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Comparative Study of Bacterial Groups within the Human Cecal and Fecal Microbiota

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The composition of the human cecal microbiota is poorly known because of sampling difficulties. Samples of cecal fluid from eight subjects were collected via an intestinal tube. Feces were also collected. Total anaerobes, facultative anaerobes, bifidobacteria, and *Bacteroides* were enumerated by culture methods, and the predominant phylogenetic groups were quantified by molecular hybridization using a set of six rRNA-targeted probes. The numbers of strict anaerobes, bifidobacteria, *Bacteroides*, and members of the *Clostridium coccoides* group and *Clostridium leptum* subgroup were lower in the cecum. Facultative anaerobes represented 25% of total bacteria in the cecum versus 1% in the feces.

The indigenous microbiota plays an important part in colonic physiology and may be involved in the pathogenesis of large-bowel diseases, such as cancer and Crohn's disease (7, 10, 23, 24). It is well established that microbiota patterns differ depending on the gastrointestinal site, with differences clearly observed between the stomach, upper small bowel, lower small bowel, and rectum (9, 10, 11, 17). The ecological conditions in the cecum (i.e., the initial part of the right colon) differ from those encountered in the distal colon (based on fecal samples). The cecum receives substrates which escape digestion in the small bowel, has higher concentrations of volatile fatty acids, and has a lower pH (9, 11, 16, 17). Few authors have attempted to describe the composition of the human cecal microbiota (4, 13, 16, 19), owing to sampling difficulties and the need to maintain anaerobic conditions from sampling to counting. Several methods have been used, such as sampling of sudden-death victims (16), use of a pyxigraphy capsule (19), needle probing during surgery (4), and intestinal intubation with a long tube (13). These techniques have inherent advantages and limitations. Only intubation and pyxigraphy can be performed in healthy subjects, and both can be repeated to study the stability of the flora or the influence of various parameters on its composition. Colonic samples of sufficient volume are difficult to obtain because of their viscosity and the difficulties in ensuring adequate anaerobic conditions. It was previously shown that a long tube could be used to sample the chyme in the right colon (11, 17). Using an intubation technique, Gorbach et al. demonstrated in three subjects that the cecum contained 100 times more bacteria than the terminal ileum (13). This study aimed to compare the composition of the cecal and fecal microbiota in healthy humans. Recent studies of the fecal microbiota indicate the necessity of molecular evaluation of this ecosystem, and until now such methods have not been employed for cecal analysis.

Eight healthy volunteers (four men and four women aged 22 to 27 years) gave informed written consent to the study protocol, which was approved by the local Ethics Committee. None had a history of gastrointestinal disease, laxative use, or recent treatment with antibiotics. They were asked to avoid fermented dairy products for 15 days and were otherwise allowed normal diets. The day before sampling, at 0900 they were nasally intubated with a double-lumen tube. The distal extremity of the first lumen had a rubber bag containing 30 g of mercury which could be inflated with air to accelerate its progression through the small bowel (11, 17). The sampling lumen consisted of a vinyl tube with an internal diameter of 3.5 mm (Portex, Hythe Kent, United Kingdom). It was multiperforated at its extremity, which was located 3 cm above the rubber bag. Its other end was closed with a stopcock. Progression of the tube was monitored fluoroscopically. When the sampling port had reached the ileum, the sampling lumen was flushed with 30 ml of nitrogen gas and the stopcock was closed; when it had reached the cecum (approximately 6 to 10 h after introduction), the tube was fixed to the nose, the rubber bag was deflated, the sampling lumen was flushed again with 30 ml of nitrogen gas, and subjects were asked to remain in a semirecumbent position to avoid further progression of the tube. The subjects received standard meals at 1200 and 1930, and the following day they received a standard breakfast at 800 and a meal at 1200. The meals consisted of two eggs in the evening and 100 g of steak for lunch, mashed potatoes, 30 g of cheese (Gruyere), 100 g of white bread, and 30 g of butter. Breakfast consisted of 250 ml of coffee, 10 g of hydrolyzed milk, 10 g of sucrose, 100 g of white bread, 30 g of butter, and 30 g of strawberry jam. The volunteers were allowed to drink water or tea ad libitum; smoking was not permitted. At 1400, on the second day, 2 to 10 ml of cecal contents was sampled by suction using a sterile syringe equipped with a stopcock connected to the stopcock of the sampling lumen. When sampling was un-

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TABLE 1. Probes used in this study

