Community of actinomycetes in 42 species of animal feces

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Abstract

The study on animal fecal actinomycete, as a source for discovering new drug laeds, is a few in the past. There is a great deal of and un-exlroited actinomycete resources in animal feces. To provide new sources for discovering new drug leads, the diversity and bioactivities of cultivable actinobacteria from animal feces have been studied. 42 species of animal fecal samples were collected from Yunnan Wild Animal Park and other habitats. The purified cultures of actinobacteria were isolated from these samples by using 5 media. 3049 pure strains were isolated. The 16S rRNA gene sequences of 1869 selected strains of them were determined, the phylogenetic analysis was carried out, and anti-microbial anti-tumor, enzyme activities as well as toxin to mice were determined. 51 genera (including a new genus, *Enteractinococcus*) of actinobacteria from the 42 species of animal feces were identified. Results of this study indicate that there is a great deal of and un-exlroited actinomycete resources in animal feces. Animal fecal actinomycete is considered as a new resource for discovering drug leads, agricultural chemicals and other industry products.

KEYWORDS Actinobacteria; diversity; animal feces.

1. Introduction

Actinomycetes (Actinobacteria) have recently received much attention, as these bacteria produce a variety of natural drugs and other bioactive metabolites, including antibiotics, enzyme inhibitors and enzymes. More than 22,000 bioactive secondary metabolites (including antibiotics) from microorganisms have been identified and published in the scientific and patent literature, and approximately half of these compounds are produced by actinomycetes. Currently, approximately 160 antibiotics have been used in human therapy and agriculture, and 100-120 of these compounds, including streptomycin, erythromycin, gentamicin, avermectin, etc.), are produced by actinomycetes [1]. However, the use of general approaches to develop new drugs from actinomycetes growing in common habitats is extremely difficult [2]. Although several microorganisms have been identified, described, screened and used in many applications, more than 90% of all microorganisms remain unknown [3 — 10]Abdelnasser et al., 2012), and these unknown microbes might offer hope for the development of new drugs.

Actinomycetes, as human and animal pathogens, have been widely studied [11].

However, until recently, there have been few studies concerning the use of fecal actinomycetes as a source for the discovery of novel drug. To obtain more unknown actinomycetes for the discovery of new bioactive metabolites, fecal samples from 42 species of carnivorous, herbivorous and omnivorous animals, including primates, mammals (perissodactyla, artiodactyla and ruminant), birds, amphibians, fishes and insects, were collected. The actinomycetes in fecal were isolated, cultivated and identified. Diversity of actinomycete was study. A part of results is report here.

2. Methods for the isolation of animal fecal actinomycetes

2.1. Collection and pretreatment of samples

Fresh fecal samples were collected from 42 selected animal species in Yunnan Wild Animal Park, Kunming, and South China Sea, China. Some samples were collected from the original animal habitats. The samples were immediately transferred to sterile dishes and dried for 10 days at 28 ºC. 2 g of each dried sample was pre-treated at 80 ºC for 1 hour and subsequently dissolved in 18 ml of sterile water containing 0.1 % $Na_4P_2O_5$, followed by shaking at 220 rpm/min for 60 min. The suspension was treated with ultrasound waves for 40s at 150W before coating [12]. The suspension was diluted from 10^{-1} to 10^{-8}, and three dilutions, 10^{-5}, 10^{-6} and 10^{-7}, were used for isolating actinomycetes.

2.1. Isolation medium for actinobacteria (per liter)

HV medium [13].

YIM 171: Glycerol 10 g, asparagine 1 g, $K_2HPO_4 \cdot H_2O$ 1 g, $MgSO_4 \cdot 7H_2O$ 0.5 g, $CaCO_3$ 0.3 g, Vit mixture of HV medium 3.7 mg, and agar 15 g, pH 7.2.

YIM 212: Mycose 5 g， proline 1 g， $(NH_4)_2SO_4$ 1 g, NaCl 1 g, $CaCl_2$ 2 g, K_2HPO_4 1 g, $MgSO_4 \cdot 7H_2O$ 1 g, Vit mixture of HV medium 3.7 mg, and agar 15 g, pH 7.2.

YIM 47: Na_2HPO_4 0.5 g, KCL 1.7 g, $MgSO_4 \cdot 7H_2O$ 0.05 g; $FeSO_4 \cdot 7H_2O$ 0.01 g, $CaCl_2$ 1 g, soy bean flour 0.2 g, lignin 1 g, Vit mixture of HV medium 3.7 mg, soil extract 100 ml, and water 900 ml, pH 7.5.

