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Comparison of Fecal Microflora of Elderly Persons in Rural and Urban Areas of Japan

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The Fecal microflora of 15 healthy elderly persons with a median age of 84 years in a rural area whose inhabitants tend to be long-lived (Yuzurihara-area, Uenohara, Yamanashi Prefecture) was compared with the microflora of individuals with a median age of 68 years in an urban area (Tokyo). The diet of the elderly persons in the Yuzurihara area is characterized by a high intake of dietary fiber. Total numbers of anaerobic bacteria were significantly smaller in the elderly persons in the Yuzurihara area than those in the Tokyo area. A significantly large number of bifidobacteria, but not of lecithinase-negative clostridia, was observed in the elderly persons in the Yuzurihara area. Large numbers and high incidences of bacilli and lecithinase-positive clostridia (mainly *Clostridium perfringens*) were found in the elderly persons in the Tokyo area. Twenty-five genera and over 81 species were isolated from the elderly persons in the Yuzurihara area, and 25 genera and over 92 species were isolated from the elderly persons in the Tokyo area. Furthermore, significantly larger numbers of *Bifidobacterium adolescentis* and *Fusobacterium mortiferum* strains were found in the Yuzurihara group, but significant reductions in the *Bacteroides buccae-oris* group, *B. thetaiotaomicron*, *Bacteroides* spp., *C. coccoides*, *C. paraputrificum*, and *Clostridium* spp. were observed in the same group. A significantly higher isolation rate of *Bacillus subtilis* was observed in the elderly persons in the Tokyo area. The difference in the fecal microflora between elderly persons in Yuzurihara and those in the Tokyo area might be due to a difference in the intake of dietary fiber.

Epidemiologic studies (4, 32, 38) suggest that decreases in dietary fiber intake increase the incidence of several colonic disorders including diverticular disease, cancer, and constipation. High dietary fiber, fibrous foods, whole-grain diets, and cruciferous vegetables have emerged as factors that may prevent a wide variety of ailments. The fermentation of dietary fiber in human intestines has been thoroughly studied (6). Some of the predominant bacteria in human intestinal contents ferment various type of hemicelluloses (33, 34, 36). However, there are few studies of the effect of dietary fiber on human intestinal microflora. It is known that elderly persons in Yuzurihara, Uenohara, Yamanashi Prefecture, Japan, an area whose inhabitants tend to be long-lived, have a higher intake of dietary fiber than elderly persons in the Tokyo area (35). It is of interest to determine the effects, if any, of dietary fiber on the fecal microflora and longevity of humans.

The present study was designed to compare further the number and type of organisms making up the fecal microflora of elderly persons in this rural area with the microflora of those in an urban area (Tokyo).

MATERIALS AND METHODS

Subjects. The subjects studied consisted of 15 healthy elderly persons on Yuzurihara and 15 healthy elderly persons in Tokyo. The elderly persons (six males and nine females) in Yuzurihara ranged in age from 75 to 90 years, with a median age of 84 years. The urban elderly persons (5 males and 10 females) ranged in age from 65 to 76 years, with a median age of 68 years. Dietary histories (35) indicated that the total intake of dietary fiber in Yuzurihara (28.8 g/day) was significantly higher than that in a rural area in Iwate Prefecture (13.1 g/day). The major dietary items of the elderly persons in the Yuzurihara area included boiled barley and rice, stewed noodles, soba (buckwheat noodles), baked rice cakes, corn, taros, Konjac (paste made from devil's tongue), miso (bean paste) soup, vegetables (including Japanese white radishes, Chinese cabbage, mushrooms, turnips, burdock, carrots, yams, butterburn, and pumpkins), bracken, buckhorns, dried fish, nori (seaweed), tofu (bean curd), eggs, manju (dumpling) fermented with sake, pickled vegetables, and Japanese green tea. The elderly persons in the Tokyo area lived on a modern Japanese diet. Boiled rice, miso soup, and pickled vegatables were eaten at every meal. Other dietary items included noodles, soba, Japanese white radishes, Chinese cabbage, mushrooms, carrots, burdock, seaweed, bean curd, milk, milk products, meat, eggs, fruit, bread, coffee, and Japanese green tea. None of the subjects had active intestinal obstructions. Each individual gave informed consent. None had been on antimicrobial treatment or other therapy for at least 4 weeks prior to collection of the stool specimens. No laxatives or other cathartic agents were used within 2 weeks before and after stool collection.

Microbial sampling and media. Fresh fecal specimens were collected in tubes containing transport medium (28) and kept at 4°C until processed. Samples (approximately 1 g [wet weight]) were suspended in diluent B (27), and serial 10-fold dilutions from 10^{-1} to 10^{-8} were prepared.

