International Journal of Microbiology Research and Reviews Vol. 1 (4), pp.076-084, April, 2013. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Cultivable actinomycete communities in mammal feces of three diet habits

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Accepted 21 March, 2013

Microbial symbionts play important roles in food digestion and absorption, immunity, pathogens resistance, and health maintaining of their hosts by co-evolution. To provide new sources for discovering new leader compounds of drugs, the diversity and bioactivities of cultivable actinobacteria of animal feces have been studied. Fecal samples of 7 species of carnivorous, omnivorous and phytophagous animal were collected from Yunnan Wild Animal Park. The purified cultures of actinobacteria were isolated from these samples by using 5 media. The 16S rRNA gene sequences of 623 selected strains were analyzed, and the phylogenetic analysis was carried out. The study results shown that actinomycete community of each animal feces were different from each other; 13 genera of actinobacetria were identified from Giant panda (Ailuropoda melanoleuca) feces, and total 31 genera of actinobacteria were identified from the 7 species of animal feces. Fecal actinobacteria, a possibility as a new source for discovering drug leader, agricultural chemicals and other industry products, are argued widely. Selective isolation methods for fecal actinomycetes are described.

Key words：Actinomycete community, diversity of fecal microorganisms, animal feces.

INTRODUCTION

Actinomycetes (Actinobacteria) have been paid a great attention owing to their production of various natural drugs and other bioactive metabolites including important antibiotics, enzyme inhibitors and enzymes for a long-term. Over 22,000 bioactive secondary metabolites were published in scientific and patent literature, and about a half of them were produced by actinomycetes. About 160 antibiotics have being applied in human therapy and agriculture now; 100-120 of them were produced by actinomycetes (Berdy, J., 2005). Actinomycete is still an

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source for new natural drugs development (Berdy, J., 2012; Tiwari, K., et al., 2012). So Baltz showed a proposition of “Renaissance in antibacterial discovery from actinomycetes” (Baltz, R. H., 2008). However, the development of new drugs from actinomycetes in common habitats is more and more difficult (Jiang, Y., et al., 2009). In order to overcome these challenges, some new concepts based on genome was described, that is “new habitats, new methods, new species, new gene cluster, new products and new use” (Jiang, Y., et al., 2009; Goodfellow, M., et al., 2010; Jensen, P. R., 2010; Xu, L. H., et al., 2010). In other words, novel microbial species should contain new gene cluster synthesizing new secondary metabolites, so far as getting new species is an important premise for obtaining new compounds. Many laboratories and companies focused on new actinomycete resources from new habitats, such as oceans, extreme environment and plants, for development of new drugs (Bull, A.T., et al., 2007; Jensen P. R.,et al., 2006; Maldonado, L. A., 2009; Lam, K. S., 2007; Wen, C. Y., et al., 2004; Yi, X. H., 2009) .

Actinomycete, as a pathogen of human and animal, had been studied widely before (Beman, B. L., 1983). But up to now, the research work on fecal actinomycetes as a source for discovery of novel drug leads is very few in the world. In order to get much more unknown actinomycetes from animal feces for discovering new bioactive metabolites, 7 species of animal which belong to the mammal with three diet habits, were selected. The actinomycetes in the feces samples were isolated, cultivated and identified.

MATERIALS AND METHODS

Collection and preparation of fecal samples

7 species of mammal were selected depended on diet habit, carnivorous, omnivorous and phytophagous animal. Fresh fecal samples were collected from the 7 species of animals which lives in the Yunnan Wild Animal Park, Kunming, China and Malaysia (Table 1). 2 to 6 individuals of each animal were chosen, for collecting the fresh feces, and mixed into one sample. Each sample was put in sterile dish immediately, and dried for 10 days at 28 ºC. 2g of each dried sample were pre-treated at 80 ºC for 1 hour, and respectively put in 18 ml sterile water with 0.1 % Na4P2O5, and shaken for 60 min at 220 rpm/min. The suspension was treated by ultrasound wave for 40’ at 150W (Jiang, Y., et al., 2010), and diluted from 10-1 to 10-8.

