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Fecal Bacterial Diversity in a Wild Gorilla†

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We describe the bacterial diversity in fecal samples of a wild gorilla by use of a 16S rRNA gene clone library and terminal-restriction fragment length polymorphism (T-RFLP). Clones were classified as *Firmicutes*, *Verrucomicrobia*, *Actinobacteria*, *Lentisphaerae*, *Bacteroidetes*, *Spirochetes*, and *Planctomycetes*. Our data suggest that fecal populations did not change temporally, as determined by T-RFLP.

About half of the world's 740 remaining mountain gorillas live in Bwindi Impenetrable National Park, Uganda. Although there are reports of pathogenic bacteria in gorilla feces (13, 20), the normal gastrointestinal microbiota of these endangered apes remains unexplored. The purpose of this study was to describe the fecal bacterial diversity of a wild Bwindi gorilla (*Gorilla beringei*).

Fecal samples (taken ≤12 h after defecation) were collected monthly from September to December 2002 from the night nests (23) of one silverback gorilla (Zeus) in Bwindi Impenetrable National Park. Zeus, the dominant silverback in the Kyagurilo group of gorillas ($n = 15$), was at least 30 years old and in apparently good health. His feces were collected from night nests and distinguished from the feces of others by size, the presence of silver hairs, and nest location. The study site (3, 11) and experimental protocols (6) are described in detail elsewhere. A 16S rRNA gene clone library was constructed from the December fecal sample, and terminal-restriction fragment length polymorphism (T-RFLP) analyses (18) were performed to investigate the temporal dynamics of fecal microbial populations. The bacterial specific primers 27F and 1492R (16) were used for library construction, whereas universal primers 515F (16) and 1391R (24) were used for T-RFLP.

The 5′-end sequences (~600 bp) of 93 clones were determined. Clones >99% identical to one another were described as an operational taxonomic unit (OTU). Forty-six OTUs were

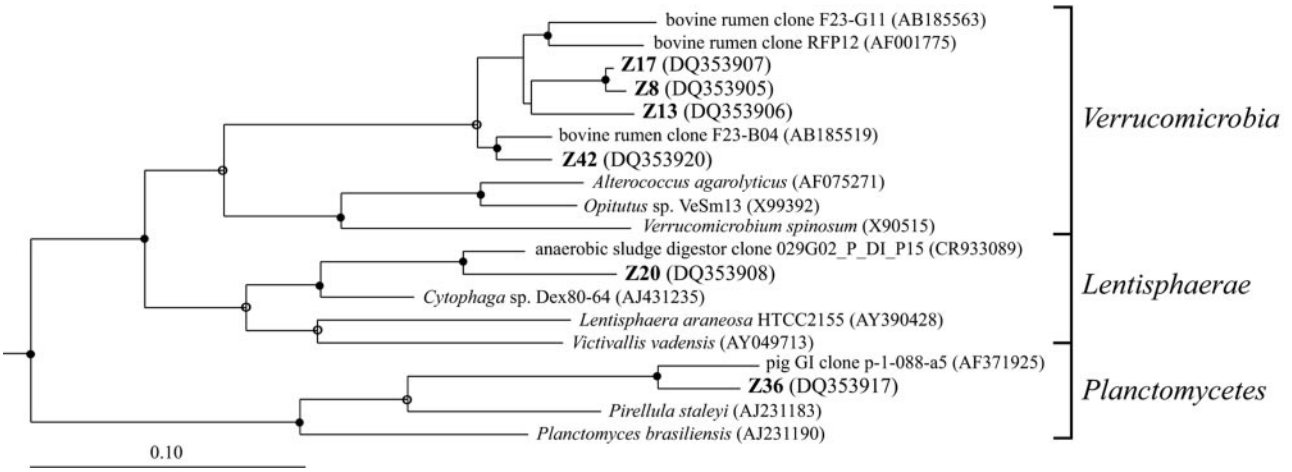

FIG. 1. Maximum-likelihood dendrogram demonstrating the affiliation of gorilla 16S rRNA gene sequences (bold) with the *Verrucomicrobia*, *Lentisphaerae*, and *Planctomycetes*. Branching points supported by ≥90% of bootstrap replicates are indicated with closed circles, and those supported by 75 to 90% are indicated by open circles. The scale bar represents 10% sequence divergence.