Culture media and methods. The method for bacterial analysis of the fecal microflora in this study was essentially that of Mitsuoka et al. (26, 27). After thorough mixing, a series of 10-fold dilutions (10^{-1} to 10^{-8}) was prepared in anaerobic diluent (26). From appropriate dilutions, 0.05-ml aliquots were spread on 4 nonselective agar plates (modified medium 10 [M10] for fastidious anaerobes [26], modified

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Eggerth-Gagnon [EG] agar for anaerobes [27], glucose-liver-blood [BL] agar for anaerobes [27], and Trypticase soy [TS] agar [BBL Microbiology Systems, Cockeysville, Md.] with 5% horse blood for aerobes [27]), as well as 11 selective agar plates (bifidobacteria selective [BS] agar for bifidobacteria [27], eubacteria selective [ES] agar for eubacteria [26], neomycin-brilliant green-taurocholate-blood [NBGT] agar for *Bacteroides* spp. [27], neomycin-Nagler [NN] agar for lecithinase-positive clostridia [27], modified veillonella selective [VS] agar for veillonellae and megasphaerae [27], modified lactobacilli selective [LBS] agar for lactobacilli [27], triphenyltetrazolium chloride-acridine orange-thallous sulfate-esculin-crystal violet [TATAC] agar for streptococci [27], phenylalcohol-egg yolk suspension [PEES] agar for staphylococci [27], potato dextrose [PD] agar [Difco Laboratories, Detroit, Mich.] for yeasts and molds [27], deoxycholate-hydrogen sulfide-lactose [DHL] agar [Eiken, Tokyo, Japan] for enterobacteria [27], and nalixidic acid-cetrimide [NAC] agar [Eiken] for pseudomonads). M10 was used in the plate-in-bottle method (25) at 37°C for 5 days. Eight agar plates (EG, BL, BS, NBGT, ES, NN, VS, and LBS agars) were incubated at 37°C for 3 days in an anaerobic steel wool jar (31) filled with oxygen-free CO_2. Four agar plates (TATAC, PEES, PD, and NAC agars) were incubated aerobically at 37°C for 48 h; TS and DHL agar plates were incubated at 37°C for 24 h. The dilutions were also heated in an 80°C water bath for 10 min prior to inoculation to detect spore formers. The heated dilutions were cultured on the surfaces of EG, BL, and TS agar plates as nonselective media and NN agar as selective medium.

After incubation, each plate was examined for bacterial colonies. Colonies from the anaerobic agar plates were subcultured on EG or BL agar plates in duplicate to allow aerobic and anaerobic incubation. Colonies from M10 were subcultured on new M10. In addition, the cultures on M10 were further transferred to EG agar. Thus, these isolates were identified as extremely oxygen-sensitive anaerobes if they did not grow on EG agar during anaerobic incubation by the steel wool jar method. Microorganisms growing only on an anaerobic agar plate were identified as strictly anaerobic bacteria. All of the strains isolated were maintained on prereduced EG liver agar slants with H_2CO_3-CO_2 buffer (27) and stored at 4°C. Transfers were usually made at 1-month intervals.

Identification of isolates. All anaerobic isolates except bifidobacteria and lactobacilli were identified by the following method. For preparation of inocula for biochemical tests of anaerobes, peptone-yeast extract (PY) broth (16) with 4% (vol/vol) Fildes peptic digest of horse blood (Fildes solution [9]) and 1% (wt/vol) glucose (designated as PYFG broth) was used. The medium was autoclaved at 115°C for 20 min and was aseptically supplemented with 0.05 ml of ascorbic acid-cysteine solution per 3 ml immediately before use. This solution was prepared by adding 37.0 g of ascorbic acid, 10.6 g of Na_2CO_3, and 2.0 g of L-cysteine hydrochloride · H_2O to 100 ml of distilled water; it was then autoclaved and stored at 4°C under a 100% CO_2 gas phase. The inocula were prepared by one or two preliminary transfers in PYFG broth. Incubation was carried out in an anaerobic steel wool jar.

The basal medium for biochemical tests was PY broth with 4% (vol/vol) Fildes solution added (designated as PYF broth). For the biochemical tests, 0.1 ml of inoculum per 3 ml of test medium was used. All liquid media were kept in a boiling-water bath for 10 min before use. Incubation was carried out at 37°C in steel wool jars in an atmosphere of 90% N_2–10% CO_2 for 7 days unless otherwise stated.