Isolation medium of actinobacteria

Following media were used for isolating actinobacteria in fecal samples:

HV medium (Hayakawa, M., et al., 1987).

YIM 171: Glycerol 10 g, asparagine 1 g, K2HPO4•H2O 1 g, MgSO4•7H2O 0.5 g, CaCO3 0.3 g, Vit mixture of HV medium 3.7 mg (Hayakawa, M., et al., 1987), agar 15 g, water 1000 ml, pH 7.2.

YIM 212: Mycose 5 g，proline 1 g，(NH4)2SO4 1 g, NaCl 1 g, CaCl2 2 g, K2HPO4 1 g, MgSO4•7H2O 1 g, Vit mixture of HV medium 3.7 mg, agar 15 g, water 1000 ml, pH 7.2.

YIM 47: Soy bean flour 0.2 g, lignin 1 g, Na2HPO4 0.5 g, KCl 1.7 g, MgSO4•7H2O 0.05 g, FeSO4•7H2O 0.01 g, CaCl2 1 g, Vit mixture of HV medium 3.7 mg, soil extract 100 ml, water 900 ml, pH 7.5.

YIM 601: Soluble starch 10 g, casein 0.3 g, KNO3 2 g, MgSO4•7H2O 0.05 g, NaCl 2 g, K2HPO4 2 g, CaCO3 0.02 g, FeSO4 10 mg, Vit mixture of HV medium 3.7 mg, agar 15 g, water 1000 ml, pH 7.2~7.4.

All media were supplemented with 4 groups of sterilized mixture inhibitors to inhibit fungi and Gram negative bacteria: 1, 50 mg cycloheximide, 50 mg nystatin, 20 mg nalidixic acid, 3 mg penicillin; 2, 100 mg cycloheximide, 100 mg nystatin, 40 mg nalidixic acid, 5 mg penicillin; 3, 50 mg K2Cr2O7, 5 mg penicillin; 4, 75mg K2Cr2O7, 5 mg penicillin for 1000 ml medium.

Table 1. Names and related data of sampling animals.

Animal name	Diet habits	Protection grand of China	List of international organization	Number of pure strains	Number of identified strains
Panthera tigris altaica	carnivore	I	CITES and IUCN	302	107
Panthera tigris	carnivore	I	CITES	258	128
Ailuropoda melanoleuca	herbivorous	I	CITES and IUCN	330	133
Viverra zibetha	herbivorous	II	CITES and IUCN	88	58
Ursus thibetanus	herbivorous	II	CITES and IUCN	112	32
Cervus nippon	omnivorous	I	IUCN	377	117
Vicugna pacos	omnivorous	Rearing		87	38
Total				1554	623

CITES= Convention on International Trade in Endangered Species of Wild Fauna and Flora；IUCN= World Conservation Union=International Union for Conservation of Nature and Natural Resources; Manchurian tiger＝*Panthera tigris altaica*； Bengal Tiger＝*Panthera tigris*; Giant panda= *Ailuropoda melanoleuca*; Zibet= *Viverra zibetha*; Asiatic black bears= *Ursus thibetanus*; Shansi Sika= *Cervus nippon*; Vicuna= *Vicugna pacos*

Table 2. Effect of selective isolation for actinobacteria from fecal samples of 7 species of animals with five media (Amount of strains obtained).

Sample Source	HV	47	171	212	601	Total
Panthera tigris altaica	11	6	15	21	19	72
Panthera tigris	12	9	22	23	32	98
Ailuropoda melanoleuca	15	16	18	21	17	87
Viverra zibetha	12	10	12	19	9	62
Ursus thibetanus	19	9	18	33	12	91
Cervus Nippon	33	22	18	36	19	128
Vicugna pacos	38	39	27	36	16	156
Total	140	111	130	189	124	694

Plate dilution method was used for isolating actinobacteria. 0.1ml of suspensions of 10-5, 10-6, 10-7 dilutions for each sample were coated on the medium plates, and cultivated for 7 to 35 days at 28 ºC, then take count of colonies, and pick up actinobacteria to slant of the same isolation medium.