Probe	Specificity	Sequence (5′-3′)	Name (OPD nomenclature)[a]	Reference
Univ1390	Universal	GACGGGCGGTGTGTACAA	S-*-Univ-1390-a-A-18	25
Eub338	Domain *Bacteria*	GCTGCCTCCCGTAGGAGT	S-D-Bact-0338-a-A-18	2
Erec482	*C. coccoides-Eubacterium rectale* group	GCTTCTTAGTCA(A/G)GTACCG	S-*-Erec-0482-a-A-19	12
Clept1240	*C. leptum* group	GTTTT(A/G)TCAACGGCAGTC	S-G-Clept-1240-a-A-18	22
Bacto1080	*Bacteroides* cluster	GCACTTAAGCCGACACCT	S-*-Bacto-1080-a-A-18	8
Bif228	*Bifidobacterium*	GATAGGACGCGACCCCAT	S-G-Bif-228-a-A-18	I. Mangin, Y. Bouhnik, A. Suan, V. Rochet, L. Raskine, P. Crenn, and J. Doŕe, unpublished data
E.coli1513	*E. coli*	CACCGTAGTGCCTCGTCATCA		20
Lab158	*Lactobacillus-Enterococcus*	GGTATTAGCA(C/T)CTGTTTCCA	S-G-Lab-0158-a-A-20	14

[a] Probe names are in accordance with the Oligonucleotide Probe Database (OPD) (1).

successful, 5 to 10 ml of nitrogen gas was flushed into the sampling lumen to ensure its patency and gentle suction was again applied every 10 min until a sample was obtained. Fecal samples were obtained on the same day, before cecal sampling. The subjects voided their feces into a box, which allowed immediate introduction of stools into an anaerobic system (Anaerocult; Merck, Darmstadt, Germany). The closed boxes containing feces and the tight syringes containing the cecal samples were transferred within 10 min to an anaerobic chamber. Samples were diluted using anaerobic dilution solution containing (in grams/liter): NaCl, 5; glucose, 2; cysteine-HCl, 0.3. They were homogenized by magnetic stirring to give a 10-fold dilution (wet weight/volume), which was then serially diluted down to 10^{-11} in anaerobic dilution solution. Aliquots (100 μl) of each dilution were evenly spread on plates of freshly prepared media. Bifidobacteria were counted using Beerens' medium (3). Plates were incubated in anaerobic jars for 5 days at 37°C using the Anaerocult procedure (Merck) prior to counting. Bifidobacteria colonies were identified by their fructose-6-phosphate-phospho-ketolase activity (21). Facultative anaerobes and *Bacteroides* were counted on brain heart agar (Bio-Rad, Marne la Coquette, France), to which 1% of hemin solution (500 μg/ml) had been added. Facultative anaerobes were counted directly after 5 days of incubation at 37°C in aerobic conditions. For counting of *Bacteroides* bacteria, brain heart agar plates were exposed for 1 h to air to select less extremely oxygen-sensitive microorganisms prior to incubation in strict anaerobic conditions at 37°C for 48 h (5). Colonies were transferred onto 1% sodium dodecyl sulfate-impregnated Nytran N membranes (Schleicher & Schuell, Ecquevilly, France) and hybridized with a *Bacteroides* group-specific probe as previously described (8). To count total viable anaerobes, 1 ml of each 10-fold dilution was inoculated in duplicate in 15 ml of Wilkins-Chalgren agar (Difco, Detroit, Mich.) at 45°C. The inoculated medium was then poured into 8- by 400-mm tubes, which were immediately cooled to ensure prompt solidification and incubated for 14 days at 37°C (18).

Total RNA was extracted from frozen fecal (200 mg) and cecal (400 μl) material, as described by Doré et al. (8), using 0.1 g of zirconium beads. RNA concentration of extracts was determined by dot blot hybridization with the universal oligonucleotide probe (8) using a standard *Escherichia coli* RNA control (rRNA standards; Roche Diagnostics, Meylan, France). 16S rRNA-targeted oligonucleotide probes are detailed in Table 1. Dot blots were achieved as previously described (8): serial dilutions of control RNA (2 to 250 ng) from pure cultures (*Bacteroides vulgatus* [ATCC 8482], *Bifidobacterium longum* [ATCC 15707], *E. coli* [Boehringer Mannheim rRNA standards], *Lactobacillus acidophilus* [ATCC 4356], *Eubacterium siraeum* [ATCC 29066], *Ruminococcus productus* [ATCC 27340]), and 100 to 200 ng of total RNA extracted from fecal and cecal samples were blotted and hybridized with purified 32P-5′ end-labeled probes overnight at 42°C. Stringent washing was performed twice for 30 min with 1× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), 1% sodium dodecyl sulfate buffer at an experimentally previously defined temperature depending on each probe (Table 1). Hybridization signals on dot blots were measured using radioimaging with the Instant Imager (Packard Instruments). Microbial quantification for each group was expressed as the percentage of the total bacterial 16S rRNA (mean of duplicate measurements). Microorganism counts expressed as log 10 are reported per gram (wet weight) of cecal or fecal contents. Data are expressed as means, with 95% confidence intervals in brackets. Comparisons between the cecal and fecal flora were made using the Wilcoxon test. Statistical significance was achieved if the P value was <0.05.

