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Intestinal Floras of Populations That Have a High Risk of Colon Cancer

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The fecal floras of polyp patients, Japanese-Hawaiians, North American Caucasians, rural native Japanese, and rural native Africans were compared. The polyp patients and Japanese-Hawaiians were considered to be groups at high risk of colon cancer, and the rural native Japanese and rural native Africans were considered to be groups at low risk. The North American Caucasians were found to have a flora composition intermediate between these two groups. Fifteen bacterial taxa from the human fecal flora were significantly associated with high risk of colon cancer, and five were significantly associated with low risk of colon cancer. Total concentrations of *Bacteroides* species and, surprisingly, *Bifidobacterium* species were generally positively associated with increased risk of colon cancer. Some *Lactobacillus* species and *Eubacterium aerofaciens*, which also produces major amounts of lactic acid, showed closest associations with low risk of colon cancer.

It often has been suggested that intestinal bacteria may play a role in the initiation of colon cancer through production of carcinogens, cocarcinogens, or procarcinogens (2). In an attempt to determine which, if any, bacterial species are directly associated with high risk of colon cancer, in 1971 the National Institutes of Health supported the Japan-Hawaii Cancer Study in which we analyzed the fecal floras of people with different risks of colon cancer. Eighteen Japanese-Hawaiian polyp patients, 15 Japanese-Hawaiian adults on a western diet, and 17 Caucasians on a western diet represented populations at high risk of colon cancer. Twenty-two rural Japanese natives and 16 rural African natives on their normal diets represented two low-risk populations. Strictly anaerobic culture techniques recovered cultural counts that were comparable to direct microscopic counts, indicating that most of the bacteria present were detected. Isolated colonies were picked in a randomized manner to obtain a representative cross section of the bacterial population of fecal specimens from each subject. Each isolate was restreaked and analyzed by conventional biochemical tests.

Three studies (4, 8, 14) established the complexity of the human intestinal flora. Not only were there approximately 300 species in the human fecal flora (a conservative estimate), but many of the species were undescribed. When this information became available, the National Institutes of Health study section realized the complexity of the human intestinal flora and concluded that the data were too complex to interpret. The project was discontinued.

During the funded period, the study section also questioned whether the fecal flora was representative of the colonic flora. As a result of this concern, we examined the flora of the entire intestinal tract of several cadavers within 4 h of death and found that the flora of the ascending, transverse, and descending colon and rectum is constant in numbers and in composition and represents the maximum stationary phase of growth (15). Thus, we are assured that the fecal flora is representative of the colonic flora. Although our comparative studies were discontinued before completion, we had preserved all of the isolates as freeze-dried cultures. Until now, no comparisons of these floras have been published.

During the intervening years, some major changes occurred. Personal computers which facilitate the analysis and management of vast amounts of data were developed, we obtained new statistical methods to compare bacterial floras (5, 6), and we developed new analytical methods to differentiate and identify bacteria, including polyacrylamide gel electrophoresis of soluble cellular proteins (13) and a library of reference patterns of cellular fatty acids for species identification (10). Within the last 18 months, we have revived the freeze-dried cultures and updated their identifications by use of electrophoretic patterns of cellular proteins, cellular fatty acid analyses, and, where needed, repeated phenotypic tests to improve and confirm species identifications. We also updated the nomenclature with names of species that have been described since the original work. Computer programs were used to compare the floras of the different risk groups. The results are reported here.

MATERIALS AND METHODS

Subjects. No subject had received antibiotics within several weeks prior to sampling. Fecal specimens were obtained from 22 healthy Japanese from a rural mountain village in Akita prefecture, Japan, and from 16 African natives of the Tswana tribe in the village of Tlaseng, about 100 miles from Johannesburg, South Africa. Both of these populations are known to have very low risk of colon cancer and very little red meat in their diets. Two moderate- to high-risk groups included 15 Japanese-Hawaiians who consumed a western-type diet and 17 Hawaiian and continental U.S. Caucasians on typical western diets. Fecal specimens from 18 polyp patients were used to determine the floras of individuals with approximately four times greater risk of colon cancer than that of the normal Japanese-Hawaiians or the normal Caucasians on western diets. The polyp patients were selected by Gary Glober of the National Institutes of Health Japan-Hawaii Cancer Study. These individuals had recovered from recent surgical removal of one or more adenomatous polyps.