Identification of pure cultivated actinobacteria

Total 1554 pure strains were isolated from the 32 animal feces samples, 623 strains of them were selected after throwing away the duplicates strains based on morphological and cultural characteristics. The DNA of pure strains was extracted for 16S rDNA analysis (Orsini, M., et al., 2001). PCR amplification of the 16S rDNA, purification and sequence of the PCR products were done as described previously (Cui, X. L., et al., 2001). 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 resultant sequences were manually aligned with available sequences from public databases. Phylogenetic trees

Table 3. cfu /g* of actinobacteria on YIM 171 medium at different dilution.

Dilution times	Actinobacteria		Other bacteria	Fungi
	Mixture fecal samples of 7 species of animal in Table 1	Fecal sample of *Vicugna pacos*		
4th	1634×10^5	1324×10^5	132×10^5	0
5th	204×10^6	188×10^6	87×10^6	0
6th	133×10^7	98×10^7	32×10^7	0
7th	66×10^8	22×10^8	11×10^8	0
8th	21×10^9	7×10^9	6×10^9	0
CK*	17×10^8		486×10^8	11×10^7

cfu /g*= Colony-Forming Units

**CK=without inhibitors at dilution 7^{th}, and cannot pick up the single colony of actinomycetes.

were inferred by using the neighbour-joining (Saitou, N., et al., 1987) and maximum-likelihood methods (Felsenstein, J., 1981). All pure cultivated strains were identified at a genus level.

RESULTS

Effect of selective isolating actinobacteria with five media

The effect of selective isolating actinobacteria from feces were 1010, and fungi were not grown in YIM 171 medium containing inhibitors (Table 3).

Type and concentration of inhibitors for isolating actinobacteria from feces were tested several times, the optimum composition were K2Cr2O7 50 mg/L + penicillin sodium 5 mg/L or mixture solution of nystatin 50 mg/L, nalidixic acid 20 mg/L and penicillin sodium 5 mg/L, most part of Gram negative bacteria were inhibited, and no fungi grown on all five medium plates. Different dilutions of sample suspension were tested with YIM 171 medium many times. The optimum dilutions were 10-5, 10-6, and 10-7, in which about 21 to 133 colonies grown on the plates, and it was very easy to pick up single colony (Table 3). However, we suggest that the optimum dilution samples of 7 species of animals with five media was shown in Table 2. Total 694 pure cultivated strains of actinobacteria were isolated. 128 and 156 strains were isolated respectively from Cervus Nippon and Vicugna pacos respectively. 72 and 98 strains were isolated from Panthera tigris altaica and Panthera tigris. The medium YIM 212, HV and YIM 171 were better for isolating actinobacteria and obtained 189, 140 and 130 strains of actinobacteria respectively.

The mean cfu (Colony-Forming Units) of actinomycetes for 7 species animal feces were about 1010, other bacteria concentration for each animal fecal sample should be tested beforeformal isolation all alone.

Actinobacterium Communities

Manchurian tiger (Panthera tigris altaica). Manchurian tiger is listed in directory at I class protect animal by China, Convention on International Trade in endangered Species of Wild Fauna and Flora (CITES) and International Union for Conservation of Nature and Natural Resources (IUCN), and is carnivorous animal of the family Felidae. Fresh feces samples of four Manchurian tiger individuals were collected, and total 302 of pure cultured actnomycete strains were isolated. 107 of them

