



Full Length Article

Evaluation of Antagonistic Activity of Actinobacteria in Saxicolous Lichens

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Abstract

Actinobacteria are important producers of novel bioactive compounds. New sources need to be explored for isolating previously unknown bioactive compound-producing actinobacteria. We evaluated the potential of saxicolous lichens as a natural source of novel bioactive actinobacterial species. Saxicolous lichen samples were collected from three climatic belts at different elevations (1600–3400 m) on Qin Mountain, Shaanxi Province, China. Actinobacteria were cultivated, enumerated, and isolated using serial dilution and spread-plate techniques under normal nutrient and oligotrophic conditions. Antimicrobial activity of actinobacterial isolates was analyzed using an agar block method against fifteen typical bacterial and fungal species and plant pathogens. The dominant isolates and isolates with broad-spectrum antagonistic activity were identified by 16S rRNA-based sequence analysis. Results showed that colony counts, total number of species, and the number of bioactive species of actinobacteria in saxicolous lichens were higher in elevation <3000 m than in elevation >3000 m. The dominant isolates were classified into 42 species of 10 genera. Antagonistic activities were detected in approximately 66% of the actinobacteria isolates. Of these, 42 isolates (28%) showed broad-spectrum antagonistic activity against ≥ 5 microorganisms tested. In conclusion, the saxicolous lichen possesses a high diversity of actinobacteria and serves as a natural source of bioactive compound-producing actinobacteria. © 2016 Friends Science Publishers

Keywords: 16S rRNA; Gause's synthetic agar; Modified humid acid agar; Oligotrophic media; Qin mountain

Introduction

Actinobacteria are the main source of antibiotics which have produced 100–120 out of 150 clinical and agricultural antibiotics (Bérdy, 2005). Isolation of novel bioactive compound-producing actinobacteria is a fundamental work for research and development of new drugs. Actinobacteria are widely distributed in natural ecosystems. Extensive studies have explored soils, sediments and water bodies from which numerous bioactive actinobacterial species were isolated. Representative genera are *Streptomyces* and *Micromonospora* (Bérdy, 2005). In recent decades, it has become increasingly difficult in finding novel bioactive actinobacteria in the above-mentioned natural ecosystems. Exploring new sources is urgent. In this context, alternative ecosystems have attracted substantial attention of researchers for actinobacteria screening, including extreme environments (Okoro *et al.*, 2009), animal feces (Jiang *et al.*, 2013), beehives (Promnuan *et al.*, 2009), wasp and swallow mud nest (Kumar *et al.*, 2012), termite guts (Watanabe *et al.*, 2003), the internal and external environments of plants (Verma *et al.*, 2009) and microbial symbioses (Suzuki *et al.*, 2016).

It is hypothesized that microbial bioactive compounds

might be involved in microbe-microbe and microbe-host communication (Yim *et al.*, 2007; Yoon and Nodwell, 2014). Lichens are symbiotic associations consisting of fungi and photosynthetic partners like green algae and cyanobacteria. They are abundant as epiphytes on plant, bare rock and exposed soil surfaces in various environments including some of the most extreme environments on Earth, such as high mountains, hot deserts and arctic tundra. Saxicolous lichens could adapt more harsh conditions than those colonizing on other media because bare rock surface is extraordinarily oligotrophic, dry in most time of the year and irradiated strongly by ultraviolet. Although a number of individual bioactive isolates or species in lichens have been described (Singh *et al.*, 1997; Davies *et al.*, 2005; Motohashi *et al.*, 2010; Brana *et al.*, 2015), little is known about the diversity of the microbial community especially actinobacteria inhabiting saxicolous lichens. Recently, González *et al.* (2005) have isolated a wide diversity of actinobacteria from saxicolous and arboricolous lichens, of which many isolates possessed biosynthetic genes. Knowledge remains lacking regarding diversity and antagonistic activities against plant pathogenic microorganisms of actinobacteria in saxicolous lichens at different elevations.

