



Analysis of the microbial diversity in the fecal material of the critically endangered African wild dog, *Lycaon pictus*

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Abstract

The African wild dog (AWD) (*Lycaon pictus*) is a critically endangered species. These animals are hypercarnivores, hunting mostly medium-sized antelope. In this study, using bacterial tag-encoded FLX-Titanium amplicon pyrosequencing (bTEFAP[®]), the microbiota in the fecal material of AWDs living in the Great Plains Zoo & Delbridge Museum of Natural History was investigated. In both samples, the most predominant bacterial phylum was the Firmicutes with members of the genus *Blautia* spp. being the most dominant bacteria.

Keywords *Lycaon pictus* · African wild dog · Fecal material · bTEFAP[®] · *Blautia* spp.

Introduction

African wild dogs (AWDs) (*Lycaon pictus*) are a type of canine that are highly specialized in hunting, and they have a unique dental morphology suggesting they are able to eat bones (Creel and Creel 2002). These animals feed primarily on antelope. Larger prey animals are hunted in packs, whereas smaller prey animals, such as hares or rodents are hunted by an individual dog (Woodroffe et al. 2007). It is known that they are very social animals, in fact they are able to communicate through sneezing (Hayward et al. 2006). Physically these canines have unique features, such as having only four toes, (absence of dewclaws) (Hartstone-Rose et al. 2010), and a colorful coat in which individual animals differ in their pigmentation pattern and color (Shumba et al. 2017). In the wild, AWD populations have undergone a sustained decline over the past half-century. This is driven in large part by rapid and severe habitat loss as well as fragmentation (Woodroffe and Sillero-Zubiri 2020; Woodroffe

and Ginsberg 1998). As a result, this animal is listed in the IUCN Red Data Book as a critically endangered species, with only 1409 mature individuals remaining (Woodroffe and Sillero-Zubiri 2020).

Very complex microbial communities colonize the digestive tract of a mammal at birth (Milani et al. 2017). Then throughout life the microbiota, such as the fecal microbiota, which includes bacteria, fungus, and other microbes, plays an important role in the maintenance of health and disease resistance by facilitating many different biological processes (Sekirot et al. 2010; Lynch and Hsiao 2019). The fecal microbiome is influenced by the genome, sex, living environment, and disease state of a host (Stephens et al. 2016; Chen et al. 2017; Wu et al. 2017; Zhu et al. 2018). Very importantly our human microbiome is also associated with different pathophysiological conditions (Rajendiran et al. 2020; Ghosh et al. 2021).

Since it has been reported that microbial diversity plays an important role in maintaining the health of the host (Wu et al. 2016), the focus of this research was to examine the bacterial diversity present in the fecal material of the AWD. An inventory of the community composition of the fecal microbiota of two AWDs was made using bacterial tag-encoded FLX-Titanium amplicon pyrosequencing (bTEFAP[®]). The bacterial diversity was then compared to other closely related *Cuon* and *Canis* species described in other studies. To our knowledge, this is the first study analyzing the microbiota present in AWDs. Understanding their gastrointestinal flora could have a positive impact on

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understanding the physiology health of these animals, which could aid in their preservation.

Materials and methods

Ethics statement

IACUC approval was granted by the Great Plains Zoo & Delbridge Museum of Natural History for the collection of fecal samples.

Animals and sample collection

The two African wild dogs, living in the Great Plains Zoo & Delbridge Museum of Natural History, Sioux Falls SD, used in this study were Monkey Wrench #4979, Male, DOB 11/17/2016, weight 29.9 kg and CJJ #4977, Male, DOB 11/17/2016, weight 34 kg. Their diets consisted primarily of Nebraska Canine, a carnivore food diet, along with deer legs or rabbits. The fecal material from the dogs, one sample/dog, were collected in March 2019. The two samples were collected within 3 h after defecation off the floor and were then placed into separate containers. The two fecal samples were then transported to a -40°C freezer and stored until they were used for laboratory experiments.