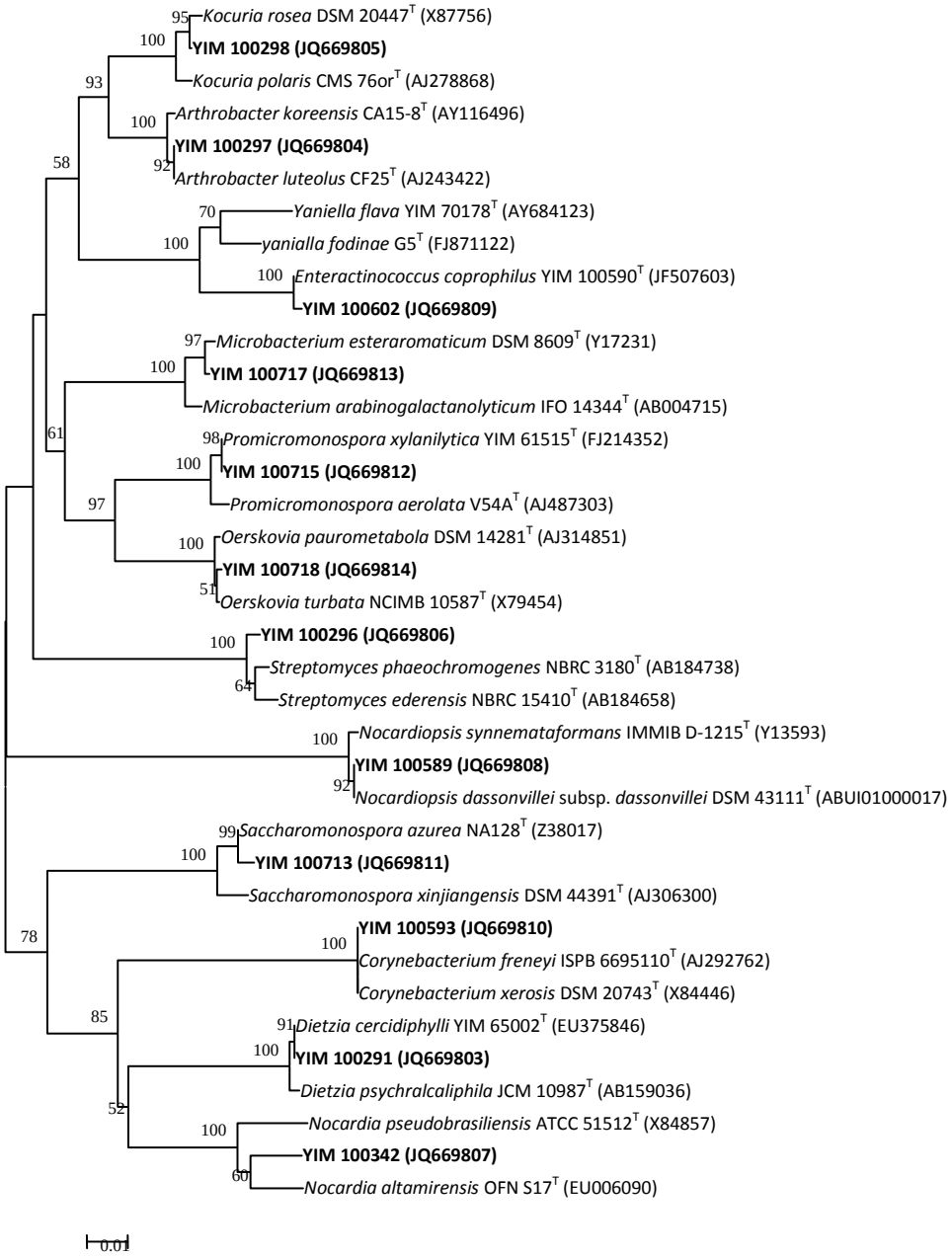

Figure 1. Neighbour-joining tree showing the phylogenetic relationships based on 16S rRNA gene sequences of culturable Actinomycetes from fecal samples of *Panthera tigris*. Sequences obtained in this work are in bold. Bootstrap values (expressed as percentages of 1,000 replications) greater than 50% are given at the nodes. Bar, 1 nt substitution per 100 nt.

were selected after throwing away many duplicates strains based on morphological and cultural characteristics.

