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Community structure and distribution of culturable bacteria in soil along an altitudinal gradient of Tianshan Mountains, China

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ABSTRACT

The Tianshan Mountains hold important and complex ecosystems. They host some unique biological resources, especially some microbes that have very crucial application values. In this study, the composition and distribution of bacterial communities from soil at different elevations of the Tianshan Mountains, China, were investigated using culture-based and molecular-based methods. The relationship between the abundance of culturable bacteria and environmental factors was also analysed. A total of 121 isolates showing different phenotypic characteristics were obtained from soil and identified by 16S rRNA gene sequencing analysis. All strains were assigned to four phyla: Proteobacteria, Actinobacteria, Bacteroidetes and Firmicutes. Actinobacteria and Proteobacteria were the dominating bacterial phyla; *Arthrobacter*, *Pseudomonas* and *Sphingomonas* were the most prevalent genera. The community structures of culturable bacteria were significantly different at different elevations, there being some common bacterial communities and some unique groups at each altitude. The principal component analysis (PCA) showed that the abundance of culturable bacterial communities has a significant positive correlation with the NO_3^- content in the soil. It indicated that NO_3^- was a better predictor of soil culturable bacterial abundance in this region. The strains isolated from alpine soil in this study are not only an invaluable part of microbial diversity, but also good candidates for further elucidating the interaction relationship between plants and microorganisms.

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Culturable bacterial community; structure; distribution; environmental factor; altitudinal gradient

Introduction

Mountains are special ecological systems that are rich in biodiversity resources. They have always attracted the attention of researchers, because of the sensitivity to global climate change [1]. Altitudinal gradients in the high mountain are often characterized by dramatic changes of the physical and biotic environment at short geographical distances [2]. Environmental factors such as temperature, precipitation, soil ion and radiation concurrently change along the altitudinal gradients under field conditions [3,4]. Many previous studies on biogeography along an altitudinal gradient have focused on macroorganisms; yet, the recent microbial distribution patterns have suggested that the ecological rules of microorganisms are different from those of macroorganisms [2,5]. The investigation of the change in soil bacterial structure and distribution along the altitudinal gradient may be correlated with the gradient of temperature, precipitation, soil ion and vegetation and would shed light on the ecological roles of bacteria [6].

In recent years, viable microbes are being identified in numerous cold environments, such as glacial, snow, ice core, permafrost and alpine subnival plants [7–10]. In the source area of the Urumqi River in the Tianshan Mountains, the microbial community structure and function of permafrost and subnival plants have been studied, but these researches mainly focus on the horizontal distribution of microorganisms [11–13]. In fact, the elevation distribution of bacteria could prove better in detangling the effects of environmental factors on the soil bacterial community distribution and composition [14,15]. Nevertheless, there are only very few reports about bacterial elevation distribution patterns, with provides us limited knowledge about how bacterial communities change along with the gradient of environmental factors caused by different altitude.

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Therefore, we selected a typical altitudinal gradient along the source area of the Urumqi River in the Tianshan Mountains, China, to study the soil bacterial community structure and distribution pattern by using a traditional culture-based approach and molecular techniques, and explore the relationship between bacterial communities and extreme environmental factors. With the advancement of molecular biological technologies for identifying microoganisms, the use of culture-independent approaches in describing bacterial communities has opened up new fields in microbial ecology [16]. Although these approaches avoid some of the limitations of traditional culture-based techniques, the isolation and cultivation of microbes are still required for physiological and functional research on microorganisms and other relevant biotechnological developments. In this research, the results are essential for understanding the microbial diversity in alpine soil and the ecological roles of microorganisms. The strains that are isolated from soil are especially good candidates for revealing the microbial cold adaptation mechanisms and further studying the interaction relationship between plants and microorganisms. This study will also reveal some phenomena and relationships among soil, microorganisms and the environment.

Materials and methods

Site description and soil sampling

The research was conducted in the source area of Urumqi River in the Tianshan Mountains (2130–3800 m, 74–95° E, 40–45° N), where the vegetation is sparse, and the annual average temperature is less than 5 °C during the day and −4 °C at night. The ecosystem in this region is very sensitive to climate change because it is on the border of a glacier with frequently fluctuating and freezing temperatures, strong wind, high ultraviolet (UV) exposure and low concentrations of oxygen and nutrients [11,13,17]. Along the increasing altitudinal gradient from 2130 to 3800 m, the mean annual temperature decreases from 6.8 to −6.0 °C, and the mean annual precipitation increases from 290 to 550 mm [18]. Therefore, it is an ideal location to study soil microorganisms and subnival plants.

Soil samples were collected from around the roots of four plants (*Pedicularis* sp., *Saxifraga stolonifera*, *Leontopodium leontopodioides* and *Polygonum viviparum*) at six different altitudes (2130, 3550, 3650, 3700, 3750 and 3800 m) along the source area of the Urumqi River in the Tianshan Mountains in July 2011. At each site, three replicated samples were collected from soil depth (0–10 cm) and five soil samples by mixing three soil cores. The samples were separately placed in an aseptic aluminum container and the tins were sealed and kept in ice during transport to the laboratory, then stored at −20 °C.