Bacteriology. Culture methods (8, 14) and media (7) were described earlier. Briefly, within 5 min of collection, individual fecal samples from healthy adults or polyp patients were placed in a plastic bag that was flushed with oxygen-free CO_2, and the feces were mixed thoroughly by kneading. A subsample of approximately 1 g (wet weight) was transferred to a tared tube containing 9.0 ml of a prereduced anaerobically sterilized dilution fluid and glass beads, flushed with oxygen-free CO_2, restoppered, weighed, dispersed by shaking, and serially diluted with vigorous shaking in tubes containing 9.0 ml of prereduced salts solution (7) under oxygen-free CO_2. Duplicate samples from 10^8, 10^9, and 10^{10} dilutions were cultured in roll tubes (25 by 142 mm) of rumen fluid-glucose-cellobiose-agar (7) and incubated for 16 to 72 h before air transport to Blacksburg, Va. Slides were prepared from 0.01 ml of a 10^4 dilution placed in 1-cm^2 areas for staining and determination of direct microscopic counts. Cultures were hand carried or shipped in gimbal boxes (so they remained upright during

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TABLE 1. Calculation of minimum percent identities of flora compositions of persons

Species	% Isolates from:		Minimum similarity (%)[a]
	Person 1	Person 2	
A	3[a]	1	1
B	0	5	0
C	2	4	2
D	7	0	0
E	6	4	4
●	●	●	●
●	●	●	●
n	●	●	●
Sample summation			28[b]

[a] Example percent of flora of this person represented by species A.
[b] Example summation of 28% represents the total minimum percent similarity (sum of all numbers in this column).

transport) at ambient temperature. After a total of 5 days at 37°C, 55 to 60 isolated colonies were picked in succession, regardless of appearance, into Sweet E broth (7). All cultures were restreaked to Sweet E agar or to supplemented brain heart infusion agar (7). If multiple colony types or morphotypes were recovered, all were identified. All cultures were characterized for identification by methods described in the *Anaerobe Laboratory Manual* (7) and more recently by electrophoresis of soluble cellular whole proteins (13) and cellular fatty acid analysis (10), with repeated conventional biochemical tests when needed. For cellular fatty acid analysis, gram-negative cultures were grown in peptone-yeast extract-glucose broth (7), and gram-positive cultures were grown in the same medium plus 0.02% Tween 80 (7). Cells grown in Tween 80 were washed once by resuspension in 0.7% $MgSO_4$ solution.

Statistics. The bacterial compositions of the fecal floras were compared by the lambda analysis of I. J. Good (6), which provides an estimate of the probability that the observed differences in composition occur by chance alone. Briefly, this test is done by comparing the similarity of the composition of the flora of each person with the flora of every other person in the two populations to be compared. The minimum percent identity is usually used (geometric mean percent similarities give similar results) and calculated as shown in Table 1.

A matrix is formed of all of the minimum percent similarities of the floras of all subjects as compared with those of all other subjects, as shown in Table 2. Example values might be 26% mean similarity between populations and 31% mean similarity within populations. This ratio (0.8387) is called the observed lambda value.