At least 5 ml (5 to 50 ml) of cecal contents could be sampled in all experiments. The sample could be obtained 2 h after the meal in 7 out of 11 experiments and between 2.5 and 3 h after the meal in the other 4 experiments. We chose to sample the cecal chime 2 to 3 h after a standard meal, as proposed by Gorbach et al. (13), based on previous reports that sampling was not always possible after an overnight fast (17). Total viable anaerobe counts as well as those of facultative anaerobes, bifidobacteria, and *Bacteroides* in cecal and fecal samples are shown in Table 2. Counts of total viable anaerobes, bi-

TABLE 2. Counts of total anaerobes, facultative anaerobes, bifidobacteria, and *Bacteroides* in the cecal and fecal microbiota of eight healthy humans

Organism	Counts (log 10 CFU/ml − means, ± SE) in:		*P* value
	Cecum	Feces	
Total anaerobes	8.0 ± 0.22	10.4 ± 0.12	0.02
Facultative anaerobes	7.4 ± 0.23	7.8 ± 0.29	0.38
Bifidobacteria	6.7 ± 0.45	8.9 ± 0.22	0.02
Bacteroides	7.4 ± 0.33	8.9 ± 0.42	0.05

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TABLE 3. In vivo experiments using molecular probes in the cecal and fecal microbiota of eight healthy humans

Probe	% RNA (mean ± SE)		*P* value
	Cecum	Feces	
Bacto1080	1.2 ± 0.22	8.0 ± 0.32	<0.001
Bif228	5.8 ± 0.37	3.2 ± 0.55	0.06
Erec482	10.0 ± 0.55	22.8 ± 2.2	0.14
Clept1240	1.4 ± 0.11	13.0 ± 0.78	0.003
E.coli1513	26.8 ± 7.4	0.02 ± 0.02	0.18
Lab185	22.8 ± 2.1	6.6 ± 0.23	0.02

fidobacteria, and *Bacteroides* were consistently lower in the cecal samples compared to those of feces. Counts of facultative anaerobes did not differ significantly between the cecal and fecal samples. Facultative anaerobes represented 25% versus 1% of the total anaerobic flora in the cecum and feces, respectively. Relative quantification of the fecal and cecal microbial populations achieved by dot blot hybridization using 16S rRNA-targeted oligonucleotide probes specific to microbial group, genus, or species is shown in Table 3. Strict anaerobic bacterial populations represented by the *Bacteroides*, *Clostridium leptum*, and *Clostridium coccoides* groups were significantly lower in the cecum. Facultative anaerobes represented by the *Lactobacillus-Enterococcus* group and *E. coli* species showed much higher rRNA proportions in cecal contents.

This study shows, using culture-dependent and culture-independent methods, that the cecal flora differs greatly from the fecal flora. Meals flush fluids, substrates, and bacteria from the upper gastrointestinal tract into the cecum, and this may influence the cecal flora. The bacterial density in the upper gastrointestinal tract (increasing from 10^5 CFU/ml in the jejunum to 10^6 CFU/ml in the ileum [24]) is low compared to that of the cecum (10^8 CFU/ml). Assuming that 500 ml of ileal fluid entered the cecum after the meal and that water absorption led to a final volume of 50 to 100 ml (6), bacterial concentration would be below 10^7 CFU/ml. In other words, endogenous bacteria passively flushed from the small intestine would account for less than 10% of the bacteria in the cecum. The total anaerobe counts were 100 times lower in the cecal contents than in the feces. Similar results have been obtained with other sampling techniques used to date (4, 13, 16, 19). Water absorption in the colon may partly explain the increased bacterial density in the feces relative to that of the cecum. However, the increase in dry matter between the cecum and feces is only 10-fold (17), whereas bacterial density increases 100-fold. Bacterial growth must therefore occur within and downstream of the right colon. Some microorganisms, such as methanogens, represent <0.003 to 0.03% of all bacteria in the right colon, compared to 5 to 12% or more in the feces (16, 19). On the other hand, the numbers of facultative anaerobes were similar in the cecal and fecal flora. Results obtained using molecular hybridization were consistent with those obtained from classical studies. Strict anaerobes analyzed using probes specific for the *Bacteroides* (*Bacteroides*, *Porphyromonas*, and *Prevotella* spp.) and *Clostridium* groups (*Clostridium*, *Eubacterium*, and *Ruminococcus* spp., essentially) represented 44% of fecal bacterial rRNA and only 13% of cecal bacterial rRNA. rRNA from *E. coli* and the *Lactobacillus-Enterococcus* group represented 50% of the cecal bacteria rRNA and only 7% of fecal bacterial rRNA. Facultative anaerobes, i.e., enterobacteria (mainly *E. coli*) and enterococci, are usually thought to have little physiological relevance because of their low densities in the feces relative to that of the dominant flora, except when the latter is weakened (for example, following antibiotic use) (23). As we observed that facultative anaerobes belong to the dominant microbiota in the cecum, this suggests that they may have an important physiological role at this site.

In conclusion, the human cecal flora differs quantitatively and qualitatively from the fecal flora. It harbors 100 times fewer anaerobes, while facultative anaerobes represent an important part. Studying the right-sided colonic flora would, thus, be more appropriate than studying feces for functions occurring in the cecum, such as fermentation of dietary fibers and endogenous substrates, or for diseases involving the right part of the colon, such as ileocecal Crohn's disease.

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