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Gut microbiota of the scimitar-horned oryx, *Oryx dammah*

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Abstract. Our aim was to obtain details of the condition of the scimitar-horned oryx (*Oryx dammah*) and to elucidate the gut microbial diversity of captive individuals of this species. For the first time, 16S rRNA barcoding was used to characterise the faecal microbiota of five captive scimitar-horned oryx individuals. A total of 15 prokaryotic phyla were identified in the five samples. Including *Firmicutes* (53.40-72.01 %), *Bacteroidetes* (12.94-23.72 %) and *Proteobacteria* (1.03-31.74 %), accounting for > 96 % of all the sequences. At the family level, there were < 3.5 % unclassified sequences. In conclusion, these data revealed similarities and differences in gut microbial diversity across the scimitar-horned oryx individuals. These observations advance the current understanding of the bacterial ecosystems in these endangered animals under captivity.

Key words: diversity, *Firmicutes*, high-throughput sequencing, 16S rRNA

Introduction

There are three oryx species (Bovidae) in northern Africa: scimitar-horned oryx (*Oryx dammah*), Cape oryx (*O. gazella*) and Arabian oryx (*O. leucoryx*). Amongst these, the scimitar-horned oryx is listed as Extinct in the Wild in the International Union for Conservation of Nature Red List because there has been no confirmed evidence of the survival of this species since the early 1990s despite extensive surveys dedicated to the detection of Sahelo-Saharan antelopes conducted in Chad and Niger. This species is well adapted for survival in dry areas and is a social bovid.

Herbivores prefer to consume fresh shoots because these usually contain low concentrations of secondary plant compounds and are protein-rich, making them more palatable, easier to digest and more nourishing (Villalba et al. 2002, 2004). Wild scimitar-horned oryx inhabited steppe and desert ecosystems, where they consumed foliage, grass, herbs, shrubs, succulent plants, legumes, juicy roots, buds and fruits. However, replicating their natural diet in captivity is challenging as food items vary greatly in terms of availability, palatability and nutrient content throughout the year. Nonetheless, captive diets are often designed to mimic the natural diet of animals.

Gut microbiota plays a vital role in nutrient uptake, food digestion, energy harvesting and vitamin synthesis in humans and other animals (Hooper et al. 2002, Bäckhed et al. 2005). Previous studies have revealed that *Firmicutes* and *Bacteroidetes* bacteria are predominant in the rumen and faeces of Bovidae (Shanks et al. 2011, Singh et al. 2012, Thoetkiattikul et al. 2013, Xue et al. 2016). The documentation of concurrent shifts in gut microbiota will aid the establishment of baseline rates of changes in the composition of gut microbial communities across different species and diets as well as the assessment of the relationship of such gut microbial shifts with species and diet. The composition, structure and role of the gut microbiota of the scimitar-horned oryx remain largely unknown. In the present study, next-generation sequencing targeting the V3-V4 region of the bacterial 16S rRNA gene was employed to characterise the gut microbiota in faecal samples of captive scimitar-horned oryx.

Material and Methods

The study was approved by the Binzhou University Animal Care and Use Committee. Fresh faecal samples (~150 g) of five healthy scimitar-horned oryx

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individuals at the Dongying Wild Animal Park, China, were collected in the late morning during March 2018 (Table 1). No animal had received anti-inflammatory or anti-microbial drugs within the past four months, and none suffered from gastrointestinal diseases. The samples were collected from the ground immediately after defecation and instantly transferred into separate plastic tubes. The samples were stored at 4 °C, with long-term storage at –80 °C, for further analysis.

DNA extraction, 16S rRNA gene PCR and sequencing were performed. Briefly, total genomic DNA was isolated from the faecal samples using a QIAamp DNA Stool Mini Kit (Qiagen, Germany) according to the manufacturer's protocol. A UV-vis spectrophotometer (NanoDrop 2000c, U.S.A.) was used to determine DNA concentration and quality. Only samples with DNA concentrations of > 20 ng/µl were selected for PCR amplification of the V3-V4 hypervariable region of the 16S rRNA gene using the primers 341F (CCTAYGGGRBGCASCAG) and 806R (GGACTACNNGGGTATCTAAT) (Barfod et al. 2013). Amplification was performed using a final volume of 30 µl containing 15 µl of Phusion Master Mix (2×), 1.5 µl of each primer and 10 µl of microbial genomic DNA. PCR conditions were as follows: denaturation for 1 min at 98 °C, followed by denaturation with 35 cycles of 10 s at 98 °C, annealing for 30 s at 55 °C and elongation for 30 s at 72 °C and final extension for 5 min at 72 °C. The PCR products were stored at 4 °C until further analysis (within two hours) using electrophoresis in 2 % agarose gel and purification using the Qiagen Gel Extraction Kit (Qiagen, Germany). Sequence libraries were constructed using TruSeq® DNA PCR-Free Sample Preparation Kit (Illumina, U.S.A.) according to the manufacturer's protocol, and index codes were added. Library quality was assessed using Qubit® 2.0 Fluorometer (Thermo Scientific) and Agilent Bioanalyzer 2100. Finally, the libraries were sequenced using an Illumina HiSeq 2500 platform,