To this end, the present study investigated actinobacterial populations residing in saxicolous lichens at different elevations under normal and oligotrophic conditions. We evaluated the potential for isolating novel bioactive compound-producing actinobacteria from the special ecosystem of saxicolous lichens by colony counts and species numbers of actinobacteria, and evaluation of their antagonistic activity followed by identification of dominant and valued actinobacterial species.

Materials and Methods

Bark Sampling

This study was carried out in the north Qin Mountain (33°57'–34°58'N, 107°45'–107°53'E), Shaanxi Province, China. Ten saxicolous lichen samples were collected from 3 climatic belts: 5 from alpine cold temperate zone (elevations of 1500–3000 m), 3 from alpine subfrigid zone (elevations of 3000–3350 m) and 2 from alpine frigid zone (elevations above 3350 m). The samples were collected with sterile blades, individually sealed in sterile polyethylene bags, transported to the laboratory within 7 h, and stored in the dark at 4°C until use.

Actinobacteria Isolation

Saxicolous lichen samples were grinded using sterilized pestles and mortars. Serial dilution and spread-plate techniques (Williams and Davies, 1965) were used to isolate actinobacteria. Serial dilutions were prepared by adding the 3.0 g of grinded lichen to 27.0 mL of sterile distilled water (10^{-1}) in a conical flask, followed by oscillation at 160 rpm for 10 min and further dilution to 10^{-3} . The dilutions (0.05 mL aliquots) were inoculated to the agar media by spread plating. Four agar media were tested: Gause's synthetic agar (soluble starch 20.0 g; KNO_3 1.0 g; K_2HPO_4 0.5 g; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.5 g; NaCl 0.5 g; FeSO_4 0.01 g; agar 10.0 g; distilled water 1000 mL), modified humic acid agar (humic acid 10.0 g; Na_2HPO_4 0.5 g; KCl 1.0 g; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.05 g; CaCl_2 1.0 g; agar 10.0 g; distilled water: 1000 mL), oligotrophic Gause's synthetic agar (Gause's synthetic agar at one fifty the recommended concentration) and water agar (agar 10.0 g; distilled water: 1000 mL). All media were supplemented with 80 mg/L potassium dichromate to inhibit the growth of bacteria and fungi. After inoculation, all plates were incubated at 28°C for 15 days. Actinobacterial colonies were identified by visual examination of the cultural and morphological characteristics; microscopic examination was performed if needed. Morphologically distinct colonies were transferred onto Gause's synthetic agar slants separately, incubated at 28°C for 7 days, and then stored in the dark at 4°C.

All experiments were performed in triplicate. The average number of actinobacteria colonies on each plate was

counted. Data are reported as colony-forming-unit (CFU)/g stove-dry lichen. The colony numbers were compared between different elevations by t-test in SAS 9.0 statistical software (SAS Institute Inc., Cary, NC, USA).

Antimicrobial Activity Assay

Antimicrobial activity of actinobacteria isolates was analyzed using an agar block method against four bacterial species and eleven fungal species provided by the Microbiology Laboratory in Shanxi Normal University. The bacterial species included *Escherichia coli* E1, *Staphylococcus aureus* S4, and two pathogens of konjac soft rot, *Serratia* sp. H1 and *Dickeya dadantii* subsp. *Dadantii* D3; the fungal species included *Penicillium* sp. P1, *Candida tropicalis* C1, and nine plant pathogens, *Verticillium dahliae* V2, *Fusarium oxysporum* FO1, *F. solani* (Mart.) Sacc FSS1, *F. sulphureum* FS1, *F. oxysporum* f. sp. *cucumerinum* FOC1, *F. oxysporum* f. sp. *niveum* FON1, *F. solani* FS3, *F. oxysporum* f. sp. *vasinfectum* FOV1, and *Didymella bryoniae* DB1.