To determine whether the observed greater similarity within populations as compared with the similarity between populations could have occurred by chance alone, the subjects are assigned at random to two populations of the same sizes as the original populations and the ratio is recalculated. Randomization and recalculation are done a thousand times, as in a Monte Carlo analysis. If no calculated ratio is as low as or lower than the observed ratio, then the probability of the observed ratio is 1/1,002 (allowing for LaPlace's law of succession by adding 1 to the numerator and 2 to the denominator) or 0.000998. If, for example, an equal or lower value is obtained three times, then $P = 0.003992$. Note that if more than one sample from each person is used (as was done in the periodontal work where this analysis was first applied), the samples must be combined before the calculations are done; otherwise, similarity within people is superimposed on the similarity within populations and the randomization comparison of populations is not valid.

Student's *t* test was used to compare the distributions of individual species in the different populations. This was done by using the percentage value for each species in each person. Thus, there were 38 values (ranging from 0 to 49.09) for percentages of isolates represented by *Bacteroides vulgatus* in the 39 low-risk population samples and 33 values (ranging from 0 to 36.67) for percentages of isolates represented by *Bacteroides vulgatus* in the 33 high-risk population samples. A separate *t* test was done for each species detected in more than two people. The actual number of isolates of the species, instead of the percentage of the isolates represented by the species, may be used if the sample sizes for different individuals are similar.

TABLE 2. Matrix formed of all minimum percent similarities of floras of all subjects[a]

Person[b]	Person[b]												
	1	2	3	4	5	6	7	8	9	●	●	●	*n*
1		X	X	X	X	X	X	X	X	X	X	X	X
2			X	X	X	X	X	X	X	X	X	X	X
3				X	X	X	X	X	X	X	X	X	X
4					X	X	X	X	X	X	X	X	X
5						X	X	X	X	X	X	X	X
6							X	X	X	X	X	X	X
7								X	X	X	X	X	X
8									X	X	X	X	X
9										X	X	X	X
●											X	X	X
●												X	X
●													X
n													

[a] In this example, persons 1 to 5 equal the first population and persons 6 to *N* equal the second population. The mean of all underlined values equals the minimum similarity within populations, and the mean of all values not underlined equals the mean similarity between the two populations.
[b] —, successive number of persons in the sequence.

TABLE 3. Differences of fecal floras of populations at low and high risk of colon cancer as determined by the lambda analysis

Population[a,b]	*P*[c]				
	Low-risk groups[a]		High-risk groups[b]		
	NA	NJ	CAU	JH	PP
NA		0.004	0.001	0.001	0.001
NJ	0.004		0.001	0.013	0.001
CAU	0.001	0.001		0.102[d]	0.011
JH	0.001	0.013	0.102[d]		0.33[d]
PP	0.001	0.001	0.011	0.33[d]	

[a] NA, rural native Africans; NJ, rural native Japanese. Both populations consumed a native diet.
[b] CAU, North American Caucasians; JH, Japanese-Hawaiians; PP, polyp patients. All consumed a western diet.
[c] Probability that the observed flora composition difference would occur by chance alone.
[d] No significant difference in flora composition.

RESULTS

From 88 fecal specimens, 5,350 bacterial isolates representing 371 taxa were detected. Of these taxa, 177 were seen only once. Thus, according to Good's coverage analysis (5), the 371 taxa represent approximately 96.7% of the kinds of bacteria in the fecal floras of the total population of subjects examined. The remaining 3.3% of the kinds of bacteria that were not detected may include many more species that proportionately are very rare. The most frequent species detected was *Eubacterium aerofaciens* type 1, which is like the type strain and which accounted for 8.43% of the bacterial isolates. *Bacteroides vulgatus* was the second most frequent species, at 7.79% of the isolates. *Escherichia coli* was 22nd most common, at 1.21%. While most of the more common species have been formally described, there were 261 phenotypically distinct and recognizable groups that represent new species yet to be named (in another lifetime of work).