Table 1. Basic information on scimitar-horned oryx used in this study.

Scimitar-horned oryx	Sex	Age (years)	Health condition
B1	male	5	health
B2	male	5	health
B3	female	6	health
B4	male	5	health
B5	female	6	health

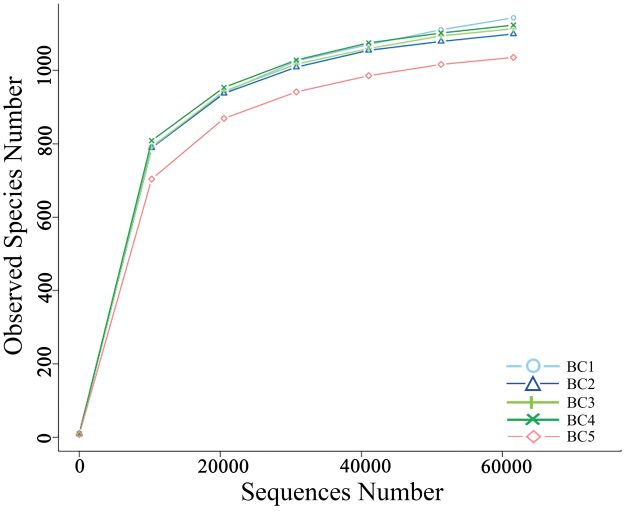

Fig. 1. Rarefaction curve analysis of the five samples.

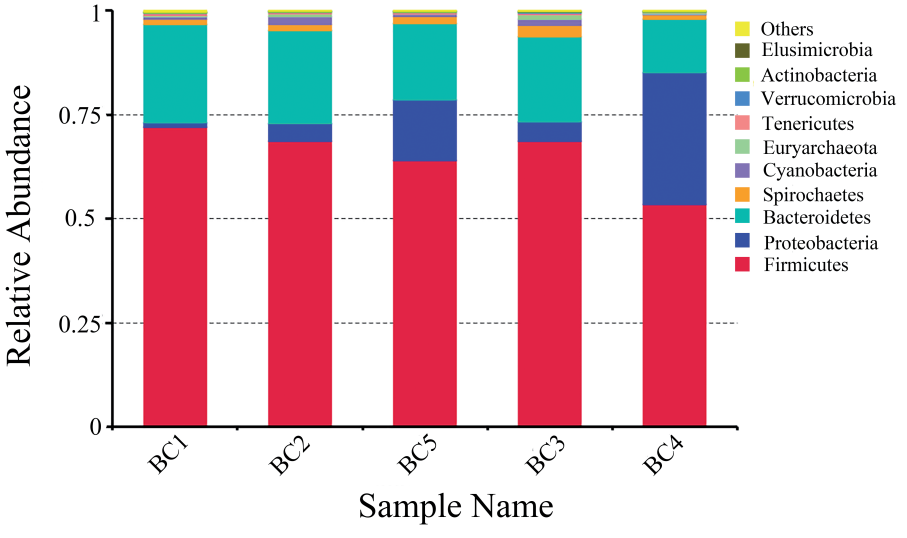

Fig. 2. Gut bacterial composition at the phylum level per sample.

and 250-bp paired-end reads were generated. The data are publicly available in the NCBI Sequence Read Archive (accession number PRJNA507332).

