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Gut Microflora of Vervet and Samango Monkeys in Relation to Diet

M. R. BRUORTON,[1] C. L. DAVIS,[2]†* AND M. R. PERRIN[1]

Department of Zoology[1] and Entomology and Department of Microbiology and Plant Pathology,[2] University of Natal, P.O. Box 375, Pietermaritzburg 3200, South Africa

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The microflora in the gastrointestinal tracts of wild vervet and samango monkeys (*Cercopithecus aethiops* and *C. mitis*, respectively) were studied, using fermentation acid analysis, electron microscopy, and culturing methods. The diets of the two species of monkey differ considerably, with that of the samango including a greater proportion of cellulose-rich leaf material, and this is reflected in the microflora. Volatile fatty acid measurements along the gut of both species showed that these end products of bacterial metabolism were concentrated in the cecum and colon. Electron microscopy indicated that morphologically similar bacteria were present in the cecum and colon of both species, but the samango possessed a distinct stomach microflora. Bacteria in the lumina of the four main regions of the gut of the monkeys (stomach, small intestine, cecum, and colon) were plated on a number of anaerobic media (Mann, Rogosa, and Sharp; clostridial basal; and complex media). The cecum and colon were found to contain higher numbers of microbes per gram (wet weight) of gut content than the stomach and small intestine. Microbial isolates were able to catabolize carboxymethyl cellulose and other polymers. This may aid the monkeys, particularly samangos, in the digestion of fibrous dietary components such as leaves.

Large numbers and many species of microorganisms are known to inhabit the gastrointestinal tract (GIT) of mammalian herbivores, and microbial fermentations may contribute greatly to their nutrition. Two categories can be differentiated for herbivores according to their digestive strategies, and the site of the gut can be enlarged to accommodate microbial fermentation. These are the foregut fermenters (including the ruminants) in which the stomach is enlarged and sacculated and the hindgut fermenters in which the cecum and/or colon is the major site of fermentation activity. In mammals with enlarged forestomachs, microbial activity precedes regular gastrointestinal digestion and protein absorption; in hindgut fermenters, the situation is reversed. Species of primates exemplify both adaptive strategies, but hindgut fermenters predominate. Old World colobine monkeys possess a large and structurally complex stomach (2), while in the cercopithecine monkeys most species possess typically unilocular, grandular stomachs and often an enlarged, sacculated cecum and colon (5).

In southern Africa, the vervet monkey *Cercopithecus aethiops* and the samango monkey *C. mitis* occur sympatrically in a number of areas. The samango is confined to forest habitats, but vervets, while predominantly savanna woodland species, have a very wide habitat tolerance (23). The diets of the two species vary considerably. The vervet is considered an omnivore, and a typical diet would include fruit, flowers, and invertebrates, with ingestion of leaves being significant only in the winter months (10, 12, 28). By contrast, leaves and other foliar material are an important component of the samango diet of predominantly fruit, as well as some insects (8, 12a, 22, 22a).

Foliar material is generally fibrous and cellulose rich and requires microbial degradation for the utilization of cellulose components. The samango, therefore, is likely to possess specialized chambers along the gut within which microbial fermentation may occur, as well as specific bacteria with an ability to catabolize cellulose. A study was initiated to investigate whether the samango does indeed have a gut morphology, physiology, and microflora more suited to the digestion of cellulose-rich material than the more omnivorous vervet. An initial morphological study of the stomach and cecum of samangos (3) and a later comparative morphometric examination of the GIT of samangos and vervets (2a) have been carried out. We present the microbiological results. The pH along the GIT of both species was examined, and the presence of bacterial fermentation end products was demonstrated. Electron micrographs are presented that show the wide variety of microorganisms found, and a bacterial cultural study is reported. This represents one of the few studies performed on the natural GIT microflora of primates living in the wild.

MATERIALS AND METHODS

Animals and sampling methods. The wild vervets and samangos examined in this study were taken from an area consisting of mature montane forest, bordered by commercially planted black wattle (*Acacia mearnsii*) plantations and cash crops (predominantly yams, *Dioscorea* sp.) in the Karkloof area of Natal, South Africa. Animals were shot (with the permission of the Parks Board) within 1 h after feeding and were dissected in the field immediately after death. Nine samangos and eight vervets were used for the pH examination, and four of each species were used for the volatile fatty acid (VFA) analyses. Seven samangos and six vervets were used for electron microscopic examinations of the bacteria along the GIT. The GITs of both adult and juvenile individuals were examined microscopically, and cultural studies were carried out with samples of the GIT of one samango and one vervet, respectively.

pH measurements were performed in the field with a glass electrode inserted into the various regions of the GIT (fundus, pylorus, proximal and distal small intestine, cecum, and proximal and distal colon). For VFA examination, contents from the GIT were removed and immediately refrigerated.

