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Diversity of Aquatic Actinomycetes in Lakes of the Middle Plateau, Yunnan, China

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A total of 749 sediment and water samples were collected from 12 lakes of the Middle Plateau of Yunnan from 1983 to 1993. The diversity and biological characteristics of the aquatic actinomycetes in these lakes were studied. Sixteen genera of actinomycetes were isolated from these samples. Micromonospores assumed a notable dominance (from 39 to 89%) in the actinomycete populations of these lake sediments. Streptomycetes were the second most abundant organisms. The diversity and counts of actinomycetes varied with the season. Thermophilic actinomycetes have a wide distribution in these lakes, but their counts were smaller. The cell wall compositions of certain *Micromonospora* and *Streptomyces* strains from an alkaline lake revealed an unusual combination of glycine and isomers of diaminopimelic acid. It seems that aquatic actinomycetes play a significant role in the decomposition of organic substances, including some toxic compounds such as phenol, in these lakes. It also appears that aquatic actinomycetes are one of the important resources for screening useful enzymes and metabolites.

Though soil actinomycete diversity has been extensively studied, relatively few efforts with aquatic actinomycetes have been attempted. Early research of actinomycetes from Wisconsin lakes pioneered aquatic actinomycete exploration (1, 6, 20). Some microbiologists have investigated the distribution, biological characteristics, and ecological effects of aquatic actinomycetes (2–5, 7–9, 14, 17, 20–22). Since aquatic environments differ greatly from terrestrial habitats, the biological characteristics of aquatic actinomycetes and their distribution are expected to be different from those of soil actinomycetes. Research of the biodiversity of aquatic actinomycetes is not only important for basic studies but also necessary in practice for its exploitation. Yunnan is situated in the southwestern part of China, between latitudes 20°09′ and 29°15′N and longitudes 97°39′ and 106°12′E. This province covers 436,200 km^2. The major populated area of Yunnan is from Qujing to Jianchuan and from the Jinsha River to the Honghe River and is called the Middle Plateau of Yunnan, with an average elevation of approximately 2,000 m above sea level and a subtropical climate. There are about 30 large and small lakes on this wide and hilly plateau. Since 1983, we have investigated the actinomycete populations of 12 selected lakes (Fig. 1) with the intention of screening these isolates for potentially useful enzymes and metabolites. Here we present the diversity of the actinomycetes in these lakes.

MATERIALS AND METHODS

Test samples. Sediment and water samples (total of 749) were collected from 12 Yunnan Middle Plateau lakes with a heavy-hammer sampler, once in April (before the end of the dry season) and once in October (rainy season). The sampling plots in every lake were distinguished by their geological environments and ecological conditions, such as the entrance or exit of rivers, depth, nutrients, aquatic plants, and human interventions (Table 1). Samples were bottled and kept in sterilized plastic flasks. The actinomycetes in samples were isolated 0 to 5 days after sampling.

Isolation and enumeration of actinomycetes. Actinomycetes were isolated by spreading dilutions of samples on colloidal chitin agar (19), starch casein agar (11), and glycerol-asparagine agar at pH 7.2 or 10.5, with phosphate buffer used to isolate actinomycetes from Chenghai Lake samples. Plates were incubated at 28°C for 15 days before the colonies were counted and picked. To isolate thermophilic actinomycetes, air dried sediment samples were pretreated at 120°C for 1 h. Dilutions of samples were spreaded on half-strength nutrient agar, yeast extract-malt extract agar, and glycerol-asparagine agar, and plates were incubated at 55°C for 5 to 7 days. The colonies which appeared on plates were counted and tentatively identified under a microscope. The colonies of some streptomycetes and micromonospores and all rare actinomycetes on plates were picked up and transferred to yeast extract-malt extract agar slants. Pure cultures were obtained from selected colonies for further identification. Pure cultures were preserved in 20% glycerol at −70°C for screening useful metabolites in our laboratories.

Taxonomic grouping of isolates and computer analysis. Taxonomic grouping of isolates and computer analysis were carried out by procedures described previously (23).