Comparisons by lambda analysis (6) showed that the flora composition of polyp patients with known highest risk of colon cancer was not significantly different from that of Japanese-Hawaiians on a western-type diet, who have a moderate to high risk of colon cancer (Table 3). The flora of Caucasians on a western diet, who also have a moderate to high risk of colon cancer, was significantly different from that of polyp patients but not from that of the Japanese-Hawaiians. The two low-risk populations had floras of different compositions from each

TABLE 4. Species significantly associated with high risk group (polyp patients and Japanese-Hawaiians)

Species[a]	Total no.		*P* of high vs. low[b]	% Of isolates in population[c]:				
	Isolates	Samples		PP	JH	CAU	NJ	NA
Bacteroides vulgatus	417	67	0.0010	13.12	9.78	8.21	7.52	0.48
Eubacterium rectale 1	75	20	0.0013	2.68	1.56	2.93	0.08	0.10
Ruminococcus torques	27	15	0.0018	0.65	1.33	0.68	0.08	
Streptococcus hansenii	15	11	0.0091	0.65	0.67	0.10	0.08	
Bifidobacterium longum	60	24	0.010	1.57	1.22	2.64	0.38	
Ruminococcus albus	29	13	0.018	1.02	1.11	0.78		
Peptostreptococcus productus 1	12	9	0.018	0.46	0.11	0.59		
Bacteroides stercoris	102	32	0.018	3.42	3.00	1.56	1.53	0.19
Bifidobacterium angulatum	52	14	0.023	2.31	1.89	0.10	0.69	
Eubacterium eligens	70	25	0.024	1.29	3.00	1.47	0.84	0.29
Eubacterium eligens 2	12	8	0.027	0.37	0.44	0.39		
Ruminococcus gnavus	90	30	0.028	3.23	2.78	0.59	1.53	0.38
Fusobacterium prausnitzii	241	63	0.036	6.01	7.00	3.03	3.30	3.74
Eubacterium cylindroides	10	6	0.037	0.37	0.22	0.39		
Eubacterium rectale 2	166	41	0.042	4.16	3.22	5.87	0.77	2.11
Total no. of isolates				1,082	900	1,023	1,303	1,042
Total no. of samples				18	15	17	22	16

[a] See Table 7, footnote *a*.
[b] Probability that the observed difference between the high- and low-risk groups could occur by chance alone.
[c] PP, Polyp patients; JH, Japanese-Hawaiians; CAU, North American caucasians; NJ, rural native Japanese; NA, rural native Africans.

other and from the other groups. These results suggested that the Caucasian population might be intermediate between the highest-risk and lowest-risk populations and led us to compare the floras of three groups. The rural African and Japanese populations formed one low-risk group, the Japanese-Hawaiians and polyp patients formed a high-risk group, and the Caucasians formed a third, intermediate group. Lambda analysis of these groups indicated that the flora composition of the Caucasians indeed was intermediate between those of the high- and low-risk groups and not significantly different from either (low-risk group versus high-risk group, $P = 0.001$; low-risk group versus Caucasian group, $P = 0.11$; and high-risk group versus Caucasian group, $P = 0.15$).

Although the rural Japanese and African low-risk populations had floras of significantly different compositions, they both represent floras that are rarely associated with colon cancer and therefore may be considered as one group. Statistically, it is conservative to combine these two groups for comparisons by lambda analyses because the analyses compare the ratio of mean similarity within groups (the combined group should be more heterogeneous) with the mean similarity between groups. For comparisons of the distribution of individual species by *t* test, the combined groups again may provide for a conservative analysis to give direct evidence concerning which, if any, bacterial species are associated with high or low risk of colon cancer. Results from the intermediate population may tend to confirm the extremes or, conversely, might give little further information because the composition of flora in this intermediate group is not significantly different from that of either the low-risk or high-risk group.

Student's *t* test of individual species distributions in high-risk, low-risk, and Caucasian floras showed that 15 relatively common species were significantly associated with high risk of colon cancer (Table 4) and 6 species were significantly associated with low risk of colon cancer (Table 5).