For bioinformatic analyses, paired-end reads were assigned to samples based on their unique barcodes and truncated by cutting off the barcode and primer sequence. The paired-end reads were merged using FLASH (v1.2.7) (Magoč & Salzberg 2011) to obtain the raw tags. Quality filtering of the raw tags was performed under specific filtering conditions to obtain high-quality clean tags (Bokulich et al. 2013) using QIIME (v1.7.0) (Caporaso et al. 2010). The tags were compared with the reference database (Gold database) using the UCHIME algorithm (UCHIME algorithm) (Edgar et al. 2011) to detect chimaeras, which were removed (Haas et al. 2011) to finally obtain the effective tags. Sequence analyses were performed using UPARSE (v7.0.1001) and multiple sequence alignment was conducted using MUSCLE (version 3.8.31) (Edgar 2004). For each representative sequence, the Greengene database (DeSantis et al. 2006) was used with the RDP Classifier (v2.2) algorithm (Wang et al. 2007) to annotate taxonomic information. Subsequent analyses of alpha diversities were performed using the normalised output data. Alpha diversity was applied to analyse the complexity of species diversity through six indices, including observed-species, Chao1, Shannon, Simpson, ACE and Good's coverage. All indices were calculated using QIIME (v1.7.0) and analysed using R (v2.15.3).

Results and Discussion

Similar to other herbivores, the scimitar-horned oryx relies upon symbiosis with microorganisms in the digestive system to utilise cellulose and hemicellulose. In the present study, for the first time, the gut microbiota of the scimitar-horned oryx was characterised using faecal samples from five captive

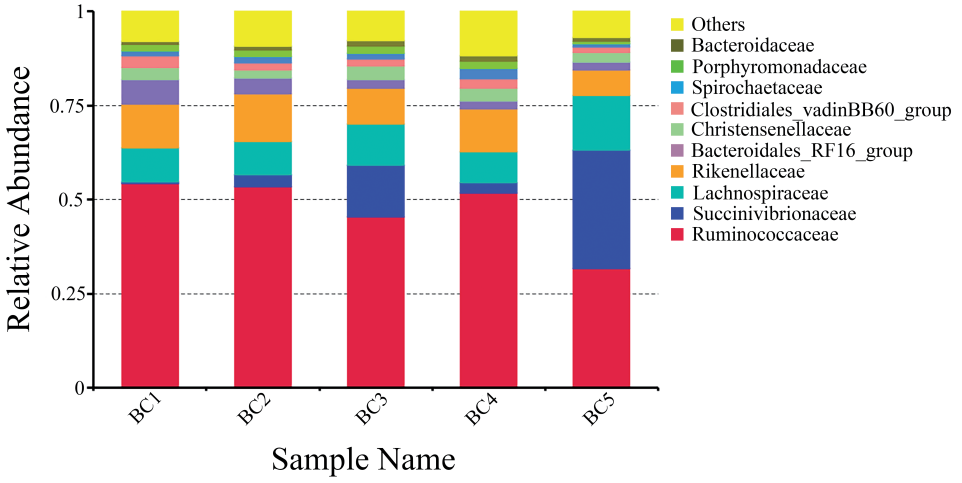

Fig. 3. Gut bacterial composition at the family level per sample.

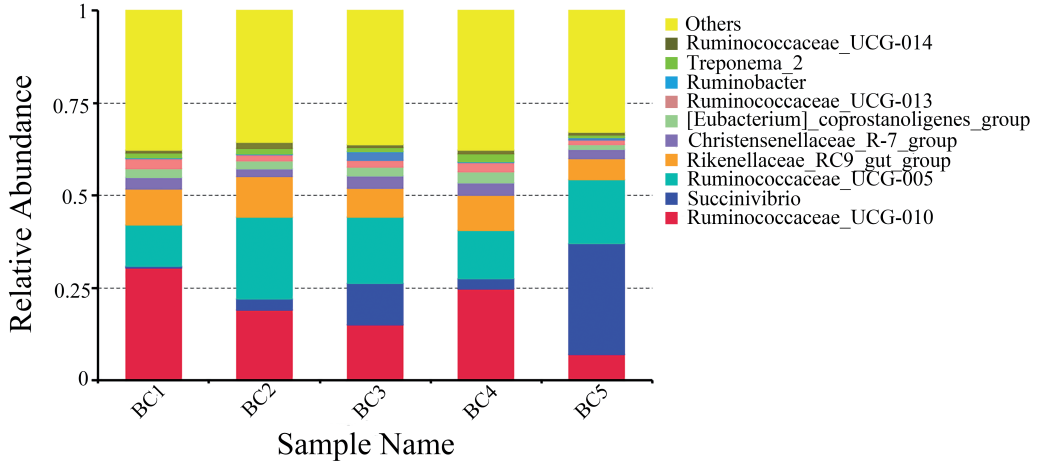

Fig. 4. Gut bacterial composition at the genera level per sample.

Table 2. Bacterial community diversity of five samples measured using different indexes.

