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In-depth diversity analysis of the bacterial community resident in the camel rumen

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ABSTRACT

The rumen compartment of the ruminant digestive tract is an enlarged fermentation chamber which houses a diverse collection of symbiotic microorganisms that provide the host animal with a remarkable ability to digest plant lignocellulosic materials. Characterization of the ruminal microbial community provides opportunities to improve animal food digestion efficiency, mitigate methane emission, and develop efficient fermentation systems to convert plant biomasses into biofuels. In this study, 16S rRNA gene amplicon pyrosequencing was applied in order to explore the structure of the bacterial community inhabiting the camel rumen. Using 76,333 quality-checked, chimera- and singleton-filtered reads, 4954 operational taxonomic units (OTUs) were identified at a 97% species level sequence identity. At the phylum level, more than 96% of the reads were affiliated to OTUs belonging to *Bacteroidetes* (51%), *Firmicutes* (31%), *Proteobacteria* (4.8%), *Spirochaetes* (3.5%), *Fibrobacteres* (3.1%), *Verrucomicrobia* (2.7%), and *Tenericutes* (0.95%). A total of 15% of the OTUs (746) that contained representative sequences from all major taxa were shared by all animals and they were considered as candidate members of the core camel rumen microbiome. Analysis of microbial composition through the solid and liquid fractions of rumen digesta revealed differential enrichment of members of *Fibrobacter*, *Clostridium*, *Ruminococcus*, and *Treponema* in the solid fraction, as well as members of *Prevotella*, *Verrucomicrobia*, *Cyanobacteria*, and *Succinivibrio* in the liquid fraction. The results clearly showed that the camel rumen microbiome was structurally similar but compositionally distinct from that of other ruminants, such as the cow. The unique characteristic of the camel rumen microbiome that differentiated it from those of other ruminants was the significant enrichment for cellulolytic bacteria.

Introduction

The camel digestive tract differs anatomically from that of true ruminants, such as cows, goats and sheep, which have a four chambered forestomach. The camel is a pseudo-ruminant with a forestomach differentiated into three compartments referred to as C1 (equivalent to the rumen), C2 (reticulum), and the gastric secreting compartment C3 (abomasum) [57]. Similar to that of other ruminants, the camel rumen is an enlarged anaerobic fermentation chamber which houses a complex microbial community consisting of bacteria, archaea, protozoa and fungi [13,49]. This symbiotic microbial community is able to decompose plant polysaccharides in feed particles into their oligomers and monomers, and further ferments the released molecules to short-chain volatile fatty acids, which are subsequently absorbed from the rumen and utilized as a source of energy by the host animal [27]. Indeed, the complex microbial community inhabiting the rumen has an obligate symbiotic association with the host animal, since plant lignocellulosic compounds by themselves are indigestible for the host digestive system. In addition to being critical for the animal's nutrition, the rumen microbiota makes a large contribution to the promotion of the health, productivity, and immunity of the host animal.

Camels live in deserts and dry lands, and usually graze on low quality natural forages and woody shrubs, which are highly fibrous and have a greater content of antinutritional compounds, such as tannins, saponins and lignins. Camels can survive in harsh and inhospitable environmental conditions, such as high salinity and drought with the minimal use of water, and their digestive system has evolved in order to adapt to these unfavorable conditions [48].

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Compared to cows and sheep, the retention time of feed particles in the camel forestomach is longer, which prolongs the exposure of plant biomasses to the symbiotic microorganisms and helps in efficient digestion of low quality fibrous diets [28]. In addition, the camel ruminal pH is largely constant and close to neutral, which further provides a suitable condition for growth and colonization of cellulolytic microorganisms [47,48]. These unique characteristics of the camel digestive system, along with the distinct microbial community inhabiting its rumen, allow the animal to digest, ferment and extract the nutrients efficiently from plant lignocellulosic material.

The microbial community in the rumen is largely populated and dominated by bacteria, and it is thought that they make the greatest contribution to decomposition and fermentation of feedstuffs in order to absorb volatile fatty acids easily [25,53]. In addition, the bacterial cells are subsequently transferred, along with the rumen digesta, to the gastric secreting compartment (abomasum) where they are subjected to HCl and host digestive enzymes, and their released digesta are utilized by the host animal as a source of amino acids and proteins [53]. The structure and composition of the bacterial community that inhabits the camel rumen is largely unknown. However, to date, a few culture-independent approaches have been applied to explore this particular bacterial community. In one study, Samsudin et al. sequenced 267 bacterial 16S rRNA gene clones and clustered the sequences into 151 operational taxonomic units (OTUs) at a 99% sequence identity level [48]. The majority of OTUs were annotated to the phyla *Firmicutes* (67%) and *Bacteroidetes* (25%), and were dominated by members of the families *Eubacteriaceae*, *Clostridiaceae*, *Prevotellaceae*, *Lachnospiraceae*, and *Rikenellaceae*. In another study, the same group inspected the camel rumen microbiota for the presence of cellulolytic bacteria by inoculating the rumen digesta in three different enrichment media [49]. Using 283 16S rRNA gene clones, 33 OTUs affiliated to the genera *Butyrivibrio*, *Clostridium*, *Eubacterium*, *Pseudobutyrivibrio*, *Schwartzia*, *Selenomonas*, *Anaerobiospirillum*, *Shigella*, and *Succinivibrio* were identified. However, none of these studies could define the details of the structure and composition of the core bacterial community resident in the camel rumen.