YIM 601: Solution starch 10 g, casein 0.3 g, KNO_3 2 g, $MgSO_4 \cdot 7H_2O$ 0.05 g, NaCl 2 g, K_2HPO_4 2 g, $CaCO_3$ 0.02 g, $FeSO_4$ 10 mg, Vit mixture of HV medium 3.7 mg, and agar 15 g, pH 7.2~7.4.

Inhibitors. All media were supplemented with 4 filter-sterilized mixtures or single solutions containing inhibitors against fungi and Gram-negative bacteria (per liter): 1. 50 mg cycloheximide, 50 mg nystatin, 20 mg nalidixic acid and 3 mg penicillin; 2. 100 mg cycloheximide, 100 mg nystatin, 40 mg nalidixic acid and 5 mg penicillin; 3. 50 mg $K_2Cr_2O_7$ and 5 mg penicillin; and 4. 75 mg $K_2Cr_2O_7$ and 5 mg penicillin.

The plate dilution method was used to isolate actinobacteria from the sample suspension. Approximately 0.1 ml of each sample (10^{-5}, 10^{-6}, and 10^{-7} dilutions) was used to coat the plates and cultivated for 7 to 35 days at 28 ºC. Subsequently, the colonies were counted, and a single actinobacteria colony was picked to inoculate a slant with the same isolation medium. The pure strains were cultured at 4℃and in 20% of glycerol at -20℃.

2.3. Effect of isolation media

The quantity of cultivable actinomycetes in mixed samples containing 8 species of animal feces was 7-21×10^9, and that of other bacteria was 6×10^9; most of the growth for the Gram-negative bacteria was suppressed using inhibitors. The optimum fecal suspension dilutions for isolating actinobacteria were 10^{-6} and 10^{-7}, in which approximately 22 to 133 colonies were observed on the isolation plates. However, the optimum concentration for each animal fecal sample should be determined in advance.

Total 1,123 pure cultivated strains of actinomycetes were isolated from 8 species of animal feces. 156 and 140 strains were isolated from *Vicugna pacos* and *Rhinoceros sondaicus*, respectively. Only 20 strains were isolated from *Testudo elephantopus*. YIM 212, HV and YIM 171 media were better for isolating actinobacteria, resulting in the identification of 289, 247 and 239 strains of actinobacteria, respectively.

2.4. Key points for isolating actinobacteria from animal feces

The abundance of Gram-negative bacteria in animal feces presents a major challenge for the isolation of fecal actinobacteria. To eliminate Gram-negative bacteria and fungi and obtain more unknown actinobacteria for discovering novel lead compounds, some key points for sampling and isolation should be considered.

First, based on the results of previous experiments, it is best to collect fresh fecal samples from wild animals living in original habitats; second, the fresh samples should be dried at 25-28 ºC for 7 to 10 days; third, the dried samples should be treated for 60 min at 80ºC, and the fecal suspension should be treated with ultrasound waves for 40s at 150W before coating (Jiang et al., 2010); fourth, potassium bichromate 50 mg and 5 mg penicillin or nystatin 50 mg, nalidixic acid 20 mg and 5 mg penicillin should be added per liter of isolation medium to inhibit the growth of fungi and Gram-negative bacteria; fifth, the samples should be diluted to 10^{-5}, 10^{-6}, and 10^{-7}, and the optimum dilution concentration for each animal fecal sample should be determined in advance; sixth, YIM 212, YIM 171 and HV medium are better for the isolation of fecal actinobacteria, and these media should be improved and constantly updated with respect to different samples; and seventh, all experiments should be performed under strict sterile conditions for avoiding spread of pathogen.

Animal fecal actinomycetes represent a new field of study. The physiological features of these bacteria are not understood. Therefore, the method for the selective isolation of actinomycetes (including the isolation media, pH, inhibitors, sample pretreatment, and culture temperatures) should be become different from those used to isolate bacteria from soil, sea and plant samples, and this method should be continually improved and updated for isolation from different fecal samples.

3. Diversity of animal fecal actinomycetes

3.1. Identification of pure cultivated actinobacteria

A total 2668 pure strains were isolated from the feces samples obtained from 42 animal species; 1,649 strains were obtained after eliminating duplicate strains based on morphological and cultural characteristics. The DNA was extracted from pure strains for 16S rDNA analysis [14]. PCR amplification of the 16S rDNA, followed by purification and sequencing of the PCR products were performed as previously described [15]. The forward primer F8 (8±27; 5'-GAG AGT TTG ATC CTG GCT CAG-3') and the reverse primer (1510±1492; 5'-GGT TAC CTT GTT ACG ACT T-3') were used. The resulting sequences were manually aligned using the sequences from available, public databases. Phylogenetic trees were inferred using neighbor-joining [16] and maximum-likelihood methods [17]. All pure cultivated strains were identified at a genus and species level.

