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Human Fecal Flora: The Normal Flora of 20 Japanese-Hawaiians

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Quantitative and qualitative examination of the fecal flora of 20 clinically healthy Japanese-Hawaiian males was carried out by using anaerobic tube culture techniques. Cultural counts were 93% of the microscopic clump counts. Isolated colonies were selected in a randomized manner to give an unbiased sampling of the viable bacterial types. Each isolate was characterized for species identification. From a total of 1,147 isolates, 113 distinct types of organisms were observed. Statistical estimates indicate that these types account for 94% of the viable cells in the feces. The quantitative composition of the flora of this group of people, together with differential characteristics of previously unreported species, is presented for those kinds of bacteria which each represented at least 0.05% of the flora.

As a result of suggestions by workers at the Wright-Fleming Institute of possible relationships between the bacteria of the human colon and the incidence of colon or other cancer (2-4, 35-37), there has been increased interest in the distribution of different kinds of bacteria in feces.

Several hundred species have been reported from the fecal flora since Escherich (22) first described "Bacterium coli commune," and there have been innumerable reports of the distribution of various morphological or cultural bacterial groups (1, 5-9, 13, 15-20, 23-28, 30-34, 42-48, 50-62). Still, many questions remain concerning the identity and frequency of individual bacterial species in the flora, the variation in their relative numbers in different specimens or subjects, and the factors which may change the composition of the flora that can now be cultured.

Van Houte and Gibbons (58) reported microscopic counts of 3.2×10^{11} bacterial cells per g (wet weight) of feces. This would be about 10^{12} to 1.6×10^{12} per g (dry weight). Since the average bacterial cell of the fecal population occupies about 1 μm^3, the cells occupy more than 30% of the total wet volume. In the last decade, it has been demonstrated repeatedly that more than 50% of the human fecal flora is cultivable if strictly anaerobic techniques similar to those developed by Hungate (40) are employed (21, 49).

The present work is an effort to describe and quantitate those presently cultivable species, each of which comprises at least 0.05% of the normal fecal flora of this human population. These data are to be used subsequently for comparisons with flora of other groups of people and to evaluate conditions that may affect the relative concentrations of bacterial species which are numerically most significant.

MATERIALS AND METHODS

Feces from 20 clinically healthy Japanese-Hawaiian males, ages 60 to 80, whose normal diets range from Oriental to Western, were studied. These individuals were in an epidemiological health study conducted by the National Cancer Institute of the National Institutes of Health. The regular weekly diet of most individuals included western foods, such as beef, bacon, preserved meats (Spam, wieners, etc.), fresh vegetables (lettuce, tomatoes, corn), and Oriental foods, such as rice, fish, tofu, and Oriental-style vegetables. Ten of the 20 ate raw fish; 13 drank milk; 18 ate eggs; and 11 of 12 questioned ate chicken.

Previous work in this laboratory on specimens from North Americans indicated that different methods for shipping feces produced changes in the composition and decreases in the statistical "coverage" values (29) of the viable flora. Methods tested included freezing in liquid nitrogen or dry ice and alcohol, with or without glycerol diluent (14). Storage or shipping at ambient temperature (20 to 30 C) under CO_2 for 2 days produced the least change of any method tested, with no statistical difference from results obtained with immediate culture. However, to eliminate any possible changes in fecal specimens during shipment, all specimens were cultured immediately after collection.

Preliminary trials indicated that different 1-g subsamples of the stool often differed in bacterial compo-

sition. Therefore, each entire stool was mixed to obtain an estimate of the bacterial population. The entire fecal specimen was collected in a plastic bag and was immediately transported to the laboratory. Processing was initiated within 5 min of defecation. The bag was flushed with O_2-free CO_2, and the stool was thoroughly mixed by kneading. Approximately 1 g of the mixed feces was transferred to 9 ml of prereduced dilution salts solution (38) under O_2-free CO_2. Actual sample weight was determined from the increased weight of the first dilution tube. After vigorous shaking to completely disperse the sample, serial 10-fold dilutions were prepared with vigorous shaking of each restoppered tube before subsampling.

Direct microscopic clump counts were made from duplicate smears of 0.01 ml of the 10^4 dilution spread over 1 cm^2. Smears were heat-fixed and gently Gram-stained. Six edge fields and four center fields were counted by two people on each of the two squares. Both microscopic and cultural counts were correlated for the actual sample size.

Duplicate rumen fluid-glucose-cellobiose agar (RGCA)$_1$ roll tubes (25 × 142 mm) were inoculated under O_2-free CO_2 with 1 ml each of 10^8, 10^9, and 10^{10} dilutions and incubated at 37 C. After incubation for 16 to 72 h, the tubes were transported in an upright position from Honolulu, Hawaii, to Blacksburg, Va. Transport time was 24 h. After a total of 5 days of incubation at 37 C, roll tubes were marked with spiral lines, and the colonies were counted by at least two individuals. Counts between 30 and 300 colonies per tube were used to estimate the cultural count.

Moisture content of the fecal specimens was determined on 10-g samples of each stool (in duplicate for each specimen of sufficient size). Samples for this determination, and for chemical analyses to be reported elsewhere, were quick frozen in sealed plastic vials in liquid nitrogen and kept frozen until analyzed. Weighed samples were lyophilized for 6 h and reweighed to determine percent dry matter by difference. Direct microscopic clump counts and cultural counts were then corrected to a dry weight basis.

In an attempt to determine the ratio of different kinds of organisms in the normal flora, every well isolated colony, regardless of appearance, was picked in succession (top to bottom of the tube) until 55 colonies had been picked. Colonies that were so close together that they could not be picked separately or that were under an obvious area of surface spreading were not picked. The colonies were observed under a dissecting microscope (10 to 15 ×) as they were picked, and the roll tubes were flushed with O_2-free CO_2 during the picking procedure by using a CO_2 cannula and picking needle guide attached to the dissecting microscope stage (Bellco Glass Co., Vineland, N.J.). Colonies were picked into 3 ml of Sweet E broth (38) because, in studies not included here, it was found that many of the isolates required one or another disaccharide for growth.

All cultures resulting from the original single colonies were streaked on supplemented brain heart infusion agar (BHIA) or Sweet E agar streak tubes and again picked into 3 ml of Sweet E broth. If more than one colony type or more than one morphotype was observed, an attempt was made to isolate and characterize each. Each isolate was characterized according to procedures described previously (38). For most isolates, about 40 different media were inoculated and analyzed. In addition to usual biochemical tests and analyses for metabolic alcohols and acids, many isolates were tested for their ability to produce hydrogen. This characteristic was found to be useful for differentiating species with otherwise similar reactions. To detect hydrogen, 1 ml of head gas (usually from a fructose broth culture), taken with a needle through the edge of the rubber stopper, was chromatogrammed on a 6-ft by 1/16 in (182.88 cm by 0.48 cm), 80 to 100-mesh silicic acid column at 40 C with 16 ml of N_2 carrier gas per minute, using a thermal conductivity detector. Relative amounts of hydrogen were scored according to peak height. "Four plus" values represent more than 2% hydrogen in the head gas. Hydrogen was produced by many strains that did not produce obvious gas in deep agar tubes.

Isolates were identified according to criteria published in the Anaerobe Laboratory Manual (38), companion manuscripts (11, 12, 39), and as discussed below.

The data have been analyzed statistically to determine the composition of the flora of these people as a population and therefore do not consider person-to-person variation and day-to-day variation within persons. These variables will be discussed in a later publication.

RESULTS AND DISCUSSION

Microscopic and cultural counts. The microscopic and cultural counts obtained on the 20 specimens are given in Table 1. The microscopic count we obtained is not as reproducible as the cultural count. For the microscopic estimates, we used stained slides rather than a counting chamber, because the prepared slides were shipped from Honolulu to Blacksburg for counting. Our values for direct microscopic counts are generally lower than those reported by van Houte and Gibbons (58). This may result from loss of cells from the slide during staining, even though care was used to minimize losses by very gentle rinsing and Gram's staining procedures, or because we report a clump count rather than a single cell count. Pairs and short chains of cells that appeared to be firmly attached to each other and might be expected to give rise to a single colony were counted as one, as in the standard methods for examination of dairy products. Van Houte and Gibbons counted cells in a Petroff-Hausser counting chamber with simple stain, which might produce slightly high values because of the difficulty of distinguishing organisms from stained debris. The same error can occur on stained slides, but may be decreased when a

decolorization step is included. Van Houte and Gibbons' counts, averaging 31.6×10^{10} per g (wet weight) may be a better estimate than ours, which averaged 11.2×10^{10} per g (wet weight). However, we have found that the average moisture content of Japanese-Hawaiian feces exceeds that of North American feces. The moisture content of specimens examined by the two laboratories might account for nearly all of the difference observed in the total microscopic counts. The dry matter content of individual specimens in the present study had nearly a threefold variation, as shown in Table 1. If we assume that the count of van Houte and Gibbons is based on specimens of equal moisture content, and upon clump count rather than single cell count, 31.3% of their average microscopic count was cultured in the present study, whereas 87.96% of our microscopic count (per gram, wet weight) was cultured. The true cultural recovery probably falls within these extremes.