16S rDNA sequences of the 107 pure cultivated strains from fecal samples of Manchurian tiger were determined. The phylogenetic analysis was carried out.

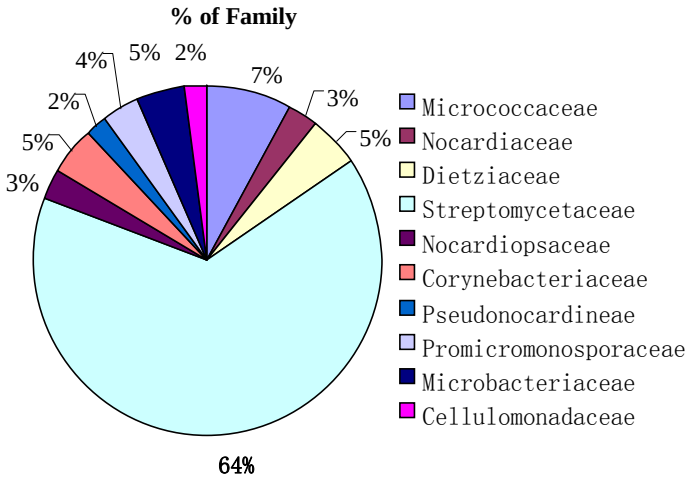

Figure 2. Composition of 10 families of actinobacteria in *Panthera tigris tigris* feces.

Strains were identified at a genus level. Total 9 genera of actinobacteria were identified from this species of animal fecal samples. They are Arthrobacter，Enteractinococcus, Microbacterium，Nocardia，Oerskovia，Promicromonospora，Saccharomonospora, Streptomyces, Yaniella. Enteractinococcus is a novel genus found from another closed in kinship, Panthera tigris amoyensis resently (Cao, Y. R., et al., 2012).

Bengal tiger (Panthera tigris). Bengal Tiger also belongs to carnivorous animal, and I class protect animal of China, CITES and IUCN. They are living in the same forest with Manchurian tiger. Total 258 strains of actinomycetes were isolated and identified from fresh fecal samples of Bengal tiger. They belong to 12 genera of 10 families, Arthrobacter，Corynebacterium，Dietzia，Enteractinococcus，Kocuria，Microbacterium，Nocardia，Nocardiopsis，Oerskovia，Promicromonospora，Saccharomonospora and Streptomyces (Figure 1). Corynebacterium，Dietzia，Kocuriaand and Nocardiopsis were not found from feces of Manchurian tiger.

Cfu/g dried sample of Streptomyces was 2×105 to 176×107 in different fecal samples, and is the first preponderant actinomycetes; 39 species of the genus were identified; Streptomyces albus, S. albidoflavus, S. griseus, S. hygroscopicus, S. rutgersensis, S. tendae, and S. violaceoruber were occurred a high frequency. Actinomycete composition of 10 families of Bengal Tigers feces is showed in Figure 2.

Members of Rhodococcus were isolated and identified, were the second genus of the widest distribution and most of the amount. Rhodococcus coprophilus, Rh. corynebacterioides, Rh. corynebacterioides, Rh. Equi, Rh. pyridinivorans and Rh. zopfii were occurred a high frequency.

Giant panda (Ailuropoda melanoleuca). Giant panda is national rare animal, and is listed in directory at I class protect animal by China, IUCN and CITES. It is only living in a very limited area of northern Sichuan, China, and belongs to omnivorous animal. But its main food is bamboo. Five sampling individuals just came from National Giant Panda Protect Region of Sichuan. Total 330 pure cultured actinomycete strains were isolated.

Table 4. Distribution of different genera in the 7 species of animal feces.