Soil physicochemical characteristics

Soil moisture was gravimetrically determined with drying at 105 °C for 12 h. Soil pH was measured in distilled water (1/10, *w/v*) with a portable pH meter (PHBJ–260, Shanghai, China). Conductivity was assayed using a conductivity meter (DDSJ–308A, Shanghai, China). Soil organic carbon (OC) was determined using the $K_2Cr_2O_7$ oxidation method described by Walkley and Black [19]. Total nitrogen (TN) was determined using the semi-micro Kjeldahl method [20]. Available phosphorus was determined using the Olsen method and soil-soluble anions and cations were determined using ion chromatography (Dionex ICS-2500, Dionex/America) [21].

Isolation and physiological characteristic determination of soil bacteria

The isolation conditions including incubation temperature and medium type were tested in order to harvest a maximum diversity of culturable bacteria. It was found that the bacteria diversity was richest and grew well on TSA and R_2A media at 20 °C.

Five soil samples of each site (altitude) were mixed well before isolation, then diluted by aseptically weighing 10 g of soil into a triangle sterilization bottle containing 90 mL sterile phosphate buffer saline (PBS) solution with glass beads and shaking at 250 r/min for 30 min. Serial dilutions were made and the 10^{-3}, 10^{-4} and 10^{-5} diluted aliquots were plated onto tryptic soy agar (TSA) and Reasoner's 2A agar (R_2A) media with triplicates [13]. Plates were incubated at 20 °C for 5–7 days, and then colony morphology and quantities were recorded. Colony-forming units (CFUs) were counted and similar bacterial isolates were divided into groups based on morphological characteristics, including shape, pigmentation, margin, surface and texture. Representative isolates from each of the identified groups were then selected for restriction fragment length polymorphism (RFLP) analysis.

Bacterial DNA extraction

Genomic bacterial DNA was extracted using the cetyltrimethyl ammonium bromide (CTAB) method [22] and stored at −80 °C. The ten-fold diluted DNA was used as template in the downstream polymerase chain reaction (PCR) and PCR−RFLP analyses.

16S rRNA gene amplification and PCR-RFLP analyses

The 16S rRNA genes were amplified with the primers 27F and 1492R [23]. A 50 μL final volume of PCR system contained 1 μL template DNA, 0.2 μmol/L of each primer, 200 μmol/L of each deoxyribonucleoside triphosphate (dNTP), 5 μL of 10 $\times$ PCR buffer, and 2 U of *Taq* DNA polymerase. A negative control (PCR mixture without template DNA) was included in all PCR amplifications. The PCRs were carried out as follows: after an initial 3-min denaturation at 95 °C, 34 cycles were run at 95 °C for 30 s, 55 °C for 30 s and 72 °C for 100 s, followed by a final extension step at 72 °C for 5 min (Bio-Rad C1000^{TM} thermal cyclers, America). For RFLP analysis, the conditions of RFLP, including the time (11, 12 and 13 h), temperature (36 and 37 °C) and restriction enzyme type (e.g. *Hae* III, *Hinf y*, *Alu* I, *Rsa* I and *Hin*-6I), were explored and optimized prior to all PCR amplification programs. Producing specific band patterns of condition was optimal and applied to all PCR products. Finally, 5 μL of PCR products were digested for 12 h at 37 °C with *Hae* III and *Hinf y* restriction enzymes simultaneously. The restriction fragments were separated in 2.0% agarose gel prepared with 0.5 $\times$ TBE (tris–borate–ethylenediaminetetraacetic acid) buffer. The 16S rDNA sequences of all major different band patterns were further sequenced.

Sequence and phylogenetic analysis

The 16S rRNA gene sequences of the isolates were compared using the Basic Local Alignment Search Tool (BLAST) in the GenBank database. We employed the Molecular Evolutionary Genetics Analysis software package MEGA 6, using the Kimura two-parameter method for distance matrix calculations and the neighbour-joining method and deduction system relations, and researched the sequences and genetic relationship between similar sequences [24]. The nucleotide sequences of the 16S rRNA genes of the culturable bacteria were deposited in GenBank under accession numbers JQ976985–JQ977054.

Statistical analyses

The soil physicochemical data were expressed as mean values with standard deviation ($\pm$SD). Statistical analyses were performed to examine the relationships between altitude and soil physiochemical factors using SPSS 22.0 software (SPSS, Chicago, IL, USA). The relationships between bacterial communities and environmental factors were evaluated using the principal component analysis (PCA). The PCA was processed using CANOCO software 4.5 [25]. R software was also used to perform statistical analysis.

Results and discussion

Isolation and identification of culturable bacteria

A total of 121 isolates were obtained from six altitudes after phenotypic analysis and the majority of those were gram-positive bacteria and pigmented. The abundance of culturable bacteria varied from 2.44 $\times$ 10^6 to 2.14 $\times$ 10^7 CFU/g among different altitudes. The results indicated that the soil of the source area of the Urumqi River possessed a high abundance of culturable bacteria (Table 1). The soil of this region proved rich in microbial resource based on the cultivation-dependent approach, although this method generally has some limitations in reflecting bacterial diversity. However, the method can provide actual strains for further research into their function and mechanism of cold tolerance, and the relationship among soil, bacteria, plants and environmental factors, which is also part of the aim of this work. Two strains with plant-growth promoting function at 4 °C and 20 °C have been used to study plant–bacteria interaction (data not shown). Therefore, the cultivation-dependent approach was chosen as an advantageous starting point for the purposes of this study.

