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Study of Microbial Distribution in the Arid Desert Terrain, Beishan Mountains Area, Gansu

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In order to explore the microbial flora characteristics of rhizosphere soils in Gansu Beishan mountains area, rhizosphere soils alongside of typical plants were selected respectively from dense and sparse vegetation zones as materials. The quantity and distribution of three groups of microorganism were investigated by dilution method of plate counting. Additionally, 16S rDNA sequence analysis was used to identify the dominant bacteria. The results showed that microorganism quantity of arid soils in Beishan was similar to normal environment. Microbial number of non-rhizosphere soil in dense vegetation zone (49.2×10^5 CFU/g) was more than that in sparse vegetation zone (35.5×10^5 CFU/g). The total population of microorganism were bacteria> actinomycetes> fungi. Furthermore, both volume and range of microorganism were decreased with the increasing soil depth in the same area. The microbial number of dense vegetation zone exhibited that *Salsola abrotanoides*>*Asterothamnus centrali-asiaticus*> *Nitraria sphaerocarpa*> *Ephedra przewalskii*, while the microbial number of sparse vegetation zone exhibited that *Salsola abuscula*>*Sympegma regelii*. The change in the number might be related to the plant root exudates. In detail, *Salsola abrotanoides* and *Asterothamnus centrali-asiaticus* could promote the growth of fungi and improve the soil alkaline condition. Whereas, the root exudates of *Salsola abuscula* and *Sympegma regelii* respectively existed mainly at the depth of 20~50 cm and 10~20 cm, could inhibit the growth of soil microorganism. By the analysis of 16S rDNA sequences, eight dominant strains were respectively identified as *Kocuria Polaris*, *Bacillus niacin*, *Arthrobacter crystallopoietes*, *Paenibacillus tarimensis*, *Nocardiopsis lucentensis*, *Arthrobacter agilis*, *Bacillus idriensis,* and *Promicromonospora kroppenstedtii*.

Key words: Beishan mountains, Desert plants, Microbial flora, 16S rDNA sequence analysis.

In Beishan mountains area, the climate is extremely dry, the terrain relatively flat, and the hydrographic net is not well developed. Annual precipitation is less than 100 mm for whole region, less than 50 mm for heart land, and even no rainfall throughout the year for some districts (RenTing X, 2006). Geomorphology is mainly made up of massifs, deserts and micro-basins. Mountain bedrock is exposed or semi-naked, mostly covered by coarse debris, with a small part of fine aeolian deposits or sands. Greenery in this area is rare and mainly comprised of *Nitraria sphaerocarpa*, *Ephedra przewalskii*, *Salsola abrotanoides*, *Asterothamnus centrali-asiaticus*, *Kalidium caspicum*, *Stipa caucasica subsp. glareosa*, *Salsola abuscula*, *Sympegma regelii*, and *Zygophyllum xanthoxylon*.

Soil microbes are the most active components in terrestrial ecosystem. Their duty is

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to decompose the residual body of plants and animals, to promote the circulation of materials and energy. Rhizosphere is a special habitat for soil microorganism, the structure of microbial community there is special too, especially in an extreme arid environment (YuHai Y *et al*., 2010). There is a relationship of interdependence and mutual restriction between rhizosphere microorganism and host plants. And this would lead to different microbial floras for different rhizospheres.

Exploring the change of rhizosphere soil microbial number and community structure has a prime importance for revealing the relations among plants, soil and microorganism. At present, many improvements on the behavior of arid environments, only take soil moisture, pH and vegetation into account, without the consideration of microbes. The research about microbial population structures in arid deserts, the dominant strains, and the behaviors of microorganism for the work of soil improvements is rare. Moreover, Beishan is a preselected area of high-level radioactive waste repository. Investigating the microbial flora there can provide certain theoretical bases for the inspection of biological effects on high-level radioactive waste repository (Xiaoming C *et al*., 2013). The former researches focused currently on geology, headwaters, rainfall, etc., while the aspects related to microbes are still blank. Therefore, carrying out work in this field has an important significance.

Research sample collection

Samples were collected from Beishan mountains area, Gansu (Longitude $97^{\circ}36^{2}\,9^{3}$ and Latitude $40^{\circ}50^{2}\,32^{3}$) in September 2012.