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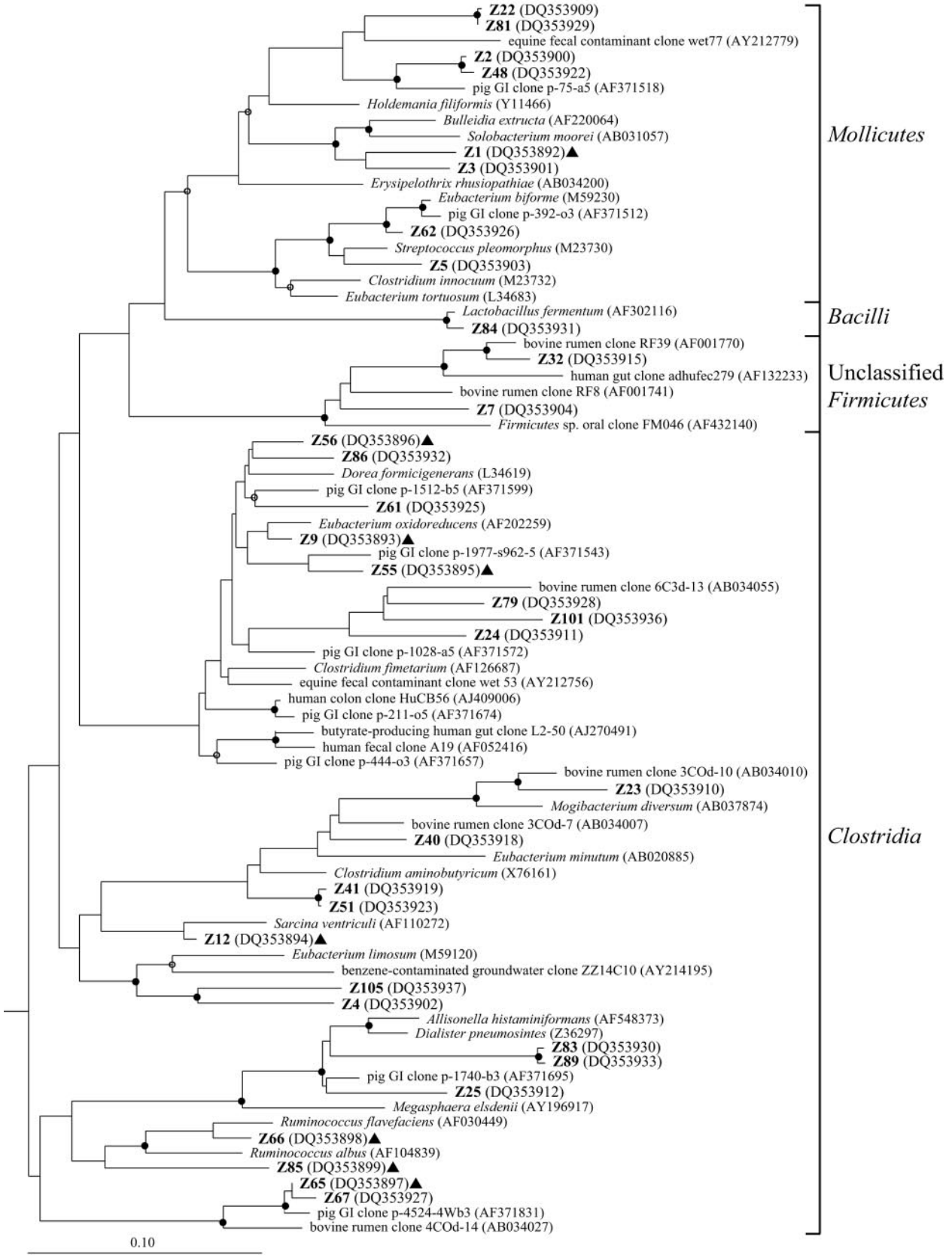

FIG. 2. Maximum-likelihood dendrogram demonstrating the distribution of gorilla 16S rRNA gene sequences (bold) within the *Firmicutes*. The four classes of this phylum are listed to the right. Branching points supported by ≥90% of bootstrap replicates are indicated with closed circles, and those supported by 75 to 90% are indicated by open circles. Partial sequences are indicated by a closed triangle. The scale bar represents 10% sequence divergence.