The isolates were identified by PCR-RFLP and 16S rRNA gene sequencing analysis, and then a dendrogram representing the phylogenetic relationships of the soil isolates and their closest related matches was generated. The results showed that the bacterial communities belonged to four phyla and 23 genera. Actinobacteria were the most abundant and diverse, accounting for 44.21% of all isolates, while Proteobacteria, which included three major lineages: alpha, beta and gamma subdivisions, was the biggest developmental lineage, accounting for 30.14%. Bacteroidetes and Firmicutes were just 5.04% and 0.23% of the total isolated strains, respectively. At the genus level, *Arthrobacter* and *Pseudomonas* were the dominant population, accounting for 49.67% and 19.15%, respectively (Figure 1). In short, we obtained a wide variety of phylogenetic groups by using RFLP analysis, as it is a useful technique to investigate the genetic diversity of bacteria, and can provide data on the bacterial community composition quickly and effectively. The above-mentioned bacterial community pattern was similar to those in other studies conducted in the low-temperature soil environment [26–30]. On the other hand, we found that many isolates were closely related to bacteria isolated from snow, glaciers and permafrost when the sequences were aligned in the GenBank databases [12,26,31–33]. However, there were considerable differences from the culturable bacteria from Antarctica, Siberian Permafrost, alpine grasslands on the Tibetan Plateau and other forest soils [4,32,34,35]. This may be due to the selectivity of large ecological

Table 1. Sequence accession number and characteristics analysis of soil bacteria.