Air: Samples of air microorganism were gathered using the natural sedimentation plate method. The petri dishes with LB, SC or PDA media were put at the top of slopes, and the lids were opened for 15 minutes.

Non-rhizosphere soils: According to the vegetation cover situation in the test area, non-rhizosphere soils of dense and sparse vegetation zones were selected as materials (Pictures A and B in Fig.1). The plants were uprooted, topsoil was scraped off, and soil samples at the depths of 10 cm, 20 cm and 50 cm were collected from bottom to top (Picture C in Fig.1).

Rhizosphere soils: According to the plant species, rhizosphere soils of *Nitraria sphaerocarpa*, *Ephedra przewalskii*, *Salsola abrotanoides*, *Asterothamnus centrali-asiaticus* from dense vegetation zone, and *Salsola abuscula*, *Sympegma regelii* from spare vegetation zone were selected as materials (Pictures D~I in Fig.1). The soils attached to the root surfaces were collected using the root shaking method (Riley D *et al*., 1969). Soil samples from different sites were collected and individually sealed in sterile plastic bags, immediately taken to the laboratory, and stored at 4°C.

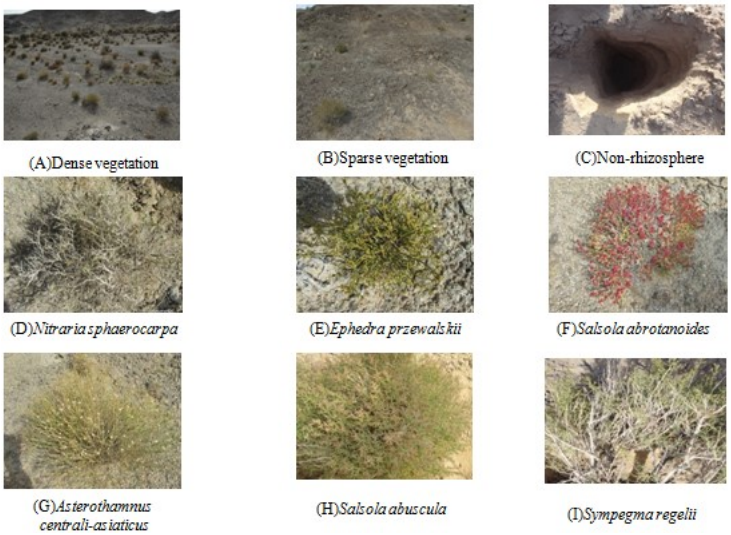

Fig. 1. Photos of soil sample collection

Main reagents and instruments

Media: LB medium (yeast extract, 5 g; peptone, 10 g; NaCl, 10 g; agar, 15 g; deionized water, 1 L; pH was adjusted to 7.2~7.4). SC medium (starch, 10 g; casein, 0.3 g; KNO_3, 2 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; K_2HPO_4, 2 g; $CaCO_3$, 0.02 g; $FeSO_4 \cdot 7H_2O$, 0.01 g; agar, 20 g; deionized water, 1 L; pH was adjusted to 7.0~7.5). PDA medium (glucose, 20 g; agar, 20 g; 20% potato extract, 1 L; natural pH). PCR primers: They were synthesized by Shanghai Sangon Biotech Co., Ltd. (27F:52 - AGAGTTTGATCCTGGCTCAG-32 ; 1492R:52 - GGTTACCTTGTTACGACTT-32). PCR purification kits (TaKaRa, Dalian).

Instruments: Automated spiral plater (AP5000, Spiral Biotech), Refrigerated centrifuge (Eppendof Centrifuge 5415R, Germany), Real-time system (CFX Connect, Bio-RAD), Agarose gel electrophoresis (Power Pac, Bio-RAD), Gel imaging system (Gel Doc XR+, Bio-RAD).

Analysis of soil physical and chemical characteristics

Physical and chemical characteristics of the investigated soils were analyzed according to the standard procedures described in reference (Hafez EE *et al*., 2009). These characteristics included soil pH in 1:5 soil–water suspension, and electrical conductivity (EC) in 1:5 soil–water extract. Soil moisture content was measured using oven drying method (105 °C, 4 h).