Comparison of van Houte and Gibbons' cultural counts with those obtained here indicates that strict anaerobic techniques and special media are essential. By using blood agar plates and anaerobe jars, they obtained an average cultural count of 8.3×10^{9} colonies per g (wet weight). With RGCA in pre-reduced roll tubes, we obtained an average cultural count of 9.9×10^{10} on a wet weight basis.

Reports of counts corrected for moisture as given by Attebery et al. (5) may provide a better basis for comparing results. Cultural counts in the present study averaged $10^{11.67}$ (4.75×10^{11})/g dry matter, 93.87% of the average microscopic count/g dry matter. This compares very favorably with an average on normal specimens of $10^{11.36}$ (2.27×10^{11})/g dry matter obtained by Attebery et al. (5) by using glove box procedures.

Except for specimen C-3, 95.4% of the total original "colonies" picked were characterized. Because recovery was poor from the first 55 "colonies" picked from specimen C-3, colonies from additional tubes were picked after 7 days of incubation. In all, only 44 viable subcultures were recovered from the 100 colonies picked from C-3. This fecal specimen had a moisture content of 87.5% and the appearance of diarrhea. However, the subject stated that this stool was of normal consistency for him. There were no unusual problems with cultural recovery from the other 19 specimens, some of which also had high moisture levels.

With the exception of specimen C-3, only occasional original colonies did not grow on subculture in Sweet E. Many of these "colonies" were originally described as "unusual" in appearance or "less than 0.2 mm in diameter." Some may have been unmelted bits of agar in the original roll tube or particles of debris in the rumen fluid of the RGCA. Some probably were bacterial types that we could not subculture. Also, because many isolates were obtained at the same time, it was sometimes necessary to store some of them. Isolates were lyophilized and/or frozen at −80 C and held at room temperature in Sweet E and chopped meat; a few of these did not survive any of these storage conditions.

TABLE 1. *Microscopic and cultural counts from 20 fecal specimens*[a]

Subject[b]	Dry matter (%)	Counts/g of dry matter[c]	
		DMCC[d]	Cultural
1	31.6	26.2	35.2
2	25.8	32.0	47.6
3	12.5	38.0	45.6
6		34.2	23.5[e]
7		49.1	56.0[e]
8	31.2	28.5	22.5
9	20.0	39.1	42.5
10	16.5	18.1	52.9
11	20.2	130.1	58.8
12	22.1	142.8	60.2
13	23.6	117.5	43.1
14	32.2	30.1	20.3
15	35.1	80.9	49.7
16	16.8	44.3	70.1
17	12.4	47.3	54.3
18	19.9	88.5	136.4
19	15.4	22.0	44.2
21	14.6	17.6	18.0
22	24.0	18.7	37.3
23	12.6	6.9	32.2
Average	21.5	50.6	47.5
Standard deviation		39.7	25.4

[a] Paired t test between microscopic and cultural counts, $t = 0.38$ (19 df = ns).

[b] Samples 4 and 5 were duplicate specimens used in shipping studies; specimen 20 was from a different treatment population. Therefore, they are not included in this study.

[c] Figures shown to be multiplied times 10^{10}.

[d] DMCC, Direct microscopic clump count.

[e] The estimate is based on 21.5% dry matter (the average for this population). There was insufficient sample to make a direct determination of the percentage of moisture.

Kinds of bacteria detected. The species and subspecies (kinds) of bacteria detected in individual fecal samples are given in Table 2. According to the formula of Good (29), the kinds

TABLE 2. *Occurrence of bacterial groups within individuals*

Specimen no.	No. of isolates	*B. fragilis* ss. *vulgatus*	*B. fragilis* ss. *thetaiotaomicron*	*B. fragilis* ss. *distasonis*	*F. prausnitzii*	*B. adolescentis*	*E. aerofaciens*	*P. productus*-II	*E. eligens*	*P. productus*-I	*E. biforme*	*E. aerofaciens*-III	*E. rectale*-I	Additional species at ca. 5% or more	Additional species at ca. 5% or less
1	62*	5	2	1	1	3	8	5	2	1			7	3: *B. fragilis* ss. a, *B. hypermegas*	2: *R. bromii*, *Peptococcus*-M, budding coccus 1: *E. rectale*-II, -III-H, *E. ventriosum*, *E. formicigenerans*, *E. hallii*, *Eubacterium*-AG, -AK, *L. leichmannii*, *C. sporosphaeroides*, *Bacteroides*-G, *R. albus*, *C. catus*, *C. comes*, *R. torques*, *Ruminococcus*-P
2	49	4	2			14	5	2						5: *B. longum* 3: *R. bromii*, budding coccus	2: *Eubacterium*-AK, *C. catus* 1: *Eubacterium*-N, *Escherichia* 'group', *B. fragilis* ss. a, e, f, *Bacteroides*-L, *R. callidus*
3	44				6		4	3	2	1	2			4: *L. acidophilus*, budding coccus	2: *E. rectale*-IV, *E. ventriosum*, *F. russii*, *R. bromii* 1: *E. rectale*-II, -III-F, *E. ruminantium*, *Eubacterium*-T, *L. leichmannii*, *L. minutus*, *Bacteroides*-J, -L, -L-3, *A. fermentans*
6	61	7			8	3		6	2		4	1	2	4: *B. fragilis* ss. c 3: *C. catus*	2: *E. aerofaciens*-II, *Eubacterium*-U, *Clostridium*-D 1: *E. rectale*-II, *Eubacterium*-AB, *Bifidobacterium*-C, *L. casei* var. *alactosus*, *L. leichmannii*, *E. hafnia*, *K. pneumoniae*, *S. faecalis* var. *liquefaciens*, *S. salivarius*, *S. epidermidis*, *B. fragilis* ss. a, *R. albus*, *R. bromii*, *P. asaccharolyticus*, *C. comes*
7	62	7	1		4	5		4		3		9	1	7: *E. rectale*-II 5: *S. hansenii*, *Ruminococcus*-AB	2: budding coccus 1: *E. aerofaciens*-II, *E. ventriosum*, *L. acidophilus*, *B. fragilis* ss. d, f, *Bacteroides*-K, *F. mortiferum*, *C. nexile*, *Ruminococcus*-AQ
8	60	9	2		1	5	15	3			1	1	3	3: *C. eutactus*	2: *B. bifidum*, *Bacteroides*-L 1: *E. aerofaciens*-II, *E. rectale*-III-F, *E. ruminantium*, *E. formicigenerans*, *Eubacterium*-AK, *B. fragilis* ss. a, d, e, f, g, *R. albus*, *Ruminococcus*-AJ, -AU
9	61	3	1	1	1	9	1	3	1	3	5	3	1	11: *B. infantis* 3: *B. longum*	2: *Eubacterium*-N-1, *S. faecalis*, *S. hansenii* 1: *Eubacterium*-AE, *B. bifidum*, *L. acidophilus*, *B. fragilis* ss. a, e, *F. symbiosum*, *Fusobacterium*-O, *Ruminococcus*-AL, *S. faecalis* var. *liquefaciens*
10	69	9	2	4	7	2	9	5		8	9		3	3: *B. longum*	2: *E. rectale*-II, *L. acidophilus* 1: *Eubacterium*-Z, *B. infantis*, *C. ramosum*-I, *B. fragilis* ss. a
11	51	4	5	1	4	2	2	2		2				5: *E. rectale*-II, *B. breve*, *B. fragilis* ss. *ovatus* 4: *L. salivarius* var. *salicinius* 3: *R. torques*	2: *B. infantis* 1: *E. aerofaciens*-II, *E. rectale*-IV, *Eubacterium*-AN, *B. furcosus*, *Coprococcus*-AN-H
12	56	5	2		8			5	4	1	1	1	1	5: *E. coli* 4: *E. rectale*-III-H, *R. albus* 3: *B. breve*	2: *E. ventriosum*, *R. bromii* 1: *E. rectale*-IV, *L. acidophilus*, *B. capillosus*, *B. fragilis* ss. a, g, *Bacteroides*-L-1, *F. russii*, *R. callidus*