Determination of enzyme and antimicrobial activities. The hydrolytical activities of enzymes were analyzed by mixing cellulose, chitin, fibrin, and mannan with agar on individual assay plates and visualized by clear zone formation (18). The other hydrolytic enzyme assays were based on the methods of Shirling and Gottlieb (19). Broths from the isolates inoculated in soybean meal-glucose medium were analyzed for their antimicrobial activities by the inhibition zone method (18).

Resistance to toxic substances. Tubes containing sterilized yeast extract-malt extract broth were spiked with a given concentration of phenol, hydrofluoric acid, or mercurous chloride and then inoculated with purified isotates. Tubes were incubated at 28°C for 15 days, and growth inhibition was recorded.

RESULTS AND DISCUSSION

Actinomycetes isolated from lake sediments. The results of isolation and enumeration of mesophilic actinomycetes in sediment samples from 12 lakes were analyzed (Table 2) by cluster analysis for generic diversity. According to their similarities in actinomycete diversity, these samples were divided into four groups (Fig. 2). The first group included actinomycetes from the sediments of lakes Datun, Lugu, Cibi, Jilu, Xingyun, Yilong, and Yangzong at an 88% similarity level. *Streptomyces*, *Micromonospora*, and *Nocardia* strains were isolated from Datun and Lugu sediments. In Jilu and Xingyun sediments, *Saccharopolyspora* strains were isolated in addition to the strains isolated from Datun and Lugu sediments. *Actinomadura* strains were found in Cibi sediments along with the above-mentioned strains. No *Nocardia* strain was isolated from Yilong sediment. *Microbispora* strains were found only in Yangzong sediment. In all, six genera were isolated from sediment samples of these

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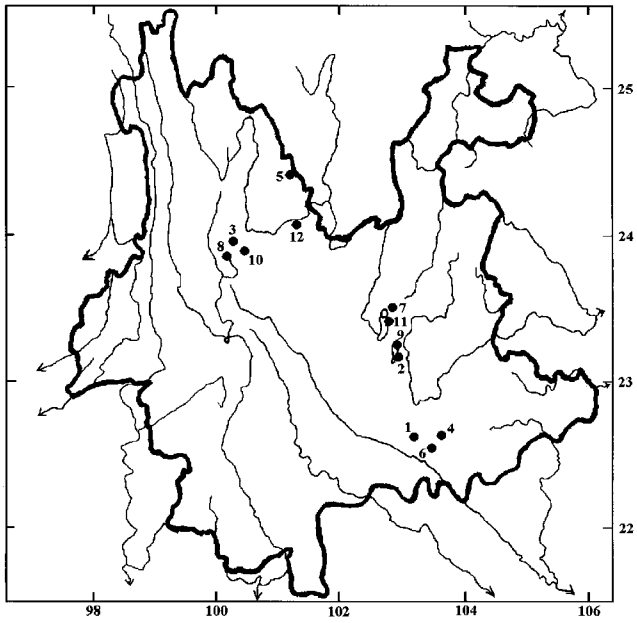

FIG. 1. Distribution of 12 Yunnan Middle Plateau lakes. 1, Jilu; 2, Xingyun; 3, Cibi; 4, Datun; 5, Lugu; 6, Yilong; 7, Yangzong; 8, Jian; 9, Fuxian; 10, Erhai; 11, Dianchi; 12, Chenghai.

seven lakes. *Micromonospora* strains were 39 to 86% of the population, with an average of 69%. Streptomycetes were 9 to 60% of the population, with an average of 28%. It is worth noting that the Datun, Jilu, and Yilong lakes, rich in organic matter and aquatic plants, were shallow and had previously dried up for 20 days in 1981. The numbers of actinomycetes in these three lakes increased greatly (up to 3- to 10-fold) compared with those in the other four lakes of this group. As a result of aridity, the aeration and heat of exposed bottom sludges facilitated the reproduction of actinomycetes. Nevertheless, *Micromonospora* strains were still a dominant proportion of the actinomycete population (Table 2).

