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Cellulolytic Bacteria in the Foregut of the Dromedary Camel (*Camelus dromedarius*)

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Foregut digesta from five feral dromedary camels were inoculated into three different enrichment media: cotton thread, filter paper, and neutral detergent fiber. A total of 283 16S rRNA gene sequences were assigned to 33 operational taxonomic units by using 99% species-level identity. LIBSHUFF revealed significant differences in the community composition across all three libraries.

In semiarid and arid lands in Australia, feral dromedary camels (*Camelus dromedarius*) have solitary access to feed supplies because they have adapted to harsh environmental conditions, which are unfavorable to other species of animals, especially domesticated livestock. Camels can utilize the abundance of low-quality shrubs and trees, such as mulga (*Acacia aneura*), broad-leaved whitewood (*Atalaya hemiglauca*), capper bushes (*Capparis spinosa*), and emu bush (*Eremophila* sp.) (14). In addition, camels can often browse on a broad spectrum of plants like thorny bushes, halophytes, and aromatic types, which are mostly avoided by domestic ruminants (7). The high content of lignocellulose in the cell wall, combined with the antinutritional factors of these shrubs and trees, has prevented cattle and sheep from using these plants as a feed source.

Like ruminants, camels have a complex gut microbiome that includes bacteria, archaea, protozoa, and fungi to coordinate plant biomass breakdown. The aim of the present work was to carry out 16S rRNA gene sequence analysis to identify fiber-digesting bacteria from the foregut of the dromedary camel that were able to utilize the different fiber types supplied in the enrichment medium as a carbon source.

Adult dromedary camels that were browsing native plants in the Queensland outback were taken to a commercial slaughterhouse, where digesta contents (500 ml) were collected from the foreguts of five random animals. This study was conducted according to the animal ethics guidelines set by The University of Queensland Animal Ethics Committee (AEC approval number SAS/069/08/UQ).

One milliliter of the rumen digesta was used to inoculate culture tubes containing prereduced basal medium 10 (1) that was enriched with one of three different fiber types, either filter paper (FP) (10), cotton thread (CT), or neutral detergent fiber (NDF) from Lucerne hay (22). The medium was prepared under anaerobic conditions and dispensed into Hungate tubes containing the fiber source in triplicate under carbon dioxide. The tubes were incubated at 37°C and subsampled into a fresh fiber enrichment medium every 7 days. After 21 days of incubation, approximately 1.5 ml from each enrichment medium was collected, and genomic DNA was extracted using an established protocol (25).

The bacterial 16S rRNA genes from the different enrichment media were amplified with the primer pairs 27f and 1492r (8) using the protocol of Samsudin et al. (15). Amplicons from the same enrichment media were pooled and cloned using a TOPO TA cloning kit according to the manufacturer's instructions (Invitrogen Corporation, San Diego, CA). A total of 300 16S rRNA clones were sequenced using the M13 forward and reverse primers and the BigDye Terminator apparatus and a 48-capillary ABI 3730 genetic analyzer (Applied Biosystems). All sequences were manually edited using CHROMAS Lite (v. 2.0) and assembled using SEQMAN (v. 3.34; DNASTAR). Chimeric sequences were identified by using the program Bellerophon (6) and were then excluded from further analyses. The 16S rRNA gene sequences were aligned using Greengenes (4), and the nearest neighbor sequences were imported into the ARB software package (9) for further alignment. MOTHUR (16) was used to assign sequences to operational taxonomic units (OTUs) based on 99% sequence identity criterion and to estimate bacterial diversity and population composition between clone libraries by using Shannon index (18) and LIBSHUFF (19) analyses, respectively.

In the CT 16S rRNA gene clone library, 100 clones were sequenced and analyzed, revealing 17 chimeras. The remaining 83 sequences were assigned to 11 OTUs (Table 1). Of these, eight OTUs (CAMbact152 to -155, 157 to -159, and -161) were from the phylum *Firmicutes* and were affiliated with *Butyrivibrio*, *Clostridium*, *Eubacterium*, *Pseudobutyrivibrio*, *Schwartzia*, *Selenomonas*, and uncultured bacteria from the bovine rumen and the hoatzin crop. Two OTUs (CAMbact160 and CAMbact162) were from the phylum *Proteobacteria* and were affiliated with *Anaerobiospirillum*, *Shigella*, *Succinivibrio*, and an uncultured bovine rumen clone. The remaining OTU (CAMbact156) was from the phylum *Synergistetes* and grouped with *Pyramidobacter*.