TABLE 2. *cont'd.*

Specimen no.	No. of isolates	*B. fragilis* ss. *vulgatus*	*B. fragilis* ss. *thetaiotaomicron*	*B. fragilis* ss. *distasonis*	*F. prausnitzii*	*B. adolescentis*	*E. aerofaciens*	*P. productus*-II	*E. eligens*	*P. productus*-I	*E. biforme*	*E. aerofaciens*-III	*E. rectale*-I	Additional species at ca. 5% or more	Additional species at ca. 5% or less
13	58	20		4	12		4	2		1			1	4: *E. rectale*-II 3: *E. rectale*-IV	2: *L. leichmannii*, *B. fragilis* ss. a, *B. ruminicola* ss. *brevis* 1: *R. albus*
14	57	5	1	3		8	10	4		1		1		6: *B. breve* 4: *B. longum*	2: *Eubacterium*-U, *C. ramosum*-I, *B. fragilis* ss. d 1: *E. ventriosum*, *E. formicigenerans*, *B. pseudolongum*, *P. acnes*, *B. fragilis* ss. *fragilis*, *F. russii*, *Peptococcus*-M, *R. torques*
15	63	1						6	5		8	7		14: *E. rectale*-IV 4: *L. acidophilus* 3: *C. comes*, *R. callidus*	2: *E. rectale*-II, *Eubacterium*-U, *B. bifidum* 1: *E. ventriosum*, *Eubacterium*-AG, *B. capillosus*, *B. fragilis* ss. d, budding coccus, *C. eutactus*
16	62	6	8	4	9	11	3	1		1				3: *R. bromii*	2: *E. rectale*-II, *B. infantis*, *B. clostridiiformis*, *R. albus*, budding coccus 1: *E. ventriosum*, *B. longum*, *L. rogosae*, *B. fragilis* ss. h, *F. symbiosum*, *A. fermentans*
17	57	7	1	1		1		3	3	5	2			5: *B. fragilis* ss. b 4: *E. rectale*-III-F 3: *L. acidophilus*	2: *R. bromii* 1: *E. rectale*-IV, *A. fermentans*, *L. leichmannii*, *L. rogosae*, *Clostridium*-A, budding coccus, *C. comes*
18	56	10	1	4	2	1	1	1	5	2			9	6: budding coccus 5: *B. fragilis* ss. b	2: *B. infantis*, *B. hypermegas* 1: *E. rectale*-III-F, *B. fragilis* ss. h, *R. bromii*, *S. intermedius*, *C. eutactus*
19	54	9	8	1				5	3	3	1	3		10: *B. fragilis* ss. a	2: *Ruminococcus*-P 1: *E. rectale*-III-F, *E. formicigenerans*, *P. acnes*, *B. fragilis* ss. *fragilis*, *B. eggerthii*, *R. bromii*, *S. hansenii*, *Coprococcus*-BN-1, *Ruminococcus*-BP
21	61	15	8	4			1	1	1	5		3		5: *B. fragilis* ss. *fragilis*, *B. fragilis* ss. a	2: *C. innocuum*, *B. clostridiiformis* 1: *E. limosum*, *E. rectale*-II, -III-F, *Eubacterium*-AY, *B. longum*, *C. ramosum*, *Bacteroides*-G, *R. torques*, *S. hansenii*
22	54	8	4		3	3	7		6	2	5			3: *S. faecalis* var. *liquefaciens*, *R. albus*	2: *R. torques* 1: *E. rectale*-II, -III-F, *Eubacterium*-AQ, *P. acnes*, *B. fragilis* ss. *fragilis*, ss.*ovatus*, *S. intermedius*, *Ruminococcus*-AJ
23	50	6	4		4	8		4	9					5: *B. longum* 3: *B. fragilis* ss. *ovatus*	1: *E. aerofaciens*-II, *E. rectale*-IV, *S. salivarius*, *S. intermedius*, *Bacteroides*-AA, *S. epidermidis*, *R. torques*

* Number of isolates

of organisms isolated (based on 44 to 69 isolates from individual specimens) account for 71 to 95% of the total viable cells (71 to 95% "coverage") in the individual fecal samples. The "% coverage" according to Good (29) = 1 - (number of kinds observed once/total number examined) × 100. The coverage of individual fecal specimens can be calculated from the observed data reported in Table 2 (e.g., for specimen C-1, % coverage = 1 - 18 [number of kinds seen once]/62 [number of isolates] × 100, which is 71%.)

There were 113 different kinds of organisms detected among the 1,147 isolates examined from the 20 people. In Table 3, the kinds of organisms are listed in order of frequency of their occurrence in this human population. The 113 kinds account for 94% of the viable cells (a 94% coverage) of the fecal flora of these individuals as a population. For this calculation of coverage and for the calculated concentrations of individual species, we used hypothesis H_9 of Good (29), in which the expected frequency is determined by S/r $(r + 1)$, where S = the number of kinds observed (113) and r = the observed frequencies of the various kinds. These calculations give more conservative estimates of the coverage and of the concentrations of individual species than would be obtained on the basis of the observed values.

The expected values obtained with hypothesis H_9 and the observed values match quite well for those kinds that were observed 10 or more times. However, the statistical estimates indicate that the number of species seen only once or twice is smaller than would be expected. One representative was observed for each of 38 species, but hypothesis H_9, which fits the rest of the data well, predicts that 56.5 kinds should be observed once. Thus, there is an indication that we may have combined in single groups some kinds which should actually be separated. Although characterization was fairly extensive, this appears to be inevitable with present methods of phenotypic characterization. Even though phenotypic differences usually indicate different genetic groups, there are even more genetically distinct organisms than now can be differentiated phenotypically. For example, Johnson (41) reported on the basis of DNA/DNA homology by competition experiments that there are at least three distinctly different genetic groups of organisms included in those that we label *Bacteroides fragilis* ss. *thetaiotaomicron*, two in ss. *ovatus*, and two in ss. *fragilis*. The genetic differences imply that there are major metabolic or chemical differences within the species or subspecies that now appear to be phenotypically uniform. Attempts to find phenotypic characteristics that differentiate the distinct genetic groups have not yet been successful.

Preliminary statistical analyses of these and additional data by I. J. Good (personal communication) indicate that the total number of different kinds of bacteria in the intestinal tract at any one time probably exceeds 400 or 500 species, but most of these are represented by less than 10^8 cells per g of feces (less than 1/1,000 of the bacterial population).

We recognize that the isolation methods used would not have detected some organisms with very specialized nutritional requirements if they, too, occurred in high numbers. However, it is reasonably certain that the isolation methods used here did not select against the facultatively anaerobic organisms in the fecal specimens: (i) because the cultural recovery accounts for such a large proportion of the total cellular population; (ii) because facultative species were isolated in numbers equal to or exceeding those reported from aerobic culture (13, 17, 23, 25-27, 31, 33, 34, 43, 45, 55-58, 62); (iii) because the organisms must be able to grow under anaerobic conditions more strict than those we are able to provide on a routine basis in vitro, if they are to be competitive in the colon; and (iv) because we have observed that some species of facultative bacteria such as *Escherichia coli*, lactobacilli, and some streptococci from other sources, including infections, can often be recovered in higher numbers under strictly anaerobic conditions than they can under aerobic conditions. In these latter cases, the microenvironment of the infection is usually anaerobic, as is the environment of feces.

It is most significant that many of the more numerous fecal organisms have not been reported from human infections. *B. fragilis* ss. *fragilis* is by far the most common anaerobe in clinical infections of soft tissue, but here it occurred at only 0.6% of the fecal flora. It is evident that the body defenses control and eliminate several hundred kinds of bacteria that may enter tissue after bowel trauma, because only a relatively few are capable of initiating infection. These present observations indicate that previous reports (49) of anaerobes occurring as predominant organisms in soft tissue infections as frequently as facultative organisms do not result from contamination detected with improved anaerobic methods. If that were the case, the more numerous (but less pathogenic) species would have been found. Direct micro-

TABLE 3. *Relative frequency of bacterial species of the normal fecal flora of 20 Japanese-Hawaiians*