Sample name	Observed species	Shannon	Simpson	Chao1	ACE	Goods coverage
BC1	1143	7.809	0.989	1521.477	1264.965	0.997
BC2	1099	7.758	0.988	1143.706	1145.836	0.998
BC3	1113	7.455	0.978	1149.093	1159.498	0.998
BC4	1123	8.123	0.992	1169.146	1171.415	0.998
BC5	1035	6.317	0.924	1070	1076.664	0.999

individuals. After removing low-quality reads and chimaeras, 367172 high-quality reads were obtained (mean 73434 reads per sample). The rarefaction curves tended to approach the saturation plateau (Fig. 1). The average Good's coverage was 99.8 %. For alpha diversity analyses, the sequence number was normalised to 61567 by random sub-sampling to standardise the sampling effort. Species diversity was measured using the Shannon and Simpson indices, and species richness was measured using Chao1 and ACE indices (Table 2).

A total of 15 prokaryotic phyla were identified in the five samples (Fig. 2), including *Firmicutes* (53.40-72.01 %), *Bacteroidetes* (12.94-23.72 %) and *Proteobacteria* (1.03-31.74 %), which accounted for > 96 % of all the sequences. However, microbial composition varied across samples. For instance, samples BC5 and BC3, which were both females, showed higher relative abundances of *Proteobacteria* (31.74 % and 14.57 %, respectively) than the remaining three samples (< 4.64 %), which were all males. In contrast, the abundances of *Bacteroidetes* in samples BC5 and BC3 (12.94 % and 18.44 %, respectively) were lower than those in the remaining three samples (20.67-23.72 %). There are many studies regarding faecal microbes in herbivores (Costa et al. 2012, Bian et al. 2013, Guan et al. 2017). According to Guan et al. (2017), *Firmicutes* (77.62 %), *Bacteroidetes* (18.28 %) and *Tenericutes* (1.34 %) were the most predominant phyla in wild sika deer, while *Firmicutes* (50.71 %) was the dominant phylum, followed by *Bacteroidetes* (31.99 %) and *Proteobacteria* (4.80 %), in captive sika deer. In a previous study, the bacterial communities in cape oryx were dominated by *Firmicutes* (42.81-55.29 %), *Bacteroidetes* (21.26-27.82 %) and *Proteobacteria* (3.05-7.14 %), represented by the families *Ruminococcaceae*, *Lachnospiraceae*, *Prevotellaceae*, *Porphyromonadaceae*, *Succinivibrionaceae* and *Rikenellaceae* (Chen et al. 2018).

At the family level, there were < 3.5 % unclassified sequences. The ten most common families are shown in Fig. 3. In samples BC5 and BC3, the predominant family was *Ruminococcaceae*, followed by *Succinivibrionaceae*, *Lachnospiraceae*, *Rikenellaceae*, *Christensenellaceae* and *Bacteroidales*_RF16_ group. Similarly, in the remaining three samples, *Ruminococcaceae* was the predominant family, followed by *Rikenellaceae*, *Lachnospiraceae*, *Bacteroidales*_RF16_group and *Christensenellaceae*. These comprised 84.69 %, 81.31 %, 85.47 %, 76.74 % and 89.12 % of the sequences in samples BC1, BC2, BC3, BC4 and BC5, respectively.

There were 159 genera detected in the five samples. Unclassified bacteria accounted respectively for 23.94 %, 21.12 %, 20.55 %, 19.69 % and 16.87 % of the sequences in samples BC1, BC2, BC3, BC4 and BC5, and the ten most common genera are shown in Fig. 4. Although the most common genera were identical in all the samples, the relative abundances of *Succinivibrio* in samples BC5 and BC3 were > 30 times higher than those in the remaining three samples. Previous studies have demonstrated that the most common genera were the bacteria responsible for cellulose digestion; thus, their higher abundances are associated with the herbivorous diet of the host (Daly et al. 2012, Jami & Mizrahi 2012).

Previous studies have shown that *Proteobacteria* are mainly associated with energy storage in humans, monkeys and mice (Bryant & Small 1956, Koren et al. 2012, Amato et al. 2014, Chevalier et al. 2015). Similarly, Sun et al. (2016) found that the relative abundances of *Proteobacteria* in the gut of Tibetan macaques were significantly higher in winter than in spring; they suspected that the increase in relative abundance during winter helps these macaques cope with the cold weather (Sun et al. 2016). Additionally, Wu et al. (2017) discovered that *Succinivibrio* spp. require carbon dioxide for anaerobic growth and ferment organic matter produced by the Krebs cycle to generate acetic and succinic acids. In this study, the high abundance of these bacteria might also be related to the energy produced by the species.

Acknowledgements

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