* Corresponding author.

† Present address: Department of Zoology, University of Cape Town, Private Bag, Rondebosch 7700, South Africa.

Microscopic examination was carried out on pieces of tissue, and contents were fixed in 3% phosphate-buffered glutaraldehyde. Samples for cultural studies were obtained by ligating and excising pieces of fundic stomach and cecum and short lengths of the small intestine and colon. These were placed in sterile screw-cap bottles and transported back to the laboratory in a sealed GasPak jar with an anaerobic atmosphere in a cooler box. The samples were further handled in an anaerobic glovebox (Forma Scientific, Marietta, Ohio), and all further culturing took place in this. Transportation from study site to laboratory took approximately 2 h.

VFA analysis. Samples were acidified with a 25% metaphosphoric acid solution and clarified by centrifugation. The analysis was performed with a Hewlett-Packard 5790 A series gas chromatograph fitted with a flame ionization detector. A Supelco glass column of 1.7 m by 3.0-mm internal diameter packed with SP1000 and 10% H_3PO_4 on Chromosorb W-AW 100/120 mesh was used.

Microscopic examination. For electron microscopy, tissues were removed from glutaraldehyde, postfixed in 2% osmium tetroxide in 0.05 M phosphate buffer, and then dehydrated in a graded alcohol series. Material was critical-point dried and coated with gold-palladium.

Isolation of bacteria. The ligated gut samples in bottles were cut to release the contents into quarter-strength Ringer solution, and these were then vigorously shaken by hand. Dilution series were performed in the buffer which had been bubbled with nitrogen gas, and samples were plated out.

Isolation and maintenance of bacteria were carried out on the following media: MRS (Mann, Rogosa, and Sharpe medium [20]), CBM (clostridial basal medium [19]), a complex medium (CO), and NA (nutrient agar; Difco). CO medium contained mineral salts solutions 1 and 11, trace metal solution [$FeSO_4 \cdot 7H_2O$, $MnSO_4 \cdot 2H_2O$, $CoCl_2$, $ZnSO_4 \cdot 5H_2O$, $CuSO_4 \cdot 5H_2O$, $AlK(SO_4)_2$, H_3BO_3, and $NaMoO_4 \cdot 2H_2O$], and a vitamin solution (biotin, folic acid, pyridoxine, thiamine, riboflavin, nicotinic acid, pantothenate, vitamin B_{12}, *p*-aminobenzoic acid, and lipoic acid) at concentrations given by Tang et al. (26); 0.2% tryptone (Difco); 0.1% yeast extract (Difco); 0.1% glucose (Merck); 0.05% starch (Merck); the VFA solution given by Ogimoto and Imai (20); and cysteine-HCl and $NaHCO_3$ (20). MRS, CBM, and CO were incubated anaerobically and NA was incubated aerobically, all at 35°C.

Screening of isolates. Isolates were obtained by streaking out all colonies within a sector of a spread plate containing 40 colonies. This was done for each type of medium, including the aerobic NA plates. Not all isolates remained viable throughout the study period.

Screening was carried out on solid media. Test substrates were added to a basal agar: PY agar (20) for anaerobes and agar containing 0.5% peptone and 0.1% yeast extract for aerobes. The test substrates were at the following concentrations: carboxymethyl cellulose (CMC), 1%; starch, 0.5%; chitin, 30 ml/liter (swollen and precipitated [21]); and skim milk, 1%. Carboxymethyl cellulase (CMCase) and chitinase activity were detected by using the method of Teather and Wood (27), and amylase was detected by using iodine (20). Urease activity was detected on agar containing PY, 2% urea, and 0.0002% phenol red, pH 7.5.

RESULTS

pH. The mean pH values varied considerably along the GIT but were similar for both species in comparable regions of the GIT (Table 1). Digesta pH was lowest in the pyloric region of the stomach and highest in the distal small intestine. The pH of the pyloric region in both species was markedly lower than that of the fundic region. The pHs of the small intestine, cecum, and colon were all above 5 for both species, with the exception of the samango cecum (pH 4.9).