Rank	Count[a]	Flora[b]	Organism(s)
	($\times 10^{10}$)		
1	5.76 (.49)	12.1 (1.0)	*B. fragilis* ss. *vulgatus*
2	3.40 (.38)	7.15 (.79)	*F. prausnitzii*
3	3.07 (.36)	6.45 (.75)	*B. adolescentis*
4	2.86 (.34)	6.02 (.72)	*E. aerofaciens*
5	2.65 (.33)	5.58 (.70)	*P. productus*-II
6	2.11 (.30)	4.45 (.62)	*B. fragilis* ss. *thetaiotaomicron*
7	1.74 (.27)	3.67 (.56)	*E. eligens* (39)
8	1.58 (.26)	3.32 (.54)	*P. productus*-I
9	1.53 (.25)	3.23 (.53)	*E. biforme*
10	1.16 (.22)	2.45 (.46)	*E. aerofaciens*-III
11–12[c]	1.12 (.22)	2.36 (.45)	*E. rectale*-I, *B. fragilis* ss. *distasonis*
13	1.08 (.21)	2.27 (.44)	*E. rectale*-II
	($\times 10^{9}$)		
14	9.97 (2.0)	2.10 (.43)	*B. fragilis* ss. a
15	9.14 (1.9)	1.92 (.41)	*E. rectale*-IV
16	8.73 (1.9)	1.84 (.40)	*B. longum*
17	8.32 (1.8)	1.75 (.39)	"Budding coccus" of Gossling
18	7.08 (1.7)	1.49 (.36)	*B. infantis*
19	6.67 (1.7)	1.40 (.35)	*R. bromii*
20	6.26 (1.6)	1.32 (.34)	*L. acidophilus*
21	5.44 (1.5)	1.14 (.32)	*B. breve*
22	5.02 (1.4)	1.06 (.30)	*R. albus*
23–24	3.80 (1.2)	.799 (.26)	*B. fragilis* ss. b, *E. rectale*-III-F
25–28	3.39 (1.2)	.713 (.25)	*E. ventriosum*, *B. fragilis* ss. *ovatus*, *R. torques* (39), *S. hansenii* (39)
29	2.98 (1.1)	.628 (.23)	*B. fragilis* ss. *fragilis*
30–34	2.17 (.94)	.458 (.20)	*E. aerofaciens*-II, *Eubacterium* U, *L. leichmannii*, *C. catus* (39), *C. comes* (39)
35–42	1.77 (.84)	.374 (.18)	*E. rectale*-III-H, *B. bifidum*, *E. coli*, *S. salivarius*, *B. fragilis* ss. d, *R. callidus* (39), *Ruminococcus* AB, *C. eutactus* (39)
43–49	1.38 (.74)	.291 (.16)	*E. formicigenerans* (39), *Eubacterium* AK, *L. salivarius* var. *salicinius*, *B. clostridiiformis*, *B. fragilis* ss. c, *Bacteroides* L, *F. russii*
	($\times 10^{8}$)		
50–58	9.94 (6.2)	.209 (.13)	*C. ramsoum*-I, *P. acnes*, *B. fragilis* ss. e f, g, *B. hypermegas*-I, *S. intermedius* (39), *Peptococcus* M, *Ruminococcus* P
59–75	6.21 (4.8)	.131 (.10)	*E. ruminantium*, *Eubacterium*-N-1, AG, *L. rogosae* (39), *C. innocuum*, *Clostridium* D, *S. faecalis*, *S. faecalis* var. *liquefaciens*, *S. epidermidis*, *B. capillosus*, *B. fragilis* ss. h, *B. hypermegas*, *B. ruminicola* ss. *brevis*, *Bacteroides* G, *F. symbiosum*, *A. fermentans*, *Ruminococcus* AJ
76–113	2.76 (3.1)	.058 (.06)	*E. limosum*, *E. hallii* (39), *Eubacterium* N, T, Z, AB, AE, AN, AQ, AY, *B. pseudolongum*, *Bifidobacterium* C, *L. casei* var. *alactosus*, *L. fermentum*, *L. minutus*, *C. sporosphaeroides*, *C. nexile* (39), *C. ramosum*, *Clostridium* A, *Escherichia* "group," *E. hafnia*, *K. pneumoniae*, *B. furcosus*, *B. eggerthii* (39), Bacteroides J, K, L-1, L-3, AA, *F. mortiferum*, *Fusobacterium* O, *P. asaccharolyticus*, *Ruminoccocus* AL, AQ, AU, BP, *Coprococcus* (39) AN-H, BN-1

[a] The estimated count per gram of fecal dry matter (± standard deviation of the estimate) is given.
[b] The percentage of fecal population (± standard deviation of the percent) is given.
[c] Where two rank numbers are listed, each organism cited was detected with equal frequency.

scopic observations of clinical material, the relatively few (1 to 7 or 8) types of bacteria isolated from such individual infections, and the more limited range of species found in clinical infections further suggest that only a minority of the organisms in the intestinal flora is pathogenic.

The extremely dense and heterogeneous population of bacteria in feces was the subject of much interest and conjecture, even before it was known that nearly all, if not all, of the bacteria observed are viable, metabolizing cells. Suggestions have been made that a major source of nutrient for these organisms includes sloughed epithelial cells and other host protein materials. However, proteolytic activity was noticeably absent or very weak among the predominant organisms encountered in the present study. Ammonium salts provide a suitable nitrogen source for several of the most numerous species, and specific carbohydrates apparently are essential for growth of many others.

On the basis of these observations, we might expect the type and amount of complex carbohydrate in the diet, or mucin, to exert an important controlling influence upon the composition of the flora. On the basis of work in our laboratory by Diwan Singh (M.S. thesis, Virginia Polytechnic Institute, Blacksburg, 1968), we may further expect the effect of changes in the diet to be quite complex. He found that several intestinal isolates of predominant species inhibit the growth of other intestinal species. Thus, changes in diet may either stimulate certain groups or fail to support growth of species that normally control others. Perhaps, as careful attention is given to individual properties and relative numbers of the bacteria present, some basic principles regarding the relationship between the flora and its host and environment can be established.

Identification of intestinal isolates. We have accepted more variation in characteristics of certain "species" than of others, because, whereas some groups of strains are extremely uniform in characteristics, other groups form a continuum of phenotypes with no obvious dividing lines. Every attempt has been made to recognize the distinctly different organisms encountered, and the descriptions reported here are based on the best information available to us now. However, they may require further modification as additional detailed studies of the isolates are performed and more examples of each are encountered.

New species. The new named species in Tables 2 and 3 include only those for which we had nine or more isolates from at least six persons in this and related studies. Descriptions of these species and of the genus *Coprococcus* are being published elsewhere (39).

Previously described species. Although we used procedures described in the Anaerobe Laboratory Manual (38) to identify the isolates, the species described there are mostly those that also occur in clinical specimens. Therefore, the number of described species and subspecies is relatively limited, and the number of tests required for their differentiation is likewise limited. There are many more species found in the intestinal flora, some of which are quite similar to those previously described, but which can be shown to differ in a number of properties if they are examined in more detail. On the basis of many new intestinal isolates in this and related studies, some additional description and differentiation of previously described species is necessary.

Eubacterium. Some strains of *Eubacterium aerofaciens* are very similar to strains of *Streptococcus intermedius*. We have differentiated these species primarily on the basis of morphology and hydrogen production. All cells of *S. intermedius* are nearly spherical, whereas some cells in cultures of *E. aerofaciens* are ovoid or definite rods. Rods with swellings in the center ("pelton de jardinier" or gardener's ball of string on a stick), as mentioned in the original description by Eggerth (18), are sometimes produced in peptone yeast extract (PY) basal medium and help in recognition of the species. All strains of *S. intermedius* produce acid from cellobiose, but this characteristic is variable in *E. aerofaciens*. A cotype strain of *S. intermedius* produces no hydrogen. Strains of *E. aerofaciens* produce copious hydrogen. Many strains of *E. aerofaciens* produce large amounts of formic acid. We recognize three phenotypic groups of *E. aerofaciens*. Those labeled "*E. aerofaciens*-II" uniformly do not ferment sucrose; those labeled "*E. aerofaciens*-III" uniformly do not ferment cellobiose and produce little or no acid in salicin.

Eubacterium biforme strains conform well to the original description by Eggerth (18). This species is well named. Definite rod-shaped cells may not appear in all cultures of each strain, but, when they do, observation of cocci attached to rods is the only assurance that the culture is not a mixture. The production of caproic acid in small amounts is a most distinguishing characteristic, but sometimes is only detected on repeated culture.

Eubacterium cylindroides: See discussion under *Fusobacterium prausnitzii* (below).

Eubacterium rectale strains fall into at least

five distinct groups. All strains ferment cellobiose, fructose, glucose, lactose, maltose, and starch. They decolorize readily, and gram-positive cells cannot be demonstrated in some strains. The rods are usually curved and usually motile or have flagella singly, in pairs, or in tufts near one or both ends of the cells. Butyric acid is a major product, together with variable amounts of lactic acid. Strains labeled "*E. rectale*-I" are slender curved rods, generally longer than the other phenotypes, frequently with large central or terminal swellings. They uniformly ferment arabinose, melezitose, melibiose, raffinose, sucrose, and xylose, and produce large quantities of hydrogen. Strains of *F. mortiferum* may be similar to "*E. rectale*-I", except that they are thicker rods showing more pleomorphism, especially on blood agar, and have no flagella.

Strains labeled "*E. rectale*-II" differ from "*E. rectale*-I" strains in that they fail to ferment melezitose and may or may not ferment melibiose and sucrose. They are generally shorter, more uniform, curved rods.

Strains labeled "*E. rectale*-III-H" differ from "*E. rectale*-II" strains in that they fail to ferment raffinose. Heat-resistant spores have been detected in some strains otherwise similar to these three types of *E. rectale*. The strains with spores have been designated as "*Clostridium*-A" (see Table 5). Many strains with swellings, however, do not resist heating at 80 C for 10 min, and are believed to be *Eubacterium* species. Strains labeled "*E. rectale*-III-F" have the same reactions as "III-H" strains, except that they produce major amounts of formic acid and no hydrogen. We have not demonstrated spores in any strains of this type. Strains labeled "*E. rectale*-IV" are similar to "*E. rectale*-III-H" strains. However, they fail to ferment xylose, may or may not ferment raffinose, and never reduce the pH in arabinose to below 5.5. The relationship of the *E. rectale* strains to *Butyrivibrio fibrisolvens* is in question. A number of the different variants of *B. fibrisolvens* from the rumen of cattle that Bryant and Small (10) described correspond closely to some *E. rectale* strains described here which do not stain gram-positive. However, the reference strains of *B. fibrisolvens* generally are even more fastidious than these isolates from humans. The isolates from humans are less aerotolerant than most other human intestinal species, as reported by Attebery et al. (5). The reference strains of *B. fibrisolvens* that we have examined are monotrichous and never stain gram-positive, even in 4- to 6-h cultures. Bryant (personal communication) states that, rarely, a rumen strain presumed to be a *Butyrivibrio* species may stain weakly gram-positive.