Acid production from 25 carbohydrates (arabinose, xylose, rhamnose, ribose, glucose, mannose, fructose, galactose, sucrose, maltose, cellobiose, lactose, trehalose, raffinose, melibiose, melezitose, soluble starch, glycogen, inulin, mannitol, sorbitol, inositol, esculin, salicin, and amygdalin) was determined by using PYF broth containing 0.5% (wt/vol) of the carbohydrate to be tested. The pH was measured directly in the culture tubes by using a combination electrode (an automatic multipoint pH measuring system [Lifetech Co. Ltd., Saitama, Japan]). The final pH was determined after incubation for 7 days in PYFG broth.

The formation of gas was detected by the appearance of crevices in PYFG broth containing 1.5% (wt/vol) Bacto-Agar (Difco). Indole production and nitrate reduction in indole-nitrate medium (BBL) were determined. The following tests were carried out with the media and methods previously described (16): gelatin liquefaction, starch and esculin hydrolysis, growth stimulation or inhibition by 20% bile, and production of ammonia and H_2S.

For the analysis of fermentation products, strains were grown for 7 days in PYFG broth. Alcohols, volatile acids, and nonvolatile acids were analyzed by a method previously described (17).

Bifidobacteria and lactobacilli were identified by a method previously described (21, 22). Enterobacteria, staphylococci, and yeasts were classified by using API systems (Analytab Products, Plainview, N.Y.). The other aerobic bacteria were identified by conventional methods (5, 19).

Microbial counting. For the bacterial species identified, the bacterial counts per gram of wet feces were calculated and converted into a logarithmic equivalent. The total viable counts were calculated from the sum of the counts of each bacterial species.

RESULTS

The same genera of strict anaerobes, facultative anaerobes, and aerobes, except for corynebacteria, were present in the feces of both groups (Table 1). The number of bifidobacteria in the feces of elderly persons in the Yuzurihara area was significantly larger ($P < 0.05$) than that in the feces of elderly persons in the Tokyo area. In contrast, significant reductions of total numbers ($P < 0.05$) and numbers of all anaerobes ($P < 0.05$) and lecithinase-negative clostridia ($P < 0.001$) were found in the feces of the elderly persons from Yuzurihara. Large numbers and high incidences of bacilli and lecithinase-positive clostridia (mainly *Clostridium perfringens*) were found in the feces of the elderly persons in the Tokyo area.

A total of 1,284 isolates from the elderly persons in the Yuzurihara area were identified as belonging to 25 genera and over 81 species. A total of 1,496 isolates from the elderly persons in the urban area were classified into 25 genera and over 92 species.

Strict anaerobes isolated from the elderly persons in the Yuzurihara area belonged to 16 genera and over 73 species, and those isolated from the urban elderly persons belonged to 13 genera and over 71 species (see Tables 2 through 5). Most anaerobic bacteria belonged to the genera *Bacteroides*, *Fusobacterium*, *Bifidobacterium*, *Eubacterium*, *Clostridium*, *Coprococcus*, *Peptostreptococcus*, *Ruminococcus*, *Veillonella*, *Megasphaera*, and *Lactobacillus*. *Megamonas*, *Mitsuokella*, *Selenomonas*, and *Acidaminococcus* were isolated from the elderly persons in the Yuzurihara area but not from the elderly persons in the Tokyo area.

Bacteroides species were recovered in large numbers from

TABLE 1. Comparison of fecal microflora of aged persons in Yuzurihara and Tokyo

Fecal microflora	No.[a] (frequency)[b] in:	
	Aged persons in Yuzurihara	Aged persons in Tokyo
Bacteroidaceae	10.8 ± 0.4 (15/15)	10.9 ± 0.2 (15/15)
Mitsuokella	8.6 ± 0.5 (3/15)	0 (0/15)
Eubacteria	10.2 ± 0.3 (15/15)	10.5 ± 0.7 (15/15)
Peptococcaceae	10.2 ± 0.4 (15/15)	10.3 ± 0.4 (15/15)
Gemmiger	9.4 ± 0.2 (3/15)	9.4 ± 0.8 (2/15)
Bifidobacteria	9.6 ± 0.5 (13/15)	9.1 ± 0.5[c] (11/15)
Veillonella	6.5 ± 2.0 (15/15)	5.6 ± 1.8 (12/15)
Megasphaera	9.0 ± 0.8 (4/15)	9.4 ± 0.6 (2/15)
Curved rods	9.7 ± 0.6 (8/15)	9.7 ± 0.1 (2/15)
Lecithinase-positive clostridia	6.6 ± 1.6 (8/15)	7.2 ± 1.8 (13/15)
Lecithinase-negative clostridia	8.7 ± 0.7 (15/15)	9.7 ± 0.6[d] (15/15)
Lactobacillus	6.7 ± 1.8 (15/15)	7.2 ± 1.8 (15/15)
Total anaerobes	10.9 ± 0.3 (15/15)	11.1 ± 0.2[c] (15/15)
Enterobacteriaceae	8.5 ± 1.0 (15/15)	8.4 ± 0.8 (15/15)
Streptococcus	7.1 ± 1.5 (13/15)	6.7 ± 1.0 (15/15)
Staphylococcus	3.3 ± 0.4 (5/15)	3.8 ± 0.9 (3/15)
Pseudomonas	3.0 (1/15)	3.0 ± 1.1 (3/15)
Corynebacteria	0 (0/15)	2.9 (1/15)
Bacilli	3.3 (1/15)	5.4 ± 1.6 (11/15)[c]
Yeasts	4.2 ± 1.0 (13/15)	4.8 ± 1.5 (12/15)
Total aerobes	8.6 ± 1.0 (15/15)	8.3 ± 0.5 (15/15)
Total bacteria	10.9 ± 0.3 (15/15)	11.1 ± 0.2[c] (15/15)