3.2． Diversity of actinobacteria

The actinomycete communities in 15 of the 42 fecal samples are described here.

1. Hoolock gibbon（*Hylobates hoolock*）. The hoolock gibbon is a member of the *Hylobatidae* primates. This primate is classified as a class I protected species according to the LPW (The Law on the Protection of the Wildlife of the People's Republic of China), the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) and the International Union for Conservation of Nature and Natural Resources (IUCN). Fresh fecal samples from two individual primates were collected at three different times, and a total 108 pure cultured strains were isolated. After eliminating several duplicates strains based on morphological and cultural characteristics, 76 strains were selected, and the 16S rDNA sequences were determined. A phylogenetic analysis was performed. The strains were identified at the genus and species levels. The isolated strains comprised 12 genera of actinobacteria: *Arthrobacter*， *Cellulosimicrobium*， *Corynebacterium, Dietzia, Gulosibacter, Kocuria*， *Microbacterium*， *Nocardia, Oerskovia*， *Rhodococcus*， *Streptomyces* and *Zimmermannella.* The molinate-degrading actinomycete *Gulosibacter,* initially identified by Célia et al. [18], currently contains three species. Ten other bacteria, *Acinetobacter, Bacillus*， *Jeotgalicoccus, Kurthia*， *Leuconostoc*， *Planococcus*， *Psychrobacter, Pseudomonas, Psychrobacillus* and *Rummeliibacillus*, were also identified.

2. Yunnan snub-nosed monkey (*Rhinopithecus bieti*). The rare Yunnan snub-nosed monkey belongs to *Cercopithecidae* primates. This monkey is classified as class I protected animal of China according to the CITES and the IUCN. Fresh fecal samples from three individuals were collected at three different times. 122 strains were isolated, and 66 strains were identified, belonging to thirteen genera of actinobacteria: *Agrococcus, Arthrobacter, Cellulosimicrobium, Citricoccus, Corynebacterium, Gordonia, Jiangella, Kocuria, Microbacterium, Mobilicoccus, Oerskovia, Rhodococcus* and *Streptomyces. Jiangella* are distributed in various habitats,

including deserts, alkaline soils, caves and plants [19－23]. Other bacteria, *Bacillus, Enterococcus*， *Leuconostoc, Paenibacillus, Catellibacterium* and *Myroides*, were also identified.

3. Assamese macaque (*Macaca assamensis*). The Assamese macaque is a member of the *Cercopithecidae* primates. This primate is as a class I protected animal in China. 93 selected strains were identified. These Actinobacteria belonged to 8 genera, including *Acinetobacter, Corynebacterium, Kocuria, Luteococcus*， *Microbacterium, Nocardiopsis, Rhodococcus* and *Streptomyces.* 11 other bacteria, namely *Achromobacter, Acinetobacter*， *Bacillus, Bordetella*， *Enterococcus*， *Escherichia, Jeotgalicoccus, Methylobacterium*，*Planococcus, Pseudomonas* and *Shigella*, were also identified.

4. Bengal tiger (*Panthera tigris*). The Bengal tiger is a member of the *Carnivore: Felidae*, and has been classified as a class I protected animal of China according to the CITES and the IUCN. These animals inhabit the same type of forest as the Manchurian tiger. 258 strains of actinomycetes were isolated from fresh fecal samples, and 177 of these strains, belonging to 13 genera, namely *Arthrobacter*， *Corynebacterium*， *Dietzia*， *Enteractinococcus*， *Kocuria*， *Microbacterium*， *Nocardia*，*Nocardiopsis*，*Oerskovia*， *Promicromonospora*，*Saccharomonospora*，*Streptomyces* and *Yaniella*, were identified. The novel genus *Enteractinococcus* was described in the International Journal of Systematic and Evolutionary Microbiology (IJSEM) [24]. The genus *Yaniella* is typically found in saline soil [25， 26]. *Streptomycetaceae* occupied 64% of actinobacteria observed, representing the predominant strain, followed by *Micrococcaceae* with 7%.