Strains labeled *E. ruminantium* and *E. ventriosum* are very similar. On the basis of reactions of reference strains, we have designated those that ferment cellobiose and produce no hydrogen as *E. ruminantium*. These strains uniformly fail to produce acid in mannose, but usually produce acid from salicin. Strains of *E. ventriosum* either do not ferment cellobiose or they produce hydrogen. They often ferment mannose and do not ferment salicin. Both of these species are coccoid to short rods. The reactions and products (major formic acid, with variable amounts of butyric, lactic, acetic, and succinic acids) are similar to those we obtained with the "budding coccus" of Gossling (J. Gossling, Abstr. Annu. Meet. Amer. Soc. Microbiol., 1972, M 7, p. 81); however, the cells of the budding coccus are more nearly spherical, as are the attached "buds." Cultures of all three species may stain weakly gram-positive. Gossling demonstrated that the cell wall structure of the "budding coccus" is gram-negative; the two *Eubacterium* species are considered to be gram-positive.

Lactobacillus. Many intestinal isolates of *Lactobacillus acidophilus*, *L. leichmannii*, and other named species of *Lactobacillus* fail to grow on aerobic plates, even after several transfers in anaerobic media. None of the reference strains of named species or strains of lactobacilli from the intestinal tract produced hydrogen. Strains of some other species (*E. aerofaciens* and *Clostridium ramosum*) may differ only slightly in morphology or cultural reactions, but they produce larger (moderate) amounts of acetic and formic acids and major amounts of hydrogen.

L. leichmannii: Also, see discussion under *C. ramosum* (below).

Bacteroides. *Bacteroides fragilis* includes a number of intermediate phenotypic groups that have not been described as subspecies previously. The differential characteristics of these groups are given in Table 4.

Bacteroides hypermegas was found in one fecal sample. In another sample, we isolated strains with the same biochemical reactions and products, including very large amounts of propionic acid, but the cells are not of the large size characteristic of *B. hypermegas*. We have labeled these smaller strains "*B. hypermegas*-I" and suspect that they are a distinct species.

Bacteroides ruminicola ss. *ruminicola* and ss. *brevis* are limited to those strains that do not produce hydrogen. A number of strains with biochemical characteristics similar to those

TABLE 4. *Characteristics of other subspecies groups of B. fragilis**

Characteristic	Subspecies group							
	'a'	'b'	'c'	'd'	'e'	'f'	'g'	'h'
No. strains	75	27	4	7	3	5	9	2
Amygdalin	v	a	−	w−	−	−w	a−	w
Arabinose	−a	a	−	aw	wa	−	a−	a
Cellobiose	−w	a	−	w−	a	−	aw	w
Esculin	a−	a−	w	w	wa	−	w−	w
Glycogen	a−	a	a	nt	nt	−	a−	a
Mannitol	−	a	−	−	−	−	−	−
Mannose	aw	a	a	aw	wa	aw	v	a
Melezitose	−	−a	−	−a	−	w	−	−
Melibiose	−w	a−	aw	w	nt	aw	aw	−
Raffinose	wa	a	a	a	a	v	a	−w
Rhamnose	wa	v	−	aw	a	w	−w	a
Ribose	v	−a	−	nt	nt	−	−	a
Salicin	−w	a	−	nt	nt	−	−a	−
Sorbitol	−	a−	−	nt	nt	−	−	−
Starch pH	a	a	a	−	a	wa	aw	a
Starch hydrolysis	+−	+	+	−	+−	−	+−	+
Trehalose	−	−	−	a−	a	a	−	aw
Xylose	aw	a	a	a	a	aw	a−	a
Gelatin digestion	−w	+−	−w	−+	−w	+−	v	+w
Indole	+	−	−	−	+	−	−	+
Hydrogen	−,2	−	nt	1	nt	nt	2,3	nt

All strains are non-motile and produce acid from fructose, glucose, lactose, maltose, and sucrose. They coagulate milk, hydrolyze esculin, grow well in PYG-bile broth, and do not reduce nitrate, produce catalase, or digest milk. No strain tested (representatives of subspecies 'a', 'b', 'c', 'f', 'g', 'h') produces acid from erythritol or inositol.

* Abbreviations and symbols: a (carbohydrate cultures), acid (below pH 5.5); +, positive reaction; −, negative reaction; nt, not tested; v, variable reaction; w, weak reaction or pH between 5.5 and 6.0; numbers (growth and gas), amount estimated on "− to 4+" scale. Where two reactions are given (e.g., aw), the first is the more usual and the second is observed less frequently.

listed for these two subspecies were found, and growth was inhibited or not stimulated by bile after transfers directly from the isolation medium. However, many of these strains produced hydrogen, and upon retesting their growth was stimulated by bile; they were therefore identified as *B. fragilis*.

Butyrivibrio. *Butyrivibrio fibrisolvens:* See discussion under *E. rectale* (above).

Fusobacterium. Based on the study of many new isolates, emended descriptions of *F. prausnitzii* and *E. cylindroides*, organisms which are quite similar in many respects, are being published elsewhere (11, 12). Strains of *F. prausnitzii* generally fail to ferment carbohydrates, although one or several may be weakly fermented. All strains hydrolyze esculin as tested by ferric ammonium citrate, and they produce little or no gas. Cells are usually stout with rounded ends, may have swellings, and often stain unevenly. They are not motile and produce no hydrogen. The major fermentation products include butyric acid with variable amounts of formic and lactic acids and often minor or trace amounts of acetic, succinic and/or pyruvic acids and ethanol.

Strains of *F. prausnitzii* are differentiated from *E. cylindroides*, which decolorizes easily, in that all strains of *E. cylindroides* produce acid (below pH 5.5) in mannose, usually in glucose and/or fructose, and sometimes in salicin and sucrose (11). *F. prausnitzii* is most easily differentiated from *F. mortiferum* in that *F. mortiferum* produces major amounts of gas and acid in a number of carbohydrate media. *F. prausnitzii* is most easily differentiated from *F. russii* in that *F. russii* does not hydrolyze esculin.

Clostridium. *Clostridium ramosum* and "*C. ramosum*-I" have similar reactions and morphology; however, strains labeled *C. ramosum* produce large amounts of hydrogen and usually ferment melibiose and raffinose, whereas "*C. ramosum*-I" does not. "*C. ramosum*-I" differs from *L. leichmannii* in producing large amounts of formic acid as the major acid product.

Although cell size is similar in many cultures, thick cells are not observed in any cultures of "*C. ramosum*-I" as they may be in young cultures of *L. leichmannii*. Both species produce D(−) lactic acid.

Cocci. *Acidaminococcus fermentans* and *Peptococcus prevotii* have a similar appearance and similar reactions and, as has been noted by others, both may stain weakly gram-positive. Strains of *P. prevotii* (including the type strain) become weakly fermentative if Tween 80 is added to glucose or fructose, whereas strains of *A. fermentans* remain nonfermentative.

Budding coccus of Gossling: See discussion under *E. ruminantium* (above).

Peptostreptococcus productus strains form a continuum of phenotypes. All strains hydrolyze esculin and ferment glucose, fructose, raffinose, and xylose and a majority of the other carbohydrates tested. Individual strains may fail to ferment a few of the other carbohydrates. Hydrogen production is variable (usually large quantities are produced). All strains produce acetic acid, usually as the major product. Most strains also produce variable amounts of succinic and lactic acids. Ethanol and pyruvic acid may be produced in small amounts. The cells in some cultures become extremely elongate and may appear as tapered rods. Typically, pointed oval (football-shaped) cells occur in chains. The strains labeled "*P. productus*-I" produce moderate to large amounts of formic acid and uniformly ferment lactose. The strains labeled "*P. productus*-II" do not uniformly ferment lactose, but, unlike *P. productus*-I, they uniformly ferment arabinose, maltose, and sucrose, and they do not produce formic acid.

S. intermedius (39): See discussion under *E. aerofaciens* (above).

The identification of facultative cocci listed here was based on direct comparison of results from labeled ATCC strains characterized in anaerobic media with results from the facultative cocci obtained in this study, using the same culture procedures as for anaerobes. Identity of the ATCC strains was verified by comparing results with published descriptions.

Groups or strains that do not belong to described species. Cultural reactions of strains not belonging to recognized species or groups discussed above are given in Table 5 and the discussion following. In addition to the characteristics given in Table 5, the strains did not: produce acid from dulcitol, grow in PY-6.5% NaCl-glucose broth, produce propionate from threonine, or produce acetylmethylcarbinol (AMC), unless specifically stated below. Also, unless otherwise indicated, neutral red was reduced, and growth was as good in routine prereduced broth medium supplemented with heme and vitamin K as it was in medium with rumen fluid (10 or 30%) or Tween 80 (0.01%) added. No strain grew on the surface of blood agar plates incubated in a candle jar or aerobic atmosphere.

In the characteristics given below, the deep agar colonies are in prereduced RGCA roll tubes after incubation for 5 days. Surface colonies were observed on brain heart infusion agar or Sweet E agar streak tubes or on anaerobic sheep blood agar plates after incubation for 1 to 2 days.

Eubacterium. *Eubacterium*-N: Deep agar colonies are pinpoint to 0.5 mm in diameter, tan, lenticular, and translucent. Agar surface colonies are 0.5 to 1 mm in diameter, circular, convex or flat, entire, opaque, dull, and smooth.

Glucose broth cultures have sediment that adheres to the bottom of the tube and usually have no turbidity; the pH is 5.8 in 5 days.