[a] Bacterial counts are expressed as mean ± standard deviation of $\log_{10}$ per gram (wet weight) of feces.
[b] Frequency of occurrence is expressed as the number of subjects with the organism detected/number of subjects examined.
[c] Statistically significant at the $P < 0.05$ level when compared with elderly persons in the rural area (Student's t test for the bacterial counts and chi-square test for the frequency of occurrence).
[d] Statistically significant at the $P < 0.001$ level when compared with elderly persons in the rural area.

TABLE 2. Fecal anaerobic gram-negative nonsporeforming bacilli isolated from aged persons in Yuzurihara and Tokyo

Species	No.[a] (frequency)[b] in:	
	Aged persons in Yuzurihara	Aged persons in Tokyo
Bacteroides		
B. capillosus	9.0 ± 0.5 (2/15)	0 (0/15)
B. bivius	0 (0/15)	9.5 ± 0.4 (3/15)
B. buccae-oris group	9.2 ± 0.4 (11/15)	10.5 ± 0.5[d] (10/15)
B. fragilis group	10.7 ± 0.3 (15/15)	10.8 ± 0.5 (15/15)
B. caccae	9.4 ± 0.5 (5/15)	9.6 ± 0.6 (6/15)
B. distasonis	9.8 ± 0.4 (14/15)	10.1 ± 0.6 (13/15)
B. fragilis	9.7 ± 0.5 (9/15)	9.6 ± 0.8 (8/15)
B. merdae	9.3 ± 0.6 (3/15)	9.8 ± 0.8 (4/15)
B. ovatus	9.6 ± 0.3 (6/15)	9.5 ± 0.7 (7/15)
B. stercoris	9.6 ± 0.3 (5/15)	9.3 ± 0.8 (3/15)
B. thetaiotaomicron	9.1 ± 0.7 (5/15)	10.1 ± 0.6[c] (8/15)
B. uniformis	9.3 (1/15)	10.0 ± 0.4 (3/15)
B. vulgatus	10.5 ± 0.3 (15/15)	10.6 ± 0.5 (15/15)
B. eggerthii	9.2 (1/15)	9.2 ± 0.5 (4/15)
B. furcosus	0 (0/15)	8.8 ± 2.1 (3/15)
B. intermedius	0 (0/15)	9.5 ± 0.2 (2/15)
B. oralis	8.9 ± 1.5 (4/15)	9.4 ± 0.6 (5/15)
B. putredinis	8.3 (1/15)	8.9 ± 1.0 (3/15)
B. splanchnics	9.6 ± 0.4 (3/15)	0 (0/15)
B. ureolyticus	8.3 (1/15)	9.4 ± 0.3 (3/15)
Bacteroides spp.[e]	9.8 ± 1.2 (14/15)	10.3 ± 0.5[d] (13/15)
Fusobacterium		
F. bullosum	0 (0/15)	9.8 (1/15)
F. glutinosum	8.6 (1/15)	0 (0/15)
F. mortiferum	9.5 ± 0.5 (7/15)	8.9 ± 1.4[c] (9/15)
F. necrogenes	0 (0/15)	9.3 ± 0.4 (3/15)
F. prausnitzii	9.9 (1/15)	0 (0/15)
F. russii	0 (0/15)	9.8 (1/15)
F. varium	9.4 ± 0.9 (4/15)	9.6 ± 1.1 (4/15)
Fusobacterium spp.[f]	9.3 (1/15)	9.3 ± 1.0 (4/15)
Megamonas hypermegas	9.3 (1/15)	0 (0/15)
Mitsuokella multiacidus	8.6 ± 0.5 (3/15)	0 (0/15)
Selenomonas spp.[g]	9.7 ± 0.6 (8/15)	9.7 ± 0.1 (2/15)