Isolates	Accession No.	C (cfu/g FW)	G^+/G^-	Similarity (%)	Closest relative species in the 16S rDNA sequence database	Source	Group
A06	JQ976985	1.14×10^6	G^-	99	*Pseudoxanthomonas* sp. R-37036	Antarctica	γ-proteobacteria
A07	JQ976986	2.50×10^4	G^-	99	*Pseudomonas* sp. RKS6-8	Roopkund Glacier of Himalayas, India	γ-proteobacteria
A10	JQ976987	2.53×10^5	G^+	99	*Rhodococcus* sp. CmLB13	Direct submission	Actinobacteria
A01	JQ976988	7.50×10^4	G^-	99	*Flavobacterium* sp. PDD-32b-56	Cloud water,1465 m a.s.l., France	Bacteroidetes
A13	JQ976989	1.13×10^5	G^-	99	Flavobacteriaceae bacterium TSBY-39	Alpine permafrost in the Tianshan Mountains	Bacteroidetes
A14	JQ976990	7.50×10^4	G^+	99	*Subtercola* sp. FB10	Arctic and antarctic microbial communities	Actinobacteria
A17	JQ976991	1.75×10^4	G^+	99	*Arthrobacter nitroguajacolicus* QT20	Qinghai–Tibet plate	Actinobacteria
B02	JQ976992	3.48×10^5	G^-	99	*Chryseobacterium* sp. QT10	Qinghai–Tibet plate	Bacteroidetes
B05	JQ976993	2.75×10^4	G^-	99	*Pedobacter kribbensis* PB93	Soil in Korea	Bacteroidetes
B08	JQ976994	1.65×10^5	G^+	99	*Arthrobacter nitroguajacolicus* T-8	Western Kunlun mountains	Actinobacteria
B09	JQ976995	4.65×10^5	G^-	99	*Flavobacterium* sp. HME6023	Direct submission	Bacteroidetes
B19	JQ976996	1.00×10^4	G^+	99	*Arthrobacter nitroguajacolicus* QT20	Qinghai–Tibet plate	Actinobacteria
C01	JQ976997	8.00×10^4	G^-	98	*Chryseobacterium* sp. NW121	Urban river	Bacteroidetes
C10	JQ976998	2.50×10^4	G^+	99	*Paenibacillus* sp. B17a	Alaskan tundra	Firmicutes
C17	JQ976999	9.50×10^5	G^+	99	*Arthrobacter nitroguajacolicus* T-8	Western Kunlun mountains	Actinobacteria
C22	JQ977000	2.50×10^3	G^+	100	*Streptomyces avidinii* 173229	Xinjiang, China	Actinobacteria
D04	JQ977001	6.50×10^4	G^+	99	*Arthrobacter* sp. Fa21	Strawberry plants	Actinobacteria
D10	JQ977002	2.45×10^5	G^-	99	*Pseudoxanthomonas* sp. R-37036	Antarctica	γ-proteobacteria
D11	JQ977003	5.50×10^4	G^-	99	*Brevundimonas* sp. XJ-412	Study of bacteria function	α-proteobacteria
D13	JQ977004	3.25×10^5	G^-	99	*Pseudomonas reactans* AMP-13	Two bioreactors	γ-proteobacteria
D19	JQ977005	1.13×10^5	G^-	99	*Chryseobacterium soli* JS6-6	Soil samples from Jeju, Korea	Bacteroidetes
E01	JQ977006	5.00×10^4	G^+	99	*Arthrobacter agilis* B1	Qinghai–Tibet plateau	Actinobacteria
E03	JQ977007	1.55×10^5	G^+	99	*Microbacterium* sp. HaLB10	Direct submission	Actinobacteria
E04	JQ977008	2.50×10^3	G^+	99	*Arthrobacter* sp. Fa21	Strawberry plants	Actinobacteria
G02	JQ977009	7.75×10^5	G^-	99	*Sphingomonas* sp. PDD-33b-12	Cloud water, 1465 m a.s.l., France	α-proteobacteria
G04	JQ977010	2.50×10^5	G^-	99	*Sphingomonas* sp. R-36583	Antarctica	α-proteobacteria
G06	JQ977011	1.50×10^6	G^-	99	*Phyllobacterium trifolii* PETP02	Spanish soils	α-proteobacteria
G08	JQ977012	5.00×10^4	G^+	100	*Bacillus simplex* Asd13	Aliaga ship dismantling zone at the Aegean Sea, Turkey	Firmicutes
G09	JQ977013	5.00×10^4	G^+	99	*Streptomyces* sp. GS11	Direct submission	Actinobacteria
G11	JQ977014	3.13×10^6	G^+	99	*Arthrobacter oxydans* 92-0600	Human clinical specimens	Actinobacteria
G13	JQ977015	3.00×10^5	G^-	99	*Sphingomonas* sp. Tibet-YD4600-2	Qinghai–Tibet plateau	α-proteobacteria
G14	JQ977016	5.75×10^5	G^-	99	*Stenotrophomonas* sp. SuP-E832	Agave root	γ-proteobacteria
G16	JQ977017	5.75×10^5	G^-	100	*Sphingomonas* sp. 14_4K	The solid waste from oil-shale chemical industry	α-proteobacteria
G17	JQ977018	2.50×10^4	G^-	98	*Mucilaginibacter* sp. PBI 162	Soil	Bacteroidetes
B18	JQ977019	3.00×10^4	G^+	99	*Paenibacillus wynnii*: LMG 22176	Antarctica	Firmicutes
C19	JQ977020	2.50×10^4	G^+	99	*Arthrobacter nitroguajacolicus* QT20	Qinghai–Tibet plate	Actinobacteria
D05	JQ977021	7.50×10^4	G^-	99	*Chryseobacterium* sp. TSE57	Glacier forefield	Bacteroidetes
E10	JQ977022	7.75×10^4	G^-	99	*Pseudomonas* sp. BLH-5B9	Bei Lu river permafrost	γ-proteobacteria
A09	JQ977023	7.00×10^4	G^-	99	*Janthinobacterium lividum* DSM 1522	Direct submission	β-proteobacteria
A12	JQ977024	2.50×10^4	G^-	99	*Pseudomonas* sp. RKS6-8	Roopkund Glacier of Himalayas	γ-proteobacteria
A15	JQ977025	8.95×10^5	G^-	99	*Brevundimonas vesicularis* EQH12	Endophytic bacteria in 13 kinds of plants	α-proteobacteria
A19	JQ977026	6.32×10^5	G^-	99	*Pseudomonas graminis* RMPP6	Soybean rhizosphere soil	γ-proteobacteria
B01	JQ977027	4.65×10^5	G^-	99	*Pseudomonas* sp. RKS6-8	Roopkund Glacier of Himalayas	γ-proteobacteria
B03	JQ977028	1.28×10^6	G^-	99	*Pseudomonas* sp. RKS6-8	Roopkund Glacier of Himalayas	γ-proteobacteria
B04	JQ977029	7.00×10^4	G^+	99	*Arthrobacter* sp. N5	Xinjiang	Actinobacteria
B06	JQ977030	6.65×10^5	G^+	99	*Rhodococcus qingshengii* S2	In floral nectar	Actinobacteria
B07	JQ977031	1.77×10^6	G^+	99	*Arthrobacter oxydans* B18	Qinghai–Tibet plateau	Actinobacteria
B10	JQ977032	1.93×10^5	G^-	99	*Pseudomonas fluorescens* LB1	Study of bacteria function	γ-proteobacteria
B13	JQ977033	7.25×10^4	G^-	99	*Flavobacterium* sp. HME6023	Direct submission	Bacteroidetes
B14	JQ977034	3.00×10^4	G^-	99	*Pseudomonas trivialis* B24	Qinghai–Tibet plateau	γ-proteobacteria

(*continued*)

Table 1. (*Continued*)