5. Giant panda (*Ailuropoda melanoleuca*). The giant panda is a rare, national treasure in China, classified as a class I protected animal according to the IUCN and the CITES. This panda only inhabits a limited area of Northern Sichuan, China, and is a member of the *Carnivore. Ailuridae* (*Ailuropodidae*), its main food is bamboo. Five individuals' feces were sampled. 330 pure cultured actinomycete strains were isolated, and 133 of these strains were identified through phylogenetic analysis of the 16S rDNA gene sequences. These strains belonged to 13 genera of actinobacteria, *Agrococcus, Arthrobacter*， *Cellulomonas*， *Cellulosimicrobium*， *Janibacter*， *Micrococcus, Micromonospora*， *Mycobacterium*， *Oerskovia, Patulibacter, Rhodococcus, Streptomyces* and *Verrucosispora.* The genus *Janibacter* was identified in 1997 [27]. *Verrucosispora* are members of the family *Micromonosporaceae*, and these bacteria were isolated from a peat bog near Gifhorn, Germany, and they typically inhabit peat bogs, lakes and oceans [28, 29]). *Patulibacter* belongs to Order *Solirubrobacterales* [30].

6. **Red panda (*Ailurus fulgens*)**. The red panda belongs the *Carnivore: Procyonidae*. This panda is classified as a class II protected animal in China. 66 selected strains were identified, belonging to 10 genera of actinobacteria, named *Agrococcus*， *Arthrobacter*， *Corynebacterium*， *Gulosibacter, Leucobacter*， *Microbacterium, Micrococcus*， *Pseudonocardia, Rhodococcus* and *Streptomyces.* Six genera of other bacteria, including *Aerococcus, Brevundimonas, Jeotgalicoccus, Planomicrobium, Pseudomonas* and *Psychrobacter*, were identified.

7. Zibet (*Viverra zibetha*). Zibet (Small Indian civet, Viverra indica， Viverricula malaccensis thai, Viverricula hanensis, Viverricula pallida taivana and Viverra pallida) lives in tropical and subtropical rain forests and evergreen broadleaf forests. This omnivorous species are classified as class II protected animals in China according to the IUCN and the CITES. The zibet is named for producing musk. Zibet fecal samples were collected from three individuals in a wild animal park in Malaysia twice. Total 88 strains of Actinobacteria were isolated using 5 different media. 58 strains were identified, comprising 15 genera: *Arthrobacter*， *Cellulomonas* ， *Cellulosimicrobium* ， *Corynebacterium, Curtobacterium* ， *Enteroactinococcus, Isoptericola* ， *Kocuria* ， *Leucobacter* ， *Microbacterium* ， *Micrococcus*， *Rhodococcus*， *Saccharopolyspora, Sanguibacter* and *Streptomyce. Curtobacterium* was rarely observed in the feces of these animals. *Cellulosimicrobium* were identified as inhabitants of termites [31, 32]. Other bacteria, including *Bacillus*， *Enterococcus* ， *Flavobacterium* ， *Hansschlegelia* ， *Methylobacterium* ， *Ochrobactrum*， *Pseudomonas*， *Sporosarcina* and *Stenotrophomonas*, were also identified from the Zibet fecal samples.

8. Asiatic black bears (*Ursus thibetanus*). Asiatic black bears (Moon bear) are omnivorous animals widely distributed throughout the forests of broad areas from north to south in the eastern halfsphere of China, Russia, Iran, Pakistan, Laos, Japan, and India. These bears are classified as class II class protected animals in China according to the IUCN and the CITES. Fecal samples of three individuals were collected twice. 112 strains of actinobacteria were isolated, and 32 strains were identified. They belonged, belonging to 11 genera: *Cellulosimicrobium, Dietzia, Kribbella, Gordonia, Microlunatus*, *Microbacterium, Nocardiopsis* ， *Promicromonospora, Rhodococcus*， *Saccharomonospora* and *Streptomyces.* Five genera of other bacteria, namely *Massilia* ， *Myroides*， *Methylobacterium*， *Rhizobium* and *Stenotrophomonas* were identified.

9. Shansi Sika (*Cervus nippon*). Shansi Sika (Sika, Sika Deer, spotted deer) is a class I protected animal in China according to the IUCN. These phytophagous animals are widely distributed throughout forests and grasslands. Notably, the prevalence of these animals is rapidly decreasing. Fecal samples were obtained from 12 individuals in Kunming and Shengyang, China, and 337 pure strains were isolated. Among these, 117 strains were identified, belonging to 16 genera of actinobacteria: *Actinocorallia* ， *Agrococcus, Arthrobacter, Cellulosimicrobium, Citricoccus* ， *Isoptericola* ， *Kocuria* ， *Leucobacter, Microbacterium* ， *Mycobacterium* ， *Promicromonospora* ， *Nocardiopsis Rhodococcus* ， *Salinibacterium*，*Streptomyces* and *Tsukamurella.* Only two other bacteria, *Bosea* and *Stenotrophomonas,* were identified.