[a–d] See Table 1, footnotes *a* to *d*, respectively.
[e] Fifty-seven isolates were recovered that could not be identified to species level by using currently accepted identification protocols and presently recognized species.
[f] Four isolates (four species).
[g] Twelve isolates (four species).

both groups (Table 2). Significantly reduced numbers of the *B. buccae-oris* group ($P < 0.001$), *Bacteroides* spp. ($P < 0.001$), and *B. thetaiotaomicron* ($P < 0.05$), but not of *Fusobacterium mortiferum*, were found in the elderly persons in the Yuzurihara area.

Higher incidences of *Bifidobacterium* species were observed in elderly persons in the Yuzurihara area than those in the Tokyo area. A significantly larger number of *Bifidobacterium adolescentis* isolates was observed in the elderly persons in the Yuzurihara area. A decrease in *Eubacterium aerofaciens* was also found in these persons (Table 3).

Some *Clostridium* species were often isolated from both groups (Table 4). The feces from the elderly persons in the Yuzurihara area and the Tokyo area contained 16 and 14 *Clostridium* spp., respectively. The numbers of *C. coccoides* ($P < 0.001$), *C. paraputrificum* ($P < 0.05$), and *Clostridium* spp. ($P < 0.001$) in the elderly persons in the Yuzurihara area were significantly smaller than those in the elderly persons in the Tokyo area. Higher incidences of *C. innocuum* and *C. perfringens* were observed in the urban elderly persons.

A large number of *Veillonella parvula* isolates was found in the feces of the elderly persons from Yuzurihara (Table 5).

The facultative anaerobes and aerobes present in the feces of the elderly persons in the Yuzurihara and Tokyo areas belonged to 9 genera and over 18 species and to 12 genera and over 21 species, respectively (Table 6). The most prevalent facultative anaerobes in both groups were *Escherichia coli* and *Enterococcus faecalis*. The numbers of these microorganisms did not differ markedly between the two groups. *Bacillus subtilis* was isolated with significantly greater frequency from the elderly persons in the Tokyo area.

DISCUSSION

Incidences of colon cancer in Western countries are up to eight times those in developing countries, where the fiber intake is generally higher (3, 4, 38). Attention has been focused on the physiological significance of dietary fiber, which includes indigestible carbohydrates and carbohydrate-like components (e.g., cellulose, lignin, hemicellulose, pentosans, gums, and pectins) of foods. The incidence of colon cancer in Japan was the lowest in the world (30, 37), whereas

TABLE 3. Fecal anaerobic gram-positive nonsporeforming bacilli isolated from aged persons in Yuzurihara and Tokyo

Species	No.[a] (frequency)[b] in:	
	Aged persons in Yuzurihara	Aged persons in Tokyo
Bifidobacterium		
B. adolescentis	9.6 ± 0.6 (13/15)	9.1 ± 0.5[c] (10/15)
Biovar a	9.3 ± 0.5 (4/15)	8.9 ± 0.2 (4/15)
Biovar b	9.6 ± 0.6 (13/15)	9.1 ± 0.5[c] (10/15)
Biovar c	9.2 ± 0.6 (2/15)	8.9 ± 0.7 (5/15)
Biovar d	9.1 ± 0.4 (4/15)	9.0 ± 0.4 (6/15)
B. bifidum	9.3 ± 0.7 (2/15)	0 (0/15)
Biovar a	8.9 (1/15)	0 (0/15)
Biovar b	9.3 ± 0.7 (2/15)	0 (0/15)
B. longum	9.4 ± 0.6 (6/15)	9.0 ± 0.8 (6/15)
Biovar a	9.3 ± 0.5 (3/15)	9.1 ± 0.6 (2/15)
Biovar b	9.4 ± 0.6 (6/15)	8.7 ± 0.7 (6/15)
Bifidobacterium spp.[d]	9.2 ± 0.5 (3/15)	8.8 ± 0.9 (4/15)
Eubacterium		
E. aerofaciens	10.1 ± 0.5 (15/15)	10.3 ± 0.6 (14/15)
E. contortum	9.7 ± 0.5 (3/15)	0 (0/15)
E. cylindroides	0 (0/15)	9.3 (1/15)
E. lentum	9.0 ± 0.6 (3/15)	9.8 ± 0.5 (5/15)
E. limosum	0 (0/15)	8.9 ± 0.5 (3/15)
E. rectale	9.8 ± 0.7 (6/15)	9.7 ± 0.9 (8/15)
E. tortuosum	0 (0/15)	9.9 (1/15)
Eubacterium spp.[e]	10.1 ± 0.6 (8/15)	10.0 ± 1.2 (13/15)
Lactobacillus		
L. brevis	6.5 (1/15)	0 (0/15)
L. casei	6.2 ± 1.5 (2/15)	0 (0/15)
L. catenaforme	0 (0/15)	9.6 (1/15)
L. gasseri	6.7 ± 1.8 (13/15)	6.0 ± 2.4 (9/15)
L. plantarum	7.7 ± 1.6 (2/15)	0 (0/15)
L. reuteri	6.5 ± 1.7 (8/15)	5.5 ± 1.4 (6/15)
L. salivarius	7.8 ± 1.5 (3/15)	5.8 ± 2.9 (5/15)
Lactobacillus spp.[f]	0 (0/15)	6.6 (1/15)

