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Composition and diversity of Canidae fecal flora

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ABSTRACT

Canidae fecal flora reflects the distal gut microbial community structure. *Cuon alpinus* was captured on Qilian Mountain in Gansu province of China, and other animals, including *Canis lupus*, *Vulpes vulpes*, *Vulpes corsac* and *Nyctereutes procyonoides*, were captured at the nearby Dalai Lake Nature Reserve in Inner Mongolia. Fecal samples were collected 2 h after defecation from five healthy unrelated adult animals of each species. None of the animals had recently fed or been administered any drugs or additives that could influence the composition or diversity of the fecal flora. Using traditional culture-dependent methods, we investigated the composition and diversity of the fecal flora of these five Canidae species. Gram-positive spore bacilli, gram-positive having no spore bacilli, gram-positive coccobacteria, gram-negative coccobacteria and gram-negative bacilli were first identified through microscopic observation and then formal identification tests were carried out, including oxygen needing, methyl red and Acetyl methyl methanol test, catalase, gelatin liquefaction, KNO_3 reduction, indole, fermentation of saccharides and mellows, sodium citrate and NaCl-phily and so on. Based on the results of these physiological and biochemical tests, along with the morphological description, species from approximately 19–21 genera were identified in the feces. The number of genera in the feces was 22 in *C. lupus*, 19 in *C. alpinus*, 21 in *N. procyonoides*, 21 in *V. corsac* and 19 in *V. vulpes*, as well as some unidentified strains. Although, some strains were endemic to the Canidae gut, there were some differences in the community among individuals and species. The Canidae fecal flora comprised 10^{10}–10^{11} colony forming units/g of feces (wet weight). The amount of bacteria reached 1.442×10^{11} cfu/g in *C. lupus*, 8.330×10^{10} cfu/g in *V. vulpes*, 8.170×10^{10} cfu/g in *V. corsac*, 8.620×10^{10} cfu/g in *N. procyonoides* and 1.485×10^{11} cfu/g in *C. alpinus*. The amount of bacteria was significantly different among species ($P < 0.05$) but not among different individuals of the same species (*C. lupus:* $P = 0.19$; *V. vulpes*: $P = 0.898$; *V. corsac*: $P = 0.315$; *N. procyonoides*: $P = 0.074$; *C. alpinus:* $P = 0.197$). The percentage of shared species among different Canidaes was 65–80%, with the highest percentage between *V. vulpes* and *V. corsac*, and the lowest between *C. lupus* and *C. alpinus*. Although the proportion of shared species between *V. vulpes* and *C. alpinus* was 78.95%, the amount of bacteria was markedly different. There was no correlation between the amount and the diversity of bacteria. The most common microbes were *Escherichia*, *Enterobacter*, *Streptococcus*, *Proteus*, *Enterococcus* and *Lactobacillus*. Of these, *Escherichia* and *Enterobacter* can be considered as beneficial strains and they were found in all the Canidaes. Our findings suggest that, despite some differences, there is high similarity in the dominant fecal bacteria of different Canidaes.

1. Introduction

There are a lot of microbial in animal intestinal, their symbiotic with host, and influence each other. Mainly research concentrated in the mammals, poultry and livestock, related on gut microbes population quantity, type and metabolic activity in domestic and foreign studies [1–3]. Canidae, as the most widely distributed taxa in Carnivora. Its have different shape, and population structure, living habits and survival condition also have different characteristic. Its population diversity and complexity are very rare in existing mammals. Concerning on Canidae intestinal flora research focuses on the domestic dogs (*Canis lupus familiaris*), especially breed, age, fiber, and breeding conditions influence on the composition and amount of the intestinal flora [4,5]. Previous studies on bacterial composition and diversity of domestic dogs intestinal are largely based on the use of classical culture dependent techniques, molecular fingerprint analysis and 16S rRNA gene cloning sequencing and system development analysis [6–10]. To our knowledge, less study has assessed the composition of the microbial communities and diversity in the intestinal tracts of wild Canidae, only found applied 16S rRNA gene sequences reveals distal gut bacterial diversity of phylogenetic analysis in wild wolves (*C. lupus*) [11].

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Currently use traditional cultivation method research the intestinal bacterial of wild Canidae has not a system research. Therefore we investigated the composition and diversity of the fecal flora of these five Canidae species, and with a comparative analysis of the domestic dog intestinal flora. Based on this research we can fully displaying the composition, community structure and micro ecological balance of the intestinal normal microbial of these Canidae species, provide based material for research the feces flora of the canine animal, and scientific basis for research the micro-ecology environment and intestinal disease of the canine animal intestinal tract.

2. Materials and methods

2.1. Experimental animals and culture medium

Cuon alpinus was captured on Qilian Mountain in Gansu province of China and raised in Beijing zoo, and other animals, including *C. lupus*, *Vulpes vulpes*, *Vulpes corsac* and *Nyctereutes procyonoides*, were captured at the nearby Dalai Lake Nature Reserve in Inner Mongolia and had been raised semi-freely in Jinan National Forestry Park with raw meat and water for about 2 months before the research began. Fecal samples were collected 2 h after defecation from five healthy unrelated adult animals of each species. None of the animals had recently fed or been administered any drugs or additives that could influence the composition or diversity of the fecal flora since they were captured.

