest to determine the effects, if any, of dietary fiber on the human fecal flora.

It is widely known that the composition of fecal flora in healthy infants differed according to the mode of feeding (2, 18, 23). Although taxonomic studies on *Bifidobacterium* species present in feces of babies have been carried out (25, 32), the bulk of anaerobes and aerobes from their feces have not been identified at the species level.

To address the aforementioned, the present studies were undertaken. Thus, the composition of the fecal flora of the following subjects were compared: (i) breast-fed versus bottle-fed infants, (ii) rural Japanese with a low incidence of colon cancer, (iii) urban Canadians with a high incidence of colon cancer, (iv) Japanese volunteers given a high beef or a rice fiber diet (particularly brown rice), and (v) elderly Japanese in rural (Yuzurihara, Uenohara, Yamanashi Prefecture) and urban (Tokyo) areas of Japan, respectively.

EFFECT OF FEEDING ON INFANTS FECAL FLORA

Comparison of fecal flora in breast-fed and bottle-fed infants are shown in Table 1. These babies were aged from 28 to 46 days. Bifidobacteria were the

predominant fecal bacteria in both groups. The differences of feeding (breast-fed or bottle-fed) influenced the fecal flora of infants. The microorganism presented at the highest percentage to total fecal bacteria in breast-fed (62.2% with respect to total population) and bottle-fed (33.3%) groups was *Bifidobacterium* (designated as *Bif.*) *breve*. High percentages of *Eubacterium* (*Eub.*) *aerofaciens*, *Bacteroides* (*B.*) *vulgatus*, *Escherichia* (*E.*) *coli*, *Enterococcus* (*En.*) *faecalis*, *En. faecium* and clostridia in the bottle-fed infants were shown. These findings agreed with the results previously reported (33, 35).

COMPOSITION OF FECAL FLORA IN HEALTHY ADULT MEN

A total of 1829 isolates from 15 healthy adult men were identified as belonging to 29 genera and over 144 species (Table 2). Some anaerobic species of *B. vulgatus*, *B. distasonis*, *B. fragilis*, *B. thetaiotaomicron*, *Fusobacterium* (*F.*) *mortiferum*, *Eub. aerofaciens*, *Eub. lentum*, *Bif. adolescentis*, *Bif. longum*, *Peptostreptococcus* spp., *Veillonella parvula*, *Clostridium* (*Cl.*) *innocuum*, *Cl. paraputrificum*, and *Cl. perfringens* were recovered. These

Table 1. Comparison of fecal flora in breast-fed and bottle-fed infants^a

Breast-fed infants			Bottle-fed infants	
No. of samples	35		35	
No. of isolates	1105		1604	
Identification of isolates	21 genera and more than 103 species		20 genera and more than 97 species	
Rank				
1	<i>Bif. breve</i>	62.2	<i>Bif. breve</i>	33.3
2	<i>Bif. bifidum</i>	14.7	<i>Eub. aerofaciens</i>	20.9
3	<i>Bif. adolescentis</i>	11.2	<i>Bif. longum</i>	12.4
4	<i>Bif. longum</i>	7.8	<i>B. vulgatus</i>	10.4
5	<i>Eub. lentum</i>	0.4	<i>Bif. adolescentis</i>	8.4
6	<i>B. distasonis</i>	0.2	<i>Bif. bifidum</i>	8.4
7	<i>B. vulgatus</i>	0.2	<i>E. coli</i>	2.4
8	<i>Eub. aerofaciens</i>	0.07	<i>B. distasonis</i>	1.1
9	<i>B. ovatus</i>	0.05	<i>En. faecalis</i>	0.7
10	<i>B. fragilis</i>	0.05	<i>En. faecium</i>	0.5
11	<i>E. coli</i>	0.05	<i>V. parvula</i>	0.3
12	<i>V. parvula</i>	<0.01	<i>L. gasseri</i>	0.3
13	<i>En. faecalis</i>	<0.01	<i>K. pneumoniae</i>	0.3
14	<i>K. pneumoniae</i>	<0.01	<i>Cl. perfringens</i>	0.3
15	<i>L. gasseri</i>	<0.01	<i>Cl. paraputrificum</i>	0.1
16	<i>En. faecium</i>	<0.01	<i>Cl. difficile</i>	0.1
17	<i>Cl. butyricum</i>	<0.01	<i>Cl. sartanogoformum</i>	0.05
18	<i>St. epidermidis</i>	<0.01	<i>Cl. tertium</i>	0.05
19	<i>St. aureus</i>	<0.01	<i>St. epidermidis</i>	<0.01
20	<i>Cl. paraputrificum</i>	<0.01	<i>St. aureus</i>	<0.01