[a–c] See Table 1, footnotes *a* to *c*, respectively.
[d] Ten isolates (three species).
[e] Thirty-seven isolates (possibly representing 15 species).
[f] Two isolates (one species).

TABLE 4. Fecal *Clostridium* spp. isolated from aged persons in Yozurihara and Tokyo

Species	No.[a] (frequency)[b] in:	
	Aged persons in Yuzurihara	Aged persons in Tokyo
C. aminovalericum	7.8 (1/15)	0 (0/15)
C. beijerinckii	7.6 ± 1.5 (3/15)	8.3 ± 0.8 (3/15)
C. bifermentans	0 (0/15)	6.3 (1/15)
C butyricum	5.3 ± 0.9 (3/15)	7.8 ± 1.6 (2/15)
C. cellobioparum	0 (0/15)	6.6 (1/15)
C. clostridioforme	8.1 ± 1.7 (6/15)	5.6 ± 0.5[d] (6/15)
C. cochlearium	6.3 ± 0.7 (4/15)	0 (0/15)
C. difficile	0 (0/15)	6.3 (1/15)
C. indolis	8.8 (1/15)	0 (0/15)
C. innocuum	8.1 ± 0.6 (5/15)	8.7 ± 1.1 (10/15)
C. oroticum	2.3 (1/15)	0 (0/15)
C. paraputrificum	6.2 ± 1.3 (7/15)	8.2 ± 1.6[c] (9/15)
C. perfringens	6.6 ± 1.6 (8/15)	7.2 ± 1.8 (13/15)
C. putrefaciens	0 (0/15)	8.9 (1/15)
C ramosum	8.7 ± 0.9 (9/15)	9.2 ± 1.8 (11/15)
C. rubrum	0 (0/15)	7.0 (1/15)
C. sartagoformue	3.1 (1/15)	7.6 (1/15)
C. sordellii	4.3 (1/15)	0 (0/15)
C. sphenoides	5.9 ± 0.6 (3/15)	0 (0/15)
C. tertium	5.0 ± 2.2 (3/15)	0 (0/15)
Clostridium spp.[e]	6.2 ± 1.4 (14/15)	8.1 ± 1.3[d] (15/15)

[a–d] See Table 1, footnotes *a* to *d*, respectively.
[e] Forty-two isolates (possibly representing 24 separate species).

that in Japanese immigrants increases to that of the host country within the second or third generations (15). Dietary factors, particularly a high intake of total fat and a relatively low intake of certain dietary fibers, play important roles in the etiology of colon cancer (4, 32).

Takanohashi et al. (35) carried out physiological, geographical, histological, and nutritional studies on the elderly persons in the Yuzurihara area. The level of fiber intake in this area was higher than the average fiber intake of the Japanese. Incidences of cancer and diverticular disease were also much lower (29). We believe that the longevity in this area is due to a high intake of dietary fiber.

On the other hand, there have been few studies on the influence of fiber intake on human intestinal microflora. Drasar et al. (8) found no significant differences in numbers of fecal bacteria in humans given a diet containing high levels of wheat bran for 3 weeks. However, Fuchs et al. (11) noted changes in the numbers of some intestinal bacteria when volunteers were fed wheat bran containing 5.4 g of crude fiber per day as a source of dietary fiber. Our results showed that a high intake of dietary fiber causes changes in the microbial populations, especially a significantly larger number of bifidobacteria and a significantly smaller number of lecithinase-negative clostridia.