Antagonistic Potentiality Assay of Lichen Actinobacteria

Antagonistic potentiality of lichen actinobacteria (APLA) was calculated by considering the number and antimicrobial spectrum of actinobacterial isolates in the lichen ecosystem using the following equation (Zhu *et al.*, 2011):

$$APLA(\%) = \frac{\sum_1^n T_n}{\sum_1^m \sum_1^n T_n} \times 100$$

Where m and n are the numbers of tested lichen samples and actinobacterial isolates with antagonistic activity, respectively; T_n is the number of target microorganisms to which the actinobacterial isolate is antagonistic.

Identification of Actinobacteria Isolates

The dominant actinobacteria isolates and isolates with broad-spectrum antagonistic activity were identified by 16S rRNA-based sequence analysis. Actinobacteria DNA was extracted from pure isolates using the method described by Saito and Miura (1963). Partial 16S rRNA gene fragments were amplified by polymerase chain reaction (PCR) using the bacterial primers 27F: 5'-AGAGTTTGATCCTGGCTCAG-3' and 1541R: 5'-AAGGAGGTGATCCAGCCGCA-3'. Amplification was carried out in a DNA Engine thermal cycler (BIO-RAD, USA), using a 50 μL reaction mixture containing 4 μL Taq DNA polymerase (2.5 U/ μL , Genscript, Nanjing), 5 μL 10 \times buffer (Transgene, Beijing), 1 μL 20 mM deoxynucleoside triphosphate (Transgene), 37 μL of sterile distilled water, 1 μL of each primer (50 μM), and 1 μL of template. The PCR thermo cycling conditions were as follows: initial denaturation at 94°C for 4 min;

30 cycles at 94°C for 1 min, 56°C for 1 min, 72°C for 2 min; and a final elongation at 72°C for 10 min. PCR reactions were purified and sequenced by Genscript Biotech (Nanjing) Co., Ltd, China. The obtained sequences were compared with available reference sequences in the EMBL/GenBank/DDBJ databases and deposited in Genbank under the accession Nos. KF554146-KF554243.

Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 4.0 (Tamura *et al.*, 2007). The 16S rRNA sequences of obtained in this study were aligned using CLUSTAL W (Thompson *et al.*, 1994) program against the corresponding reference nucleotide sequences retrieved from Genbank database. Tree topologies were evaluated by bootstrap analysis based on 1,000 resampling of the neighbor-joining dataset.

Results

Colony Counts and Species Number of Actinobacteria under Normal Nutrient Condition

A wide diversity of actinobacteria was found in saxicolous lichens. Under normal nutrient condition, actinobacteria counts and species numbers were higher in saxicolous lichens from elevation <3000 m, compared to saxicolous lichens from higher elevations. Actinobacteria colony counts and species number were different between G and H media (Table 1).

On Gause's synthetic agar, colony count of actinobacteria in lichen sample from elevation of 1600 m was significantly more than those of other elevations ($P<0.05$). Colony count of actinobacteria in lichen sample from elevation of 3424 m was least in all elevations. Total number of actinobacteria species in lichen samples was 18 in sample from elevation of 1600 m vs. 4 in samples from elevations of 3331 m and 3424 m (Table 1).

On modified humic acid agar, colony count of actinobacteria in lichen sample from elevation of 2614 m was significantly more than those of other elevations ($P<0.05$). Colony count of actinobacteria in lichen sample from elevation of 3491 m was least in all elevations. Total number of actinobacteria species in lichen samples was 12 in sample from elevation of 2252 m vs. 3 in sample from elevation of 3491 m (Table 1).

For actinobacteria from different elevations, the utilization of two tested carbon compounds was different. Compared to humic acid-utilizing actinobacteria, colony counts of starch-utilizing actinobacteria were significantly more in saxicolous lichens from elevations of 1600 m, 3003 m and 3165 m, while significantly less in samples from elevations of 1917-2823 m ($P<0.05$). There was no significant difference in samples from elevations of 3331-3491 m ($P>0.05$). The species numbers of starch-utilizing actinobacteria were more in 7 out of 10 samples, compared to humic acid-utilizing actinobacteria (Table 1).