TABLE 1. Measurements of pH and VFA concentration in the GIT of vervet and samango monkeys[a]

GIT region[b]	Samango		Vervet	
	pH (n = 9)	VFA (n = 4) (mmol/liter)	pH (n = 8)	VFA (n = 4) (mmol/liter)
Fundus	5.0 ± 0.5a	27 ± 8h	4.7 ± 0.5d	11 ± 3h
Pylorus	2.5 ± 0.7a	7 ± 3	2.3 ± 0.6d	5 ± 2
SI 1	6.0 ± 0.7	20 ± 8	6.0 ± 0.4	18 ± 4
SI 2	6.2 ± 0.7b	18 ± 7	6.7 ± 0.4e	12 ± 6
Cecum	4.9 ± 0.4b	199 ± 14	5.1 ± 0.6e	229 ± 24
Colon 1	5.0 ± 0.4c	174 ± 6	5.2 ± 0.6f	190 ± 15
Colon 2	5.9 ± 0.5c	122 ± 15i	5.8 ± 0.4f	190 ± 42i

[a] Values are means ± standard deviations. Values with common letters (in the same column) differ significantly, using the paired t test ($P < 0.001$; for f:f, $P < 0.02$). Values with common letters (in the same gut region) differ significantly, using the Mann-Whitney U test ($P < 0.05$).

[b] SI 1 and SI 2 represent proximal and distal halves of the small intestine, respectively. Colon 1 and colon 2 represent proximal and distal halves of the colon, respectively.

VFA. VFA occurred along the GIT of both species in various concentrations (Table 1). The lowest concentrations were found in the fundic stomach and the small intestine, and the highest concentrations occurred in the cecum and colon.

Microscopic examination of the gut. (i) Stomach. Very few bacteria were observed on the gastric epithelium of vervets. However, the surface and contents of the stomach in all samangos examined (with the exception of a juvenile) were completely covered by a dense mat of bacteria (Fig. 1a and b). The morphology of the dominant bacteria in the stomach appeared similar in samples taken from as far afield as Northern Transvaal, Cape Vidal on the coast, and the Natal midlands. They consisted predominantly of long bacilli, with an average length of 12 to 14 μm, although very long (80-μm) filamentous forms were observed. Glycocalyx-like filaments were observed on the bacteria.

(ii) Small intestine. Although substantial numbers of viable bacteria were recorded from the contents of the small intestine of both species (Table 2), few were observed by scanning electron microscopy. In general, no densely populated regions of tissue were found in either species. However, in some vervets a region of the distal small intestine (ileum) was found to be covered by extremely long (up to 80 μm), segmented, filamentous rods. All filaments appeared to be basally attached to the epithelial surface (Fig. 1c).

(iii) Cecum. The digesta in the cecum of both species consisted of densely packed areas of bacteria. Bacterial populations were varied, with many morphological types present (Fig. 1d and e). The bacteria consisted of two groups, lumenal unattached species and bacteria attached to the epithelium.

In all samangos and vervets examined, the cecal surface was covered by a dense mass of thin, flagellated spiral bacteria. These were attached to the epithelial surface and formed a dense "fuzzy" layer over the surfaces of the columnar epithelial and goblet cells. The spiral bacteria were approximately 3 to 4 μm long.