Investigation of air microorganism

The airborne microbial samples were cultured for 3 days at 37 °C. The concentrations of airborne microbes were calculated by Omegna formula (Qi L *et al*., 2008): $C = N \times \frac{100}{A} \times \frac{5}{t} \times \frac{1000}{10} = \frac{50000N}{A \cdot t}$. In the formula: C means total number of microorganism in each cubic meter of air (CFU/m^3); N means number of colonies on the plate; A means base area of the plate (cm^2); T means exposure time (min).

Investigation of soil microorganism

Viable aerobic microbes in soil were enumerated by dilution plate count method. Soil (5 g) was poured into an erlenmeyer flask with sterile water (45 mL) and a few glass beads to get dissolved. Then the soil suspension was diluted with fresh sterile water by gradient dilution method, and evenly coated on agar medium (100 µL) with the automated spiral plater. LB plates were inverted in the incubator at 37 °C for 3~5 days. SC and PDA plates were inverted at 28 °C for 10~14 and 5~7 days, respectively. The calculation formula for the number of microorganism: N = A×F×10× (1+M). In this formula: N means number of colony forming units (CFU/g); A means average number of colonies on the plates; F means dilution factor; M means moisture content.

The number of microorganism in three categories was recorded for each sample respectively. Data were expressed as mean ± standard error. To get the microbial flora, the microorganism in same sampling point at different depths and different sampling points were taken to make a comparative analysis. At the same time, the number of colonies for each kind of microorganism was determined, and those in larger numbers were marked as predominant strains. Distinct colony types based on color, size, and morphology were noted, picked, and streaked onto fresh media for isolation. The cultures were preserved by refrigerating on slant media at 4 °C.

Identification of dominant strains: Microbial cultures were subjected to phylogenetic analyses by sequencing the 16S rDNA genes. Colony PCR mixtures (50 µl) contained 4 µl of template, 2 µL each primer, 25 µL of 2×PCR mix, and 17 µL of ddH_2O. Reaction mixtures were incubated in the Real-time system at 94 °C for 5 min, followed by 30 cycles at 94 °C for 1 min, 55 °C for 1 min, and 72 °C for 2 min, and then a final extension of 10 min at 72 °C. rDNAs were amplified with universal primers 27F and 1492R. PCR-amplified fragments were verified by agarose gel electrophoresis, shipped to Sangon Biotech (Shanghai) Co., Ltd., where products were sequenced in both directions by using BigDye terminator v3.1 and 3730xl DNA Analyzer (ABI, USA). Sequence reads were analyzed against the Ribosomal Database Project database by BLAST (http://blast.ncbi.nlm.nih.gov).

Physical and chemical characteristics of the soil

Beishan mountains area is located north of the Hexi Corridor, close to the Tengger Desert and Badain Jaran Desert, where the ground is mainly composed of brown desert soil. The results of soil analysis were shown in Table 1 and Table 2.

The analysis results showed very low moisture concentrations in all soils, the minimum value is less than 1%. The low moisture concentration is a prominent problem in arid

Table 1. Physical and chemical characteristics of the soils at 10 cm for the dense vegetation zone

Sample sites	Moisture(%)	pH	Eh(mV)	Ec(μs/cm)
Non-rhizosphere	3.210	9.36	-137.0	146.3
Nitraria sphaerocarpa	0.668	9.22	-129.6	154.8
Ephedra przewalskii	0.723	9.02	-131.4	258.0
Salsola abrotanoides	0.561	9.31	-134.4	209.0
Asterothamnus centrali-asiaticus	1.058	8.88	-122.9	393.0

Table 2. Physical and chemical characteristics of the soils in the sparse vegetation zone

Sample sites	Depth(cm)	Moisture(%)	pH	Eh(mV)	Ec(μs/cm)
Non-rhizosphere	10	2.911	8.80	-107.2	1 746.6
Non-rhizosphere	20	3.530	8.50	-96.6	1 665.0
Non-rhizosphere	50	3.573	8.66	-105.2	1 947.0
Salsola abuscula	10	0.893	9.17	-140.0	818.0
Salsola abuscula	20	3.799	7.95	-67.6	5 590.0
Salsola abuscula	50	7.552	7.82	-60.1	3 870.0
Sympegma regelii	10	4.218	8.31	-89.2	6 750.0
Sympegma regelii	20	3.006	7.98	-69.7	4 660.0
Sympegma regelii	50	3.609	7.38	-58.4	6 193.3