Colony Counts and Species Number of Actinobacteria under Oligotrophic Condition

Actinobacteria recovered on oligotrophic Gause's synthetic agar were oligotrophy-tolerant species. On oligotrophic Gause's synthetic agar, colony count of actinobacteria in saxicolous lichen from elevation of 2614 m was more than those of other elevations ($P<0.05$). Colony count of actinobacteria in sample from elevation of 3491 m was less than those of other elevations. The differences reached significant ($P<0.05$) compared to 5 samples. Among all elevations, numbers of actinobacteria species were highest in saxicolous lichens from elevations of 2252 m and 3165 m and lowest in sample from elevation of 3491 m (Table 1).

We defined actinobacteria recovered on water agar as extreme oligotrophy-tolerant species for water agar was more oligotrophic than oligotrophic Gause's synthetic agar. On water agar, colony count of actinobacteria was highest in saxicolous lichen from elevation of 1917 m ($P<0.05$) and lowest in sample from elevation of 3424 m among all elevations. Among all elevations, numbers of actinobacteria species were highest in samples from elevations of 1917 m, 2252 m and 3165 m and lowest in sample from elevation of 3491 m (Table 1).

Colony counts and species numbers of oligotrophy-tolerant actinobacteria and extreme oligotrophy-tolerant species in saxicolous lichens were different. Compared to extreme oligotrophy-tolerant actinobacteria, colony counts of oligotrophy-tolerant species were more in elevations of 1600-3165 m ($P<0.05$ for 1600 m and 2823-3165 m) and less in elevations of 3331-3491 m ($P<0.05$). These results indicated that the oligotrophy-tolerant degree of actinobacteria increased with elevations. Generally, species numbers of oligotrophy-tolerant actinobacteria were more than extreme oligotrophy-tolerant species (Table 1).

Colony counts and species numbers of actinobacteria in saxicolous lichens were different under different nutrient conditions. Compared to normal nutrient condition, colony counts of actinobacteria were less in lichens from elevations of 1600-2614 m and more in lichens from elevations of 2823-3165 m under oligotrophic condition ($P<0.05$) (Table 1).

Dominant Actinobacteria Species

A total of 223 actinobacteria isolates were obtained from the saxicolous lichen samples on four media. The dominant isolates were classified into 42 species of 10 genera: *Streptomyces* spp. (64.3%), *Pseudonocardia* spp. (9.5%), *Micromonospora* spp. (7.1%), *Nocardopsis* spp. (4.8%), *Actinoplanes* spp. (2.4%), *Kribbella* spp. (2.4%), *Rhodococcus* spp. (2.4%), *Nocardia* spp. (2.4%), *Arthrobacter* spp. (2.4%) and *Umezawaea* spp. (2.4%) (Table 2).

Table 1: Colony counts (10^3 CFU/g dry lichen) and species numbers of actinobacteria isolates in saxicolous lichens from different elevations

Elevations (m)	Media ^a				Species numbers			
	G	H	OG	W	G	H	OG	W
3491	0.6±0.1e(b) ^b	0.4±0.2e(b)	0.22±0.0d(b)	0.9±0.2c(a)	5	3	5	2
3424	0.4±0.1e(a)	0.8±0.4e(a)	0.4±0.2d(a)	0.8±0.3c(a)	4	5	6	6
3331	0.5±0.1e(b)	0.44±0.2e(b)	1.5±0.3d(b)	2.9±1.1c(a)	4	4	6	5
3165	6.7±2.4e(b)	1.5±0.2e(c)	10.7±2.7d(a)	6.3±0.8c(b)	10	7	14	10
3003	45.8±2.2e(b)	8.3±0.9e(d)	60.6±7.1d(a)	21.9±4.1c(c)	11	5	11	9
2823	119.3±16.7d(c)	222.8±32.9de(b)	381.7±42.4ab(a)	204.1±15.9b(b)	8	5	12	6
2614	305.3±27.2c(b)	890.1±356.6a(a)	457.6±12.2a(b)	170.4±17.4b(b)	11	7	11	7
2252	481.1±93.7b(b)	593.4±21.1bc(a)	178.7±40.3c(c)	102.4±30.9bc(c)	11	12	14	10
1917	460.3±9.9b(b)	788.9±109.3ab(a)	395.2±119.5ab(b)	340.9±197.3a(b)	6	10	10	10
1600	571.1±82.2a(a)	400.6±91.0cd(b)	374.4±49.4b(b)	182.1±26.4b(c)	18	7	11	9