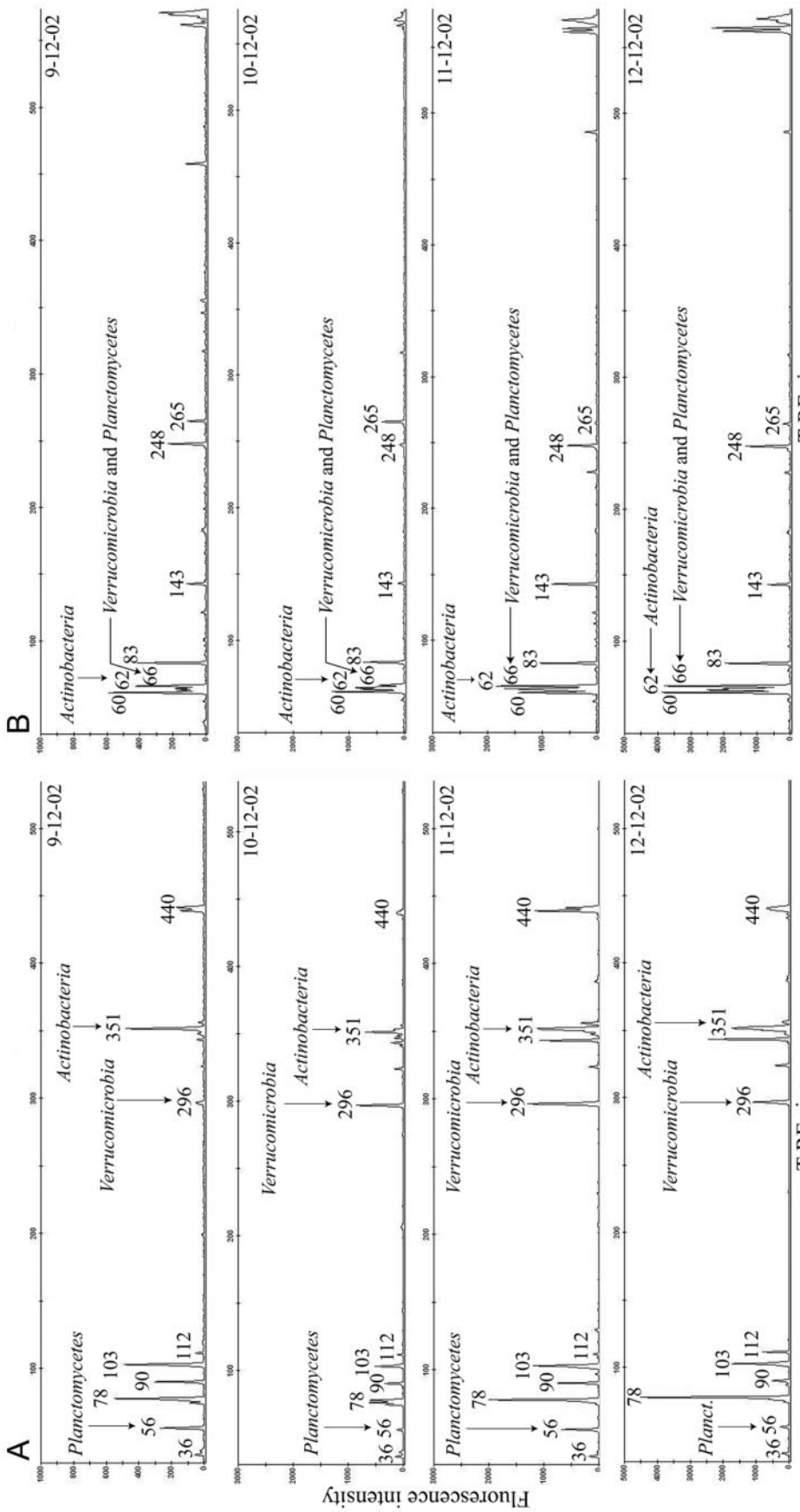

FIG. 3. Terminal-restriction fragment length polymorphism electropherograms signifying microbial community diversity in the gorilla feces for the months of September, October, November, and December of 2002. Panels to the left (A) were generated with the enzyme MseI, whereas panels to the right (B) were generated with the enzyme HhaI. The *x* axis represents the T-RF length (bases), and the *y* axis represents fluorescence intensity (in arbitrary units). T-RFs common to all 4 months in the same enzyme panel are indicated by T-RF size above each peak. Putative associations between T-RFs and the *Actinobacteria*, *Planctomycetes*, and *Verrucomicrobia* phyla are shown.

TABLE 1. Comparison of gastrointestinal clone libraries generated from herbivores and omnivores

Bacterial phylum	% of phylogenetic lineage[a]									
	Gorilla	Human[b]	Vegetarian woman[c]	Holstein cows, fluid fraction[d]	Holstein cows, solid fraction[d]	Yak[e]	Jinnan cows[e]	Reindeer[f]	Horse[g]	Pig[h]
Actinobacteria	5.3	0.2	0.5						1.0	1.1
Bacteriodetes	1.1	47.7	6.0	38.1	26.2	33.3	45.9	32.4	20.0	11.2
Fibrobacter						3.3	3.7			
Firmicutes	71.0	50.8	90.2	52.4	71.4	58.3	22.6	67.6	72.0	81.3
Flexistipes										0.3
Fusobacteria		0.08								
Lentisphaerae	3.2									
Planctomycetes	1.1									0.3
Proteobacteria		0.6	3.3	4.7			27.3		1.0	5.3
Spirochetes	1.1			2.4	2.4	5.1	0.5		3.0	0.5
Verrucomicrobia	17.2	0.6							3.0	
Unclassified		0.02		2.4						

[a] Values are proportions of phylogenetic lineages reported for each clone library.
[b] Eckburg et al. (5). Data represent the means of the results for three individuals.
[c] Hayashi et al. (8).