Genus	1	2	3	4	5	6	7	Genus	1	2	3	4	5	6	7
Agrococcus			√			√		*Micromonospora*			√				
Arthrobacter	√	√	√	√		√	√	*Mycobacterium*			√				
Cellulomonas			√					*Nocardia*	√	√					
Cellulosimicrobium			√	√			√	*Nocardiopsis*		√			√	√	√
Citricoccus						√		*Oerskovia*	√	√	√				
Corynebacterium		√						*Patulibacter*			√				
Curtobacterium				√				*Promicromonospora*	√	√			√		
Dietzia		√			√		√	*Rhodococcus*			√	√	√	√	√
Enteractinococcus	√	√						*Saccharomonospora*	√	√			√		√
Gordonia					√			*Salinibacterium*						√	
Isoptericola				√			√	*Sanguibacter*				√			
Janibacter			√					*Streptomyces*	√	√	√	√	√	√	√
Kocuria		√		√		√	√	*Tsukamurella*						√	
Leucobacter						√		*Verrucosispora*			√				
Microbacterium	√	√		√	√	√		*Yaniella*	√						
Micrococcus			√	√				**Total 32**	**9**	**12**	**13**	**10**	**8**	**11**	**9**

1=*Panthera tigris altaica;* 2=*Panthera tigris tigris*; 3=*Ailuropoda melanoleuca*; 4=*Viverra zibetha*; 5= *Ursus thibetanus*; 6=*Cervus nippon*; 7= *Vicugna pacos*

√=having

133 of them were identified by using phylogenetic belonged to 13 genera of actinobacteria, Agrococcus, Arthrobacter，Cellulomonas，Cellulosimicrobium，Janibacter (Martin, K., et al., 1997)，Micrococcus, Micromonospora，Mycobacterium，Oerskovia, Patulibacter, Rhodococcus, Streptomyces and Verrucosispora. Verrucosispora is a genus of the family Micromonosporaceae which prefer to live in sediment of lake.

Zibet (Viverra zibetha). Zibet (Small Indian civet, Viverra indica，Viverricula malaccensis thai, Viverricula hanensis, Viverricula pallida taivana and Viverra pallida) is living in tropical and subtropical rain forest and every green broadleaved forest, belongs to omnivorous animal, and is listed in directory at II class protect animal by China, IUCN and CITES. It is named after producing musk. The test fecal samples of Zibet were collected from three individuals for two times in a wild animal park in Malaysia. Total 88 strains of actinobacteria were isolated with 5 media. 58 strains of them were identified. They belonged to Arthrobacter, Cellulosimicrobium, Curtobacterium, Isoptericola, Kocuria, Microbacterium, Micrococcus, Rhodococcus，Sanguibacter and Streptomyces. Curtobacterium was rare in animal feces. Isoptericola was found from inhabitant of termites (Bakalidou, A., et al., 2002; Stackebrantd, E., et al., 2004).

Asiatic black bears (Ursus thibetanus). Asiatic black bears distribute widely in forest of broad areas from north to south in eastern halfsphere, China, Russia, Iran, Pakistan, Laos, Japan, and India，and belongs to omnivorous animal. It is listed in directory at II class protect animal by China, IUCN and CITES. Fecal sample of three individuals were collected for two times. 112 strains of actinobacteria were isolated. 32 strains of them were identified. They belonged to Dietzia, Gordonia, Microbacterium, Nocardiopsis，Promicromonospora, Saccharomonospora，Rhodococcus and Streptomyces.

Shansi Sika (Cervus nippon). Shansi Sika (Sika, Sika Deer, spotted deer) is I class protect of China and IUCN. It widely distributes in forest and grass lands, and belongs to phytophagous animal. But its amount is decreasing rapid. Fecal samples of 12 individuals were collected from Kunming, Shengyang, China respectively. 337 pure strains were isolated and 117 strains of them were identified. They belonged to 11 genera of actinobacteria, grococcus, Arthrobacter, Citricoccus，Kocuria，Leucobacter, Microbacterium，Nocardiopsis, Rhodococcus，Salinibacterium，Streptomyces and Tsukamurella.