Species that have been thought to relate to colon cancer or protect from colon cancer are listed in Table 6. The predominant species in each population are listed in Table 7 for purposes of direct comparison.

DISCUSSION

We might expect *Bacteroides* species to be highly associated with high risk of colon cancer because consumption of red

TABLE 5. Species significantly associated with low-risk group (rural native Japanese and rural native Africans)

Species[a]	Total no.		*P* of low vs. high[b]	% Of isolates in population[c]:				
	Isolates	Samples		PP	JH	CAU	NJ	NA
Lactobacillus S06	26	11	0.0043			0.10	0.54	1.73
Fusobacterium AB	17	11	0.0055			0.20	0.38	0.96
Eubacterium aerofaciens 1	451	71	0.0066	3.97	7.22	8.50	7.52	15.16
Eubacterium BN	37	12	0.032	0.18	0.11		0.54	2.59
Eubacterium aerofaciens 2	137	37	0.034	0.28	2.89	1.47	3.76	4.22
Peptostreptococcus DZ2	22	9	0.048	0.09		0.20	1.30	0.19
Total no. of isolates				1,082	900	1,023	1,303	1,042
Total no. of samples				18	15	17	22	16

[a] See Table 7, footnote *a*.
[b] Probability that the observed difference between the high- and low-risk groups could occur by chance alone.
[c] PP, Polyp patients; JH, Japanese-Hawaiians; CAU, North American caucasians; NJ, rural native Japanese; NA, rural native Africans.

TABLE 6. Distribution of selected species among populations with different risk of colon cancer

Taxon[a]	Distribution of species (% of isolates) in[b]:				
	PP	JH	CAU	NJ	NA
Bacteroides					
B. caccae	0.74	1.22	1.37	0.69	—[c]
B. distasonis	1.66	1.33	1.27	0.61	2.02
B. distasonis 2	0.18	0.22	—	—	—
B. eggerthii	0.18	0.22	0.29	—	—
B. fragilis	1.94	1.67	0.49	2.46	8.73
B. merdae	0.37	0.22	0.20	0.23	—
B. ovatus	2.59	1.22	0.39	2.38	0.29
B. ovatus 2	0.74	—	0.29	0.08	—
B. splanchnicus	—	—	0.10	—	—
B. stercoris	3.42	3.00	1.56	1.53	0.19
B. thetaiotaomicron	1.48	1.44	1.37	3.15	0.96
B. thetaiotaomicron 3	0.09	0.33	0.49	—	—
B. uniformis	3.42	1.11	1.27	1.92	1.25
B. uniformis 2	0.37	0.44	1.08	0.77	0.38
B. vulgatus	13.12	9.78	8.21	7.52	0.48
Bacteroides FB	1.57	3.89	0.59	5.99	2.30
Bacteroides FO	0.74	1.11	0.98	1.38	0.19
Other	1.38 (3)[d]	—	0.30 (2)	0.16 (2)	—
Subtotal	33.99	27.20	20.25	28.87	16.79
Bifidobacterium					
B. adolescentis	3.05	2.67	2.15	5.53	2.98
B. angulatum	2.31	1.89	0.10	0.69	—
B. bifidum	0.09	0.22	—	0.08	—
B. breve	—	0.89	—	—	—
B. dentium	0.09	0.22	—	—	—
B. longum	1.57	1.22	2.64	0.38	—
Other	—	0.11 (1)	—	—	—
Subtotal	7.11	7.22	4.89	6.68	2.98
Lactobacillus					
L. acidophilus 5	0.09	1.33	1.08	1.00	2.21
L. gasseri	—	—	0.10	—	0.10
L. rogosae	0.37	0.11	0.29	0.15	0.10
L. salivarius	—	0.44	—	1.69	—
Lactobacillus S06	—	—	0.10	0.54	1.73
Other	0.46 (5)	0.55 (2)	0.40 (3)	1.23 (6)	0.20 (2)
Subtotal	0.92	2.43	1.97	4.61	4.34