Broth Medium, Beef extract-peptone medium, fermentation medium, Sodium citrate medium, Peptone Water Medium, Dextrose Peptone Water medium, Gelatin liquefaction medium, Produce hydrogen sulfide medium, Triple Sugar Iron Agar medium, Nitrate reducing medium and various salt solution medium and so on, all of the medium were prepared from microbiology laboratory, college of life science, Qufu Normal University. Blood plate medium purchased from Nan Jing bianzhen biological technology Co., Ltd.

2.2. Isolation of feces flora

An extensive series of dilutions sample were plated onto several blood plate medium and incubated at 37 °C for 24 h as appropriate. After recording morphological characters, combined with microscope, distinguish, classified and counted different colonies, and screened advantage strains. Then as many picked different forms of single strains to obtain pure cultures, and use microscope examine strains to consistent.

2.3. Identification of feces flora

Strains that gram-positive were plated on the nutrition deficient medium, incubated at 37 °C for 24 h as appropriate. Then dyed to strains and microscope generated spores or not.

The isolates were subjected to biochemical tests. Mainly includes: oxygen needing, methyl red and Acetyl methyl methanol test, catalase, gelatin liquefaction, KNO_3 reduction, indole, fermentation of saccharides and mellows, sodium citrate and NaCl-phily and so on. Based on the results of these physiological and biochemical tests, along with the morphological description, reference "Common Bacteria System Identification Manual" and "Bergey's Manual of Determinative Bacteriology. 8th ed.", and related information about identification of bacterial.

2.4. Data analysis

According to the colonies countability to choose the dilution degrees, calculate the contents of bacterial count per gram, and with its numerical said. Faecal flora formed unit formula:

$$\text{Colony forming units/g of feces(wet weight)} = \frac{\text{the mean number flat plate colonies} \times \text{dilution factor}}{\text{the drops of bacterium fluid}}$$

According to the following two conditions to determine the advantage strains: one is the strain is visible on the solid culture medium, second is the strain on the number of their colonies solid culture. The rate advantage calculation method:

$$P_i = \frac{n}{N} \times 100\%$$

Among them: *Pi* represents a strain of bacterium group in feces detection rate; *n* is for solid culture medium a visible strains number; *N* is for solid culture medium on the total number of feces flora. If the strains that accords with afore-mentioned two conditions, detection rate is 100%.

The percentage of shared species among different Canidaes represents that the same strain in two species appear in feces flora of frequency. The percentage of shared species calculation method:

$$P_i = \frac{2n}{N1 + N2} \times 100\%$$

Among them: *Pj* represent two species feces flora matching degree; *n* is for two species feces flora strains of same number; *N*1 represents the first species of feces flora strains number; *N*2 represents the second species of feces flora strains of the number.

Application the software SPSS 17.0 Statistics data processing and analysis.

3. Result

3.1. Five kinds of canine animal feces flora composition

Based on the results of morphological description, Gram's staining properties and microscope, preliminary species from 45 strains were isolated in feces flora of *C. lupus*, 33 strains were isolated in feces flora of *V. vulpes*, 27 strains were isolated in feces flora of *V. corsac*, 38 strains were isolated in feces flora of *N. procyonoides*, 39 strains were isolated in feces flora of *C. alpinus*. Along with the physiological and biochemical tests, finally we find the number of genera in the feces was 22 in *C. lupus*, 19 in *C. alpinus*, 21 in *N. procyonoides*, 21 in *V. corsac* and 19 in *V. vulpes* (Table 1). From Table 1 we can see five kinds of canine animal feces flora was contain of *Escherichia*, *Enterobacter*, *Enterococcus*, *Staphylococcus*, *Klebsiella*, *Proteus*, *Citrobacter*, *Streptococcus*, *Bacillus*, *Agrobacterium* and *Edwardsiella* for *C. lupus* of feces flora respectively and *C. alpinus* feces flora unique. Some of them in different canine animal feces strains of bacteria exhibiting high similarity in the same species, some strains different individual feces flora is endless also and same, detection for individual differences ($P > 0.05$).

3.2. Five kinds of canine animal feces flora amount and multiple comparison analysis

The Canidae fecal flora comprised 10^{10}–10^{11} colony forming units/g of feces (wet weight). The amount of bacteria reached 1.442×10^{11} cfu/g in *C. lupus*, 8.330×10^{10} cfu/g in *V. vulpes*, 8.170×10^{10} cfu/g in *V. corsac*, 8.620×10^{10} cfu/g in *N. procyonoides* and 1.485×10^{11} cfu/g in *C. alpinus*. Of them, the amount of bacteria in fecal of *C. alpinus* was most, second was *C. lupus*, the number of others has certain stability. From Table 2 draw, *C. alpinus* and *C. lupus* feces flora amount was no significant differences ($P = 0.304$), the amount of bacteria in fecal of *V. vulpes*, *V. corsac* and *N. procyonoides* has no significant difference ($P > 0.05$), but not among different individuals of the same species ($P > 0.05$). The amount of bacteria in fecal of *C. alpinus* and *C. lupus*, between the others