^a Data were expressed as a percentage of total bacteria. *B.*, *Bacteroides*; *Bif.*, *Bifidobacterium*; *C.*, *Coprococcus*; *Cl.*, *Clostridium*; *E.*, *Escherichia*; *En.*, *Enterococcus*; *Eub.*, *Eubacterium*; *K.*, *Klebsiella*; *L.*, *Lactobacillus*; *S.*, *Streptococcus*; *St.*, *Staphylococcus*; *V.*, *Veillonella*.

results in our study were similar to the data described previously (13, 21, 28).

EFFECT OF A HIGH BEEF DIET ON HUMAN FECAL FLORA

The 762 isolates from nine rural Japanese (high fiber group) belonged to 23 genera and over 75 species or biovars, while the 618 isolates from eight urban Canadians (high beef group) represented 18 genera and over 66 species or biovars (4). Strict anaerobes were found in similar diversity in the feces of Japanese (13 genera, over 61 species or biovars) and urban Canadians (11 genera, over 55 species or biovars). Details of the relatively high percentage of anaerobes are given in Table 2. Most anaerobic bacteria belonged to the genera *Bacteroides*, *Fusobacterium*, *Bifidobacterium*, *Eubacterium*, *Lactobacillus*, *Clostridium*, *Peptostreptococcus*, and *Veillonella*. *Bacteroides* spp. were recovered in large percentages from both populations. The relative percentages of *B. vulgatus*, *B. uniformis*, the *B. buccae/oris* group, *Cl. innocuum* and *Cl. ramosum* were higher in the Canadians than in the Japanese; there were lower percentages of *Eub. aerofaciens* and *Bif. adolescentis* in the feces of the Canadians. The most prevalent facultative aerobes in either populations were *Escherichia* (*E.*) *coli* and *Enterococcus faecalis*.

The effect of a high beef diet on fecal flora of six healthy adult men was the increasing percentage (compared to the total bacterial population) of *B. fragilis*, *B. uniformis*, *B. stercoris*, *Cl. paraputrificum*, *Cl. innocuum* and *Cl. ramosum* and the decreasing percentages of *Bif. adolescentis*, *Eub. aerofaciens* and *Bif. longum* (Table 2).

Incidences of colon cancer in Western countries are up to eight times those in developing countries, where the fiber intake is generally higher (6, 7). While the incidence of colon cancer in Japan was the lowest in the world (29, 36), in Japanese immigrants it increases to that of the host country by the second or third generations (5, 19). Dietary factors, particularly a high intake of total fat and a relatively low intake of certain dietary fibers, play important roles in colon cancer etiology (7, 30).

It is widely known that the fecal flora are strongly influenced by the diet. One line of evidence concerning the effect of diet has been reported from comparisons of the fecal flora of populations that habitually consume different diet. The purpose of this study was to detect any differences in the fecal flora between high meat eaters and high fiber eaters, which might be correlated with the high incidence of colon cancer in urban Canadians and the low incidence in rural Japanese. Several studies (13, 14, 26) have demonstrated that certain significant differences in the composition of the fecal flora between high- and low-risk populations of colon cancer are not apparent. The present investigation showed that the fecal flora, represented by anaerobic gram-positive nonsporeforming bacteria, in particular, *Bifidobacterium* spp. and *Eubacterium* spp., is dominant in rural Japanese.

Koornhof et al (21) showed that clostridial species are more numerous in the fecal flora of Africans than of patients with colonic polyps. On the other hand, *Cl. paraputrificum* was common in feces from subjects in England, Scotland, and U.S.A., but rare in the samples from Hong Kong, Uganda, and Japan (10).