10. Vicuna (*Vicugna pacos*). Vicunas were originally identified in frigid zones from 3650 to 4800 m above sea level in the Andes Mountains. Humans have long reared these camels, for it produce high-grade hair. Vicunas belong to *Artiodactyla: Camelidae.* Three individuals were imported from the Republic of Chile into Yunnan Wild Animal Park. Fecal samples were obtained from these animals, and 87 strains of actinomycetes were isolated. Among these, 66 strains were identified, belonging to 15

genera, namely *Arthrobacter*， *Brevundimonas*, *Cellulosimicrobium*， *Corynebacterium*, *Dietzia*，*Enteractinococcus*, *Gordonia*, *Isoptericola*，*Kocuria*，*Microbacterium*, *Micrococcus*， *Nocardiopsis*, *Rhodococcus*，*Saccharomonospora* and *Streptomyces*. The number of *Arthrobacter arilaitensis* was 80×10^5/g in the fresh fecal sample. Eleven other bacteria, including *Achromobacter*， *Advenella*， *Ancylobacter*， *Jeotgalicoccus*， *Kurthia*， *Lysobacter*， *Methylobacterium*， *Ornithinibacillus*，*Psychrobacillus*，*Shigella* and *Solibacillus*, were also isolated and identified.

11. Rhino（*Rhinoceros sondaicus*）. The rhino is a rare nationally treasured animal in China, classified as a class I protected animal according to the IUCN. The rhino belongs to both *Rhinocerotidae* and *Perissodactyla*. Fecal samples were collected from two individuals in Indonesia. 202 strains were isolated, and 112 strains were identified. Fourteen genera were identified: *Arthrobacter, Brevundimonas, Corynebacterium, Dietzia, Gulosibacter, Kocuria, Microbacterium, Micromonospora, Nocardiopsis, Promicromonospora, Pseudoclavibacter, Rhodococcus, Streptomyces* and *Tessaracoccus. Gulosibacter* and *Tessaracoccus* are rarely observed in nature. One strain (YIM 100770), identified as a novel genus, was characterized using polyphasic taxonomic procedures [33]. Nineteen other bacteria, including *Achromobacter, Alcaligenes, Ancylobacter, Bacillus, Hansschlegelia, Kurthia, Luteimonas, Methylobacterium, Massilia, Novosphingobium, Paracoccus, Pseudomonas, Psychrobacillus, Rhizobium, Shigella, Solibacillus, Sphingobacterium, Staphylococcus* and *Stenotrophomonas*, were also identified.

12. Indian elephant（*Elephas maximus*）. The Indian elephant is typically found in south China and Asia, and the prevalence of this rare, nationally treasured animal is rapidly decreasing. Thus, this elephant is classified as a class I protected animal in China according to the IUCN. The Indian elephant belong to *Proboscidea: Elephantidae.* Fresh fecal samples were obtained from four individuals in the Xiaomemgyang National Natural Protect area and Yunnan Wild Animal Park. 121 strains were isolated, and 68 strains were identified, comprising 15 genera of actinobacteria, including *Arthrobacter*， *Cellulomonas, Cellulosimicrobium*， *Citricoccus*，*Janibacter*，*Kocuria*，*Leucobacter*，*Microbacterium*，*Micrococcus*，*Micromonospora*，*Promicromonospora, Rhodococcus*，*Sphaerobacter, Streptomyces* and *Verrucosispora*. Three genera of Gram-negative bacteria, namely *Bacillus, Devosia* and *Planococcus*, were identified.

13. Chinese bamboo rat（*Rhizomys sinensis*）. Humans have long reared the Chinese bamboo rat, for producing high protein. The bamboo rat is a member of *Rodentia: Rhizomyidae*. Fresh fecal samples were obtained from 6 individuals, and actinomycetes were isolated from these samples at two different times. 306 strains were isolated, and 104 strains were identified, belonging to 13 genera, namely *Agrococcus*，*Arthrobacter*，*Brachybacterium, Corynebacterium, Dietzia, Gordonia, Labedella*， *Microbacterium, Oerskovia*， *Rhodococcus*， *Sanguibacter*， *Streptomyces* and *Williamsia.* Three genera of Gram-negative bacteria, *Comamonas, Flavobacterium* and *Psychrobacter*, were identified.

