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Large numbers of new bacterial taxa found by Yunnan Institute of Microbiology

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Extreme environments, primeval forest, sea sediments and plants in China yielded members of a new suborder, Jiangellineae, a new family Sinobacteraceae, and 23 new genera of actinomycetes and other bacteria. Three suggestions were made for further discoveries of new actinomycetes. First, natural habitats such as old growth forests, and extreme environments, should be given sampling priority; second, culturing procedures need to be constantly improved to mimic natural habitats; third, less than 98.5% similarity of 16S rRNA sequence to that of a known species could be used as an indicator of a new species.

bacteria, new taxon, suborder Jiangellineae, family Sinobacteraceae

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Extensive hypersaline and alkaline soils, deserts, and more than 100 salt lakes are found in Xinjiang Uyghur Autonomous Region, Qinghai and Gansu provinces, northwestern China. We collected 535 soil and lake sediment samples from these provinces and autonomous region between 1993 and 2008. Actinomycetes and other bacteria were isolated from these samples using dilution plate techniques involving 12 different media. Isolates were purified, and then identified using a polyphasic taxonomic approach.

Large numbers of new bacterial taxa were discovered from the isolates, described and published. Members of a new suborder, Jiangellineae [1], were found in primeval desert soil in Gansu Province (Figure 1). 16S rRNA signature nucleotides are at positions 127: 234 (G-C), 598: 640 (C-G), 672: 734 (G-C), 831: 855 (U-A), 833: 853 (G-C), 840: 846 (A-U), 950: 1231 (G-C), 952: 1229 (G-C), 955: 1225 (G-U), 986: 1219 (U-G) and 987: 1218 (C-G). This is the highest level bacterial taxon established by a Chinese microbiologist. We named the family Jiangellaceae within this suborder. The type genus is *Jiangella* Song et al. [2]. Members of 8 new genera, *Yania* [3], *Myceligenerans* [4], *Zhihengliuella* [5], *Haloactinospora* [6], *Haloglycomyces* [7], *Amycolicicoccus* [8], *Yimella* [9] and *Sinococcus* [10] were discovered in saline soil. Members of 6 new genera, *Streptomonospora* [11], *Sediminimonas* [12], *Haloactinopolyspora* [1], *Alkalibacillus* [13], *Salinimicrobium* [14] and *Aidingimonas* [15] were found in salt lakes and mines. We conclude that new bacterial taxa are surprisingly common in extreme environments, especially in hypersaline and alkaline habitats (Table 1).

There are extensive natural forests, which are well-protected from the actions of humans, in and around Yunnan Province. Members of 3 new genera, *Actinobispora* [16], *Actinomycetospora* [17] and *Planosporangium* [18] were found in soil samples collected from tropical rain forest in Xishuangbanna, Yunnan. A member of a new genus, *Naxibacter* [19], was found in coniferous forest at an elevation of 3700 m in Lijiang, northwestern Yunnan. A

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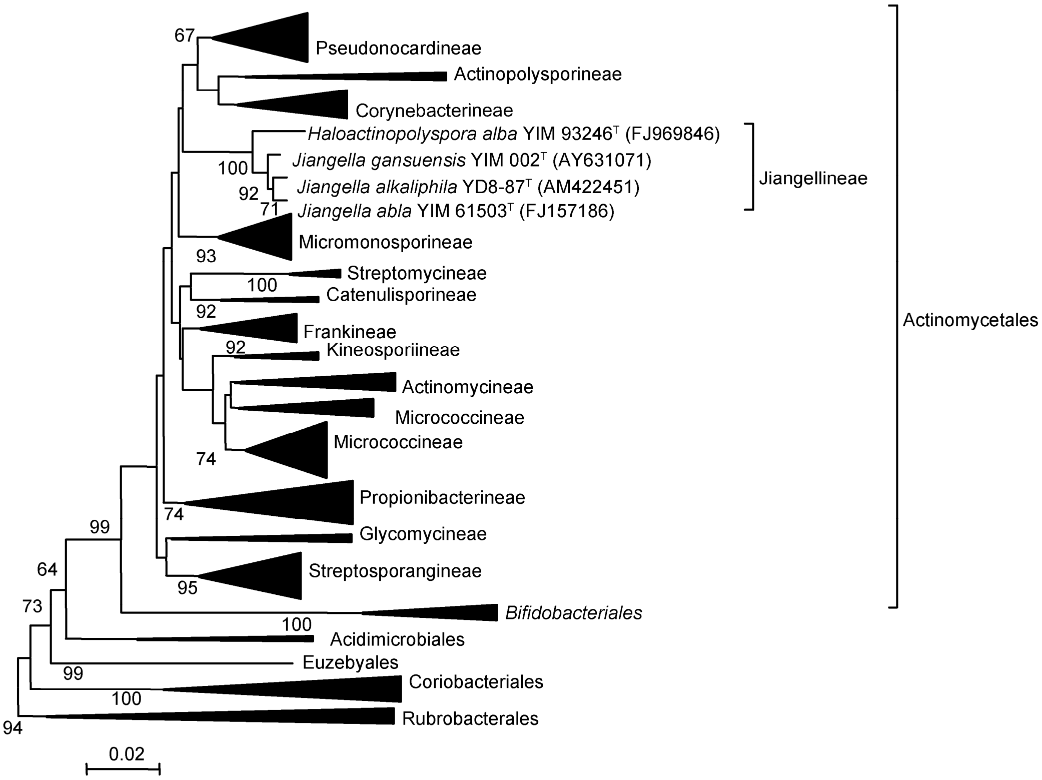