[a] See Table 7, footnote *a*.
[b] PP, Polyp patients; JH, Japanese-Hawaiians; CAU, North American caucasians; NJ, rural native Japanese; NA, rural native Africans.
[c] —, not detected.
[d] Values in parentheses indicate number of taxa.

meat and a high-fat diet is reported to be associated with high risk of colon cancer (2, 3) and fat stimulates bile flow, which in turn specifically stimulates *Bacteroides* species. This possibility has been supported by studies that show that many strains of *Bacteroides* species convert bile to metabolites and fecapentaenes, which have been thought to be cocarcinogens or mutagens (9). Only two *Bacteroides* species, *Bacteroides vulgatus* and *Bacteroides stercoris*, showed significant association with high risk as opposed to low risk (Table 4). Total concentrations of *Bacteroides* species showed only a slight increase of these species with increasing risk (Table 6).

Bifidobacterium longum and *Bifidobacterium angulatum* also were significantly associated with high risk of colon cancer (Table 4), and total concentrations of bifidobacteria increased with higher risk of colon cancer (Table 6). This is contrary to some suggestions by commercial companies that ingestion of bifidobacterium cultures to increase the numbers of these bacteria in the intestinal flora might offer increased protection against colon cancer. However, our finding of increased numbers of bifidobacteria in the flora of the high-risk populations is in line with the observation of Finegold et al. (4), who reported that *Bifidobacterium infantis* is more numerous in the feces of 18 people who consumed a Western diet than in the feces of 15 native Japanese who subsisted primarily on a native Japanese diet.

Lactobacillus S06 (see Table 7, footnote *a*, for explanation of designation following genus name) and *Eubacterium aerofaciens* were significantly associated with a low risk of colon cancer (Table 5), and total lactobacillus concentrations also were inversely related to risk (Table 6). *Lactobacillus acidophilus* 5 has phenotypic characteristics of *Lactobacillus acidophilus* but appears to be different from the type strain in cellular fatty acid composition.

Lactobacillus S06 is a species that, to our knowledge, is yet to be described. Strains grow best anaerobically, produce short rods and oval cells in short chains similar to those of *Lactobacillus minutus*, and produce acid from glucose and fructose, usually from mannose, and occasionally from trehalose. Milk is coagulated, and good growth occurs in 20% bile–glucose medium. Lactic acid is the major product, and small amounts of acetic and formic acids are produced in peptone-yeast extract-glucose broth cultures (7). No hydrogen gas is produced, and no acid is produced from amygdalin, arabinose, cellobiose, erythritol, esculin, glycogen, inositol, lactose, maltose, mannitol, melezitose, melibiose, raffinose, rhamnose, ribose, salicin, sorbitol, starch, sucrose, or xylose. Esculin usually is not hydrolyzed. Starch is not hydrolyzed, and gelatin is not liquefied. Nitrate is not reduced, and indole is not produced.

The incidence of *Eubacterium aerofaciens* showed an inverse relationship with risk of colon cancer among all of the populations tested. *Eubacterium aerofaciens* is anaerobic and produces major amounts of lactic acid with large amounts of acetic acid and traces of formic acid. Cells are short rods with oval and some swollen cells in short chains. Strains ferment glucose, fructose, maltose, mannose, and salicin. Most strains ferment cellobiose, lactose, and sucrose and hydrolyze esculin. A few strains ferment amygdalin, esculin, mannitol, melibiose, raffinose, ribose, starch, or trehalose. Milk is acidified and may be coagulated. Good growth usually is produced in 20% bile–glucose broth. No strain ferments arabinose, erythritol, glycogen, inositol, melezitose, rhamnose, sorbitol, or xylose. All strains produce hydrogen gas, which differentiates them from lactobacilli and bifidobacteria. Without the test for hydrogen gas production, strains of *Eubacterium aerofaciens* may be mistaken phenotypically for species of lactobacilli. *Eubacterium aerofaciens* 1 and *Eubacterium aerofaciens* 2 probably are closely related subspecies. They form two close clusters of strains by cellular fatty acid analysis.