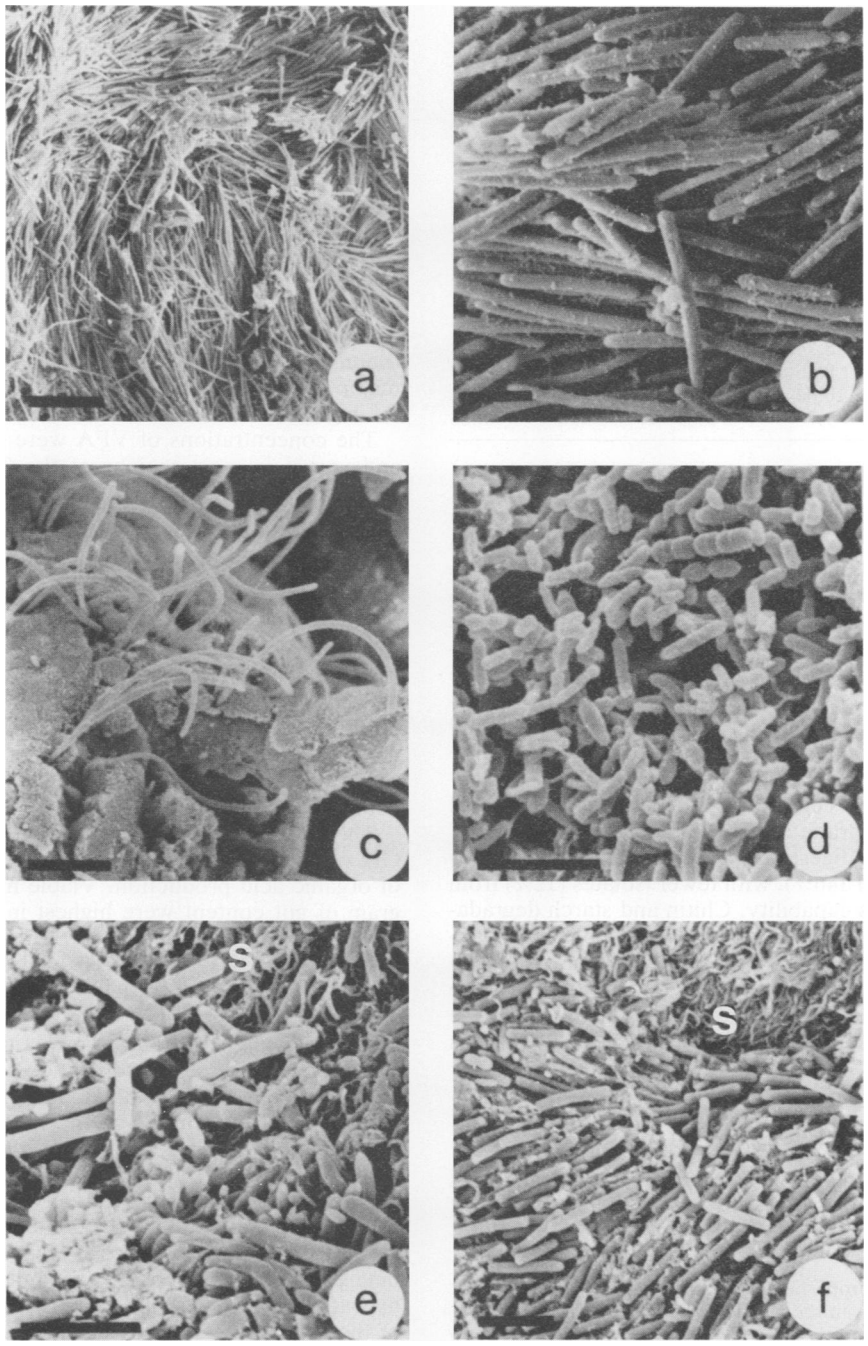

FIG. 1. Microscopic examination of the gut. (a and b) Bacteria in the stomach of a samango. Bars, 25 (a) and 5 (b) μm. (c) Segmented filamentous bacteria in the small intestine of a vervet. Bar, 10 μm. (d and e) Bacteria from the cecum of a samango, showing morphological diversity of the bacteria. Note the spiral bacteria (S) in panel e. Bars, 5 μm. (f) Bacteria in the colon of the samango, showing a predominance of rods and spiral bacteria (S). Bar, 5 μm.

(iv) Colon. High numbers of unattached bacteria were apparent in the colonic contents (Fig. 1f) and were frequently found with digesta trapped within the colonic crypts. In many samangos, the predominant unattached bacteria were rod-shaped bacilli. The entire surface of the colon in both species was covered by a lawn of spiral attached bacteria, similar to those observed in the cecum (Fig. 1f).

Bacterial culture. Numerous culturable bacteria were present in the different regions of the GIT of both monkey species (Table 2). In the samango stomach, plate counts on MRS medium were highest, indicative of the presence of lactobacilli. This was also the case in the samango small intestine, although the difference in numbers on the three anaerobic media was not as great as in the stomach. The samango cecum and colon had higher culturable bacterial numbers than the stomach and small intestine. Bacteria from