Therefore, in the current study, high-throughput 16S rRNA gene amplicon pyrosequencing was applied in order to explore the structure and composition of the bacterial community inhabiting the camel rumen in more detail. In contrast to traditional cloning based methods, this approach has the capacity to sequence thousands of 16S rRNA amplicons and therefore provides greater coverage of microbial diversity.

Materials and methods

Sampling of rumen digesta, microbial cell collection and DNA extraction

Rumen digesta were collected from three mature female single-humped camels aged between 2 to 5 years. Each animal was randomly selected from a group of camels freely browsing on native plants. The dominant native plants browsed by selected animals were species from *Tamarix*, *Salsola*, *Alhagi camelorum*, *Aristida*, and *Cenchrus*. Animals were transferred to the nearest commercial slaughterhouse, where the liquid and solid fractions of the rumen digesta were sampled in sterile containers. The fractions were kept in liquid nitrogen during transfer to the laboratory, where they were stored at −80 °C.

To collect liquid-borne microbes, 40 mL of the liquid digesta were first centrifuged for 5 min at 500 × *g* (at 4 °C) in order to remove the remaining solid particles. Then, 20–30 mL of the liquid fraction were centrifuged for 10 min at 12,000 × *g* (at 4 °C) and the cell pellet was immediately resuspended in 1.4 mL of stool lysis buffer (ASL) from the QiaAmp® DNA Stool Mini Kit (Qiagen, Valencia, CA, USA). DNA was extracted according to the manufacturer's instructions based on the protocol for isolation of DNA from stools for pathogen detection. Particle-adherent microbes were recovered as described previously [41]. Briefly, 10–15 g of the rumen solid fractions were centrifuged at 12,000 × *g* for 5 min to remove the residual liquid fractions. Solid particles were then resuspended in 15–30 mL dissociation buffer (0.1% Tween 80, 1% methanol and 1% tertiary butanol; vol:vol, pH 2). The mixture was vigorously vortexed for 1–3 min, centrifuged for 5 min (500 × *g* and 4 °C) and the liquid phase was collected in a sterile container. This step was repeated three times, the collected supernatants were pooled and the microbial cells were recovered by centrifugation at 12,000 × *g* for 10 min. Finally, the DNA was extracted as explained above.

PCR amplification of 16S rRNA using barcoded primers

The primer pair S-D-Bact-0341-b-S-17 (5′-CCTACGGGNGGCWGCAG-3′) and S-D-Bact-0785-a-A-21 (5′-GACTACHVGGGTATCTAATCC-3′) was used for amplification of a 464 bp fragment corresponding to the V3–V4 regions of the 16S rRNA gene [26]. To barcode the PCR amplicons, a unique sequence of 10 nt (multiple identifier (MID) was added to the 5′ end of the forward primer. Finally, the sequence of the 454 A-key primer (5′-CGTATCGCCTCCCTCGCGCCATCAG-3′) was added to the 5′ end of the barcoded forward primers. In addition, the sequence of the B-key primer (5′-CTATGCGCCTTGCCAGCCCGCTCAG-3′) was also added to the 5′ end of the reverse primer. PCR was performed in triplicate in a 50 μL reaction volume under the following running conditions: 94 °C for 4 min followed by 30 cycles of 94 °C for 30 s, 55 °C for 40 s, and 72 °C for 1 min, and a final extension at 72 °C for 7 min. The PCR amplicons were gel recovered using the Qiaquick® Gel Extraction Kit (Qiagen) and sequenced using Roche 454 GS FLX titanium (Macrogen Inc. Seoul, Korea). The raw sequencing reads have been deposited at the EBI Sequence Read Archive (SRA) under study accession number: PRJEB6946.

Analysis of the Roche 454 GS FLX generated amplicon sequences

GS FLX data processing was performed using the Roche GS FLX software (v 2.9). Bacterial 16S rRNA gene amplicon sequences were processed for quality-filtering and phylogenetic analysis using the QIIME pipeline (Version 1.8.0) [6]. First, sequences were screened, trimmed, and filtered using the split_libraries.py script according to the default setting, which filters out reads smaller than 200 and longer than 1000 nt, reads containing more than 6 nt ambiguous bases, reads containing a missing qual score and a mismatch in the primer, reads with a mean qual score below a minimum of 25, and reads containing homopolymers longer than 6 nt. The remaining sequences were then subjected to alignment and OTU picking. Sequences were clustered into OTUs using Uclust software [12] based on their sequence similarity (97%). Chimeric sequences were filtered out using ChimeraSlayer [18]. Representative sequences were then aligned against the Greengenes core set [9] using the PyNAST aligner [5]. Taxonomies were assigned to representative sequences using the RDP naïve Bayesian classifier [58] with a confidence value of 0.8 against most recent Greengenes OTUs [9]. The alignments were then filtered to remove gaps and hypervariable regions, and define conserved and non-conserved positions using a Lane mask [6]. A phylogenetic tree representing the relationship between OTUs was constructed using FastTree [44]. The OTU table was then filtered for singleton OTUs. Therefore, those OTUs that were derived from singleton reads (or represented less than 0.002% of the total sequences), which were largely sequence artifacts, were discarded. The details of the number of reads per sample before