[d] Tajima et al. (27). Fluid and solid fractions of ruminal contents were used for library construction.
[e] An et al. (1). Archaeal clones represented 7.2% and 1.5% of the clone libraries derived from ruminal samples obtained from yaks and from Jinnan cows, respectively. All values presented in columns 7 and 8 have been corrected to show the relative proportions of bacterial sequences recovered in each of these libraries.
[f] Sundset et al. (25a). Reindeer were fed late-summer natural pasture.
[g] Daly et al. (4).
[h] Leser et al. (17).

identified. For OTUs <95% identical to a previously described rRNA gene, a representative clone was sequenced in its entirety (~1,400 bp). No chimeras were detected. Coverage according to Good's method (7) was calculated to be 67%. The fecal bacterial community was diverse, with clones falling within seven bacterial lineages: *Firmicutes* (71% of the clones), *Verrucomicrobia* (17.2%), *Actinobacteria* (5.3%), *Lentisphaerae* (3.2%), *Bacteroidetes* (1.1%), *Spirochetes* (1.1%), and *Planctomycetes* (1.1%) (see the supplemental material).

A notable finding was the presence of clones grouping within the *Verrucomicrobia* phylum (12) (Fig. 1). 16S rRNAs from the *Verrucomicrobia* are commonly found in soils (2). Although Bwindi gorillas may accidentally or intentionally eat soil (22), it is unlikely that ingested *Verrucomicrobia* bacteria just passing through the gastrointestinal tract would be detected in large numbers in our analyses. Therefore, members of the *Verrucomicrobia* phylum are likely part of the normal intestinal microbiota of gorillas. *Verrucomicrobia*-affiliated sequences have also been obtained from the gastrointestinal tracts of diverse herbivores (4, 19, 27) and humans (5, 10, 25).