Table 2. Effects of high fat/high

	Healthy adults		Rural Japanese (Low fat & high fiber)		Urban Canadians (High fat & low fiber)	
No. of samples	15		9		8	
No. of isolates	1829		762		618	
Identification of isolates	29 genera and more than 144 species		23 genera and more than 75 species		18 genera and more than 66 species	
Rank						
1	<i>B. vulgatus</i>	27.8	<i>Eub. aerofaciens</i>	25.1	<i>B. vulgatus</i>	33.3
2	<i>Bif. adolescentis</i>	14.1	<i>Bif. adolescentis</i>	15.8	<i>B. distasonis</i>	9.8
3	<i>B. distasonis</i>	10.9	<i>B. vulgatus</i>	15.8	<i>B. ovatus</i>	7.5
4	<i>Eub. aerofaciens</i>	8.9	<i>B. ovatus</i>	7.1	<i>B. uniformis</i>	7.5
5	<i>B. thetaiotaomicron</i>	5.2	<i>B. distasonis</i>	6.3	<i>B. buccae/oris</i> group	6.7
6	<i>Bif. longum</i>	5.2	<i>B. fragilis</i>	6.3	<i>B. fragilis</i>	5.2
7	<i>B. buccae/oris</i> group	3.6	<i>P. productus</i>	3.9	<i>Bif. adolescentis</i>	5.2
8	<i>B. uniformis</i>	3.6	<i>Bif. longum</i>	3.2	<i>Bif. bifidum</i>	5.2
9	<i>B. ovatus</i>	2.8	<i>Bif. bifidum</i>	2.5	<i>Eub. aerofaciens</i>	5.2
10	<i>P. prevotii</i>	2.8	<i>R. lactaris</i>	2.5	<i>B. thetaiotaomicron</i>	3.3
11	<i>Eub. rectale</i>	2.2	<i>F. varium</i>	1.9	<i>Eub. lentum</i>	3.3
12	<i>Bif. bifidum</i>	2.2	<i>Eub. rectale</i>	1.9	<i>P. productus</i>	2.0
13	<i>C. comes</i>	1.8	<i>S. intermedius</i>	1.9	<i>F. varium</i>	1.7
14	<i>B. veroralis</i>	1.4	<i>B. splanchnicus</i>	1.6	<i>Eub. rectale</i>	1.3
15	<i>R. lactaris</i>	1.4	<i>F. mortiferum</i>	1.6	<i>Cl. innocuum</i>	0.8
16	<i>P. productus</i>	1.1	<i>B. thetaiotaomicron</i>	1.2	<i>F. mortiferum</i>	0.7
17	<i>F. varium</i>	0.9	<i>Eub. lentum</i>	1.2	<i>Cl. ramosum</i>	0.2
18	<i>P. micros</i>	0.7	<i>B. buccae/oris</i> group	0.04	<i>E. coli</i>	0.05
19	<i>B. fragilis</i>	0.5	<i>E. coli</i>	0.03	<i>En. faecalis</i>	<0.01
20	<i>F. mortiferum</i>	0.5	<i>En. faecalis</i>	<0.01	<i>En. faecium</i>	<0.01

^a See the footnote of Table 1.

F., *Fusobacterium*; *M.*, *Mitsuokella*; *Me.*, *Megasphaera*; *P.*, *Peptostreptococcus*; *R.*, *Ruminococcus*.

Mastromarino et al (22) also indicated that the numbers and incidence of clostridia, i.e., *Cl. butyricum*, *Cl. innocuum*, *Cl. indolis*, *Cl. paraputrificum*, *Cl. tertium*, and *Cl. sartanogoforum*, constitute a greater percentage of the fecal flora in patients with colon cancer. These clostridia have been shown to be capable of exhibiting nuclear dehydrogenation, an important reaction in the formation of carcinogens from bile acids (11, 17). In this study, an increasing percentage of *Cl. innocuum*, *Cl. ramosum*, and *Cl. paraputrificum* with a high beef intake was observed. High recovery of these microorganisms lend support to the findings of Hill and his colleagues (1, 20).

EFFECT OF DIETARY FIBER ON HUMAN FECAL FLORA

Comparison of the fecal flora of five healthy adult men on a polished rice or brown rice diet for 14 days is shown in Table 2. By genera, the same strict anaerobes, facultative anaerobes, and aerobes were present in the feces throughout this