Figure 1 Phylogenetic position of strain YIM 93246^T and members of the genus *Jiangella* within the class *Actinobacteria* based on 16S rRNA gene sequence analysis.

Table 1 Habitats of new bacterial taxa

New taxon	Habitat	Reference
New genera		
Haloactinopolyspora	Salt lake sediments in Xinjiang	[1]
Jiangella	Desert soil in Sunan, Gansu	[2]
Yania	Saline soil in Xinjiang	[3]
Myceligenerans	Alkaline salt marsh in Qinghai	[4]
Zhihengliuella	Saline soil in Qinghai	[5]
Haloactinospora	Hypersaline soil in Xinjiang	[6]
Haloglycomyces	Hypersaline soil in Xinjiang	[7]
Amycolicicoccus	Saline soil contaminated by crude oil in Xinjiang	[8]
Yimella	Saline soil in Xinjiang	[9]
Sinococcus	Saline soil in Qinghai	[10]
Streptomonospora	Salt lake sediments in Xinjiang	[11]
Sediminimonas	Ancient salt mines in Yunnan	[12]
Alkalibacillus	Salt lake sediments in Qinghai	[13]
Salinimicrobium	Salt lake sediments in Qinghai	[14]
Aidingimonas	Salt lake sediments in Xinjiang	[15]
Actinobispora	Natural forest in Weixin, Yunnan	[16]
Actinomycetospora	Natural forest in Xishuangbanna, Yunnan	[17]
Planosporangium	Natural forest in Menghai, Yunnan	[18]
Naxibacter	Natural forest in Lijiang, Yunnan	[19]
Plantactinospora	Root of *Maytenus austroyunnanensis* in Xishuangbanna, Yunnan	[20]
Sciscionella	Sediments in northern South China Sea	[21]
Marinactinospora	Sediments in northern South China Sea	[22]
Sinobacter	Farmland soil polluted by chemicals	[22]
New family		
Sinobacteraceae	Farmland soil polluted by chemicals	[23]

member of a new genus, *Plantactinospora* [20], was found in root tissue of *Maytenus austroyunnanensis* in Xishuangbanna, Yunnan. In addition, members of 2 new genera, *Sciscionella* [21] and *Marinactinospora* [22], were found in sediment samples collected from the northern South China Sea, and a member of a new family, *Sinobacteraceae* [23], and a new genus S*inobacter* [22], were also found from farmland soil polluted by chemicals and considered an artificial extreme environment. In total, more than 120 new species of bacteria were isolated from western China. In the last 10 years, our laboratory has become a world leader in the discovery of new bacterial taxa.

Drugs such as antibiotics and anticancer agents have made enormous contributions to human life over the last 100 years. Actinomycetes are one of the groups of microorganisms that are key producers of antibiotics. About 75% of the antibiotics used in medicine and agriculture are produced by actinomycetes, and these the pharmaceutical development of, however, has met with tremendous difficulties in the past. One of the difficulties is avoiding retesting of known microbial species and compounds. Based on our research and that of others overseas, it appears that more than 90% of the microorganisms in the natural world have not yet been obtained in pure culture [24–26]. From genomic research, it is evident that every new microbial taxon has the potential to contain new genes or gene clusters that synthesize new secondary metabolites. Obtaining new microbial species, particularly those in new genera, families or orders, and finding ways to culture previously uncultured microorganisms, are key to the discovery of new compounds and the development of new drugs [27]. We believe that the challenge to develop the genetic resources of uncultured microorganisms using metagenomic procedures will be aided by the following.