14. Peacock（Pavo cristatus）. The peacock, a member of *Galliformes:*

Phasianidae, is classified as a class I protected animal according to the LPW, the IUCN and the CITES. Fecal samples were obtained from twelve individuals, and the actinomycetes were isolated for three times. 188 strains were isolated, and 118 selected strains were identified, comprising eighteen genera of actinobacteria, namely *Arthrobacter*， *Brevibacterium* ， *Cellulosimicrobium*， *Curtobacterium*， *Dietzia*， *Gordonia* ， *Isoptericola* ， *Janibacter* ， *Kineococcus* ， *Kocuria* ， *Leucobacter* ， *Microbacterium* ， *Nocardiopsis* ， *Oerskovia* ， *Rhodococcus* ， *Pseudonocardia* ， *Sanguibacter* and *Streptomyces*, representing the largest genus observed in the present study. Other bacteria, including *Bacillus* ， *Devosia* ， *Lysinibacillus* ， *Methylobacterium*， *Planococcus*， *Planomicrobium*， *Shigella* and *Staphylococcus*, were also identified.

15. Common black-headed Gull (Larus ridibundus). The common black-headed gull belongs to *Ciconiiformes: Laridae.* It is listed in the directory by LPW and IUCN. The common black-headed gull, a typical migrant bird, is typically found in Siberia, Russia. Over thirty thousand *Larus ridibundus* migrate to Dian Lake, Kunming from Siberia between December and March every year. 37 selected strains were identified from the fecal samples of these birds, belonging to ten genera of actinobacteria, namely *Arthrobacter* ， *Blastococcus* ， *Devosia* ， *Microbacterium* ， *Oerskovia* ， *Paracoccus* ， *Plantibacter* ， *Promicromonospora* ， *Pseudoclavibacter* and *Streptomyces.*

3.3. Diverse features of animal fecal actinomycetes

222 genera, 53 families and 23 orders have been described in Begey's Manual of Systematic Bacteriology [34]. Fifty-one genera of pure culturable Actinobacteria were isolated and identified in fecal samples collected from 42 animal species. These Actinobacteria belong to 27 families of 12 orders, representing is 24% of 222 genera, 51% of 53 families, and 52 % of 23 orders. An incertae sedis, *Sphaerobacter*， was isolated from Indian elephant feces. Thus, these results showed that the diversity of animal fecal actinobacteria is very rich. The actinobacteria identified herein are listed in Table 1.

Table 1. Component of Phylum Actinobacteria in the fecal samples of 42 animal species

Class I	**Family**	**Genus**
Actinobacteria		
Corynebacteriales	*Corynebacteriaceae*	*Corynebacterium*
	Dietziaceae	*Dietzia*
	Mycobacteriaceae	*Mycobacterium*
	Nocardiaceae	*Gordonia, Nocardia，Rhodococcus, Williamsia*
	Tsukamurellaceae	*Tsukamurella*
Frankiales	*Frankiaceae*	*Blastococcus，*
	Geodermatophilaceae	*Mobilicoccus*
Jiangellales	*Jiangellaceae*	*Jiangelle*
Kineosporiales	*Kineosporiaceae*	*Kineococcus，*
Micrococcales	*Micrococcaceae*	*Arthrobacter, Citricoccus, Enteractinococcus, Kocuria, Micrococcus，Yaniella,*
	Brevibacteriaceae	*Brevibacterium*
	Cellulomonadaceae	*Cellulomonas*
	Dermabacteraceae	*Brachybacterium*
	Intrasporangiaceae	*Janibacter，*
	Promicromonosporaceae	*Cellulosimicrobium, Isoptericola, Promicromonospora*
	Microbacteriaceae	*Agrococcus, Curtobacterium, Gulosibacter, Labedella, Leucobacter, Microbacterium, Plantibacter，Pseudoclavibacter，Salinibacterium, Zimmermannella*
	Cellulomonadaceae	*Oerskovia*
	Sanguibacteraceae	*Sanguibacter*
	Incertae sedis	*Actinotalea*
Micromonosporales	*Micromonosporaceae*	*Micromonospora, Verrucosispora*
Propionibacteriales	*Propionibacteriaceae*	*Luteococcus, Microlunatus, Tessaracoccu*
Pseudonocardiales	*Pseudonocardiaceae*	*Pseudonocardia，Saccharomonospora, Saccharopolyspora*
Streptomycetales	*Streptomycetaceae*	*Streptomyces*
Streptosporangiales	*Thermomonosporaceae*	*Actinocorallia*
	Nocardiopsaceae	*Nocardiopsis*
Class II		
Thermoleophilia		
Solirubrobacteriles	*Patulibacteriaceae*	*Patulibacter*
Incertae sedis	*Incertae sedis*	*Sphaerobacter，*

Forty-eight other bacteria, including *Achromobacter, Acinetobacter, Advenella, Aerococcus, Alcaligenes, Ancylobacter, Aurantimonas, Bacillus, Bordetella, Bosea，Brevundimonas, Brochothrix, Catellibacterium, Desemzia, Devosia, Enterococcus,*

Escherichia, Flavobacterium, Hansschlegelia, Jeotgalicoccus, Kurthia, Lactococcus，*Luteimonas, Leuconostoc, Lysinibacillus*，*Lysobacter, Massilia, Methylobacterium, Myroides*，*Novosphingobium, Ochrobactrum, Ornithinibacillus, Paenalcaligenes, Paenibacillus, Paracoccu, Planococcus, Planomicrobium, Pseudomonas, Psychrobacter, Psychrobacillus, Shigella, Solibacillus, Sphingobacterium, Sphingomonas, Sporosarcina, Staphylococcus, Stenotrophomonas* and *Rhizobium*, were also identified.