^aG, Gause's synthetic agar; H, modified humic acid agar; OG, oligotrophic Gause's synthetic agar; W, water agar

^bDifferent letters out of (in) the brackets in the same column (line) refer to significant difference at $P<0.05$ by t-test

Table 2: Taxonomic distribution and origin of dominant actinobacteria species

Family	Genus	Species	Isolates	Nutrient conditions		Climatic belts ^a		
				Normal	Oligotrophic	ACTZ	ASZ	AFZ
<i>Streptomycetaceae</i>	<i>Streptomyces</i>	27	75	17	17	18	15	5
<i>Micromonosporaceae</i>	<i>Micromonospora</i>	3	4	1	2	0	0	3
	<i>Actinoplanes</i>	1	1	0	1	1	0	0
<i>Nocardiaceae</i>	<i>Nocardia</i>	1	1	0	1	1	0	0
	<i>Rhodococcus</i>	1	1	0	1	0	0	1
<i>Nocardiopsaceae</i>	<i>Nocardiopsis</i>	2	2	2	0	0	1	1
<i>Pseudonocardiaceae</i>	<i>Pseudonocardia</i>	4	4	1	3	1	0	3
	<i>Umezawaea</i>	1	4	1	0	1	0	0
<i>Nocardioidaceae</i>	<i>Kribbella</i>	1	5	1	1	1	1	0
<i>Micrococcaceae</i>	<i>Arthrobacter</i>	1	1	1	1	1	0	0
Total: 7	10	42	98	24	27	24	17	13

^aACTZ, alpine cold temperate zone; ASZ, alpine subfrigid zone; AFZ, alpine frigid zone

Antagonistic Activity of Actinobacteria Isolates

A total of 148 out of 223 bark actinobacteria isolates were found antagonistic to at least one of 15 microorganisms tested. The number of antagonistic isolates decreased with increasing elevation. The total number of antagonistic isolates on Gause's synthetic agar was highest in four media. The ratio of antagonistic isolates in samples from elevation of 2252 m was higher than those of other elevations, while in samples from elevation 3424 m was lowest. Among four media, ratio of antagonistic isolates was highest on water agar and lowest on modified humic acid agar (Table 3).

Antagonistic Potentiality

The APLA substantially varied in samples from different elevations. APLA in sample from 1917 m was higher than those of other elevations. APLA in sample from 3491 m was lower than those of other elevations. APLA recovered on Gause's synthetic agar was 18.8% - 21.0% higher than those on other media.

Distribution of Antagonistic Lichen Actinobacteria

Numbers of isolates antagonistic to each target

microorganism differed in lichen samples from different elevations. Generally, the numbers of antagonistic isolates were higher in samples from lower elevations. Specifically, the numbers of isolates antagonistic to five target microorganisms, *S. aureus* (G⁺), *E. coli* (G⁻), *C. tropicalis*, *F. solani* and *F. oxysporum* f. sp. *vasinfectum*, in sample from elevation of 1917 m were higher than those of other elevations. The numbers of isolates antagonistic to five target microorganisms, *Penicillium* sp., *Fusarium oxysporum*, *F. solani* (Mart.) Sacc, *F. sulphureum* and *Didymella bryoniae*, in sample from elevation of 1600 m were higher than those of other elevations. The numbers of isolates antagonistic to *Dickeya dadantii* subsp. *Dadantii* in samples from elevation of 1917 m and 2823 m were higher than those of other elevations. The numbers of isolates antagonistic to *F. oxysporum* f.sp. *cucumerinum* in samples from elevation of 1600 m and 2252 m were higher than those of other elevations. The number of isolates antagonistic to *F. oxysporum* f. sp. *niveum* in sample from elevation of 2252 m was higher than those of other elevations. The number of isolates antagonistic to *Serratia* sp. in sample from elevation of 3003 m was higher than those of other elevations. The number of isolates antagonistic to *V. dahliae* in sample from elevation of 3165 m was higher than those of other elevations (Table 4).