In the FP 16S rRNA gene clone library, 100 sequences were assigned to 18 OTUs (Table 1). Of these, 13 OTUs (CAMbact152, -153, -158, -163, -164, -165 to -167, -169, -172, -173, -175, and -176) represented the phylum *Firmicutes*, with sequences from *Anaerovorax*, *Butyrivibrio*, *Clostridium*, *Eubacterium*, *Mogibacterium*, *Oscillobacter*, *Pseudobutyrivibrio*, *Schwartzia*, *Streptococcus*, *Succiniclasticum*, uncultured rumen bacteria from domesticated

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TABLE 1 Bacterial 16S rRNA sequences from the foregut of dromedary camels, cultured from three different fiber enrichment media

16S rRNA gene OTU	No. of clones[a]	GenBank accession no.	Fiber enrichment(s)[b]	Nearest valid taxon	% sequence similarity
CAMbact152	48	HQ008599	CT/FP	*Eubacterium* sp. F1	98.3
CAMbact153	42	HQ008600	CT/FP	*Pseudobutyrivibrio ruminis* M80	98.9
CAMbact154	4	HQ008601	CT	*Selenomonas ruminantium* S4	99.2
CAMbact155	4	HQ008602	CT	*Selenomonas ruminantium* L1	98.8
CAMbact156	14	HQ008603	CT/FP	*Pyramidobacter piscolens* W5455	95.6
CAMbact157	2	HQ008604	CT	*Selenomonas ruminantium* L14	97.8
CAMbact158	19	HQ008605	CT/FP/NDF	*Clostridium bifermentans* JCM 7832	99.9
CAMbact159	6	HQ008606	CT	*Selenomonas ruminantium* S36	98.4
CAMbact160	1	HQ008607	CT	*Anaerobiospirillum thomasii* DSM11806	92.0
CAMbact161	3	HQ008608	CT	*Schwatrzia succinivorans* S1-1	99.8
CAMbact162	3	HQ008609	CT/NDF	*Shigella boydii* 5216-70	100.0
CAMbact163	22	HQ008610	FP/NDF	*Streptococcus bovis*	100.0
CAMbact164	3	HQ008611	FP	*Succiniclasticum ruminis* SE10	92.6
CAMbact165	1	HQ008614	FP	*Butyrivibrio hungatei* Su6	97.1
CAMbact166	3	HQ008617	FP	*Anaerovorax* sp.	93.7
CAMbact167	8	HQ008618	FP	*Clostridium* sp. AP81	93.0
CAMbact168	5	HQ008620	FP	*Prevotella ruminicola* 23	97.0
CAMbact169	1	HQ008621	FP	*Clostridium* sp. P6	91.0
CAMbact170	1	HQ008622	FP	*Succinivibrio dextrinosolvens* 0544	99.7
CAMbact171	6	HQ008623	FP	*Fibrobacter succinogenes* RS223	99.5
CAMbact172	1	HQ008624	FP	*Schwartzia succinivorans* DSM10502T	90.0
CAMbact173	1	HQ008625	FP	*Mogibacterium neglectum* P9a-h	92.8
CAMbact174	1	HQ008626	FP	*Fibrobacter intestinalis* JG1	99.2
CAMbact175	6	HQ008627	FP	*Clostridium butyricum* IDCC 5101	99.8
CAMbact176	1	HQ008628	FP	*Oscillobacter valerigenes* Sjm18-20	91.0
CAMbact177	36	HQ008631	NDF	*Ruminobacter amylophilus* H18	97.4
CAMbact178	3	HQ008633	NDF	*Citrobacter amalonaticus*	99.8
CAMbact179	18	HQ008634	NDF	*Escherichia coli* O111:H-11128	99.9
CAMbact180	4	HQ008635	NDF	*Shigella* sp. 29 (2010)	99.9
CAMbact181	6	HQ008637	NDF	*Enterococcus faecium* L118	99.9
CAMbact182	4	HQ008640	NDF	*Escherichia coli* 4106	98.9
CAMbact183	2	HQ008643	NDF	*Citrobacter koseri* CDC8132-86	98.1
CAMbact184	4	HQ008645	NDF	*Citrobacter freundii* JCM24066	99.5