Eightteen genera of actinobacteria were identified from fecal samples of Peacock, and the actinomycete component was the complexest; second was sixteen genera in Shansi Sika； third was fiveteen genera in Zibet，Vicuna and Indian elephant; least was only three genera from *Tragelaphus buxtoni* and *Python reticulates* (un-known).

Members of the genus *Streptomyces* were isolated from 100% samples, representing the most predominant microbes, and the cfu (colony-forming units)/g fresh sample ranged from 2×10^5 to 176×10^7 in different fecal samples. *Streptomyces albus, S. albidoflavus, S. griseus, S. hygroscopicus, S. rutgersensis, S. tendae*, and *S. violaceoruber*, etc., were observed at a high frequency.

Members of *Rhodococcus* were isolated and identified in the fecal samples from all animal species, representing the second most prevalent and widely distributed genus. *Rhodococcus coprophilus, Rh. corynebacterioides, Rh. corynebacterioides, Rh. equi, Rh. pyridinivorans* and *Rh. zopfii* were most frequently observed.

The genome sizes of *Streptomyces* and *Rhodococcus*, up to 9×10^7 base pairs, are the largest genomes in actinobacteria, and some species contain 20 or more natural product biosynthetic gene clusters [35－37]. These results are consistent with the idea that the function of actinomycetes in the host intestinal tract is primarily influenced through bioactive substances produced by the members in these two genera.

Members of *Arthrobacter*, *Microbacterium* and *Micrococcus* were identified from most of the animal feces examined.

26 genera belong to Order *Micrococcales*, and 8 genera belong Order *Corynebacteriales* in the 42 tested animal feces.

Based on these results, two conclusions can be drawn: first, members of both *Streptomyces* and *Rhodococcus* exhibited the widest distribution and contained the largest numbers, and second, the composition of actinobacteria with Chemotype IV to IX [38, 39], globose and bacilliform shapes, particularly the Order *Micrococcales*, exhibited the richest diversity and were detected at a high frequency in most of animal feces samples. These distinct features of the fecal actinobacterium community differ from those of the soil, marine and plant communities.

Some members of rare actinobacteria, such as *Yaniella* [25, 26], were identified from the feces of two species of tigers. A strain (YIM 100708) of *Jiangella* [19], a genus widely distributed in saline and alkaline soil, desert, indoor wall material, caves and plant stems, were also isolated from the feces of *Rhinopithecus bieti*. The members of a novel genus, *Enteractinococcus*, belonging to *Micrococcaceae*, were isolated and characterized from three species of tigers [24].

Members of *Micromonospora* were isolated from the feces of *Ailuropoda melanoleuca*, *Rhinoceros sondaicus*, *Elephas maximus* and *Cygnus melancoryphus*

(not shown), Interestingly, *Nocardia* was only isolated from *Panthera tigris altaica* and *Grus japonensis*, *Saccharopolyspora* was only isolated from *Viverra zibetha* and *Verrucosispora* was only isolated from *Ailuropoda melanoleuca*. Actinomycetes possessing cell wall chemotype II (for example *Actinoplanes* and *Dactylosporaniugm*) and III (*Actinomadura* and *Streptosporangium*) are commonly found in soil and lakes, but these genera not isolated from the animal fecal samples in examined herein.

It is worth to notice that, among the 1,649 sequenced strains, 16S rDNA sequences similarities of 96 strains with valid published species were below 98.5%. Thus, nearly 6% pure cultivated strains were unknown, potentially representing novel species [33, 40].