Isolates	Accession No.	C (cfu/g FW)	G^+/G^-	Similarity (%)	Closest relative species in the 16S rDNA sequence database	Source	Group
B15	JQ977035	2.50×10^3	G^+	99	*Arthrobacter oxydans*: S32212	Paddy field soil	Actinobacteria
B16	JQ977036	2.50×10^3	G^-	99	*Pseudomonas migulae* HRT13	Qinghai–Tibet plateau	γ-proteobacteria
B20	JQ977037	2.50×10^3	G^+	99	*Streptomyces* sp. Sd-51(2011)	Qinghai–Tibet plateau	Actinobacteria
C02	JQ977038	2.25×10^5	G^-	99	*Flavobacterium* sp. WB3.3-59	Rivulet water and tufa biofilm	Bacteroidetes
C04	JQ977039	2.50×10^3	G^+	99	*Arthrobacter nitroguajacolicus* CAT5	Qinghai–Tibet plateau highway	Actinobacteria
C11	JQ977040	2.50×10^3	G^+	99	*Arthrobacter nitroguajacolicus* T-8	The Western Kunlun Mountains	Actinobacteria
C18	JQ977041	1.83×10^6	G^+	99	*Arthrobacter* sp. 108	2,4-D degradation	Actinobacteria
D01	JQ977042	3.50×10^4	G^-	99	*Janthinobacterium lividum* DSM 1522	Direct submission	β-proteobacteria
D02	JQ977043	2.50×10^4	G^-	99	*Janthinobacterium lividum* DSM 1522	Direct submission	β-proteobacteria
D08	JQ977044	3.20×10^5	G^-	99	*Flavobacterium* sp. WB 3.4.10	Hardwater creek, Harz Mountains, Germany	Bacteroidetes
D14	JQ977045	1.83×10^5	G^-	99	*Chryseobacterium soli* JS6-6	Soil samples from Jeju, Korea	Bacteroidetes
D17	JQ977046	1.50×10^4	G^-	99	*Janthinobacterium lividum* DSM 1522	Direct submission	β-proteobacteria
E02	JQ977047	1.00×10^4	G^+	99	*Arthrobacter nitroguajacolicus* QT20	Qinghai–Tibet plate	Actinobacteria
E06	JQ977048	2.53×10^5	G^-	99	*Pseudomonas graminis* RMPP6	Soybean rhizosphere soil	γ-proteobacteria
E09	JQ977049	1.50×10^4	G^-	99	*Pseudomonas trivialis* B24	Qinghai–Tibet plateau	γ-proteobacteria
E11	JQ977050	4.35×10^5	G^+	100	*Arthrobacter* sp. d9(2010)	Direct submission	Actinobacteria
E13	JQ977051	7.50×10^3	G^-	99	*Pseudomonas* sp. RKS6-8	Roopkund Glacier of Himalayas	γ-proteobacteria
E14	JQ977052	1.48×10^5	G^+	99	*Curtobacterium flaccumfaciens* A4-16	Soil crusts in the muusshadi	Actinobacteria
E16	JQ977053	1.68×10^5	G^+	99	*Kocuria* sp. WCH21	Tobacco rhizosphere	Actinobacteria
E19	JQ977054	2.50×10^3	G^-	99	*Lysobacter* sp. HX1	Soil bacteria from alpine grassland	γ-proteobacteria

Note: C, concentration of each isolate; G, Gram reaction.

environments. The most probable reason for the observed differences is that similar climatic environments breed similar microbial taxa even under different geographic conditions. In addition, this investigation has expanded our knowledge of culturable bacterial diversity and has already provided basic information on microbial diversity in extreme environments.

According to the characteristics of the culturable bacteria, the majority of the isolates were gram-positive bacteria and highly pigmented, which is in agreement with some previous studies [11,36,37] because gram-positive bacteria have strong adaptability to cold and harsh environments. In addition, pigmentation is essential to maintaining membrane fluidity and stabilization [11,36], it can help bacteria resist and adapt to environment change. The phylum Actinobacteria, including *Arthrobacter*, *Rhodococcus*, *Streptomyces* and *Lentzea*, all present high resistance to UV radiation and desiccation [38], as such representatives are well known for the production of antibiotics and other secondary metabolites (enzymes, vitamins, etc.) [39]. Similarly, within the phylum Firmicutes, *Bacillus* and *Paenibacillus* can resist extreme environments for producing spores. The phylum Bacteroidetes was represented by the classes *Flavobacterium*, *Chryseobacterium*, *Geobacteracea* and *Mucilaginibacter*. Although Bacteroidetes were regarded as a small taxon, it still had a larger proportion, and the isolates had high similarity to strains associated with cold environments [40–42]. The phylum Proteobacteria was divided into three major classes: Alphaproteobacteria, Betaproteobacteria and Gammaproteobacteria subdivisions. Proteobacteria is important for global carbon, nitrogen and sulphur cycling; therefore, this phylum is more sensitive to climate changes and plays an essential role in the soil biosphere. Within the class Alphaproteobacteria, the genus *Sphingomonas* can degrade organic compounds [43]. Therefore, these strains are considered important for the study of biological cycles and environmental restoration. Another portion of the strains had a high similarity to some functional bacteria such as nitrogen-fixing bacteria and such that find application in environmental management, bacterial biofilm treatment and biological protection [44,45]. This suggested that these isolates can survive in extreme environments, and they were likely to play a function for enhancing the stress resistance of plants.