Table 3: Numbers and ratios of antagonistic isolates in saxicolous lichens

Elevations (m)	Gause's synthetic agar		Humic acid agar		Oligotrophic Gause's synthetic agar		Water agar		Total	
	Numbers	Ratios %	Numbers	Ratios %	Numbers	Ratios %	Numbers	Ratios %	Numbers	Ratios %
3491	1	50.0	2	66.7	2	66.7	1	100.0	6	66.7
3424	2	50.0	3	60.0	2	50.0	2	50.0	9	52.9
3331	2	66.7	1	50.0	1	33.3	3	100.0	7	63.6
3165	3	50.0	4	80.0	4	44.4	4	80.0	15	60.0
3003	5	83.3	4	80.0	5	71.4	3	75.0	17	77.3
2823	4	50.0	1	25.0	2	50.0	5	100.0	12	57.1
2614	5	83.3	2	33.3	3	42.9	3	60.0	13	54.2
2252	6	75.0	6	75.0	7	100.0	4	80.0	23	82.1
1917	4	66.7	7	77.8	6	85.7	6	85.7	23	79.3
1600	13	72.2	2	28.6	4	66.7	4	66.7	23	62.2
Total	45	67.2	32	59.3	36	63.2	35	77.8	148	66.4

Table 4: Numbers of isolates which antagonistic to different microorganisms

Elevations samples (m)	Target microorganisms ^a															Total
	Typical bacteria or fungi						Plant pathogens									
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
3491	5	2	0	0	1	0	2	0	1	2	1	1	0	0	0	8
3424	5	5	1	0	4	1	4	0	0	1	0	1	0	2	2	10
3331	4	3	0	0	3	0	2	0	1	1	0	0	0	0	0	6
3165	9	11	3	2	2	0	9	0	4	2	1	2	0	0	1	11
3003	11	8	3	2	8	1	6	1	3	3	1	2	1	0	5	14
2823	9	7	2	2	3	3	2	0	4	3	0	2	0	0	5	11
2614	6	9	1	2	2	0	2	0	2	3	0	2	0	1	0	10
2252	13	15	6	6	5	0	8	3	8	7	4	6	5	2	7	14
1917	20	17	4	7	5	3	5	2	6	7	1	5	6	5	6	15
1600	18	13	8	6	7	1	8	4	9	13	4	5	3	2	10	15
Total	100	90	28	27	40	9	48	10	38	42	12	26	15	12	36	15

^aTarget microorganisms: 1. *Staphylococcus aureus* S4, 2. *Escherichia coli* E1, 3. *Penicillium* sp. P1, 4. *Candida tropicalis* C1, 5. *Serratia* sp. H1, 6. *Dickeya dadantii* subsp. *Dadantii* D3, 7. *Verticillium dahliae* V2, 8. *Fusarium oxysporum* FO1, 9. *F. solani*(Mart.)Sacc FSS1, 10. *F. sulphureum* FS1, 11. *F. oxysporum* f.sp. *Cucumerinum* FOC1, 12. *F. oxysporum* f. sp. *Niveum* FON1, 13. *F. solani* FS3, 14. *F. oxysporum* f.sp. *Vasinfestum* FOV1, 15. *Didymella bryoniae* DB1

Moreover, the numbers of isolates antagonistic to different target microorganisms varied in each lichen sample. For samples from elevations 1600 m, 1917 m, 2823 m, 3003 m, 3331 m and 3491 m, numbers of isolates antagonistic to *S. aureus* (G⁺) were more than those of other target microorganisms. For samples from elevations 2252 m, 2614 m and 3165 m, numbers of isolates antagonistic to *E. coli* (G⁻) were more than those of other target microorganisms. For sample from elevation 3424 m, numbers of isolates antagonistic to *S. aureus* (G⁺) and *E. coli* (G⁻) were more than those of other target microorganisms (Table 4).