Eubacterium-N-1: Deep agar colonies are 0.2 to 1 mm in diameter, white, lenticular, and translucent. Agar surface colonies are 0.5 to 1 mm in diameter, circular, entire, convex, opaque, dull, and smooth.

Glucose broth cultures have ropy sediment without turbidity; the pH is 5.7 to 6 in 5 days. Tween 80 (0.01%) is required for maximum growth; growth is produced in PY-6.5% NaCl-glucose broth.

Eubacterium-T: Deep agar colonies are 2 mm in diameter and rhizoid. Agar surface colonies are 4 mm in diameter, circular, entire, opaque, white, shiny, and smooth.

Glucose broth cultures have a stringy, mucoid sediment, usually with no turbidity; the pH is 5 in 5 days. AMC is produced.

Eubacterium-U: Deep agar colonies are 1 to 4 mm in diameter and of "raspberry" form. Surface agar colonies are 1 to 4 mm in diameter, circular, flat to low convex, translucent, and dull.

Glucose broth cultures have stringy (sometimes grainy or smooth) sediment and usually have no turbidity; the pH is 5 to 5.4 in 5 days. Neutral red is not reduced by three of the five strains tested.

Eubacterium-Z: Deep agar colonies are 1 to 2 mm in diameter and appear as fluffy "wooly balls." Agar surface colonies are 1 to 2 mm in diameter, circular, low convex, entire, and granular.

Glucose broth cultures are turbid with smooth or stringy sediment; the pH is 4.9 to 5.3 in 5 days. AMC is produced.

Eubacterium-AB: Deep agar colonies are 0.5 mm in diameter, white, and lenticular. Agar surface colonies are 3 to 5 mm in diameter,

TABLE 5. *Characteristics of less common bacteria of the fecal flora**

Characteristic	*Eubacterium* - N [bals] *	*Eubacterium* - N-1 [bl(a)]	*Eubacterium* - T [FA2]	*Eubacterium* - U [BFla]	*Eubacterium* - Z [Bl(s,py)]	*Eubacterium* - AB [AFls2]	*Eubacterium* - AE [B(Als,py)]	*Eubacterium* - AG [A2(ls)]	*Eubacterium* - AK [(bals)]	*Eubacterium* - AN [BL]	*Eubacterium* - AQ [Als2]
No. strains	4	6	1	5	6	2	3	3	6	4	1
Amygdalin	-	w–	w	–	–w	–w	–	–	–	w	–
Arabinose	-	–	–	–w	–	a	aw	-	-	w	–
Cellobiose	-	–	–	aw	–	a	aw	-	-	a	–
Dextrin	–	–	–	–	–	–w	–	-	–	w-	–
Erythritol	-	-	–	–	–	–	-	-	-	-	–
Esculin pH	-	–	–	–	–w	w	–	-	–	–	–
Esculin hyd.	-	–	–	+	+–	+	+	–	–	+	–
Fructose	wa	–w	–	a	a	a	w	aw	-	a	–w
Galactose	–w	–	w	a	wa	aw	-	-	-	a	–
Glucose	w–	w–	a	a	a	a	–w	–	-	a	a
Glycerol	-	-	–	–	–	–	-	-	-	-	–
Glycogen	-	–	–	–	–	–	-	–	-	-	–
Inositol	-	–	–	–	a	–	-	–	-	-	–
Inulin	-	-	–	–	aw	a	–	–	-	a	–
Lactose	-	–	–	a	a–	a	–w	–	-	v	–
Maltose	-	–	a	aw	v	a	–	–	–	a	–
Mannitol	-	–	–	–w	–w	–	-	-	-	-	–
Mannose	v	–	a	w–	a	–	-	-	-	w	–
Melezitose	-	–	–	–	–	–	-	–	-	-	–
Melibiose	-	–	–	aw	–w	w	-	–	-	-	–
Raffinose	-	–	–	a	–	a	-	–	-	-a	–
Rhamnose	-a	–	–	–	–	–	-	-	-	-	a
Ribose	–	–	–	–	–	–	-	–	-	-	a
Salicin	-w	–	–	aw	v	a	-	-	-	-	–
Sorbitol	-	–	–	v	–w	a	-	-	-	-	–
Sorbose	-	–	w	–	aw	–	–	-	-	-	–
Starch pH	-	–	–	–	–	–	-	–w	–	w–	–
Starch hyd.	-	–	–	–	–	–	-	–	–	–	–
Sucrose	-	–w	a	v	a	a	-	-	-	a	–
Trehalose	–	–	–	–	v	–	-	-	–	-	–
Xylose	–	–	–	–	aw	–	aw	–	-	a–	–
Gelatin dig.	–	–	–	–	–	–	–	–	w–	–	–
Milk	–	–	–	–	–c	–	c	–	–	–c	–
Indole	–	–	–	–	–	–	–	–	–	–	–
EYA react.	–	–	–	-	-	–	-	-	-	-	–
Hemolysis	-	-	–	-	-	-	-	-	-	-	–
H_2S	–	–	–	–	–	w	–	–	–	–	–
Gas	1	–	1	4	3,4	4	v	–	–	4	4
Hydrogen	4	–	1	4	4	4	4	–	–	4	4
PY-growth	–,1	1	2	2,1	2	2,3	–,1	1	1,–	–	2
PY-CHO gr.	2	4	4	4	4	4	2,3	2,3	1,–	4	4
PYG-bile gr.	1	3	–	4	3,4	–	2,–	2,1	–,1	4	2,3
Pyruvate	(A)	–	AF	AB	B(A)	AF	–	A(F)	–	-	A
Gluconate	-	–	–	(AB)	–		-	–	–	-	–
Motility	–	–	–	–	–	–	v	–,+	–	–	–
Morphology											
10 microns											

TABLE 5. *cont'd.*

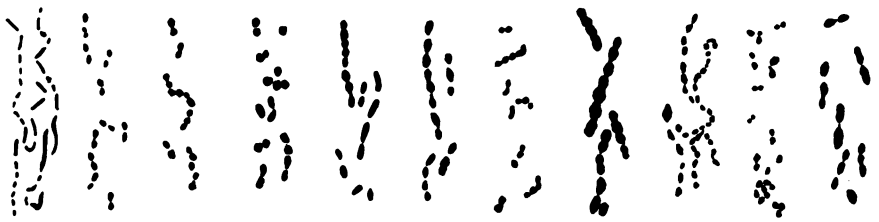
Characteristic	*Eubacterium* - AY [ASl]*	*Coprococcus* - AN-H [Fbal(s)]	*Coprococcus* - BN-1 [Fbla]	*Peptococcus* - M [CLhiwbibas(vpc')]	*Ruminococcus* - P [FA2(ls,py)]	*Ruminococcus* - AB [FA2ls(py)]	*Ruminococcus* - AJ [FA2ls(py)]	*Ruminococcus* - AL [ASl]	*Ruminococcus* - AQ [AF2(sl,py)]	*Ruminococcus* - AU [AL2s]	*Ruminococcus* - BP [AS(l,py)]
No. strains	2	1	1	4	4	5	3	1	2	1	4
Amygdalin	–	–	a	–	-	v	–	a	–	–	–
Arabinose	a	-	–	–	-	a	–w	–	–	–	aw
Cellobiose	–	-	–	–	-	–	-	a	–	–	–
Dextrin	w	w-	w	–	–	w–	-	–	–w	–	-
Erythritol	–w	-	-	–	-	–	-	-	–	–	-
Esculin pH	–	w	–	–	–	w	-	a	–w	–	–
Esculin hyd.	–	+	+	–	+	+	–	+	+	–	+
Fructose	aw	a	a	–w	aw	aw	a	a	a	aw	a
Galactose	wa	a	a	–	a	aw	-	a	a	–	aw
Glucose	aw	a	a	–	a	a	w	a	a	w–	aw
Glycerol	v	-	-	–	-	–	-	-	–	–	-
Glycogen	–	-	–	–	–	–	–	-	–	–	-
Inositol	–	-	-	–	-	–	-	-	–	–	-
Inulin	–	-	-	–	-w	wa	-	-	–w	–	a
Lactose	a–	a	-	–	-	–	-	a	a	–	a
Maltose	a	a	a	–	a	a	v	a	a	–	a
Mannitol	a	-	-	–	-	–	-	-	–	–	-
Mannose	w	a	a	–	–	–	–w	–	–	–	a
Melezitose	–	-	-	–	-	–	-	-	–	–	-
Melibiose	–	-	–	–	-	w	-	-	aw	–	a
Raffinose	–	-	–	–	-	a	-	-	v	–	a
Rhamnose	–	-	-	–	-	a	a-	-	–	–	a
Ribose	–	-	-	–	–w	a	-a	-	–	–	wa
Salicin	–	a	a	–	-	a	-	a	–w	–	-
Sorbitol	a	-	-	–	-w	–	-	-	–	–	-
Sorbose	–	-	-	–	-	–	-	-	–	–	-
Starch pH	–	w	w	–	–w	w–	-	–	–w	–	-
Starch hyd.	–	–	–	–	–	+	–	–	–	–	-
Sucrose	–	v	a	–	–w	a	-	-	–	–	–w
Trehalose	–	-	-	–	–	–	-	-	–	–	-
Xylose	wa	-	-	–	–a	aw	-	-	–	–	a
Gelatin dig.	–	–	–	–	–	–	–	w	–	–	–
Milk	–	c–	–	–	–	–	–	c	–	–	–c
Indole	–	–	–	–	–	–	–	–	–	–	–
EYA react.	–	-	–	-	-	-	-	-	-	–	-
Hemolysis	b–	–	–	-a–	–	–	-	-	-	–	–
H_2S	–	-	–	+	–	v	–	-	–	–	–
Gas	–,1	2	3	3	3	3,4	–	1	3	2,3	3
Hydrogen	4	3	2	4	4,2	4	–	–	4	4	4
PY-growth	2	1,–	1	2	1,–	1,2	1,–	–,1	2	1	1,–
PY-CHO gr.	4	4	3	4	4	4	4	4	4	4	4
PYG-bile gr.	3	3	3	v	4,–	4	–	3	4	3	4
Pyruvate	A(S)	-	-	CA+	AF	AF	-	-	AF	A	A(-)
Gluconate	–	-	-	–	-	AF	-	-	–	–	-
Motility	–	–	–	–	–	–	–	–	–	–	–
Morphology											
10 microns											