First, in natural environments, as a result of evolution, organisms have formed communities in which composition, rate of change and numbers of microorganisms should be relatively stable [28]. In general, actinomycete diversity in natural forest (especially tropical rainforest) is more complex than in secondary growth forest. We isolated 26 genera from tropical rain forest in Xishuangbanna and 17 genera from primeval forest in Grand Shangri-La. In contrast, only 5 genera were isolated from secondary growth forest in the Emei and Qingcheng Mountains (Table 2) [28]. The microbial communities of soil are remarkably different between tropical rainforest and frigid forest, and different between soils, lake sediments, sea sediments and soils hot springs. It is worth emphasizing extreme environments that have extreme acidity, alkalinity, salt, radioactivity, heat (hot springs), or cold (Polar Regions and snowy mountains); we found unique microorganisms living in these environments. Members of 19 genera were isolated from hypersaline soil in Qinghai Province (Table 2). In the search for further microorganisms, samples should thus be collected from unique environments including natural forests and extreme environments including sea sediments, plant tissues and animal feces. As treasure-houses of microbial resources, these environments should be well-protected, as National Nature Reserves or National Parks using the appropriate legislative steps, just as we protect unique animal and plant species.

Second, selective isolation methods for microorganisms need to be continually improved, with consideration given to the microorganisms' habitats of origin. Culturing variables include medium composition (e.g., carbon and nitrogen, auxin, hormones, vitamins, inhibitors and salt concentrations), pH and temperature. T3 medium (cellulose 10 g, casein 0.3 g, KNO_3 0.2 g, $CaCO_3$ 0.02 g, $FeSO_4$ 0.01 g, NaCl 150 g, KCl 20 g, $MgCl_2\cdot 6H_2O$ 30 g, $MgSO_4\cdot 7H_2O$ 5 g, K_2HPO_4 1 g, agar 20 g, pH 7.5), was designed for isolation of halophilic bacteria. Using this medium, many new taxa including members of *Amycolicicoccus*, *Haloactinopolyspora*, *Haloactinospora*, *Haloglycomyces*, *Sinococcus*, *Yimella*, *Zhihengliuella*, *Actinopolyspora*, *Saccharomonospora* and *Saccharopolyspora* were isolated from samples of saline soil and lake sediments in Xingjiang and Qinghai. Numbers of unculturable taxa rise and fall, as new taxa are found, and new culturing methods continually developed.

Third, based on DNA-DNA hybridization and 16S rRNA sequencing of many bacteria, if there is 98.5% similarity

Table 2 Diversity of cultured actinomycetes in different habitats

Area	Diversity of cultured actinomycetes
Secondary forest in Emei and Qingcheng Mountains	*Dactylosporangium*, *Mycobacterium*, *Nocardia*, *Promicromonospora*, *Streptomyces*
Tropical rain forest in Xishuangbanna	*Actinomadura*, *Actinoplanes*, *Actinopolymorpha*, *Agrococcus*, *Agromyces*, *Arthrobacter*, *Citricoccus*, *Dactylosporangium*, *Friedmanniella*, *Kribbella*, *Lentzea*, *Microbacterium*, *Micromonospora*, *Mycobacterium*, *Nocardia*, *Nocardioides*, *Nonomuraea*, *Oerskovia*, *Planosporangium*, *Promicromonospora*, *Pseudonocardia*, *Rhodococcus*, *Saccharopolyspora*, *Sphaerisporangium*, *Streptomyces*, *Streptosporangium*
Natural forest in Grand Shangri-La	*Actinomadura*, *Actinopolymorpha*, *Agromyces*, *Arthrobacter*, *Dactylosporangium*, *Kocuria*, *Lentzea*, *Mycetocola*, *Nocardia*, *Nocardioides*, *Oerskovia*, *Promicromonospora*, *Pseudonocardia*, *Rhodococcus*, *Streptomyces*, *Streptosporangium*, *Tsukamurella*
Hypersaline soil in Qinghai	*Alkalibacillus*, *Citricoccus*, *Corynebacterium*, *Isoptericola*, *Jiangella*, *Marinococcus*, *Myceligererans*, *Nesterenkonia*, *Nocardiopsis*, *Prauserella*, *Rhodococcus*, *Saccharomonospora*, *Salinimicrobium*, *Sinococcus*, *Sinocurtobacterium*, *Streptomonospora*, *Streptomyces*, *Yania*, *Zhihengliuella*

between the 16S rRNA sequences of a new isolate and a known species, the isolate has an 80% likelihood of being a new species [27]. Therefore, for any new isolate, part of the 16S rRNA sequence (about 800 bp), should be determined, and 98.5% similarity could be defined as a reasonable boundary between species (Table 3) [29].

Table 3 Similarity of 16S rDNA sequence and possibility of new species [27]

Similarity of 16S rDNA sequence	Possibility of new species (%)
99%	20~30
98.5%~99%	50
98%~98.5%	70~80
97%~98%	90
95%~97%	100 New species
95%	100 New genus

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