Additionally, total antagonistic spectra of lichen actinobacteria isolates substantially varied in different elevations. Total antagonistic spectra was 15 target microorganisms for samples from elevations 1600 m and 1917 m versus 6 target microorganisms for samples from elevations 3331 m (Table 4).

Identification of 13 Broad-spectrum Antagonistic Isolates

The phylogenies of 13 broad-spectrum antagonistic isolates were shown in Fig. 1. According to the phylogenetic characteristics, these 13 isolates were identified as *Str. griseorubiginosus* (three strains), *Str. avidinii* (three strains),

Str. cirratus (two strains), *Str. vinaceusdrappus*, *Str. sporoverrucosus*, *Str. rishiriensis*, *Str. Scopuliridis*, and *Arthrobacter nitroguajacolicus*, respectively.

Discussion

The present study was focused on the diversity and antagonistic activities of actinobacteria in saxicolous lichens from three climatic belts in the north Qin Mountain. Our results showed that a large number of actinobacteria were residing in saxicolous lichens. There were 42 dominant actinobacteria species belonging to 10 genera, in which *Nocardioopsis*, *Kribbella*, *Nocardia*, *Arthrobacter* and *Umezawaea* were not reported in previous research (González *et al.*, 2005). Among the 10 genera, *Strptomyces* were distributed in all of the three climatic belts and the species numbers were more than other genera. In addition, *S. cirratus* and *S. avidinii* were distributed widely in different elevations.

Plenty of oligotrophic-tolerant actinobacteria were undoubtedly residing in saxicolous lichens, because the rock surface is oligotrophic. Using oligotrophic media to isolate these species is helpful for the research of actinobacteria diversity in saxicolous lichens. However, the previous study of saxicolous lichens only used media with normal nutrient

condition, which was difficult to evaluate oligotrophic actinobacteria species. In this study, we got a large number of oligotrophic actinobacteria using two oligotrophic media. The dominant species belonging to 8 genera: *Streptomyces* spp., *Pseudonocardia* spp., *Micromonospora* spp., *Actinoplanes* spp., *Kribbella* spp., *Rhodococcus* spp., *Nocardia* spp. and *Umezawaea* spp. Three genera, *Actinoplanes* spp., *Nocardia* spp. and *Umezawaea* spp., were unique to oligotrophic media. Compared to actinobacteria recovered under normal nutrient condition, colony counts of oligotrophy-tolerant actinobacteria was less in samples from alpine cold temperate zone, while more in samples from alpine subfrigid zone and alpine frigid zone. Oligotrophy-tolerant degree of actinobacteria in saxicolous lichens was increased with elevations.

Another important finding of the present study was that a large number of bioactive compound-producing actinobacteria were obtained from the saxicolous lichens tested. To our knowledge, our work provides the first evidence regarding the antagonistic activity of *Str. avidinii*, *Str. malachitospinus*, *Str. stramineus*, *Str. tauricus*, *Str. laculatispora*, *Nocardiopsis listeri*, *Nocardiopsis dassonvillei* subsp. *Albirubida*, *Actinoplanes regularis*, *K. Endophytica* and *U. Tangerina* (Fig. 2). These potentially valuable strains provide alternative resources for research and development of new antibiotics.