It is widely known that the bifidobacterium count in the intestine is decreased with advancing age in humans, whereas the numbers of *C. perfringens* and members of the family *Enterobacteriaceae* are increased (13, 23). Although the number of fecal bifidobacteria isolated from the elderly persons in the Yuzurihara area was smaller than that isolated from healthy adults (26), it was larger than that isolated from the elderly persons in the Tokyo area. These findings indicate that general changes in fecal microflora with advancing age in the elderly persons in Yuzurihara were not observed even when these individuals were more than 20 years older than those in the Tokyo area. Haenel (14) demonstrated that persons more than 81 years old in Bulgaria, who lived on yogurt, milk products, and dietary fiber, had large numbers of fecal bifidobacteria. We suggest that changes in the human fecal microflora with advancing age, in particular decreased numbers of bifidobacteria and increased numbers and incidences of lecithinase-negative clostridia, can be delayed by a high fiber intake.

The composition of the fecal microflora of the elderly persons in the Yuzurihara area was similar to that of rural healthy Japanese (2) with a median age of 54 years, who lived on a typical Japanese diet with a low intake of total fat and a high intake of dietary fiber. Although a larger number of bifidobacteria was isolated from the feces of rural Japanese, the number of lecithinase-negative clostridia in the elderly persons in the Yuzurihara area was as small as that in rural Japanese. These results also indicate the effects of a typical Japanese diet with a high intake of dietary fiber.

In the present study, the numbers of the *B. buccae-oris* group, *B. thetaiotaomicron*, *Bacteroides* spp., *C. coccoides*, *C. paraputrificum*, and *Clostridium* spp. were significantly smaller in the elderly persons in the Yuzurihara area than those in the Tokyo area. In contrast, significantly larger numbers of *Bifidobacterium adolescentis* and *F. mortiferum* were noted in the elderly persons in the Yuzurihara area. Aries et al. (1) demonstrated low isolation rates of *Bacteroides* spp. from the intestines of strict vegetarians. Finegold et

TABLE 5. Fecal anaerobic cocci isolated from aged persons in Yuzurihara and Tokyo

Species	No.[a] (frequency)[b] in:	
	Aged persons in Yuzurihara	Aged persons in Tokyo
Acidaminococcus fermentans	9.6 (1/15)	0 (0/15)
Coprococcus		
C. comes	0 (0/15)	9.1 ± 0.3 (2/15)
Copracoccus spp.[c]	8.9 ± 0.6 (2/15)	9.3 ± 0.8 (4/15)
Gemmiger spp.[d]	9.4 ± 0.2 (3/15)	9.4 ± 0.9 (3/15)
Megasphaera elsdenii	9.0 ± 0.8 (4/15)	9.4 ± 0.6 (2/15)
Peptostreptococcus		
P. anaerobius	9.0 ± 0.8 (4/15)	9.9 ± 0.6 (3/15)
P. asaccharolyticus	9.3 (1/15)	0 (0/15)
P. magnus	9.1 ± 0.9 (5/15)	9.6 (1/15)
P. micros	9.6 (1/15)	0 (0/15)
P. parvulus	0 (0/15)	8.6 (1/15)
P. prevotii	0 (0/15)	9.5 (1/15)
P. productus	9.6 ± 1.2 (8/15)	9.6 ± 0.8 (10/15)
Peptostreptococcus spp.[e]	9.9 ± 0.8 (7/15)	10.2 ± 1.2 (13/15)
Ruminococcus		
R. bromii	0 (0/15)	9.8 (1/15)
R. callidus	9.3 (1/15)	9.0 (1/15)
R. gnavus	9.3 ± 1.2 (6/15)	9.3 ± 0.6 (4/15)
R. lactaris	9.3 (1/15)	0 (0/15)
Ruminococcus[f]	8.8 ± 0.8 (5/15)	9.6 (1/15)
Veillonella		
V. parvula	6.5 ± 2.0 (14/15)	5.6 ± 1.8 (12/15)
V. alacalescens	0 (0/15)	3.4 (1/15)

[a,b] See Table 1, footnotes *a* and *b*, respectively.
[c] Eight isolates (three species).
[d] Nine isolates (two species).
[e] Twenty-nine isolates (possibly representing 11 separate species).
[f] Seven isolates (possibly representing six separate species).