Bacterial community structure and distribution at different altitudes

The results suggested that there was an upward trend in the total CFU along the altitudinal gradient from 3550 to 3800 m, but the highest value appeared at 2130 m

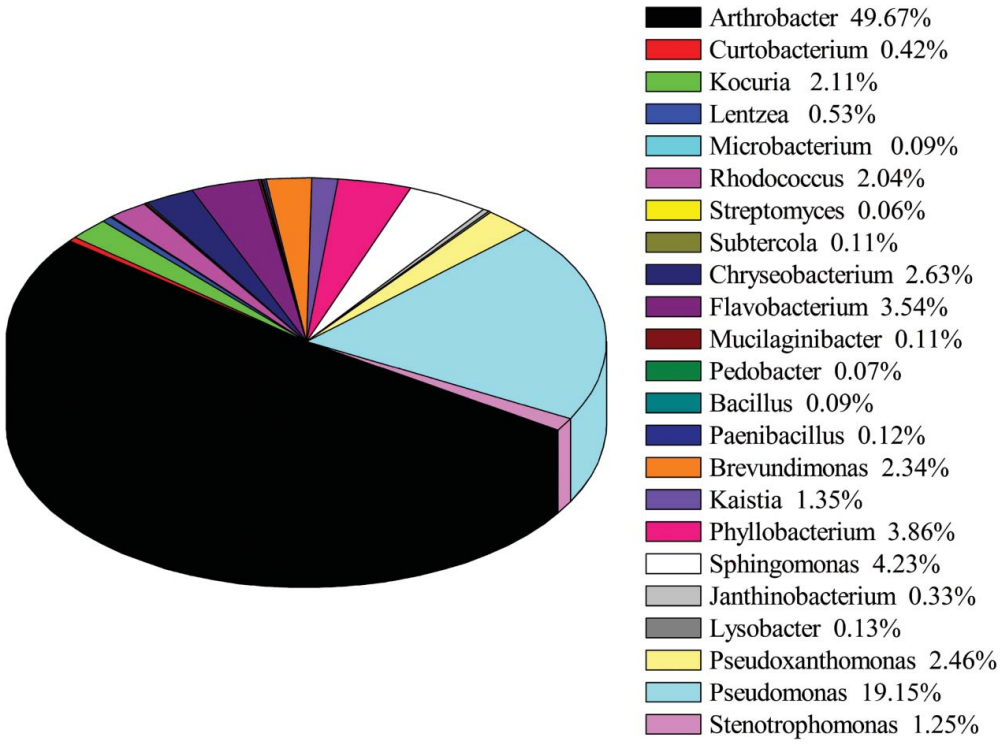

Figure 1. Diversity and composition of bacterial communities in all soil samples.

(Table 2). A few early studies also showed that the numbers of total bacteria increased with elevation increasing in the Austrian Northern and Central Alps [46,47]. However, some studies have suggested that the number of culturable bacteria significantly declined with increasing elevation [30,48]. The possible reason for these controversial results is that the environmental conditions including both abiotic factors (i.e. temperature, precipitation and atmospheric composition) and biotic factors (i.e. vegetation, biodiversity and composition) change with the altitudinal gradient and geographical location [46,48,49]. It could be speculated that the major influencing factor to microbes was most probably different in different regions.

Overall, in terms of the relative abundance of each phylum or genus, the bacterial community structure and distribution were obviously different among the six altitudes (Figure 2). Actinobacteria had a wide distribution

Table 2. Number of bacteria and physicochemical characterization of soil.

Altitude	CFU × 10^6 (CFU/g)	H_2O (%)	pH	Conductivity (μS/cm)	OC (g/kg)	TN (g/kg)	P (mg/kg)
3800 m	7.97	16.56 ± 4.88	7.38 ± 0.054	184 ± 2.87	38.49 ± 0.018	2.55 ± 0.044	13.9 ± 1.21
3750 m	5.82	34.54 ± 1.39	7.08 ± 0.029	153 ± 13.71	76.35 ± 0.84	5.17 ± 0.064	17.6 ± 0.21
3700 m	7.60	29.61 ± 0.71	7.10 ± 0.005	156 ± 7.44	73.04 ± 0.48	5.01 ± 0.080	21.0 ± 1.41
3650 m	5.45	43.04 ± 1.61	7.13 ± 0.021	159 ± 9.01	99.24 ± 0.29	7.05 ± 0.065	27.8 ± 0.47
3550 m	2.44	66.74 ± 1.34	7.15 ± 0.041	483 ± 13.59	170.81 ± 3.23	12.13 ± 0.204	43.6 ± 0.69
2130 m	21.4	27.11 ± 3.48	7.33 ± 0.029	277 ± 16.08	83.38 ± 0.53	6.03 ± 0.023	22.3 ± 0.13

	Soluble ions × 10^{-2} (g/kg)						
Altitude	Na^+	K^+	Mg^{2+}	Ca^{2+}	NH_4^+	Cl^-	NO_3^-	SO_4^{2-}
3800 m	2.217	1.533	0.286	8.619	0.527	4.078	2.273	2.776
3750 m	4.799	4.388	0.561	11.943	1.147	6.528	2.231	5.700
3700 m	6.728	4.758	0.430	9.859	1.297	9.496	4.055	3.884
3650 m	5.008	3.320	0.440	11.038	1.023	7.118	3.400	3.438
3550 m	7.181	21.773	1.378	31.164	3.389	8.748	0.000	18.432
2130 m	2.008	5.675	0.529	18.430	1.215	5.158	8.849	7.056

Note: Variables: CFU, total number of culturable bacteria; TN, total nitrogen; OC, organic carbon; H_2O, soil moisture.