[a] A total of 283 clones were examined.
[b] Some of the OTU groups were able to grow on fiber-enriched medium of more than one type.

ruminants, uncultured oral bacteria, and bacteria from nonruminant animals. Two OTUs (CAMbact171 and CAMbact174) belonged to the genus *Fibrobacter*, and the remaining three OTUs (CAMbact 156, CAMbact168, and CAMbact170) belonged to the genera *Pyramidobacter*, *Prevotella*, and *Succinivibrio*, respectively.

In the NDF 16S rRNA gene clone library, 100 sequences were assigned to 11 OTUs (Table 1). Eight OTUs (CAMbact162, CAMbaact177 to -180 and CAMbact182 to -184) were from the phylum *Proteobacteria* and were affiliated with *Citrobacter*, *Escherichia*, *Ruminobacter*, *Shigella*, and two uncultured clones from the porcine intestine and feces of the rodent family Caviidae. The remaining three OTUs (CAMbact158, CAMbact163, and CAMbact181) were from the phylum *Firmicutes* and were affiliated with *Clostridium*, *Streptococcus*, and *Enterococcus*. The presence of *Escherichia coli* in the NDF medium was unclear. It is speculated that *E. coli* probably used the fermentation products from the other bacteria for its growth and survival in that medium. The 21 days of incubation, with subculturing every 7 days, may have enriched monomer-utilizing bacteria, rather than the polymer utilizers, which may explain the detection of *E. coli* as one of the dominant organisms in these media.

In total, 283 sequences were assigned to 33 OTUs (Table 1). Six OTUs were unique only to the CT medium, 13 OTUs were unique only to the FP medium, and 8 OTUs were unique only to the NDF medium. Thirteen of the 33 OTUs had <98% identity to their nearest relatives, indicating 13 possible new species, while 4 OTUs had <92% identity, indicating four possible new genera. *Firmicutes* was the most abundant phylum in the FP and CT media, whereas the phylum *Proteobacteria* was prevalent in the NDF medium. According to the Shannon indices, the FP library (2.36 ± 0.19 [mean ± standard error of the mean]) was significantly more diverse ($P = 0.05$) than either the CT (1.73 ± 0.20) or NDF (1.68 ± 0.16) libraries. The solubility of degradable cellulose in the filter paper along with the increased susceptibility to biological attack due to the large surface area may explain why the bacterial community was more diverse in the FP medium (5). Comparison of the community compositions across the three 16S rRNA gene sequence clone libraries using the LIBSHUFF algorithm also indicated significant differences ($P < 0.0001$).

At the species level, *Pseudobutyrivibrio ruminis* was the most abundant species in the CT enrichment medium (38.5%), *Eubacterium* sp. was the most abundant species in the FP medium (32%), and *Ruminobacter amylophilus* was the most abundant species in the NDF medium (36%). The reason for the presence of