TABLE 5. *cont'd.*

Characteristic	*Bacteroides* - G [FA2(py,ls)]*	*Bacteroides* - J [SAl]	*Bacteroides* - K [A2ls(F)]	*Bacteroides* - L [(als)]	*Bacteroides* - L-1 [blasibiv]	*Bacteroides* - L-3 [a(ls)]	*Bacteroides* - AA [afls2]	*Fusobacterium* - O [bp4(as)]	*Clostridium* - A [Bl(s)]	*Clostridium* - D [Ba4(lfs)]	*Bifidobacterium* - C [Al2(f)]
No. strains	2	1	1	6	2	4	3	1	2	5	1
Amygdalin	–	–	–	-	-	–	–	–	w	–	-
Arabinose	–	-	–w	-	-	–	–w	–	w	–	-
Cellobiose	–	a	–	-	-	–	–	–	a	–	a
Dextrin	-	w	–	-	-	–	–	–	w	–	–
Erythritol	-	-	–	-	-	–	–	–	–	–	-
Esculin pH	–	–	–	-	-	–	–	–	–w	–	–
Esculin hyd.	v	+	+	-	-	–	+	+	+	–	–
Fructose	w–	-	aw	-w	-	–	a	w	a	aw	a
Galactose	–	-	a	w-	-	–	a	w	aw	a	–
Glucose	w–	-	aw	w-	-	–	a	w	a	a	–
Glycerol	-	-	–	-	-	–	–	w	–	–	-
Glycogen	–	–	–	-	-	–	–	–	–	–	–
Inositol	–	-	–	-	-	–	–	–	–	–	-
Inulin	–	-	–	-	-	–	–	–	–	–	–
Lactose	–w	a	–	–	-	–	–w	–	a	a–	–
Maltose	–	a	a	-	-	–	a	–	a	wa	-
Mannitol	–	-	–	-	-	–	–	–w	–	v	-
Mannose	w–	-	a	-	-	–	a	w	a	a	–
Melezitose	–	-	aw	-	-	–	–	–	v	–	–
Melibiose	–	-	w	-	-	–	–	–	wa	–	-
Raffinose	–w	–	a	-	-	–	a–	–	aw	–	–
Rhamnose	–	-	a	-	-	–	–	–	–	–	–
Ribose	w	-	–	-	-	–	–	–	–	–	–
Salicin	w	a	–	-	-	–	–	–	–w	–	-
Sorbitol	–	-	–	-	-	–	–	–	–	–w	–
Sorbose	-	-	–	-	-	–	–	–	–	–	–
Starch pH	–w	a	–	-	-	–	–	–	w	–	a
Starch hyd.	–	–	–	-	-	–	–	–	+	–	+
Sucrose	–	-	a	-w	-	–	a	–	a	a	–
Trehalose	–	-	w	-	-	–	wa	–	–w	–	–
Xylose	–w	–	a	-	-	–	a–	–	a	–	–
Gelatin dig.	–	–	–	+–	-	–	–	–	–	–	–
Milk	–	c	–	–	-	–	–	–	c	c–	–
Indole	–	–	–	–	–	–	+	+	–	–	–
EYA react.	-	-	–	-	-	–	–	–	-	–	-
Hemolysis	–	-	–	-	-	–	–	a	b–	a–	-
H_2S	–	-	+	–	-	+	+–	+	–	–	–
Gas	–,2	–	4	–	–	–	1	4	4	4	–
Hydrogen	–	–	4	–,1	–	2,–	–	4	4	4	–
PY-growth	1,2	–,2	3	2,–	–,1	1,3	3	3	1	1	1
PY-CHO gr.	3	4	4	2,–	–,1	1,3	4	4	4	4	4
PYG-bile gr.	2,3	–	4	–	–	1,2	4	4	1,2	4	1,3
Pyruvate	–	-	A	-	-	A(L)	A(F)	AB	BA	A	A
Gluconate	-	-	AF	-(A)	-	–	A(F)	–	–	–	AF
Motility	–	–	+	–	–	–	–	–	+	–	–
Morphology											
10 microns											

circular, slightly erose, convex, opaque, slightly mottled, smooth, shiny, and mucoid.

Glucose broth cultures have fluffy or powdery sediment and usually have no turbidity; the pH is 4.7 to 5 in 5 days. AMC is produced by one of the two strains tested.

Eubacterium-AE: Deep agar colonies are 0.5 mm in diameter, lenticular, and white. Agar surface colonies are pinpoint, circular, entire, low convex, slightly mottled, smooth, and shiny.

Glucose broth cultures are turbid and have a smooth sediment; the pH is 5.7 to 6 in 5 days. Arabinose and xylose are more strongly fermented, with a pH value of 5.2 to 5.8 in 5 days. Growth is stimulated by glucose, fructose, lactose, and cellobiose, although the pH may not decrease very much in these media.

One of the three strains is motile with one to three subpolar flagella.

Eubacterium-AG: Deep agar colonies are minute to 0.5 mm in diameter, lenticular, and white or tan. Agar surface colonies are pinpoint to 1.5 mm in diameter, circular, entire to lobate, low convex, translucent, white, shiny, and smooth.

Fructose broth cultures produce smooth sediment with no turbidity, or very little turbidity; the pH is 5 to 5.7 in 5 days.

One of the three strains is motile and peritrichous.

The utilization of pyruvate, lack of hydrogen production, and larger cell size differentiate these organisms from similar organisms (isolated in another study; manuscript in preparation), which we have designated *Eubacterium*-AG-H.

Eubacterium-AK: Deep agar colonies are 0.1 mm in diameter, white or translucent, and lenticular to bifoleate. Agar surface colonies are pinpoint to 0.5 mm in diameter, circular, and transparent.

Glucose broth cultures produce a slightly stringy sediment and often have no obvious turbidity. The cells may decolorize easily, and only some elements in a chain or some portions of a cell may stain gram-positive. Rumen fluid (30%) is required for growth.

Eubacterium-AN: Deep agar colonies are 0.5 to 1 mm in diameter, white, and fuzzy or "cauliflower-like." Agar surface colonies are granular, erose, flat to slightly umbonate, translucent, tan, and spreading.

Glucose broth cultures have a smooth sediment and only slight turbidity; the pH is 4.8 to 5 in 1 day. AMC is produced.

Eubacterium-AQ: Deep agar colonies are 0.5 mm in diameter, lenticular, and tan. Agar surface colonies are pinpoint (on anaerobic sheep blood agar plates) to 2 mm in diameter (on streaked roll tube), circular, entire, semi-opaque, shiny, and smooth.

Glucose broth cultures have smooth to stringy sediment, and often have no turbidity; the pH is 4.8 to 5.5 in 1 to 2 days. Although the cellular morphology is quite distinctive, not all fecal strains with the "O," "S," and coiled shapes have the other characteristics of this strain. Similar morphology may be observed, for example, in some strains of *C. ramosum*.

Eubacterium-AY: Deep agar colonies are 0.5 mm in diameter, opaque, and lenticular to "fuzzy." Agar surface colonies are pinpoint, circular, entire, semi-opaque, and yellowish.

Fructose broth cultures have smooth sediment and produce a slight turbidity; the pH is 5.3 to 5.6 in 5 days. Dulcitol is strongly fermented; the pH is 5.2 to 5.4 in 5 days.

Coprococcus. *Coprococcus*-AN-H: Deep agar colonies are 0.5 mm in diameter, lenticular, and tan. Agar surface colonies are 1 to 2 mm in diameter, circular, entire, semi-opaque, shiny, and smooth.

Glucose broth cultures have powdery sediment and no turbidity; the pH is 5 in 1 day. The production of AMC and growth in PY-6.5% NaCl-glucose broth are variable.