regions, and has a strong restriction on the growth of plants. In general, the more plant roots and litters, the more abundant moisture in soil, but sometimes, plant growth would led to a reduction of soil moisture resulted from the extremely dry conditions. In addition, soil moisture showed a trend of rising from the surface to the deep. The influence of the dry climate caused strong surface evaporation, so the surface soil was extremely dry. According to the pH classification standard (highly acid soil, pH◡5.0; acid soil, pH 5.0~6.5; neutral soil, pH 6.5~7.5; alkaline soil, pH 7.5~8.5; strongly alkaline soil, pH>8.5), soil in this region exhibited to be alkaline or strongly alkaline. Compared to the non-rhizosphere soils, rhizosphere soils generally indicated lower pH values. The acidification of rhizosphere soil might be related to the CO_2 exhaled in the process of plant respiration, and the secretions (amino acids, fatty acids and other organic) produced in the activities of root growth and metabolic.

The conductivity values (EC) in above tables reflected the salt conditions. Soils in the sparse vegetation zone showed higher salt concentrations, and the maximal value appeared in the rhizosphere of *Sympegma regelii* (Ec=5 867.8 μs/cm). The situation of significantly different salt contents for different plants in rhizosphere indicated that vegetation coverage was one of the important factors that affect soil salinity.

Concentration of airborne microbes

When the excavation of soil samples carried out, air samples were collected as controls to investigate the correlations between soil and airborne microbes. The concentrations of air microorganism in dense and sparse vegetation regions were shown in Table 3.

As shown above, means of airborne

Table 3. Concentration of airborne microbes in different regions ($\times 10^2$ CFU/m^3)

Sample region	Bacteria	Actinomycetes	Fungi
Dense vegetation	39.99±17.05	1.38±1.10	8.32±2.40
Sparse vegetation	12.87±2.66	0.20±0.19	3.96±1.05

bacteria, actinomycetes and fungi were 26.43×10^2, 0.79×10^2 and 6.14×10^2 CFU/m^3, respectively. The bacteria occupied substantially advantage in total concentration with the ratio of 79.23%. In addition, the concentration of airborne microbes in dense vegetation region was higher than that in sparse vegetation region. This situation might result from the various plants in dense vegetation region which are important sources of airborne microbes. Furthermore, the good air circulation in sparse vegetation region is not conducive to the growth and reproduction of microorganism.

Rhizosphere soil microbial flora of 4 typical plants from dense vegetation zone

The main plants distributed in dense vegetation region were *Nitraria sphaerocarpa*, *Ephedra przewalskii*, *Salsola abrotanoides*, *Asterothamnus centrali-asiaticus*, *Kalidium capsicum*, *Stipa caucasica subsp. Glareosa*, and so on. Rhizosphere soils of the first four plants were selected as materials. With the non-rhizosphere soil as reference, the quantity and distribution of three groups of microorganism at the depth of 10cm were investigated (Fig. 2). Results revealed that the microorganism in rhizosphere soil were significantly more than that in non-rhizosphere soil, and *Salsola abrotanoides> Asterothamnus centrali-asiaticus> Nitraria sphaerocarpa>Ephedra przewalskii*. This might be because roots secreted some biologically active substances into soil during the growth of plants. They could accumulate microbes in rhizosphere, and promote their growth and development. The microbial quantities in Beishan mountains area were similar to those in normal environments (bacteria, $n\times10^3$~$n\times10^8$ CFU/g; actinomycetes, $n\times10^6$~$n\times10^8$ CFU/g; fungi, $n\times10^3$~$n\times10^5$ CFU/g; $1\leqslant n<10$) (Jie Z *et al*., 2013). But the rhizosphere soils of *Salsola abrotanoides* and *Asterothamnus centrali-asiaticus* showed high fungi concentrations with means of 6.7×10^6 and 4.8×10^6 CFU/g, respectively. This finding would indicate that *Salsola abrotanoides* and *Asterothamnus centrali-asiaticus* herald a certain promotion for fungal growth. However, the bacteria and fungi in large numbers limited actinomycetes' growth because of the microbiological antagonism.