Currently, there is no indication that the lactic acid bacteria, including *Eubacterium aerofaciens*, have any protective effect against colon cancer. While lactic acid bacteria are associated with diets that produce lower risks of colon cancer, they may be overgrown by other bacteria when high-risk diets are consumed.

During the initial studies of the fecal flora, it became evident that individuals tended to maintain their own distinctive flora compositions. Although many bacterial species are shared, individuals generally have their own distinct predominant species combinations, even after drastic changes in diet (12). In subsequent work, this observation led us to determine the contribution that host genetics plays in the composition of the human periodontal flora. With monozygous and dizygous twins, there is evidence that host genetics exerts some control over the composition of the flora both in adolescent children (11) and adults (11a).

Some recent reports (1, 16) indicate that there is a geneti-

TABLE 7. Incidence (as percentage of isolates) of predominant species in the floras of Japanese-Hawaiian polyp patients, normal Japanese-Hawaiians on a western-type diet, normal Caucasians on a western-type diet, native rural Africans, and native rural Japanese[a]

Polyp patients		Japanese-Hawaiians		N. American Caucasians		Rural native Japanese		Rural native Africans	
Species	% Of isolates	Species	% Of isolates	Species	% Of isolates	Species	% Of isolates	Species	% Of isolates
Bacteroides vulgatus	13.1	*Bacteroides vulgatus*	9.8	*Eubacterium aerofaciens* 1	8.5	*Bacteroides vulgatus*	7.5	*Eubacterium aerofaciens* 1	15.2
Fusobacterium prausnitzii	6.0	*Eubacterium aerofaciens* 1	7.2	*Bacteroides vulgatus*	8.2	*Eubacterium aerofaciens* 1	7.5	*Bacteroides fragilis*	8.7
Eubacterium rectale 2	4.2	*Fusobacterium prausnitzii*	7.0	*Eubacterium rectale* 2	5.9	*Bacteroides* FB	6.0	*Eubacterium aerofaciens* 2	4.2
Eubacterium aerofaciens 1	4.0	*Bacteroides* FB	3.9	*Ruminococcus bromii*	5.2	*Bifidobacterium adolescentis*	5.5	*Ruminococcus bromii*	3.9
Bacteroides stercoris	3.4	*Eubacterium rectale* 2	3.2	*Fusobacterium prausnitzii*	3.0	*Eubacterium aerofaciens* 2	3.8	*Clostridium ramosum*	3.8
Bacteroides uniformis	3.4	*Eubacterium biforme*	3.1	*Eubacterium rectale* 1	2.9	*Enterococcus faecium*	3.6	*Fusobacterium prausnitzii*	3.7
Ruminococcus gnavus	3.2	*Eubacterium eligens*	3.0	*Bifidobacterium longum*	2.6	*Fusobacterium prausnitzii*	3.3	*Bifidobacterium adolescentis*	3.0
Ruminococcus bromii	3.2	*Bacteroides stercoris*	3.0	*Peptostreptococcus* DZ	2.2	*Bacteroides thetaiotaomicron*	3.2	*Eubacterium* BN	2.6
Bifidobacterium adolescentis	3.0	*Eubacterium aerofaciens* 2	2.9	*Bifidobacterium adolescentis*	2.2	*Bacteroides fragilis*	2.5	*Escherichia coli*	2.3
Eubacterium rectale 1	2.7	*Ruminococcus gnavus*	2.8	*Eubacterium biforme*	1.9	*Bacteroides ovatus*	2.4	*Bacteroides* FB	2.3