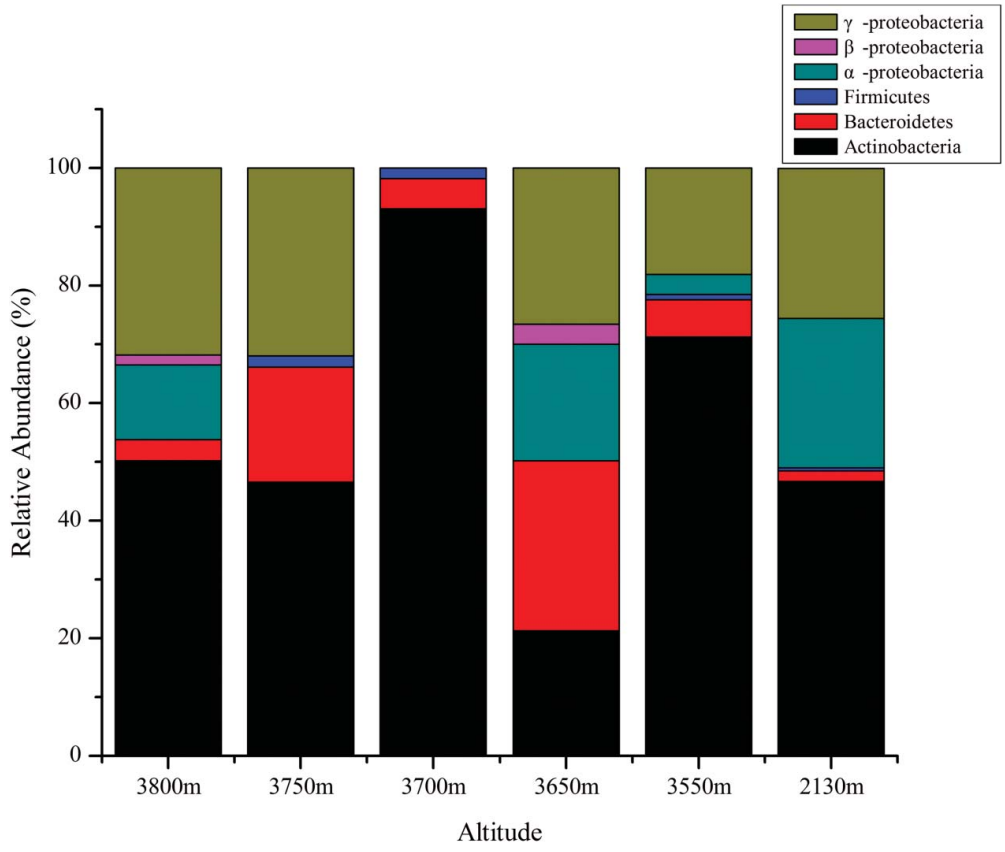

Figure 2. Relative abundance and structure of soil bacterial groups at each altitude.

and rich diversity. *Arthrobacter*, *Curtobacterium*, *Kocuria*, *Microbacterium*, *Rhodococcus* and *Streptomyces* etc. belonged to Actinobacteria. Proteobacteria was a dominant population, although it was not isolated from 3700 m. In addition, Bacteroidetes were isolated from all altitudes, although they only accounted for a small proportion of the isolates.

At the level of genus, *Arthrobacter* was one of the dominant populations; it was distributed at all altitudes. *Pseudomonas* was also a dominant population and was distributed at all altitudes except 3700 m. At the highest altitude of 3800 m, *Kocuria*, *Rhodococcus*, *Streptomyces* and *Brevundimonas* were distributed, and *Rhodococcus* and *Streptomyces* were distributed at 3750 m as well. These distribution patterns may be in response to the changes of the soil and features of the ecological environment [50]. For instance, *Arthrobacter*, *Rhodococcus* and so on, all appeared at 3800 m and higher altitudes and constituted a large proportion (Figure 3), because they are psychrotolerant and stress resistant microorganisms and can survive under cold conditions. These characteristics are due to a series of cell metabolism specifics, including the production of cold-active enzymes, anti-freezing proteins and exopolymeric substances [51]. At an altitude of 3550 m, only *Curtobacterium* and *Microbacterium* were isolated and distributed, since the environmental conditions there were relatively special (Table 2). The bacterial structure and distribution may be correlated with the type and concentration of soil ions, temperature, precipitation and the plants. These isolates have the ability to survive in such environmental conditions, for they have complex nutritional requirements. *Mucilaginibacter*, *Bacillus*, *Phyllobacterium* and *Stenotrophomonas* were only distributed at the lowest altitude of 2130 m (Figure 3), and the main reason was that some strains grow in more mild environmental conditions [13], so the lower altitude environment (higher temperature, richer nutrition etc.) was suitable for them. In summary, *Arthrobacter*, *Pseudomonas* and *Sphingomonas* were the most prevalent genera along the altitudinal gradient. Thus, the results demonstrated that there are some common bacterial communities and some unique groups at each altitude.