TABLE 6. Fecal facultative and aerobic bacteria isolated from aged persons in Yuzurihara and Tokyo

Species	No.[a] (frequency)[b] in:	
	Aged persons in Yuzurihara	Aged persons in Tokyo
Acinetobacter sp.[d]	0 (0/15)	8.2 (1/15)
Bacillus		
B. subtilis	0 (0/15)	5.4 ± 1.5 (11/15)[c]
Bacillus spp.[e]	3.3 (1/15)	4.3 ± 0.5 (3/15)
Candida		
C. albicans	4.2 ± 0.9 (12/15)	4.0 ± 1.5 (12/15)
C. tropicalis	3.5 ± 0.9 (2/15)	0 (0/15)
Candida spp.[f]	3.3 ± 1.4 (3/15)	3.9 (1/15)
Citrobacter		
C. freundii	5.3 ± 0.6 (4/15)	5.8 ± 1.5 (5/15)
Citrobacter sp.[g]	6.3 (1/15)	6.8 (1/15)
Corynebacterium sp.[d]	0 (0/15)	2.9 (1/15)
Enterococcus		
E. faecalis	7.0 ± 1.2 (12/15)	6.7 ± 0.9 (15/15)
E. faecium	6.5 ± 2.5 (8/15)	6.4 ± 0.8 (9/15)
Escherichia coli	8.5 ± 1.0 (15/15)	8.4 ± 0.8 (15/15)
Filamentous fungi	2.3 (1/15)	0 (0/15)
Klebsiella pneumoniae	7.3 (1/15)	6.8 ± 1.2 (7/15)
Micrococcus spp.[h]	0 (0/15)	3.8 ± 1.7 (4/15)
Pseudomonas aeruginosa	3.0 (1/15)	3.9 ± 1.1 (8/15)
Staphylococcus		
S. aureus	0 (0/15)	3.9 (1/15)
S. epidermidis	3.3 ± 0.5 (5/15)	3.8 ± 0.9 (3/15)
Staphylococcus spp.[i]	3.4 ± 0.8 (2/15)	3.6 ± 0.5 (2/15)
Streptococcus		
S. durans	6.2 ± 2.5 (5/15)	0 (0/15)
S. intermedius	7.4 ± 2.7 (6/15)	9.8 ± 0.8 (6/15)
S. salivarius	0 (0/15)	6.8 (1/15)
S. sanguis	0 (0/15)	4.9 (1/15)
Streptococcus[j]	6.3 ± 2.4 (6/15)	5.9 ± 1.5 (9/15)

[a–c] See Table 1, footnotes *a* to *c*, respectively.
[b] One isolate (one species).
[e] nine isolates (possibly representing five separate species).
[f] Five isolates (possibly representing three separate species).
[g] Two isolates (one species).
[h] Six isolates (possibly representing three separate species).
[i] Twelve isolates (possibly representing seven separate species).
[j] Sixteen isolates (possibly representing 10 separate species).

al. (10) also demonstrated that the fecal microflora in Seventh-Day Adventists contained higher counts of *Lactobacillus* spp. and lower incidences of *C. perfringens* and *Fusobacterium* spp. than did the fecal microflora in controls. The results of the present study were in agreement with the lower incidence of *C. perfringens* in Seventh-Day Adventists. In studies of fecal bacteria isolated from the feces of rural Japanese, high incidences of *Bifidobacterium adolescentis* and *Eubacterium aerofaciens* were detected (2, 18, 29). Our data closely agreed with the higher incidence of *Bifidobacterium adolescentis*.

Our previous study (2) noted significantly smaller numbers of *C. coccoides* and *C. tertium* and a significantly lower incidence of *C. innocuum* in rural Japanese than in urban Canadians. Mastromarino et al. (20) also indicated that lecithinase-negative clostridia, i.e., *C. butyricum*, *C. innocuum*, *C. indolis*, *C. papraputruficum*, *C. tertium*, and *C. sartagoforme*, constitute a greater proportion of fecal microflora 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 (7, 12). In the present study, decreases in *C. coccoides* and *C. paraputruficum* numbers and decreased isolations of *C. innocuum*, *C. perfringens*, and *C. clostridioforme* in the elderly persons in the Yuzurihara area may indicate the prevention of intestinal putrefaction by clostridia and the low production of carcinogens.

Mitsuoka et al. (24) demonstrated that a higher incidence of *Bifidobacterium adolescentis* and a lower incidence of *Bifidobacterium longum* in elderly persons was the characteristic composition of fecal *Bifidobacterium* spp. The results in our study closely agree with their results. The number and incidence of *Bifidobacterium adolescentis* isolates in the elderly persons in the Yuzurihara area was, however, significantly higher than that in the elderly persons in the Tokyo area. This finding shows that a reduction of *Bifidobacterium adolescentis* with advancing age may be inhibited by a high intake of dietary fiber.

In conclusion, the present investigation revealed two points: (i) the fecal microflora of elderly persons in the Yuzurihara area contained a larger number of bifidobacteria, in particular *Bifidobacterium adolescentis*, and a smaller number of lecithinase-negative clostridia, in particular *C. coccoides* and *C. paraputrificum*, than the fecal microflora of elderly persons in Tokyo did, and (ii) changes in fecal microflora with advancing age can be modified by a higher intake of dietary fiber.

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