The second group included actinomycetes from Jian and Fuxian sediments at a 75% similarity level. Eight and seven genera were isolated from Jian and Fuxian sediments, respectively. *Thermomonospora*, *Microtetraspora*, *Thermopolyspora*, and *Saccharomonospora* isolates were dominant in sediment samples from these two lakes. Fuxian Lake is the deepest lake in Yunnan, with less organic matter and fewer aquatic plants. Far fewer actinomycetes (<25%) were found in this lake than in Jian Lake. Even fewer or no actinomycetes were isolated from the deep plots of Fuxian Lake sand sediments.

The third group included samples from lakes Dianchi and Erhai at an 81% similarity level. They are the two largest lakes in Yunnan, with strong human interventions. Five and eight genera were isolated from Erhai and Dianchi sediment samples, respectively. We have previously isolated *Actinoplanes* strains many times from the soils of Xishan Mountain by Dianchi Lake; these strains were found exclusively in sediment samples from Dianchi Lake (Table 2). Members of this genus probably came from Xishan Mountain soils.

In the fourth group were actinomycetes from Chenghai Lake sediment samples. The drainage basin of Chenghai has been deforested in the past 300 years, which dramatically affects the recyling of lake water. The lake has become a dead lake, with an alkaline pH of 9.0 at present. The counts of actinomycetes in the sediment samples of this lake were much lower than those of other lakes. However, the diversity in this lake was complex, with at least nine genera present. *Micromonospora* strains were again notably dominant (89%). The actinomycete diversity in shallow-water (3- to 3.5-m) plots was rather complex, since eight genera were isolated. However, in deep-water (28- to 32-m) plots the diversity was relatively monotonous. About 72% of the 251 isolates from Chenghai Lake could be cultured in broth containing 5% NaCl; nearly all of these strains could grow at pH 9, and 50% could grow at pH 10.5. Accordingly, the strains isolated from this lake are considered to be alkaline-pH-resistant actinomycetes, with optimal growth occurring between pHs 7 and 9. Only 0.8% of the strains were alkalophilic actinomycetes, with optimal growth occurring between pHs 9 and 10.

Actinomycetes isolated from lake water. Actinomycetes isolated from lake water samples of Dianchi, Erhai, Datun, and Jilu were *Streptomyces*, *Micromonospora*, *Actinoplanes*, *Nocardia*, *Rhodococcus*, and *Mycobacterium* strains. The total actinomycete counts were 2,780 to 4,264 CFU/ml. No actinomycetes were isolated from the water samples of the other eight lakes. This observation differs from that of Johnston and Cross (8), by whom high percentages of lake water actinomycetes were isolated. We postulate that the Yunnan Middle Plateau lakes are generally clear and not as densely polluted by human activities as are lakes in England.

Thermophilic actinomycetes. The populations of thermophilic actinomycetes isolated from lake sediments were as follows: *Streptomyces* strains, 66%; *Micromonospora* strains, 25%; and *Thermoactinomyces* strains, 6%. Other genera were rare

TABLE 1. General characteristics of 12 Yunnan Middle Plateau lakes

Lake	Altitude (m)	Length (km)	Width (km)	Area (km^2)	Depth (m)		Storage capacity (10^6 m^3)	pH	Aquatic plant status	No. of samples	
					Maximum	Mean				Sediment	Water
Jilu	1,731	15.5	3.1	42.0	15.0	4.0	1.9	6.5–7.0	Rich	30	30
Xingyun	1,723	10.5	3.8	39.0	12.0	9.0	2.3	6.0–6.8	Poor	30	30
Cibi	2,150	6.5	3.0	15.0	21.0	5.0	0.75	6.4–6.7	Moderate	30	30
Datun	1,280	7.0	2.9	12.0	2.7	1.3	0.34	6.7–7.0	Extremely rich	30	30
Lugu	2,700	9.4	5.2	49.0	93.6	40.6	19.5	7.0	Poor	15	0
Yilong	1,411	13.8	3.0	42.0	7.0	3.5	1.2	6.0–7.0	Rich	30	30
Yangzong	1,770	12.7	2.5	31.0	30.0	20.0	6.0	6.0–7.0	Poor	30	30
Jian	2,015	7.4	3.0	20.0	7.0	3.5	0.7	6.4–7.0	Rich	30	30
Fuxian	1,721	32	6.7	211	155	98.6	1,890	7.0	Poor	41	20
Erhai	1,970	42	5.8	250	20.7	10.2	254	6.0–6.7	Moderate	42	42
Dianchi	1,885	32	10.5	300	8.0	5.0	157	5.5	Moderate	30	30
Chenghai	1,503	20	4	79	37	15	270	9.0	Poor	70	30