fiber diets on human fecal flora^a

High beef diet		Polished rice		Brown rice	
6		5		5	
548		404		338	
20 genera and more than 71 species		20 genera and more than 62 species		17 genera and more than 46 species	
<i>B. vulgatus</i>	27.3	<i>B. vulgatus</i>	28.1	<i>B. vulgatus</i>	27.1
<i>B. fragilis</i>	10.0	<i>B. thetaiotaomicron</i>	7.2	<i>Bif. adolescentis</i>	11.0
<i>B. distasonis</i>	10.0	<i>Bif. longum</i>	7.2	<i>R. gnavus</i>	8.8
<i>B. uniformis</i>	8.0	<i>Eub. aerofaciens</i>	7.2	<i>B. uniformis</i>	7.0
<i>B. buccae/oris</i> group	7.0	<i>B. distasonis</i>	5.7	<i>B. distasonis</i>	7.0
<i>B. veroralis</i>	7.0	<i>B. fragilis</i>	5.7	<i>P. magnus</i>	7.0
<i>F. mortiferum</i>	7.0	<i>Bacteroides</i> sp. A	4.6	<i>B. fragilis</i>	5.6
<i>B. stercoris</i>	6.5	<i>Eub. lentum</i>	4.6	<i>B. thetaiotaomicron</i>	4.3
<i>P. productus</i>	4.5	<i>Eub. rectale</i>	4.6	<i>Eub. rectale</i>	4.3
<i>Bif. adolescentis</i>	2.7	<i>P. anaerobius</i>	4.6	<i>B. ovatus</i>	3.5
<i>C. comes</i>	2.7	<i>B. ovatus</i>	3.6	<i>B. buccae/oris</i> group	3.5
<i>Eub. lentum</i>	2.2	<i>B. splanchnicus</i>	3.6	<i>Bif. longum</i>	3.5
<i>R. gnavus</i>	2.2	<i>B. uniformis</i>	3.6	<i>P. productus</i>	1.6
<i>Eub. aerofaciens</i>	1.2	<i>Bif. adolescentis</i>	1.8	<i>Bacteroides</i> sp. A	1.4
<i>Eub. rectale</i>	1.0	<i>P. prevotii</i>	1.8	<i>Cl. innocuum</i>	0.9
<i>Cl. parapatrificum</i>	0.2	<i>Cl. ramosum</i>	1.4	<i>Eub. aerofaciens</i>	0.9
<i>Cl. innocuum</i>	0.2	<i>P. magnus</i>	0.9	<i>Eub. lentum</i>	0.7
<i>Bif. longum</i>	0.2	<i>R. gnavus</i>	0.9	<i>P. anaerobius</i>	0.4
<i>Bif. bifidum</i>	0.2	<i>Me. elsdenii</i>	0.7	<i>V. parvula</i>	0.4
<i>Cl. ramosum</i>	0.02	<i>Cl. innocuum</i>	0.7	<i>En. faecalis</i>	0.2

study. A total of 404 isolates from the five persons given a polished rice diet were identified as belonging to 20 genera and over 62 species. A total of 338 isolates from the subjects on a brown rice diet were classified into 17 genera and over 46 species. The results showed that the total bacteria had decreased for the brown rice diet.

At day 14 of the brown rice diet, an increasing percentage of *Bif. adolescentis*, *Ruminococcus* (*R.*) *gnavus* and *Peptostreptococcus magnus* were observed, whereas the percentage of *Eub. aerofaciens* and *E. coli* decreased. Low percentage of all clostridia and low incidences of *Cl. parapatrificum* and *Cl. perfringens* were observed for the brown rice diet.

There have been few studies on the effect of fiber intake on the human fecal flora. Drasar et al (12) and Moore et al (27) found no significant differences in numbers of fecal flora in volunteers on a high fiber diet. On the other hand, Fuchs et al (16) noted a significantly increasing number of total bacteria with a high

Table 3. Comparison of fecal flora of elderly persons in rural and urban areas of Japan^a