Microbial diversity

The inner portion of the AWD fecal samples used in this study were analyzed using bacterial tag-encoded flexible-Titanium (FLX) amplicon pyrosequencing (bTEFAP[®]) (MR DNA Shallowater, TX). The 16S rRNA gene V4 variable region PCR primers 515/806 were used in 30 cycles using the HotStarTaq Plus Master Mix Kit (Qiagen, Germantown, USA) under the following conditions: 95°C for 5 min, followed by 30–35 cycles of 95°C for 30 s, 53°C for 40 s and 72°C for 1 min, after which a final elongation step at 72°C for 10 min was performed. After amplification, PCR products were checked in a 2% agarose gel to determine if amplification was successful and the relative intensity of the bands. Samples were multiplexed using unique dual indices were pooled together in equal proportions based on their molecular weight and DNA concentrations. Pooled samples were purified using calibrated Ampure XP beads (Agencourt Bioscience Corporation, MA, USA). Then the pooled and purified PCR product was used to prepare an Illumina DNA library (Illumina, San Diego, USA). Sequencing was performed on a MiSeq (Illumina, San Diego, USA) following the manufacturer's recommendations. Sequence data were processed using MR DNA analysis pipeline (MR DNA, Shallowater, TX, USA). In summary, sequences were joined, sequences < 150 bp removed, and sequences with ambiguous

base calls removed. Sequences were quality filtered using a maximum expected error threshold of 1.0 and dereplicated. The dereplicated or unique sequences were denoised; unique sequences identified with sequencing and PCR point errors were removed, followed by chimera removal, thereby providing a denoised sequence or zero-radius OTU (ZOTU). Final ZOTUs were taxonomically classified using BLASTn against a curated database derived from NCBI (www.ncbi.nlm.nih.gov).

Nucleotide sequence accession numbers

The NGS data have been deposited in the Sequence Read Archive under accession numbers SRX10894549 and SRX10894550.

Results and discussion

High-throughput sequencing to determine bacterial diversity present in fecal material

bTEFAP[®] has been used to determine the microbes present in the fecal material of animals, such as cattle (Hristov et al. 2012), cats and dogs (Handl et al. 2011), and whales (Guass et al. 2016). In the present study, the fecal material from two adult male African wild dogs were analyzed. A total of 64,601 quality sequences were generated. The number of ZOTUs were 172 and 165, the Chao1 index was 171.8 and 173.2 and the Shannon index was 3.5 and 3.0 for CJJ and Monkey Wrench, respectively. In a study by Ley et al. (2008), it was shown that herbivorous animals had greater bacterial diversity in their fecal material compared to carnivorous animals. The AWD being a hypercarnivorous canid showed little bacterial diversity.

The predominant bacterial phylum was Firmicutes in samples CJJ and Monkey Wrench, containing 66.4 and 95.1% of the sequences, respectively (Table 1). As well, in sample CJJ, a high abundance of Fusobacteria 19.4%

Table 1 The bacterial community composition at the phylum level

Phyla	CJJ #4977 ^a	Monkey wrench #4979 ^a
Firmicutes	66.43	95.10
Fusobacteria	19.40	0.11
Actinobacteria	7.13	3.80
Bacteroidetes	6.92	0.75
Proteobacteria	0.12	0.24

Bold indicates the most predominant phylum

^aRelative percentage of sequences

sequences, along with moderate amounts of Actinobacteria (7.1%) and Bacteroidetes (6.9%) were also detected. It is common to detect high abundances of bacteria from the phyla Firmicutes followed by Bacteroidetes in the gut of both terrestrial and marine mammals, with the exception of terrestrial carnivores. In terrestrial carnivores, the relative average abundance of Firmicutes was greater than 70% compared to 3.2% for Bacteroidetes (Nelson et al. 2013). In our study, a similar trend was found.