TABLE 2. Numbers of actinomycetes in sediment samples from 12 Yunnan Middle Plateau lakes

Lake	10^3 CFU/g (dry wt)[a]																	No. of genera
	Streptomyces	*Actinoplanes*	*Micromonospora*	*Thermomonospora*	*Microbispora*	*Microtetraspora*	*Micropolyspora*	*Actinomadura*	*Streptosporangium*	*Saccharomonospora*	*Saccharopolyspora*	*Promicromonospora*	*Nocardia*	*Rhodococcus*	*Mycobacterium*	Unidentified	Total	
Datun	421.0 (14)		2,562.0 (86)										8.0				2,991.0	3
Lugu	54.7 (20)		209.7 (78)										2.7			2.7	269.8	3
Cibi	117.0 (28)		264.0 (63)					3.0			3.0		32.0			2.9	421.9	5
Jilu	318.0 (9)		2,742.0 (77)								222.0		260.0				3,542.0	4
Xingyun	41.0 (13)		273.0 (84)								6.0		4.0				324.0	4
Yilong	1,708.0 (51)		1,538.0 (47)					20.0			38.0						3,304.0	4
Yangzong	735.0 (60)		477.0 (39)		0.05								9.0				1,221.05	4
Jian	497.0 (44)		516.0 (52)	0.05		0.3		20.0		21.0	21.0		46.0			4.8	1,126.15	8
Fuxian	157.6 (45)		177.5 (52)				0.4	2.6	0.5		0.5		0.9				340.0	7
Erhai	117.2 (14)		521.9 (63)	1.7									14.4	9.6		26.2	822.6	5
Dianchi	40.4 (6)	26.3	594.3 (76)	5.1			4.4						88.5	4.4	4.4		722.8	8
Chenghai	3.3 (7)		38.69 (89)			0.26	0.03	0.26		0.05		0.07	0.4	0.59		0.15	43.8	9

[a] Parenthetical values are percentages of the actinomycete population.

(Table 3). Most thermophilic actinomycetes from these lakes could grow at 28°C and thus should be called thermotolerant actinomycetes. Only a small number (~1%) of them could be cultured at 68°C but not at 28°C and thus should be called strictly thermophilic actinomycetes. The results demonstrate that thermophilic actinomycetes were widely distributed in lake sediments (Table 3).

Seasonal variation in actinomycete diversity. Investigation of the actinomycetes in samples from lakes Dianchi, Erhai, and Chenghai indicated that the actinomycete diversity in Dianchi and Erhai samples was more complex during the dry season than during the rainy season. Actinomycetes were also more abundant during the dry season than during the rainy season. The seasonal variation in actinomycete number and diversity in Chenghai samples is shown in Table 4. In Chenghai samples, the generic diversity in the dry season is more complex than it is in the rainy season. Nine genera were isolated from samples collected during the dry season. Only five genera were isolated during the rainy season. However, *Streptomyces* counts in the rainy season were higher than those in the dry season. This indicates that some of the streptomycetes were washed from land soils during the rainy season, instead of being original inhabitants.