	Healthy adults		Urban elderly persons		Rural elderly persons	
No. of samples	15		15		15	
No. of isolates	1829		1496		1284	
Identification of isolates	29 genera and more than 144 species		25 genera and more than 92 species		25 genera and more than 81 species	
Rank						
1	<i>B. vulgatus</i>	27.8	<i>B. vulgatus</i>	25.8	<i>B. vulgatus</i>	31.8
2	<i>Bif. adolescentis</i>	14.1	<i>B. buccae/oris</i> group	20.5	<i>Eub. aerofaciens</i>	11.8
3	<i>B. distasonis</i>	10.9	<i>Eub. aerofaciens</i>	12.9	<i>B. distasonis</i>	6.3
4	<i>Eub. aerofaciens</i>	8.9	<i>B. distasonis</i>	7.6	<i>Eub. rectale</i>	6.3
5	<i>B. thetaiotaomicron</i>	5.2	<i>B. thetaiotaomicron</i>	7.6	<i>B. fragilis</i>	5.0
6	<i>Bif. longum</i>	5.2	<i>Eub. lentum</i>	4.0	<i>Selenomonas</i> sp.	5.0
7	<i>B. buccae/oris</i> group	3.6	<i>Eub. rectale</i>	3.2	<i>B. ovatus</i>	4.0
8	<i>B. uniformis</i>	3.6	<i>B. fragilis</i>	2.6	<i>B. stercoris</i>	4.0
9	<i>B. ovatus</i>	2.8	<i>F. varium</i>	2.6	<i>M. multiacidus</i>	4.0
10	<i>P. prevotii</i>	2.8	<i>P. magnus</i>	2.6	<i>Bif. adolescentis</i>	4.0
11	<i>Eub. rectale</i>	2.2	<i>P. productus</i>	2.6	<i>P. productus</i>	4.0
12	<i>Bif. bifidum</i>	2.2	<i>B. ovatus</i>	2.1	<i>F. mortiferum</i>	3.2
13	<i>C. comes</i>	1.8	<i>B. stercoris</i>	1.3	<i>F. varium</i>	2.5
14	<i>B. veroralis</i>	1.4	<i>R. gnavus</i>	1.3	<i>Bif. bifidum</i>	2.0
15	<i>R. lactaris</i>	1.4	<i>Cl. ramosum</i>	1.0	<i>Bif. longum</i>	2.0
16	<i>P. productus</i>	1.1	<i>Bif. adolescentis</i>	0.8	<i>R. gnavus</i>	2.0
17	<i>F. varium</i>	0.9	<i>Bif. longum</i>	0.6	<i>B. buccae/oris</i> group	1.5
18	<i>P. micros</i>	0.7	<i>F. mortiferum</i>	0.5	<i>B. thetaiotaomicron</i>	1.2
19	<i>Cl. innocuum</i>	0.3	<i>P. magnus</i>	1.2	<i>P. magnus</i>	1.2
20	<i>F. mortiferum</i>	0.5	<i>E. coli</i>	0.2	<i>Eub. lentum</i>	0.9

^a See the footnote of Table 2.

fiber intake. In this study, although a reduction of the genus *Clostridium* was observed in all the subjects on a brown rice intake, marked differences at the species level of the genus were not found. Our results with the rice fiber diet were also comparable to the lower incidence of *Cl. perfringens* among Seventh-Day Adventists (15).

COMPARISON OF THE FECAL FLORA OF ELDERLY PERSONS IN RURAL AND URBAN AREAS OF JAPAN

Comparison of the fecal flora of elderly persons in rural and urban areas of Japan is given in Table 3. A total of 1496 isolates from 15 elderly urbanites ranging in age from 65 to 76 years were classified into 25 genera and over 92 species. A total of 1284 isolates from 15 elderly persons, ranging in age from 75 to 92 years, in the rural area were identified as belonging to 25 genera and over 81 species.

Higher percentages of the *B. buccae/oris* group, *B. fragilis*, *F. varium*, *R. gnavus*, *Cl. ramosum*, and *Cl. innocuum* were found in feces of the urban elderly persons compared with the healthy adults, whereas the percentage of *Bif. adolescentis* and *Bif.*

longum in elderly persons in the urban area were markedly decreased. Percentages of *Bif. adolescentis* and *Bif. longum*, but not *B. thetaiotaomicron*, in rural elderly persons were higher than those in elderly urbanites.

Mitsuoka et al (25) demonstrated that a higher incidence of *Bif. adolescentis* and a lower incidence of *Bif. longum* in elderly persons reflected the characteristic composition of fecal *Bifidobacterium* spp. Mitsuoka and Hayakawa (24) also reported that the number and incidence of *Cl. perfringens* in elderly persons were higher than those in healthy adults. Results of our study are in very good agreement with these findings.

Elderly persons in Yuzurihara, an area whose inhabitants tend to be long-lived, have a higher intake of dietary fiber than elderly residents of the Tokyo area (3). The percentage of *Bif. adolescentis* and *Bif. longum* in rural elderly persons was higher than those in elderly urbanites. This finding suggests that with advancing age the fecal flora can be modified by a higher intake of dietary fiber.

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