Saxicolous lichen is a special ecosystem, in which the knowledge regarding microbial diversity is less. For the research of diversity and bioactivity of actinobacteria, only one previous report touched upon saxicolous lichen (González *et al.*, 2005). They isolated 6 genera of actinobacteria in saxicolous lichens from torrid zone and frigid zone, in which 5 were same as our study (exclude *Actinomadura*). In their report, the antimicrobial activity of the actinobacteria isolates was evaluated against 3 microorganisms, *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*. Filamentous fungi and plant pathogens were not tested. In the present study, we tested the antagonistic activities of actinobacteria isolates using 15 target microorganisms, including 4 typical microbes which could represent for all microorganisms in the world and 11 plant pathogens, which could guide the exploring of new agricultural antibiotics and evaluate of antagonistic actinobacteria resources in saxicolous lichens more systematically.

In conclusion, our research provide new insights on ecological distribution of actinobacteria in saxicolous lichens at different elevations and comprehensively evaluated the antagonistic actinobacteria resources in saxicolous lichens. Saxicolous lichen is an important source of bioactive compound-producing actinobacteria, which should receive more attention in research and development of new antibiotics and anti-tumor agents.

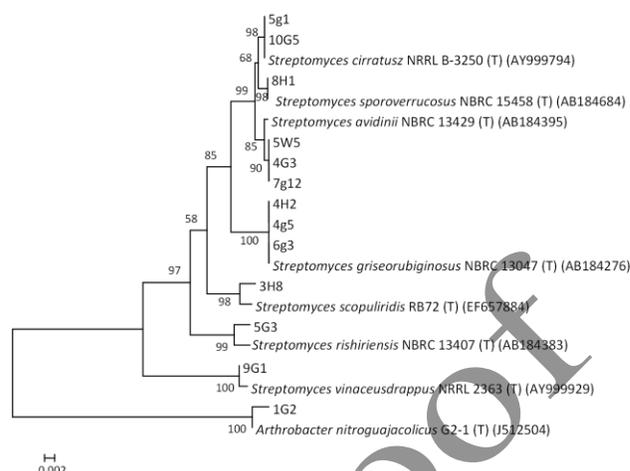


Fig. 1: Phylogenetic tree of the 13 broad-spectrum antagonistic isolates

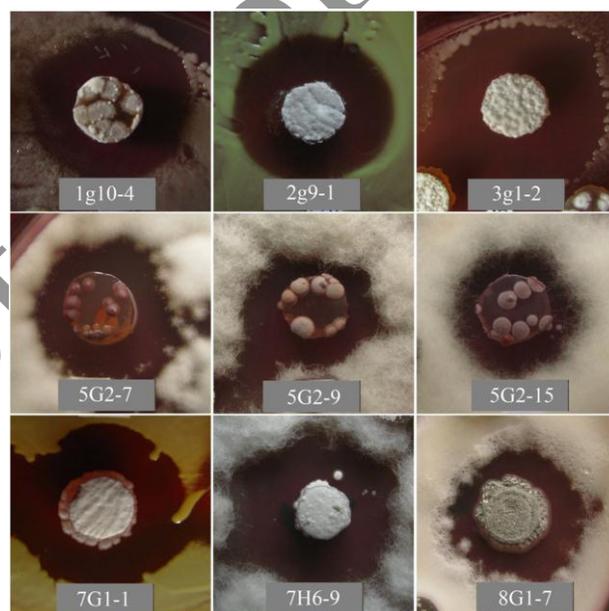


Fig. 2: Antagonistic activity of representative species unreported before. Letters before the “-” represent for the names of isolates: 1g10, *Umezawaea tangerina*; 2g9, *Streptomyces laculatispora*; 3g1, *Streptomyces stramineus*; 5G2, *Streptomyces tauricus*; 7G1, *Streptomyces spororaveus*; 7H6, *Nocardiopsis dassonvillei* subsp. *Albirubida*; 8G1, *Streptomyces malachitospinus*. Numbers behind the “-” represent for target microorganisms: 1. *Staphylococcus aureus* S4, 2. *Escherichia coli* E1, 4. *Candida tropicalis* C1, 7. *Verticillium dahliae* V2, 9. *F. solani* (Mart.) Sacc FSS1, 15. *Didymella bryoniae* DB1

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