Bacteroides ovatus	2.6	*Bifidobacterium adolescentis*	2.7	*Ruminococcus obeum*	1.6	*Bacteroides uniformis*	1.9	*Lactobacillus acidophilus* 5	2.2
Bifidobacterium angulatum	2.3	*Bifidobacterium angulatum*	1.9	*Gemmiger formicilis*	1.6	*Lactobacillus salivarius*	1.7	*Eubacterium rectale* 2	2.1
Bacteroides fragilis	1.9	*Bacteroides fragilis*	1.7	*Bacteroides stercoris*	1.6	*Ruminococcus gnavus*	1.5	*Ruminococcus obeum*	2.1
Peptostreptococcus productus 2b	1.9	*Ruminococcus obeum*	1.6	*Ruminococcus* CE	1.5	*Bacteroides stercoris*	1.5	*Bacteroides distasonis*	2.0
Bacteroides distasonis	1.7	*Eubacterium rectale* 1	1.6	*Eubacterium aerofaciens* 2	1.5	*Streptococcus* S03	1.5	*Clostridium perfringens*	1.8
Bifidobacterium longum	1.6	*Bacteroides thetaiotaomicron*	1.4	*Eubacterium eligens*	1.5	*Bacteroides* FO	1.4	*Lactobacillus* S06	1.7
Bacteroides FB	1.6	*Ruminococcus torques*	1.3	*Peptostreptococcus productus* 2b	1.5	*Ruminococcus bromii*	1.3	*Eubacterium rectale* 3f	1.6
Bacteroides thetaiotaomicron	1.5	*Ruminococcus callidus* 1	1.3	*Bacteroides thetaiotaomicron*	1.4	*Peptostreptococcus* DZ 2	1.3	*Peptostreptococcus* DZ	1.5
Eubacterium eligens	1.3	*Bacteroides distasonis*	1.3	*Bacteroides caccae*	1.4	*Peptostreptococcus productus* 2b	1.2	*Gemmiger formicilis*	1.4
Escherichia coli	1.3	*Lactobacillus acidophilus* 5	1.3	*Coprococcus comes*	1.4	*Lactobacillus acidophilus* 5	1.0	*Eubacterium rectale* 3h	1.3
Peptostreptococcus productus 1b	1.2	*Eubacterium rectale* 4	1.2	*Peptostreptococcus productus* 1b	1.4			*Ruminococcus flavefaciens*	1.2
Ruminococcus callidus 1	1.1	*Gemmiger formicilis*	1.2	*Peptostreptococcus productus* 2c	1.3			*Bacteroides uniformis*	1.2
Bacteroides FL	1.1	*Bacteroides caccae*	1.2	*Bacteroides uniformis*	1.3			*Eubacterium biforme*	1.1
Eubacterium ventriosum 1	1.0	*Bifidobacterium longum*	1.2	*Bacteroides distasonis*	1.3			*Bacteroides thetaiotaomicron*	1.0
Ruminococcus albus	1.0	*Bacteroides ovatus*	1.2	*Butyrivibrio crossotus*	1.3			*Fusobacterium* AB	1.0
		Peptostreptococcus productus 2c	1.2	*Eubacterium rectale* 3h	1.3				
		Ruminococcus albus	1.1	*Escherichia coli*	1.2				
		Bacteroides FO	1.1	*Eubacterium rectale* 3f	1.1				
		Peptostreptococcus productus 2b	1.1	*Lactobacillus acidophilus* 5	1.1				
		Bacteroides uniformis	1.1	*Bacteroides uniformis* 2	1.1				
		Ruminococcus bromii	1.0	*Bacteroides* FO	1.0				

[a] Numbers following species designations represent variants that may be equivalent to subspecies. Letters (e.g., FB) or letters and numbers (e.g., S03) following genus designations represent recognizable but undescribed species.

cally determined predisposition for colorectal cancer. It might be useful to determine whether there is host genetic control of composition of the intestinal flora that could influence risk of colorectal cancer or other intestinal diseases.

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