TABLE 2. Bacterial numbers in the GITs of samango and vervet monkeys

GIT region	Bacteria (log CFU/g [wet wt] of gut content) in given medium			
	MRS	CBM	CO	NA
Samango				
Fundic stomach	8.54	7.85	7.60	4.98
Small intestine	9.60	9.08	9.11	7.89
Cecum	10.36	9.91	10.38	7.83
Colon	9.79	10.34	9.89	7.45
Vervet				
Fundic stomach	8.34	8.38	8.30	7.08
Small intestine	8.36	8.52	8.45	6.90
Cecum	10.00	10.38	10.65	3.48
Colon	9.78	9.69	10.28	6.26

the vervet cecum and colon enumerated on plates showed a similar trend of higher numbers per gram (wet weight) than the stomach and small intestine. However, in contrast to the samango, the vervet had similar numbers of bacteria able to grow on the three anaerobic media in the stomach. The number of aerobes (presumably facultative) are also presented. These were low in the stomach and higher in the hindgut, but always comparatively lower than the anaerobes. The vervet had fewer bacteria able to grow aerobically in all regions of its GIT (exept the stomach) than the samango.

Degradative properties of isolates. The isolates were tested for their ability to degrade a range of substrates (Table 3). The property of CMC digestion was common among isolates from the samango GIT (46%), with fewer isolates (12%) from the vervet having this capability. Chitin and starch degradation was carried out by isolates from both species of monkeys. On the agar plates on which skim milk was used as a substrate, fuzzy zones of activity were seen around a large number of isolates from both monkeys, but few of these isolates created zones of complete clearing. Urea hydrolysis was not common.

DISCUSSION

The hypothesis adopted for this study was that samangos have certain morphological or physiological adaptations of the GIT related to herbivory and, more specifically, folivory, in contrast to the more omnivorous vervet. M. Bruorton (2a) has presented evidence that, although the GITs of the monkeys are morphologically similar, a major difference between the species is that the volumes of the cecum and colon (and thus the fermentation capacity) of the samango greatly exceed those of the vervet. For example, the average volumes of the samango cecum and colon are 25.8 and 175.8 cm^3, respectively, compared with 10.8 and 100.5 cm^3 for the corresponding regions in the vervet. The pH of the fundic region of the stomach of both species is suitable for the growth of certain microorganisms. The hindgut in general has a lower pH than the small intestine, and the lower pH in the cecum of both species strongly suggests that this is a major site of organic acid production. This is supported by the higher concentrations of VFA found in this region.

TABLE 3. Digestive properties of bacterial strains isolated from various regions in the samango and vervet GIT[a]

GIT region	No. of isolates tested	% of isolates positive for substrate digestion				
		CMC	Chitin	Starch	Skim milk	Urea
Samango						
Fundic stomach	90	27.8	42.2	32.2	44.4	7.8
Small intestine	79	53.2	30.4	12.7	35.4	7.6
Cecum	105	40.0	30.5	20.0	23.8	10.5
Colon	59	76.3	22.0	1.7	28.8	8.5
Vervet						
Fundic stomach	73	8.2	0	9.6	20.5	0
Small intestine	63	9.5	36.5	11.1	27.0	3.2
Cecum	24	8.3	8.3	4.2	54.2	0
Colon	21	38.0	42.8	47.6	57.1	23.8

[a] Bacteria were isolated from MRS, CBM, CO, and NA media.

The concentrations of VFA were similar in the samango and vervet in most regions of the GIT, except the fundic stomach and distal colon. This supports the contention of Clemens and Phillips (7) that herbivorous primates have higher concentrations of VFA in the stomach than omnivorous species. The VFA concentrations found in the stomach of the sykes monkey (*C. mitis*) were higher than in the omnivorous vervet and baboon (*Papio cynocephalus*) (6, 7), suggesting that fermentation occurs in the sykes' stomach. Lower levels of VFA in the distal colon of the samago than vervet may indicate that more VFAs are absorbed in the colon of the former. The quantity absorbed appears to depend primarily on the surface area available, and the samango has a greater absorptive surface area available compared with the vervet (2a).

The biomass of bacteria observed at various sites in the GIT of samangos and vervets appeared to be related to levels of organic acid production. Viable numbers of bacteria per gram of gut content were highest in the cecum and colon, and the greatest numbers and morphological variety of bacteria were observed microscopically in those regions of greatest VFA concentration. The stomach of the samango was densely colonized, predominantly by a mass of closely associated long rods, and this was noted in monkeys from geographically separate populations. These might be a true autochthonous flora, maintained in the selective environment of the stomach, where there is a low bacterial species diversity. By contrast, the vervet had no such recognizable stomach flora.