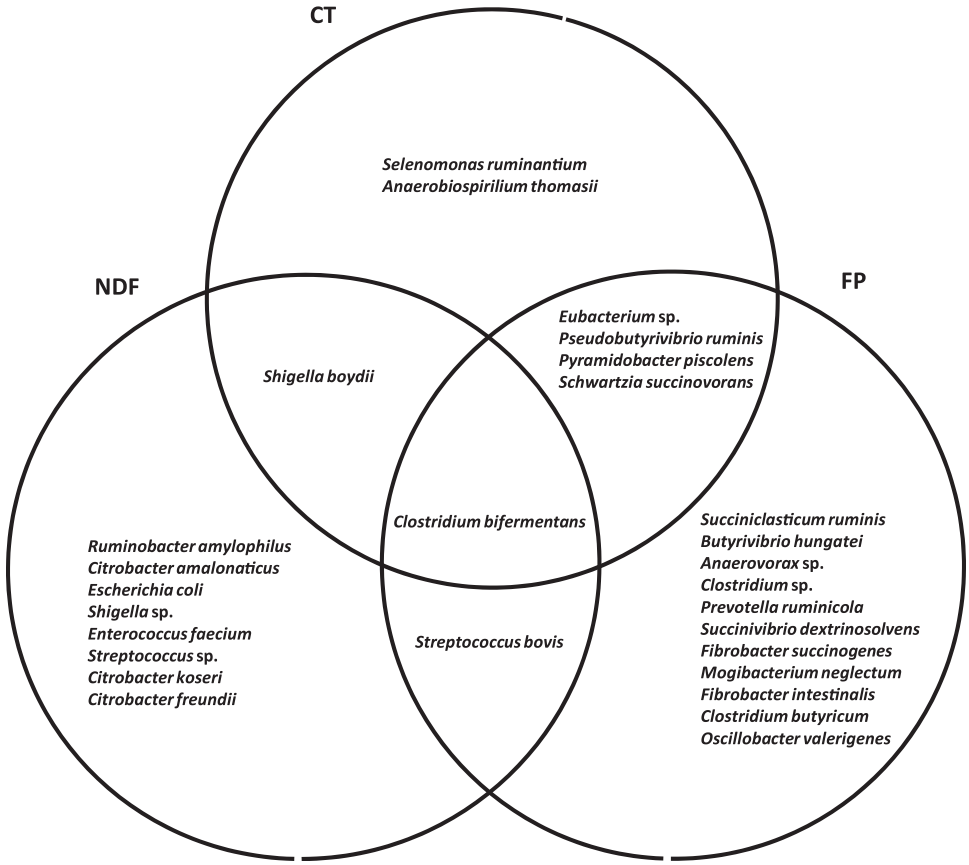

FIG 1 Venn diagram of bacterial distributions at the species level when cultured in the three enrichment media: CT, FP, and NDF.

a high number of *P. ruminis* bacteria in the CT medium remains unclear, as *P. ruminis* is a butyrate-producing bacterium that is nonxylanolytic, nonamylolytic, and nonproteolytic (22). It is likely that *Pseudobutyrivibrio* survives on other components of the medium. In contrast, *Eubacterium cellulosolvens* and members of the genus *Clostridium* are reported to possess cellulolytic activity but are usually present in relatively low numbers in the rumen (2, 3, 17). In the present study, *Clostridium bifermentans*, a species common in feces, sewage, and soil, was recovered in all three media (Fig. 1), and *Eubacterium* was the most abundant species in the FP medium and was also present in the CT medium. *Eubacterium* was also present in large numbers in foregut samples of the dromedary camel (15) and in alpacas and sheep fed with alfalfa (13).

In the rumen, *Fibrobacter succinogenes*, *Ruminococcus albus*, *Ruminococcus flavefaciens*, and *Butyrivibrio* sp. are actively involved in degrading plant polymers, like cellulose and hemicellulose. However, in the present study, a very low number of clones of *Fibrobacter succinogenes* were detected in the FP enrichment medium, and no cellulolytic ruminococci were detected from the three media. In previous studies, sequences from *F. succinogenes* were not recovered from the rumen when cattle were fed hay or hay plus concentrate (21, 24) or from clone libraries of wild African ruminants and domesticated zebu cattle (11). In the present study, competition among the fibrolytic bacteria to utilize the available substrate in the culturing medium could also have caused the low number of clones. For example, when fibrolytic bacteria are cultured together in enrichment medium with cellulose, *R. flavefaciens* is inhibited, while *R. albus* increases in numbers during 12 to 48 h of degradation, before becoming dominated by the massive growth of *F. succinogenes* after 60 to 70 h of degradation (12).

In conclusion, fiber type influences bacterial species that grow in fiber-enriched medium. The bacterial growth can therefore indicate the bacterium's fiber type preference. However, common species of bacteria that share more than one type of fiber enrichment were also identified. The identification of new bacterial species and possibly new genera reveals the significance of the results of this study. This provides an insight into the poorly researched bacterial ecosystem of the camel and lays the foundation for future studies.

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