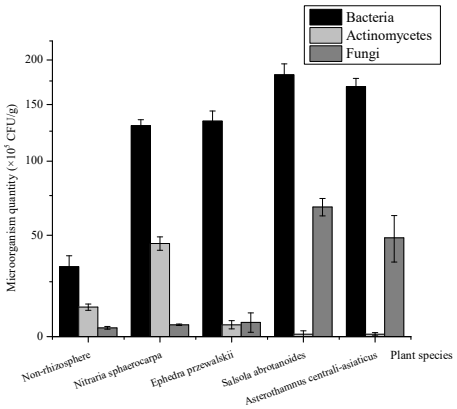

Fig. 2. Microbial distribution trend horizontally for the rhizosphere of *Nitraria sphaerocarpa*, *Ephedra przewalskii*, *Salsola abrotanoides* and *Asterothamnus centrali-asiaticus*

Rhizosphere soil microbial flora of 2 typical plants from sparse vegetation zone

Typical plants in sparse vegetation region were *Salsola abuscula* and *Sympegma regelii*. As shown in Fig. 3, the quantitative relationships among three groups of microorganism were bacteria>actinomycetes>fungi in this region. They occupied ratios of 66.20%~76.96%, 16.38%~26.57%, and 6.67%~7.23%, respectively. This finding was different from the investigation result of airborne microbes. The airborne actinomycetes took a higher rate than airborne fungi. This might be because of low-moisture, high-salt, and strong-alkali that limited the growth of fungi in soil. The total numbers of microorganism in soils were

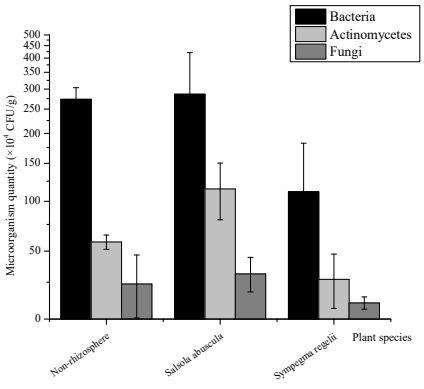

Fig. 3. Microbial distribution trend horizontally for the rhizosphere of *Salsola abuscula* and *Sympegma regelii*

Salsola abuscula rhizosphere>non-rhizosphere> *Sympegma regelii* rhizosphere. This might indicate that the root exudates of *Sympegma regelii* herald a certain inhibition for microorganism.

Fig. 2 and Fig. 3 were combined to create summaries: The total number of microorganism in non-rhizosphere soil from dense vegetation zone (49.2×10^5 CFU/g) was larger than that from sparse vegetation zone (35.5×10^5 CFU/g). And the maximum value of 250.4×10^5 CFU/g occurred in *Salsola abrotanoides* rhizosphere, the minimum value of 14.9×10^5 CFU/g occurred in *Sympegma regelii* rhizosphere. The distribution trend for bacteria was basically consistent with that for total microorganism. Bacterial maximum value of 182.0×10^5 CFU/g, and minimum value of 11.2×10^5 CFU/g also existed in *Salsola abrotanoides* rhizosphere, and *Sympegma regelii* rhizosphere, respectively. However, the ultimate value (45.3×10^5 CFU/g) for actinomycetes resided around roots of *Nitraria sphaerocarpa*, and the least value (1.0×10^5 CFU/g) around roots of *Salsola abrotanoides* and *Asterothamnus centrali-asiaticus*. The ultimate value (67.4×10^5 CFU/g) for fungi appeared in *Salsola abrotanoides* rhizosphere, and the least value (1.0×10^5 CFU/g) appeared in *Sympegma regelii* rhizosphere. The differences of microbial quantities and compositions among six rhizospheres might be because of the variety of root exudates and organic matters secreted by plants in different types and amounts.