All four classes of the *Firmicutes* were represented in the gorilla fecal samples (Fig. 2). Class distributions were as follows: *Clostridia* (51.5%), *Mollicutes* (39.4%), *Bacilli* (1.5%), and unclassified *Firmicutes* (7.6%). *Firmicutes* are prevalent in the gastrointestinal tracts of ruminants (1, 19, 27, 28), pigs (17), horses (4), and humans (5, 8–10, 25).

A high level of divergence from both cultivated bacterial and environmental 16S rRNA gene sequences was observed; only four clones (4.3%) shared ≥97% identity with sequences in GenBank. Moreover, only four gorilla fecal 16S rRNA gene sequences shared high (≥95%) identity to previously described cultured bacteria, including *Sarcina ventriculi* (clone Z12), *Lactobacillus fermentum* (Z84), *Ruminococcus flavefaciens* (Z66), and *Eubacterium oxidoreducens* (Z9).

The Bwindi gorillas consume a diet high in fiber (21, 22), and although a clone closely affiliated with the cellulolytic bacterium *R. flavefaciens* was recovered, sequences related to the *Fibrobacteria* phylum were not. However, by use of species-specific *Fibrobacter succinogenes* primers (26), an *F. succinogenes* 16S rRNA gene fragment was amplified from fecal genomic DNA, suggesting that gorillas harbor a variety of cellulolytic bacteria. Approximately 35% of foods eaten by Bwindi gorillas contain condensed tannins (21, 22). Therefore, the presence of rRNA gene sequences similar to the 16S rRNA of *Eubacterium oxidoreducens*, a bacterium known to anaerobically decarboxylate gallate, a phenolic compound found in plant flavonoids, tannins, and lignin (14, 15), suggests that intestinal bacteria play a role in tannin tolerance by gorillas.

To explore temporal changes in microbial diversity, T-RFLP analyses were performed with samples collected over the 4-month period (Fig. 3). Chi-square tests of homogeneity for MseI T-RFLPs ($P = 0.137$) and for HhaI T-RFLPs ($P = 0.172$) indicated that there was insufficient evidence to conclude that microbial diversity varied by month. In an effort to maximize the diversity observed in this study, both bacterial (clone library) and universal (T-RFLP) primer sets were used in the analyses. Both the clone library and T-RFLP analyses suggested that *Verrucomicrobia* are important members of the gorilla fecal microbiota.

As with any PCR-based method, clone libraries are subject to biases (29). With this in mind, the gorilla clone library was compared with gastrointestinal clone libraries from animals using diverse digestive strategies (Table 1). All gut libraries contained members of the *Bacteroidetes* and *Firmicutes* bacterial lineages, but like the vegetarian woman described in reference 8, the gorilla harbored a lower proportion of bacteria belonging to the *Bacteroidetes*. The proportions of members of the *Verrucomicrobia* and *Actinobacteria* lineages were higher in the gorilla feces than in the other gut clone libraries. Members of the *Proteobacteria* were not recovered in the gorilla analysis.

Planctomycetes were found only in gorilla feces and the pig gastrointestinal tract.

The gorilla fecal microbiota encompassed several major bacterial lineages, and diversity changed little over the course of 4 months. The idea of the presence of *Verrucomicrobia* was supported by the clone library and T-RFLP analyses, suggesting that this phylum plays an important role in the gastrointestinal microbiology of the gorilla. Sequences for bacteria related to lineages that degrade fiber, reduce aromatic compounds, and ferment nonstructural carbohydrates were recovered. However, the extent to which these bacteria affect digestive function, detoxification of secondary plant compounds, and food choices of gorillas remains to be determined.

Nucleotide sequence accession numbers. The 16S rRNA gene sequences obtained from the gorilla feces have been deposited in GenBank under accession numbers DQ353892 to DQ353947.

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