Vicuna (Vicugna pacos). Vicuna is originally living in frigid

zones from 3650m to 4800m of Andes Mountain. It has been reared by human for a long time owing it produces high grade hair. Three individuals were imported from Republic of Chile into Yunnan Wild Animal Park in 2008. Their feces were collected. 87 strains of actinomycetes were isolated. 38 strains were identified. They belonged to 9 genera, Arthrobacter，Cellulosimicrobium，Dietzia，Isoptericola，Kocuria，Nocardiopsis, Saccharomonospora，Rhodococcus and Streptomyces.

DISCUSSION

Table 4 summarized fecal actinobacterium community of the 7 species of animals. Manchurian tiger and Bengal Tiger are closed in kinship, and belong to carnivorous animals. Cultivable actinomycete communities were also similar. Bengal Tiger had more 3 genera than Manchurian tiger. But members of genus Yaniella were only got from Manchurian tiger (Orsini, M., et al., 2001).

Giant panda, zibet and asiatic black bear are omnivorous animals. But type and sources of diet, living habit were different from each other. The actinomycete communities of them were quite different. 13, 10 and 8 genera were identified from the three animals respectively. Actinomycete community of giant panda was the most complex. Only two genera, Streptomyces and Rhodococcusm were owned by the three species of animals. 15 genera, Curtobacterium, Dietzia, Gordonia, Isoptericola, Janibacter, Micrococcus, Micromonospora, Mycobacterium, Nocardiopsis, Oerskovia, Patulibacter, Promicromonospora, Saccharomonospora, Sanguibacter and Verrucosispora were only found from one of the three species of animals.

Shansi Sika and vicuna are phytophagous animals. But their kinship, type and sources of diet, living habit and environments were very different from each other. Five genera, Arthrobacter, Kocuria, Nocardiopsis, Rhodococcus and Streptomyces were owned by the two animals. Agrococcus, Citricoccus, Leucobacter, Microbacterium, Salinibacterium and Tsukamurella were only found from Sika deer. But Cellulosimicrobium, Dietzia, Isoptericola and Saccharomonospora were found from vicuna.

In this study, 31 genera of actinobacteria and 17 of other bacteria (not-shown) were isolated and identified from only the 7 species of animal feces. Members of Streptomyces were the first preponderant microbe, cfu/g of dried samples were up to 109, and distributed in all 7 species of animals. 39 species of the genus were identified, and Streptomyces albus, S. albidoflavus, S. griseus, S. hygroscopicus, S. rutgersensis, S. tendae, and S. violaceoruber etc., were occurred a high frequency. Members of Arthrobacter were identified in 6 species animals. Members of Rhodococcus were isolated and identified from fecal samples of 5 species of animals, and Rhodococcus coprophilus, Rh. corynebacterioides, Rh. corynebacterioides, Rh. equi, Rh. pyridinivorans and Rh. zopfii were occurred a high frequency. Microbacterium was identified from 5 species of animal feces. Total 14 genera (46%) of the Order Micrococcales were isolated, and were the widest distribution and the most at amount in the 7 species of animals. But 13 of the 31 genera, Cellulomonas, Citricoccus, Corynebacterium, Curtobacterium, Curtobacterium, Gordonia, Janibacter, Leucobacter, Micrococcus, Micromonospora, Mycobacterium, Patulibacter, Salinibacterium, Sanguibacter, Tsukamurella, Verrucosispora and Yaniella were found only from one of the 7 species of animals.

These results indicated that, first, members of the genus Streptomyces and Order Micrococcales were the widest distribution and the largest amount; second, composition of actinobacteria with Chemotype IV to IX (Lechevalier, M. P., et al., 1970; 1977; 1980) and globose and bacilliform shapes, specially Order Micrococcales, were the richest diversity, and occurred a high frequency in most part of tested animal feces. These are distinct features of cultivable fecal actinomycete community differing from those in soil, plant, and marine environment.

