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Anaerobic Fecal Bacteria of the Baboon

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The predominant bacterial genera of baboon feces were enumerated and identified by established procedures. The predominant genera isolated were *Lactobacillus, Eubacterium, Streptococcus,* and *Bacteroides.*

Previous reports on the fecal microflora of the baboon have been restricted to the facultative anaerobic bacteria (9, 11, 12). Recent studies with improved anaerobic methods have shown that obligate anaerobes greatly outnumber the facultative bacteria in feces of humans and other mammals. We have characterized the predominant genera of bacteria in baboon feces as a preliminary step to the study of the role of intestinal bacteria in neutral sterol and bile acid metabolism.

The five baboons in this study were housed in a gang cage at the Southwest Foundation for Research and Education in San Antonio, Tex. Each animal was 3 years old at the time of sampling. The animals were weaned at 4 months and since that time have been fed a baboon chow diet (Special Monkey Chow 25, Ralston-Purina, St. Louis, Mo.) supplemented with 20% saturated fat (palm and olive oils) and 0.4% cholesterol.

Feces were collected into a sterile container immediately after excretion and placed in a GasPak (Baltimore Biological Laboratory, Cockeysville, Md.) anaerobe jar. The jar was evacuated and refilled with anaerobic gas several times to insure minimum exposure of the sample to atmospheric oxygen. Transport time to the laboratory was 20 to 30 min.

All subsequent culture procedures were performed in a stainless steel-acrylic anaerobic chamber containing an atmosphere of 10% hydrogen, 5% CO_2, and 85% nitrogen and a palladium catalyst to remove trace amounts of oxygen (1). The sample was thoroughly mixed with a sterile tongue depressor, and 0.5 g of sample was added to 4.5 ml of diluent. Serial 10-fold dilutions of the feces were prepared in prereduced salts diluent (7).

Aliquots of appropriate dilutions were spread on the surface of plates of medium 10 (M10) of Caldwell and Bryant (3) modified by substitution of Casitone (Difco) for Trypticase (Baltimore Biological Laboratory), deletion of sodium sulfide and sodium carbonate, increasing the cysteine to 0.05%, and decreasing the agar concentration to 1.5%. Similar alterations of M10 were found to have little effect on the enumeration and isolation of bacteria from human feces (4). This and other media used in this study were prereduced and anaerobically sterilized.

The plates were incubated 5 days at 37°C. Cultural counts were estimated from plates containing 30 to 300 colonies. The bacterial counts, expressed as organisms per gram of dry matter (2), are shown in Table 1. The average of the total counts was 2×10^{11} organisms per g of dry matter. Total bacterial counts reported for human feces have been 2.29×10^{11} to 3.3×10^{11} organisms per g of dry matter with glove box techniques (2, 5) and 2.56×10^{11} to 4.75×10^{11} organisms per g of dry matter with the roll tube technique (6, 8). The lower counts observed in this study may well be due to the diet, animal age, and the shorter average transit time of the adolescent baboon compared with that of humans.

Fifty-five colonies were randomly selected from a countable plate (8) and streaked to the modified M10 medium to verify purity. If more than one colony type was observed, each was restreaked for isolation. Each pure isolate was inoculated into three broth media: peptone yeast extract (PY) broth as described by Holdeman and Moore (7), PYGH broth (PY plus 1% glucose plus 10 μg of hemin per ml), and PYG80 broth (PY plus 1% glucose plus 0.1% Tween 80). A Gram stain was prepared from the PY broth when turbidity was apparent. Each isolate was also streaked to a brain heart infusion agar plate and incubated 5 days to check for aerobic growth.

The short-chain volatile and nonvolatile carboxylic acids from growth in PY and PYGH or PYG80 were analyzed by gas-liquid chromatography after 5 days of incubation at 37°C. The extraction and methylation procedures were as described by Holdeman and Moore (7). Analyses

TABLE 1. *Percent dry matter and cultural count per gram of dry matter for five baboon fecal specimens*

Baboon no.	% Dry matter	Organisms ($\times 10^{11}$)/g of dry matter
X165	28.5	2.1
X241	30.3	3.6
X245	24.3	2.1
X256	26.7	1.0
X162	29.7	1.0
Avg	27.9	2.0

were made on a Shimadzu GC-3BF gas chromatograph (American Instrument Co., Silver Spring, Md.) equipped with flame ionization detectors and glass columns (3-mm ID by 2 m) packed with 10% SP-1000-1% H_3PO_4 on 100/120 Chromosorb W AW (Supelco, Inc., Bellefonte, Pa.). A 2-μl sample was routinely injected. The volatile fatty acids for most organisms and the methyl esters of the nonvolatile acids were each eluted in approximately 4 min. Formic acid is not detected by flame ionization detectors.