Despite the fact that relatively high numbers of viable bacteria were recorded in the contents of the small intestine, few lumenal microbes were seen in either species by scanning electron microscopy. Accumulation of soluble carbohydrates and partially digested protein products in the lumen of the small intestine provides ideal substrates for bacterial growth (11). Numerous fast-growing strains have been isolated from the small intestine of humans (11). Similarly, Mackie and Wilkins (16) reported that the small intestine of horses is a suitable site for bacterial proliferation. The bacteria isolated from the small intestine of the monkeys might consist of similar fast-growing strains that could be flushed out in the ileum regularly, leaving fewer microbes to be seen by scanning electron microscopy. In two of the vervets examined, the ileum was infested with extremely long segmented bacteria, which covered the villi and intervillous region with a hairlike mat in regions of heavy colonization. Similar microorganisms have been studied in the ileum of mice (4).

The largest number and greatest variety of bacteria were found in the cecum and colon of both samango and vervet.

Most of the microorganisms occurred in the lumen in complex masses, which probably reflects longer residence times of the food in these regions. The entire surface of the cecum and colon of all monkeys examined was covered by a lawn of spiral microbes, attached to the epithelial lining. These organisms have been reported colonizing the cecal and colonic epithelia of a number of primates, including the vervet (9), rhesus monkey (25), and humans (24), and such disparate animals as dogs and mice (13) and the koala bear (17). Lee (14) has stated that, despite the close proximity of the cecum and colon, the mucosa-associated microfloras at these two sites are very different, with two types of spiral organisms being recognizable. The spiral bacteria observed by transmission electron microscopy in the initial study of the samango (3) were flagellated and appeared to indent the plasma membrane only slightly. Lee and Phillips (15) claimed that they had isolated spiral organisms from the ceca of rats and mice, but the role of these bacteria in the ecology of the gut is not certain.

Bacterial isolates from both the samango and vervet were screened for some physiological properties. Although identical isolation procedures and media were used with both species, many isolates from the cecum and colon of the vervet could not be maintained after initial isolation. This resulted in a lower total number of colonies being screened for the vervet than for the samango (181 and 333 isolates, respectively). This may indicate a qualitative difference in the lumen gut microflora of the two monkey species. Cellulose is an important component in the diet of the monkeys, particularly the samango. Although CMC is not a suitable substrate for cellulases active against crystalline cellulose, many "true" cellulases are active against CMC, and this substrate was used in the study. Many isolates (46%) from the samango were active against CMC. This is in contrast to strains from the omnivorous vervet, of which 12% were CMCase positive. The presence of a high percentage of possible cellulose-digesting bacteria in the stomach of a monogastric primate has not been reported. Bauchop (1) isolated a number of bacterial strains (predominantly lactobacilli) from the stomach of the rhesus monkey but did not report the presence of cellulose digesters. Their presence has been inferred in monogastric primates on the basis of VFA measurements from the stomach (7), but this may be misleading since other noncellulolytic strains of bacteria also produce VFAs as fermentation end products. The presence of bacteria able to degrade CMC, and therefore possibly cellulose, in high numbers in the samango stomach may be a result of their herbivorous diet and probably is important in the digestion of foliar material. The occurrence of this gastric flora is also reflected by the higher VFA concentrations in the fundic stomach of samangos compared with vervets.

Chitinase activity was fairly widely distributed, and often isolates that showed CMCase activity could degrade chitin. Chitinolytic activity would potentially aid in the breakdown of insect exoskeletons, such as those ingested by the vervet.

McLean et al. (18) have reviewed bacterial urease production in the gut, and they advocate the theory that urease-producing bacteria are predominantly found adherent to the (rumen) wall. This may explain the low numbers of urease producers isolated from both species, as lumenal bacteria rather than attached bacteria were isolated with the methods described. The numbers of ureolytic bacteria may also have been underestimated due to repression of urease by nitrogen in the PY basal medium.

In conclusion, there is evidence for bacterial fermentations, particularly in the cecum and colon, in both the samango and vervet. Samangos have larger cecal and colonic volumes, and a high percentage of bacteria able to digest CMC and thus possibly cellulose, in all regions of the GIT. This may be important for the samangos, which are restricted to forest habitats where seasonal fluctuations of fruit abundance often necessitates increased specialization on leaves. Vervets possibly require less specialization of the GIT because of their omnivorous diet and ability to forage in different habitats.

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