Bergey's Manual of Systematic Bacteriology (Volume 5) collected 16 Orders, 44 Families and 216 genera of the Class Actinobacteria (Whitman, W. B., et al., 2012). Total 31 genera of actinobacteria were obtained from only 7 species of animals in this study, and occupy 14% of the 216 genera. It is worth to notice that the 16S rDNA sequence similarities of 78 of 623 sequenced strains (total 1554) with valid published species were below 98.5 %. In other words, nearly 12% sequencing strains and 5% of isolates (1554 strains) in this study were un-known, and they were possible novel species (Xu, L. H., et al., 2007). Members of the genus Enteractinococcus which was found from Panthera tigris amoyensis before (Cao, Y. R., et al., 2012), were isolated and identified from Manchurian tiger and Bengal tiger. These results showed that a large of unknown actinomycetes existed in animal feces.

Each animal has evolved a particular gastrointestinal microbial flora in the cause of organic co-evolution for a long-term. The flora is carrying the genetic order of ancestor, on the one hand, and will be changed continuously alone with living environments, food sources, age, and state of health one the other hand, so that demonstrated kaleidoscope and extremely rich diversity. There are several ten thousand of higher animal and insects in the whole world. Therefore animal is a tremendous treasure-house of microbial resources.

We and cooperative partners have isolated more than 80 bioactive secondary metabolites from some fecal actinomycete strains, including actinomycin C, abkhazomycin, cosmomycin, desertomycin，desferrioxamine E, discodermolide, emodin, erythromycins, favofungin, geldanamycin, kasugamycin, kidamycins, leucomycin, oasomycins A, puromycin, rutamycin, rhodomycinone, tirandamycin, vicenistatin, polyene macrolides etc (not-shown). These compounds have complex structure and various activities. Several novel compounds, such as sannastatin, a novel toxic macrolactam polyketide glycoside, which produced by an un-identified Streptomyces sp. YIM 100282, had been found (Yang, S. X.,et al., 2011).

In conclusion, animal fecal actinobacteria, like those in soil, oceans, extreme environments and plants, had high diversity but different composition. Enormous unknown actinobacteria widely exist in animal feces. Therefore, animal fecal actinobacteria is important resources for developing novel antibiotics, anti-tumor agents, enzyme inhibitors, immunity inhibitor, agricultural chemicals, enzymes and other useful products. Both Streptomyces and Rhodococcus were the predominant genera and the widest distribution in animal feces. Genome sizes of this two genera are up to 9×107 base pairs, one of biggest genome in actinobacteria, and some species of them contains 20 or more natural product biosynthetic gene clusters (Omura, S., et al., 2001; Bentley, S. D., et al., 2002)

. We showed further a hypothesis that first, the function of actinomycetes in intestinal tract of hosts was mainly played by bioactive substances produced from members of the two genera; second, secondary metabolites with bio-activities produced by fecal actinobacteria, except pathogen, should be no toxic or lower toxic to their hosts. We think these are very important excellence comparing with the metabolites from other microorganisms in other habitats.

Key of isolating actinobacteria from animal feces

Selective isolation methods for fecal actinomycetes, especially un-known actinomycetes, are very important, and as a research project, it should be studied, improved and renewed constantly. A large number of Gram negative bacteria, fungi and even known actinomycetes in animal feces are a main problem for selective isolation of un-known actinobacteria. In order to eliminate these troubles, and obtain much more un-known actinobacteria for discovering novel lead compounds, sampling and isolation methods are key points.

ACKNOWLEDGMENT

This work is financed by the National Natural Science Foundation of China (No. 31270001, 81072553 and 21062028), National Major scientific and technology special projects (2009ZX09302-003), National Institutes of Health USA (1P41GM086184-01A1). We thank Mr. Youlong Li for his kindly help.

ABBREVIATIONS

rRNA Ribosomal Riboucleic Acid
AIDS Acquired Immune Deficiency Syndrome
HIV Human Immunodeficiency Virus
rDNA Ribosomal Deoxyribonucleic Acid
CITES Convention on International Trade in Endangered Species of Wild Fauna and Flora
IUCN International Union for Conservation of Nature and Natural Resources

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