Phylogenetically, AWDs fall as the outgroup of the clade “wolf” of canids (Toh et al. 2005). Evolutionarily estimates suggest an average genomic divergence of 3.91 mya between *Lycaon* and the clade containing *Cuon* and *Canis* (Chavez et al. 2019). As a result, the bacterial diversity found in the AWD was compared to different hypercarnivorous *Cuon* and *Canis* species. For example, the fecal microbes present in captive dholes (*Cuon alpinus*) living in Jiangxi province China, contained a total of 19 different phyla with an average of 1196 OTUs. The core microbiota consisted of the phyla Bacteroidetes (21.63–38.97%), Firmicutes (20.97–44.01%), Proteobacteria (9.33–17.60%), Fusobacteria (9.11–17.90%), and Actinobacteria (1.22–2.87%) (Wu et al. 2016). Using the clone library method, the bacterial diversity in the fecal material of wild wolves (*Canis lupus*) living in inner Mongolia China was determined. Five different phyla were identified. The majority of the OTUs were classified within the phylum Firmicutes (60%), along with Bacteroidetes (16.9%), Proteobacteria (9.2%), Fusobacteria (9.2%) and Actinobacteria (4.6%) (Zhang and Chen 2010). Using a next generation sequencing approach, it was shown in different wolf subspecies living in China that the top five fecal microbiome OTUs (99%) were members of the phyla Bacteroidetes, Fusobacteria, Firmicutes, Proteobacteria, and Actinobacteria (Wu et al. 2017). Since AWDs are an out grouping of the clade “wolf” of canids (Toh et al. 2005), it would be expected that their bacterial community composition would be similar other closely related canid species. However, at the phylum level their bacterial diversity is more similar to the terrestrial carnivores examined in the study by Nelson et al. (2013). This study did contain the canid representative bush dogs (*Speothos venaticus*). However, there were other terrestrial carnivores which are not canids included, such the cheetah (*Acinonyx jubatus*) and the short beaked echidna (*Tachyglossus aculeatus*).

In our study at the genus level, the most frequently detected genus was *Blautia*, from the phylum Firmicutes, at 49.0 and 60.0% for CJJ and Monkey Wrench, respectively (Table S1). It has been suggested that *Blautia* may have diverse biology activity. For example, in humans, members of this genus were associated with a reduced mortality from graft-versus-host disease (Jenq et al. 2015). In young adult people, a lower-fat diet increased the abundance of both *Faecalibacterium* and *Blautia* (Wan et al.

2019). It was also found that *Blautia* was the only gut microbe which was significantly and inversely associated with visceral fat area in both men and women (Ozato et al. 2019). In piglets fed an insect species, *Hermetia illucens*, based diet an increased abundance of *Blautia* was shown, suggesting this feed supplement has a positive influence on the cecal microbiota (Biasato et al. 2020).

This genus was not a dominant genus in any of the previously mentioned *Cuon* or *Canis* studies (Wu et al. 2017, 2016; Zhang and Chen 2010). The presence of a high percentage of *Blautia* in the fecal material of the two AWDs used in this study may indicate the animals are in good health, since these animals were raised in captivity. However, in the study by Wu et al., the dholes were also raised in captivity, whereas the wolves were raised in a semi-free environment (Wu et al. 2017; Zhang and Chen 2010). Perhaps a high percentage of *Blautia* may be a good indicator of health in AWDs which may not be true of other closely related species. Another possibility is that studies involving the microbiota of wolves, dholes and AWDs are very few and the sample sizes are small. As a result, these studies may not be truly representative, but nonetheless valuable.

Conclusions

In this study, the bacterial diversity in the fecal material of two AWDs living in captivity was determined. This animal is a hypercarnivore and shows little bacterial diversity, with a high abundance of Firmicutes at the phylum level. This is in agreement with other studies involving terrestrial carnivores. At the genus level, the high abundance of members of *Blautia* spp. is very different than what was identified in closely related *Cuon* or *Canis* species. In future, we wish to further characterize the bacterial diversity found in the fecal material of AWDs living in the wild.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s00203-021-02678-9>.

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Author contributions FAS and RWM designed this project. JB provided the fecal samples and information about the African wild dogs used in this study. SED was responsible for bTEFAP® and data collecting. FAS and RWM analyzed data. FAS and RWM wrote the manuscript. All authors contributed to discussions and revisions, and approved the final manuscript.

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Data availability The NGS data have been deposited in the Sequence Read Archive under accession numbers SRX10894549 and SRX10894550.

Code availability Not applicable.

Declarations

Conflict of interest The authors declare that they have no conflict of interest. All the authors read and approved the final manuscript.

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