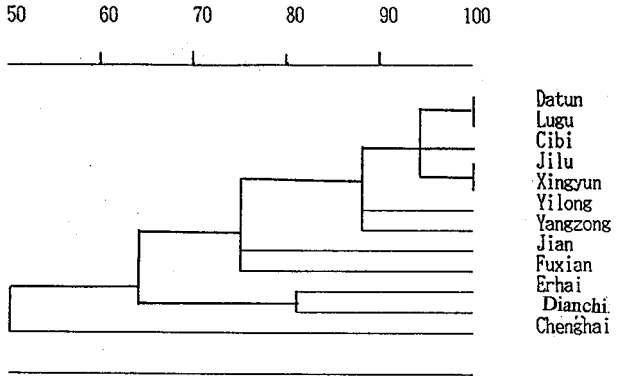

FIG. 2. Similarities (in percentages) in actinomycete diversity in samples from 12 Yunnan Middle Plateau lakes.

Cell wall composition. The cell wall of a micromonospore was initially reported to contain glycine and *meso*-diaminopimelic acid (*meso*-DAP) and was classified as wall chemotype II (12, 13). Among the 31 *Micromonospora* strains isolated from Chenghai Lake, 11 showed the presence of both L-DAP and *meso*-DAP. Interestingly, 2 of these 11 strains were dominant organisms, representing about 20% of the actinomycetes found in this lake. Of the 11 *Micromonospora* strains isolated from Fuxian Lake, two also contained both L-DAP and *meso*-DAP. Thus, we conclude that the frequency of L-DAP in the *Micromonospora* cell wall is higher in a high-salinity stream strain than in a freshwater strain, as previously noted by Kawamoto et al. (10).

The cell wall compositions of 32 isolates from Chenghai Lake able to grow on alkaline (pH 10) media were determined. They had been classified as *Streptomyces* isolates on the basis of morphology. Among these isolates, 11 were found to contain glycine and *meso*-DAP and 1 contained *meso*-DAP and 3-OH-DAP. Miyashita et al. (15) suggested that these alkalinophilic

TABLE 3. Thermophilic actinomycetes in sediment samples from nine lakes

Lake	CFU/g (dry wt)									No. of genera
	Streptomyces	*Micromonospora*	*Microbispora*	*Microtetraspora*	*Thermopolyspora*	*Saccharomonospora*	*Nocardia*	*Thermoactinomyces*	Total	
Jilu	100	100					50	50	300	4
Xingyun	100							50	150	3
Cibi	50							50	100	3
Datun	50							150	200	3
Yilong	3,950	3,400							7,350	3
Yangzong	900	50	50				100	10	1,150	6
Jian	4,150			300	50			350	4,850	5
Fuxian	233	8					58	100	1,150	5
Chenghai	17.5	0.7				0.6		35	53.8	5

TABLE 4. Seasonal variation in the number of actinomycetes in Chenghai Lake sediment samples

Season	Plot	10^2 CFU/g of dry sediment (%)														
		Micromonospora					*Streptomyces*	*Actinomadura*	*Thermopolyspora*	*Microtetraspora*	*Saccharomonospora*	*Promicromonospora*	*Nocardia*	*Rhodococcus*	Unidentified	Total
		1	2	3	4	Subtotal										
Dry	A	79.4	59.7	22.0	3.1	182.2	6.3	9.4					3.1			201.0
	B	121.6	56.5	3.4	1.7	183.2	90.7	3.4	1.7			3.4	1.7	5.1	5.1	294.3
	C	315.5	153.2	49.5		518.0	22.5							9.0		549.5
	D	205.1	179.5	4.3	4.3	392.2	12.8									405.0
	E	363.6	272.7	17.7	5.1	659.1	32.8			13.0	2.5		15.2	15.2	2.5	740.3
Mean		220.6 (50.7)	144.3 (33.1)	19.7 (4.5)	2.8 (0.6)	386.9 (88.3)	33.0 (7.3)	2.6 (0.6)	0.3 (0.07)	2.6 (0.6)	0.5 (0.1)	0.7 (0.2)	4.0 (0.9)	5.9 (1.4)	1.5 (0.3)	438.0 (100)
Rainy	A	284.3	107.8	4.9	4.9	401.9	34.3									436.2
	B	166.4	93.0	4.9	2.4	266.7	41.6							110.0		418.3
	C	254.3	120.8	4.2		379.3	162.5						58.3	8.4	4.2	612.7
	D	257.6	113.6	7.5		378.7	41.7									420.4
	E	335.1	130.7	4.7		471.5	233.4		9.3							714.2
Mean		259.7 (49.9)	113.2 (21.8)	5.2 (1.0)	1.5 (0.3)	379.6 (72.9)	102.7 (19.7)		1.9 (0.4)				11.7 (2.2)	23.7 (4.6)	0.8 (0.2)	520.4 (100)