3.4. A comparison of actinomycete diversity in different habitats

In previous studies, 72 genera of actinomycetes were identified from different habitats （Table 2）. 17 genera were isolated from soil samples collected from primeval forests in Grand Shangri-La, southwest China [41]. 13 genera were isolated from soil samples from subtropical every green forest in Gulin, Sichuan [42]. 26 genera were isolated from soil samples from tropical rain forests in Xishuangbanna, southwest China [43], and was the richest area in actinomycete diversity. 16 genera were isolated from soil samples from hypersaline soil in Qinghai, west China [44] (Table 2). *Jiangella, Myceligererans, Yaniella* and *Zhihengliuella* were novel genera which were discivered from this habitat. 15 genera of actinomycetes were identified from samples collected from the Baltic Sea, and members of *Micromonospraceae* were the most [45]. Until recently, approximately 48 genera of actinobacteria were isolated and identified from marine habitats worldwide [46]. Two genera *Streptomyces* and *Rhodococcus* were common in the 6 habitats. In the present study, 51 genera of actinobacteria were isolated from only 42 species of animal feces, and 23% of 222 genera, 51% of 53 families, and 52 % of 23 orders were classified in Bergey's Manual.

Table 2. A comparison of actinomycete diversity in different habitats

Genus	1**	2	3	4	5	6	Genus	1	2	3	4	5	6
Actinocorallia						√	***Micromonospora***		√	√		√	√
Actinomadura	√	√	√		√		***Mobilicoccus***						√
Actinoplanes			√		√		***Mycetocola***	√					
Actinopolymorpha,	√*	√	√				***Myceligererans***				√	√	
Actinotalea						√	***Mycobacterium***		√	√		√	√
Agrococcus			√			√	***Nesterenkonia***				√		
Agromyces	√		√				***Nocardia***	√	√	√			√
Amycolatopsis					√		***Nocardioides***	√	√	√			
Arthrobacter	√		√		√	√	***Nocardiopsis***				√	√	√
Blastococcus						√	***Nonomurae***		√	√			
Brachybacterium						√	***Oerskovia***	√		√			√
Brevibacterium						√	***Patulibacter***						√
Brevundimonas						√	***Planosporangium***			√			
Cellulomonas					√	√	***Plantibacter***						√
Cellulosimicrobium						√	***Prauserella***				√		
Citricoccus			√	√		√	***Promicromonospora***	√	√	√		√	√

Corynebacterium				√		√	***Pseudoclavibacter***						√
Curtobacterium						√	***Pseudonocardia***	√	√	√			√
Dactylosporangium	√		√				***Rhodococcus***	√	√	√	√	√	√
Dietzia						√	***Saccharomonospora***		√		√		√
Enteractinococcus						√	***Saccharopolyspora***			√			√
Friedmanniella			√				***Salinibacterium***						√
Gordonia						√	***Salinimicrobium***				√		
Gulosibacter,						√	***Sanguibacter***						√
Isoptericola				√	√	√	***Sphaerobacter***						√
Janibacter						√	***Sphaerisporangium***			√			
Jiangella				√		√	***Streptomyces***	√	√	√	√	√	√
Kineococcus						√	***Streptomonospora***				√		
Kocuria	√				√	√	***Streptosporangium***	√		√			
Kribbella			√				***Tessaracoccus***						√
Labedella						√	***Tsukamurella***	√					√
Lentzea	√		√				***Verrucosispora***		√				√
Leucobacter						√	***Williamsia***						√
Luteococcus						√	***Yaniella***				√		√
Marinococcus				√			***Zhihengliuella***				√		
Microbacterium			√		√	√	***Zimmermannella***						√
Micrococcus						√	**Total 72**	**17**	**13**	**26**	**16**	**15**	**51**

Reference **1= Primeval forest soil in Grand Shangri-La, Cao *et al.*, 2009(1); 2= Subtropical every-green forest soil in Sichuan, Cao *et al.*, 2010(2) ; 3= Tropical rainy forest soil in Xishuangbanna, Jiang *et al.*, 2013(3) ; 4= Hypersaline soil in Qinghai, Jiang *et al.*, 2006(4) ; 5= Baltic Sea, Jiang *et al.*, 2011(5) ; 6= This study(6)
*=having;

Therefore, Actinomycete communities of animal feces are not only complex but also different from those in soil, extreme environments, and marine environments. More than one million animal species have been identified worldwide. Thus, animal fecal actinomycetes are tremendous natural resources.

4. Conclusion

The results obtained from the seven-year study of animal fecal actinomycetes should be highlighted several key points:

The diversity of animal fecal actinomycetes is rich. 51 genera were identified from only 42 animal fecal samples. The members of the genera *Streptomyces* and *Rhodococcus*, and *Microbacterium* and *Micrococcineae* (including the genus *Arthrobacter*) were predominant. However, many of the actinomycetes present in animal feces remain unknown.

There are millions of species of animals on the earth. Similar to the actinomycetes in other habitats (soil, sea, plant), animal fecal actinomycetes represent a tremendous resource for the development of drug leads.

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