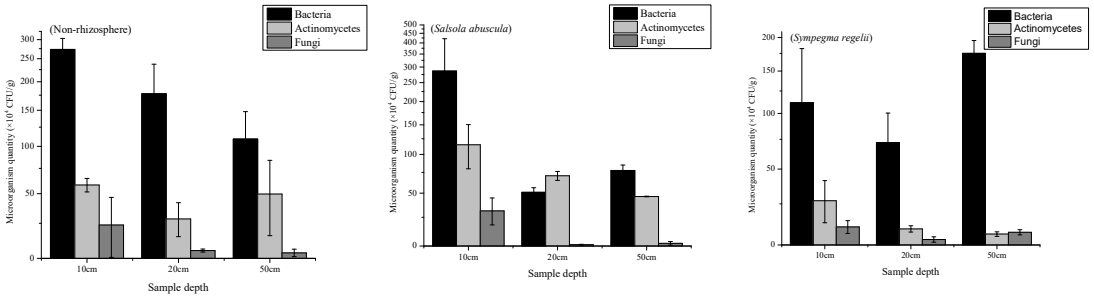

Fig. 4. Microbial distribution trend vertically for the rhizospheres of *Salsola abuscula* and *Sympegma regelii*

The vertical alteration of microbial flora in plant rhizospheres

In the present study, we took rhizospheres of *Salsola abuscula* and *Sympegma regelii* for example to examine the microbial changes with soil depths (Fig. 4).

As shown in Fig. 4, microbial numbers decreased with the increase of soil depth in the non-rhizosphere area. This situation resulted from that the increase of soil was accompanied by the reduction of organic matter, humus, and temperature. Number of actinomycetes also decreased with the increase of soil depths in both rhizospheres. But numbers of bacteria and fungi first declined and then climbed up. Previous studies have presented that the distribution of soil microorganism are closely related to root exudates and litters. In a relatively low concentration, root exudates are conducive to the growth of microorganism, but in an excessive concentration, they would become negative factors in turn. Humus soils with germination stimulating factors such as a dense mass of complex polysaccharides and organic nitrogen can alleviate bacteriostatic action, and improve the microbial numbers (Dan Z, 2005). Compared with the findings in this study, we got a speculation that root exudates of *Salsola abuscula* and *Sympegma regelii*r espectively existed mainly at the depth of 20~50 cm and 10~20 cm, could inhibit the growth of soil microorganism. While the exudates of *Sympegma regelii* existed at the depth about 50 cm was in an appropriate concentration, which could promote the growth of microorganism.

Identification results of predominant strains

Dilution plate count analysis of cultivable soil microorganism revealed 19 bacteria, 21 actinomycetes, and 8 fungi. The first eight microorganism in largest numbers were marked as predominant strains with the symbols of A, B, C, D, E, F, G and H. The colony morphologies were given in Fig. 5.

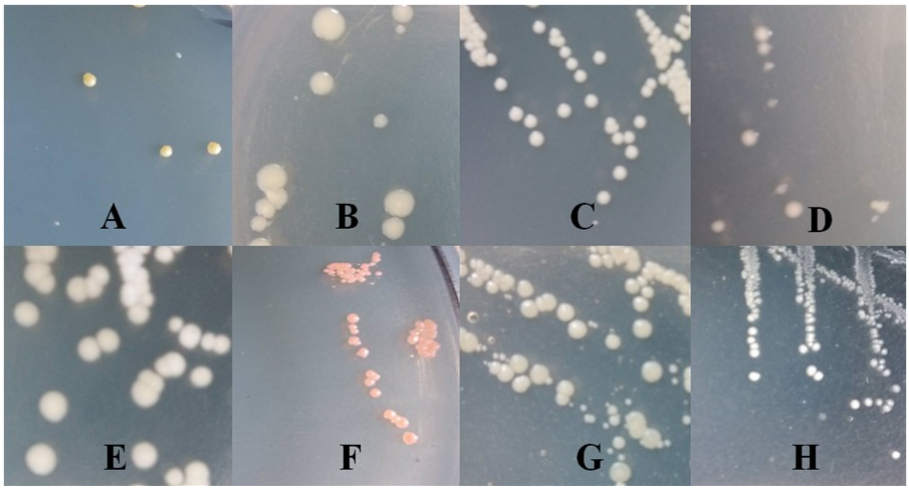

Fig. 5. Pictures of predominant strains

As shown in Table 4, the similarities between predominant strains and indexed strains were very high. Eight strains belonged to six different genera of *Kocuria*, *Bacillus*, *Arthrobacter*, *Paenibacillus*, *Nocardiopsis*, and *Promicromonospora*. *Kocuria* widely distributed in animal skins, rhizospheres, freshwater, marine sediments, and other natural environments. Its type strain *Kocuria rosea* was established by Carl Flügge in 1886 (Ran T *et al*., 2010). *Bacillus* is a kind of frequent bacteria in soil, and **possesses** high tolerability to extreme environments. *Arthrobacter* also widely exists in natural environments. Furthermore, many scholars have isolated them from harsh environments such as ancient murals, glaciers, and so on (BinLing Z *et al*., 2012; MingXia C, 2005). *Nocardiopsis* is a kind of gram-positive bacteria high in G+C content. They