TABLE 5. Some bioactivities of actinomycetes

Bioactivity	No. of strains	
	Tested	Active
Enzyme activity		
Cellulase	258	83
Chitinase	258	219
Firin olysin	1,561	352
Mannase	908	108
Rennet	908	242
Protease	246	90
Lysozyme	1,508	27
Activity against:		
B. subtilis	258	6
A. niger	1,741	118

actinomycetes of cell wall type II showed some similarities with members of the genus *Nocardiopsis*. However, a suitable taxon appears to exist for our wall chemotype II streptomycetes from Chenghai Lake. Twelve other *Streptomyces* strains with optimal growth at pH 7.0 and 14 thermophilic *Streptomyces* strains from Chenghai and Fuxian appeared with the more typical combination of glycine and L-DAP (wall chemotype I). The cell wall compositions of streptomycetes probably change in an alkaline environment.

Some biological characteristics of aquatic actinomycetes. The hydrolytic enzyme activities of isolated strains were examined, and the results are shown in Table 5. Several high-yield producers of chitinase and fibrinolysin with potential for future applications were found. The resistances to toxic substances and antimicrobial activities of aquatic actinomycetes from various genera were examined. Only 6 of 258 strains showed activities against *Bacillus subtilis*, and 118 of 1,741 strains showed activities against *Aspergillus niger*. We found that 77% of the strains tested grew well in broth containing 5 ppm of phenol, and 11 strains even grew well in the presence of 3,000 ppm of phenol. Some (51%) strains grew in 5,000 ppm of hydrofluoric acid, and 69% of strains grew well in mercurous chloride. These results indicate that actinomycetes in the lakes of Yunnan have high levels of resistance to toxic substances; they might play an important part in the ecological balance of lake aquatic systems.

Conclusions. From these observations, the following features of the aquatic actinomycetes of Yunnan Middle Plateau lakes can be described. (i) The number and generic diversity of actinomycetes in each of these 12 lakes related directly to the physical, chemical, and biological features of the lakes examined. Actinomycetes were fewer in plots of poor nutrient content, sand sediment and deep water than in regular lake environments. The numbers of actinomycetes isolated from occasionally dry lakes, such as Jilu, Yilong, and Datun, were 3- to 10-fold higher than those isolated from the other lakes. Nine genera of actinomycetes were isolated from Chenghai Lake, an alkaline lake. In all, 16 genera of actinomycetes were isolated from these 12 lakes. On the basis of the number and generic diversity of isolates, we divided the actinomycete populations of these 12 lakes into four groups with different similarity levels. (ii) *Micromonospora* strains were notably dominant (39 to 89%) in the sediment samples of all 12 lakes. This is characteristic of the aquatic actinomycete population in lakes compared with the terrestrial actinomycete population (23). Streptomycetes were the second most abundant organisms, with an average level of 6 to 60%. Some of them probably came from

land washes, and the numbers varied with the season. The numbers of all other genera in these lakes were small. (iii) The cell wall compositions of *Micromonospora* and *Streptomyces* strains from alkaline Chenghai Lake revealed an unusual combination of glycine and isomers of DAP. This was also found in other actinomycetes from alkaline environments. (iv) Actinomycetes might play a significant role in the decomposition of chitin, cellulose, protein, and some toxic substances present in these lakes. (v) Aquatic actinomycetes found in this research might have potential for fibrinolysin and chitinase production.

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