TABLE 2. *Distribution of bacterial genera within individual animals*

Genus	Glucose fermentation products[a]	Baboon no.				
		X165	X241	X245	X256	X162
Lactobacillus	L(sa)	7[b]	16	33	23	37
Eubacterium						
1	A(lsb)	3	1	1	2	4
2	b(la)	2	2	3	2	
3	a(sl)	3	1	3	1	
4	Lb(as)	5		1		1
5	AS(L)	2	1	1	1	
6	B(lap)	2				
Bacteroides						
1	Sa(l)	3	3	3		
2	a(lp)			3	3	1
3	sa	2		2		
4	L(as)			1	1	1
5	Al				2	
6	SLa	1				
Streptococcus	L	5	13	2	5	5
Fusobacterium						
1	B(a)	1			1	1
2	ba(lp)	1		1	3	1
Butyrivibrio	bL(a)	1		1		2
Leptotrichia	L(s)				1	3
Propionibacterium	Pa(Ls)			1	3	
Bifidobacterium	ALs		1		1	
Megasphaera	CB*iv*v*ib*a(p)			2		
Gemmiger	Bs				1	
Other anaerobic cocci						
1	CLab*ib*v*iv*(sp)		2	1		
2	al(s)	1			3	1
3	Ab					1
4	Las	1				
5	b(l*ib*i*v*)	2				

[a] Acids produced from glucose in PYGH or PYG80 compared to acids in PY. Capital letters indicate ≥1 meq/100 ml; small letters indicate <1 meq/100 ml. Products in parentheses were not produced by all isolates. Product abbreviations: a, acetic; b, butyric, c, caproic; *ib*, isobutyric; *iv*, isovaleric; l, lactic; p, propionic; s, succinic; v, valeric.

[b] Numbers of isolates.

Each isolate was tentatively identified to genus based on the Gram stain and carboxylic acid analysis; motility and heat resistance (spore test) were determined when indicated (6, 7, 8). The carboxylic acids used in the presumptive identification of each genus are listed in Table 2. Some genera are divided into groups based on similar acid patterns; however, we do not suggest that these groups represent a single species. Fifty-one of the 116 *Lactobacillus* were obligate anaerobes; the remainder grew poorly aerobically. The isolates identified as *Streptococcus* were facultative anaerobes and were tentatively identified as enterococci. All other isolates were obligate anaerobes. The distribution of bacterial genera within individual animals is also depicted in Table 2. The total number of isolates identified from each animal differs from the 55 colonies picked due to loss of some isolates before identification or gain of isolates due to mixed cultures.

The major genera are listed in order of frequency of their occurrence in Table 3. Although the variety of genera is similar to that reported in human feces, the predominance of *Lactobacillus* in the feces of these adolescent baboons differs from that reported for human feces. Moore and Holdeman reported about equal numbers of *Eubacterium* and *Bacteroides* in the feces of 20 Japanese-Hawaiians (8), but in 3 Caucasian male astronauts *Bacteroides* was the predominant genus (6). Finegold et al. found a predominance of *Bacteroides,* with *Eubacterium* second most numerous, in the feces of 31 Japanese-Americans and noted significant differences in the composition of the flora as a function of diet (5). Adult swine on a chow diet had a predominance of *Streptococcus* in their feces, and very few gram-negative organisms were detected (10). The high numbers of *Lactobacillus* that we recovered from baboon feces (9.2×10^{10} organisms per g of dry matter) compared with the numbers reported for human feces (10^9 to 10^{10} organisms per g of dry matter) (6, 8) seem to indicate a basic difference in the predominant bacterial flora of baboon feces. This difference may be due to diet, animal age, or perhaps intrinsic differences of the baboon.

TABLE 3. *Predominant bacterial genera of the fecal flora of five baboons*

Genus	No. of isolates	Cultural count[a] ($\times 10^{10}$)	% Flora[b]
Lactobacillus	116	9.2	46.0
Eubacterium	42	3.3	16.7
Streptococcus	30	2.4	11.9
Bacteroides	26	2.1	10.3
Anaerobic cocci	15	1.2	5.9
Fusobacterium	9	0.7	3.6
Other[c]	14	1.1	5.6

[a] Organisms per gram of dry matter.
[b] Based on total of viable organisms recovered.
[c] Four genera, each representing <2% of flora.

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