Table 4. Comparative analysis results of 16S rDNA sequences for predominant strains

No.	Source	Species	Similarity	Accession
A	*Asterothamnus centrali-asiaticus*	*Kocuria polaris*	98%	JF798390.1
B	Dense vegetation	*Bacillus niacini*	95%	AF468221.1
C	*Salsola abuscula*	*Arthrobacter crystallopoietes*	98%	KC456536.1
D	*Nitraria sphaerocarpa*	*Paenibacillus tarimensis*	97%	NR_044102.1
E	Sparse vegetation	*Nocardiopsis lucentensis*	98%	JF966688.1
F	*Salsola abrotanoides*	*Arthrobacter agilis*	99%	JQ684255.1
G	*Ephedra przewalskii*	*Bacillus idriensis*	98%	HQ699498.1
H	*Sympegma regelii*	*Promicromonospora kroppenstedtii*	96%	NR_042622.1

distribute in variety of environments, and sometimes occupy the dominant position in moderate or high salt environments (LingLing *et al*., 2007).

DISCUSSIONS

Previous investigations have shown that plant roots play an important role in microbial distributions, especially for the desert ecosystem (Shamir *et al*., 2007). And this study suggested that numbers of microorganism in rhizosphere were significantly different from that in non-rhizosphere. This situation resulted from the frequent exchange of substances such as nutrients, moisture, and oxygen between roots and soils during the growth of the plants (YuHai *et al*., 2010). Meanwhile, enzymes produced by rhizosphere microorganism could decompose, mineralize organic matter, improve nutrient content of soil, and then increase

the absorption of nutrient, promote the growth of plants in turn (ZiXiong L *et al*., 2005).

The number of microorganism was affected by many elements. Although moisture affected the living environment of soil microorganism, it was not the decisive factor for microbial activities in the studied area. In roots distributed layers, organic matter providing energy sources was a key element for the growth of microorganism. Previous studies have suggested that fungi perform vigorous growth under acidic conditions, while bacteria and actinomycetes under neutral or alkaline conditions (Yang SS *et al*., 2003). This study found that pH value exercised a powerful influence on fungi, and a gentle one on bacteria and actinomycetes. To distinguish the soil factors that made effects on microorganism in exact degrees, control experiments need to be carried out in the future.

The number and distribution of microorganism closely reflect soil fertility and plant nutrition. So the conditions of soil, plants and climate could be reflected by them. On the other hand, microbial extracellular metabolites always act as binders which could play an important role in reducing sand and restoring ecosystem (DongMei X *et al*., 2007; XinRong Z *et al*., 2001; Bruggen AHC *et al*., 2000). In this study, the growths of *Nitraria sphaerocarpa*, *Ephedra przewalskii*, *Salsola abrotanoides*, *Asterothamnus centrali-asiaticus*, and *Salsola abuscula* were conducive to microbial enrichment. Furthermore, *Salsola abrotanoides* and *Asterothamnus centrali-asiaticus* could promote the growth of fungi. So these plants would be taken into account in the improvements on the behavior of arid environments in the future.

In general, the ratio of fungi to bacteria is often used as a soil index of acidity. The raise of this ratio may be accompanied by the descent of pH value (Franciska TD *et al*., 2006). This study found that rate of fungi/bacteria in rhizosphere were higher than that in non- rhizosphere for *Salsola abrotanoides, Asterothamnus centrali-asiaticus, Salsola abuscula*, and *Sympegma regelii*. And the pH values decreased in some degree. So we inferred that these four plants could promote the growth of fungi and improve the soil alkaline condition in Beishan mountains area.

ACKNOWLEDGMENTS

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