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* Acids and alcohols produced in PY-carbohydrate (usually glucose or fructose) broth cultures are given in brackets after the name of the organism. Those produced from pyruvate and lactate are indicated in the table. Capital letters indicate 1 meq (or more) per 100 ml; small letters indicate less than 1 meq/100 ml. Products in parentheses are not uniformly detected. Abbreviations for products: a, acetic acid; b, butyric acid; c, caproic acid; c', caprylic acid; f, formic acid; h, heptanoic acid; ib, isobutyric acid; iv, isovaleric acid; l, lactic acid; p, propionic acid; py, pyruvic acid; s, succinic acid; v, valeric acid; 2, ethanol; 4, butanol.

Abbreviations and symbols for other reactions: a (carbohydrate cultures), acid (below pH 5.5); a (hemolysis), alpha; b (hemolysis), beta; c (milk), curd; +, positive reaction; −, negative reaction; -, no visible growth or only slight growth and negative reaction; nt, not tested; v, variable reaction; w, weak reaction or pH between 5.5 and 6.0; numbers (growth and gas), amount estimated on "− to 4+" scale. Where two reactions are given (e.g., 'aw'), the first is the more usual and the second is observed less frequently. EYA, Egg yolk agar (modified McClung-Toabe).

No strain tested produces acid from adonitol, utilizes lactate, produces catalase, reduces nitrate, or digests meat.

Coprococcus-BN-1: Agar surface colonies are 0.5 to 2 mm, circular, entire, flat to umbonate, colorless, shiny, and smooth.

Glucose broth cultures are turbid and have a smooth sediment; the pH is 5 in 1 day.

Peptococcus. *Peptococcus*-M: Deep agar colonies are 0.5 to 1 mm in diameter, white, and lenticular. Agar surface colonies are 0.5 to 1 mm in diameter, circular to irregular, convex, entire to erose, and dull.

Glucose broth cultures are turbid and have a ropy sediment.

Ruminococcus. *Ruminococcus*-P: Deep agar colonies are 0.5 to 1.5 mm in diameter, lenticular, translucent, and tan. Agar surface colonies are 1 to 5 mm in diameter, translucent to opaque, buff to white, entire to erose, convex to umbonate, and smooth.

Glucose cultures are turbid and have a smooth to stringy sediment; the pH is 5 to 5.5 in 1 to 2 days. Growth in PY-6.5% NaCl-glucose broth is variable.

Ruminococcus-AB: Deep agar colonies are 0.5 to 2 mm in diameter, white, and lenticular. Agar surface colonies are 0.5 to 1 mm in diameter, circular, entire, low convex, and translucent.

Glucose broth cultures are turbid and have a heavy smooth sediment; the pH is 4.8 to 5 in 1 to 2 days. Dulcitol is weakly fermented by two of the five strains.

Ruminococcus-AJ: Deep agar colonies are 0.5 to 1 mm in diameter, lenticular, tan, and translucent. Agar surface colonies are 0.5 to 1.5 mm in diameter, circular to slightly irregular, convex or slightly raised, translucent, shiny, and smooth.

Fructose broth cultures are turbid and have a smooth sediment; the pH is 4.7 in 5 days.

Ruminococcus-AL: Deep agar colonies are 1 mm in diameter and lenticular. Agar surface colonies are 1 to 2 mm in diameter, circular, entire, convex, granular, dull, and opaque.

Glucose broth cultures have flocculent sediment and no turbidity; the pH is 4.8 in 1 day.

Ruminococcus-AQ: Deep agar colonies are 2 to 5 mm in diameter, lenticular, with opaque, tan centers and irregular translucent edges. Agar surface colonies are 0.5 mm in diameter, circular, entire to erose, low convex, slightly granular, semi-opaque, and buff.

Glucose broth cultures have a flocculent to ropy sediment and sometimes have no turbidity; the pH is 5.2 to 5.5 in 1 day. Hippurate is hydrolyzed.

Ruminococcus-AU: Deep agar colonies are 1 mm in diameter, white, and lenticular. Agar surface colonies are 0.5 mm in diameter, circular, entire, convex, grayish, and smooth.

Fructose broth cultures have a smooth sediment and no turbidity; the pH is 5 after incubation for 5 days.

Ruminococcus-BP: Deep agar colonies are 1 mm in diameter, lenticular, and tan. Agar surface colonies are 0.5 to 1.5 mm in diameter, circular, entire, low convex to flat, translucent, and smooth.

Glucose broth cultures have smooth sediment and no turbidity; the pH is 5 to 5.2 in 1 day.

Bacteroides. *Bacteroides*-G: Deep agar colonies are 0.5 mm in diameter, white, and lenticular. Agar surface colonies on BHIA are 0.5 to 1 mm in diameter, circular, entire, low convex, translucent, smooth, and shiny; on sheep blood agar plates at 4 days, there is poor growth. Colonies are 0.5 mm in diameter, circular, erose, tan, fried egg-form, and have clear granular edges and dense translucent centers. The cultures produce no hemolysis.

Glucose broth cultures have a crusty sediment and no turbidity; the pH is 5.8 to 6 in 5 days.

Bacteroides-J: Deep agar colonies are 1 mm in diameter and lenticular. Agar surface colonies are 1 to 2 mm in diameter, circular, entire, convex, opaque, dull, granular, and smooth or bumpy.

Lactose broth cultures have a flocculent sediment and very little turbidity; the pH is 5.2 to 5.3 in 5 days. Hippurate is hydrolyzed.

Bacteroides-K: Deep agar colonies are 1 mm in diameter, white, and lenticular. Agar surface colonies are 0.5 to 1 mm in diameter, circular, entire, convex, translucent, shiny, and smooth.

Glucose broth cultures are turbid and have a smooth sediment; the pH is 5.1 in 1 day. The cells are motile and have a single lateral or subpolar flagellum.

Bacteroides-L: Deep agar colonies are minute to 0.5 mm in diameter, transparent to translucent, lenticular, and white to tan. Agar surface colonies are pinpoint to 1.5 mm in diameter, circular to slightly irregular, flat to low convex, transparent to translucent, smooth, and shiny.

Glucose broth cultures have stringy to flocculent sediment and usually no turbidity. Rumen fluid (30%) somewhat enhances the growth of most strains. Gelatin liquefaction probably is dependent on the amount of growth. Several strains did not withstand lyophilization or freezing at −70 C.

The cellular morphology, generally better (although poor) growth, slight fermentation of a few carbohydrates, and lack of isobutyrate or

isovalerate production distinguish this group from *Bacteroides* L-1. Lack of pyruvate utilization, poorer growth, and the slight carbohydrate fermentation distinguish the group from *Bacteroides*-L-3.

Bacteroides-L-1: Deep agar colonies are 0.2 mm in diameter and lenticular. We have been unable to obtain surface growth of these strains.

Glucose broth cultures have very slight or no turbidity, but cells can be seen in Gram stains and wet mounts.

Bacteroides-L-3: Deep agar colonies are 0.5 to 1 mm in diameter, white to tan, and lenticular. Agar surface colonies are 0.5 to 1 mm in diameter, circular to slightly irregular, slightly erose, raised, translucent, and yellowish.

Glucose broth cultures have a smooth sediment and usually are turbid. The production of H_2S (SIM) and utilization of pyruvate distinguish this group from *Bacteroides* L and *Bacteroides* L-1.

Bacteroides-AA: Deep agar colonies are 0.5 mm in diameter, lenticular, and translucent. Agar surface colonies are 0.5 mm in diameter, circular, entire, convex, and translucent.

Glucose broth cultures are turbid and have a smooth sediment; the pH is 5.4 in 5 days. AMC is produced.

Fusobacterium. *Fusobacterium*-O: Deep agar colonies are minute, white, and lenticular. Agar surface colonies are 2 to 4 mm in diameter, irregular, lobate, raised with concave center, and translucent.

Glucose broth cultures have a smooth sediment and little turbidity; the pH is 5.7 to 5.9 in 1 to 2 days. Threonine is converted to propionate.

This organism differs from *F. nucleatum* by morphological characteristics of the cells and by hydrolyzing esculin.

Clostridium. *Clostridium*-A: Deep agar colonies are 0.5 to 1 mm in diameter, tan, and transparent. Agar surface colonies are 1 to 2 mm in diameter, circular, entire to erose, low convex, beige, and semi-opaque.

Glucose broth cultures are turbid with a smooth sediment; the pH is 5.2 to 5.5 in 5 days.

Oval, terminal (sometimes subterminal) spores are produced. The spores survive heating at 70 C for 10 min. One of the two strains survived heating at 80 C for 10 min. Cells are motile and have polar tufts of flagella.

Clostridium-D: Deep agar colonies are 2 to 3 mm in diameter, white, and lenticular. Agar surface colonies are 2 mm in diameter, circular, entire, convex, semi-opaque, grayish-white, and smooth.

Glucose broth cultures are turbid and have a smooth sediment; the pH is 5.2 in 5 days. Cultures survive heating at 80 C for 10 min, but no spores have been seen in stained smears.

Bifidobacterium. *Bifidobacterium*-C: Deep agar colonies are 0.5 mm in diameter, white, and lenticular. Agar surface colonies are 0.5 to 1 mm in diameter, circular, erose, convex, granular, and translucent.

Fructose broth cultures have a powdery sediment and some turbidity; the pH is 5 in 5 days.

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