Relationship between bacterial communities and environmental factors

The results from this study showed that the alpine soil in the Tianshan Mountains is a unique environment, in which

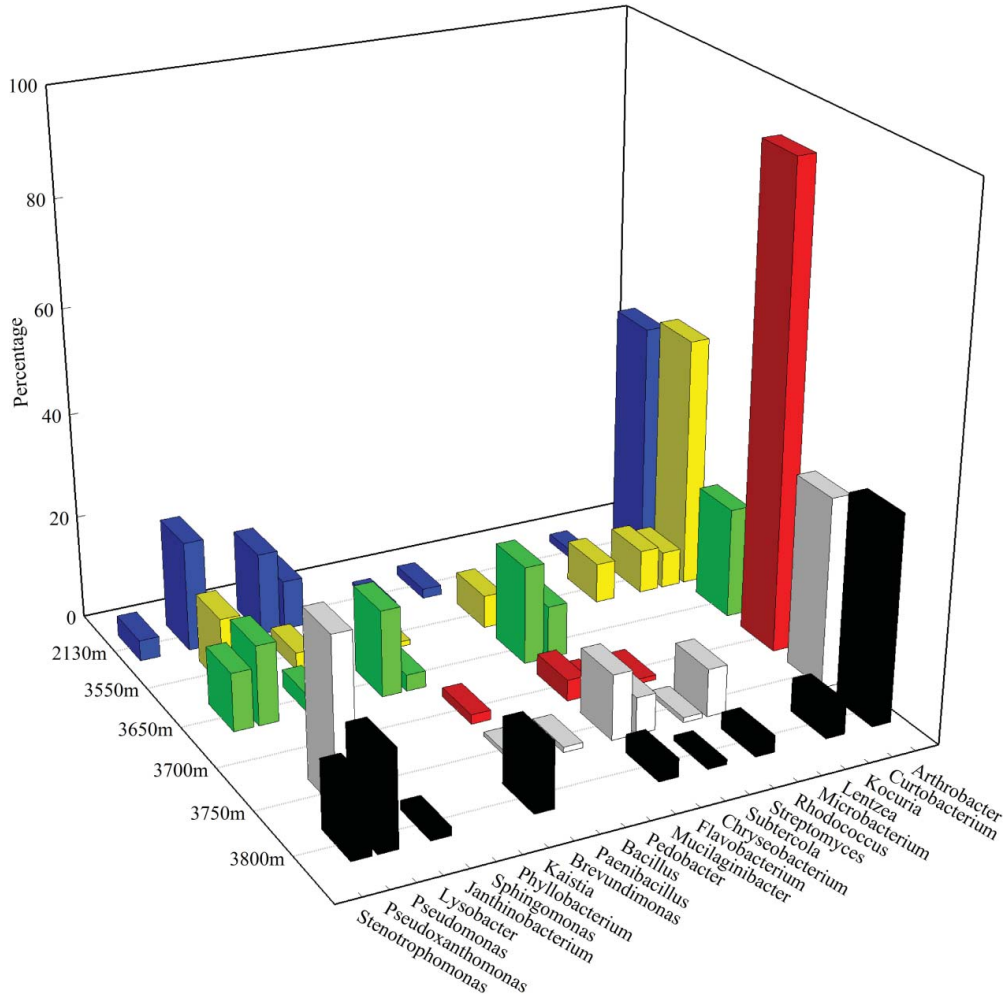

Figure 3. Distribution for each group of soil bacteria at each altitude.

the organic matter and ions were abundant. The numbers of bacterial groups were remarkably different among altitudes, and exhibited no regularity from a high altitude to a low altitude. The region is also an approved and explored niche for microbes [50]. Moreover, it was surprising that the minimum number of culturable bacteria appeared at 3550 m (Table 2), where the soil moisture content, conductivity, NH_4^+, OC, Na^+, K^+ and Mg^{2+} concentrations reached the highest values, but the NO_3^- content was zero. Thus, the absence of nitrate but abundance of ammonia at this altitude suggested that ammonium and nitrite-oxidizing bacteria are poorly represented. This could be confirmed by searching for *Nitrobacter* or *Nitrosomonas* spp. specifically. In fact, since we have actually obtained an isolate library, it would be relatively straightforward to screen these isolates for the presence of specific enzymes involved in the nitrogen cycle.

The relationship between the bacterial abundance and environmental factors (Table 2) was examined using PCA (Figure 4). The first principal component (Axis 1) explained 68.9%, and the second one (Axis 2) explained 22.1% of the total variance. The results showed that CFU was significantly positively correlated with the NO_3^- content. Thus, it appeared that NO_3^- was the key driver of the soil bacterial communities' abundance in the studied region. Compared with previous studies, the results showed that temperature, precipitation, carbon supply and soil pH led to differences in bacterial communities in different regions [3,5,49,52]. We speculate that this may be related to the unique environment in the studied area. Moreover, NO_3^- is an important electron acceptor in the denitrification process and it is essential in the nitrogen cycle. The differences in the NO_3^- status at different elevations might have also contributed to

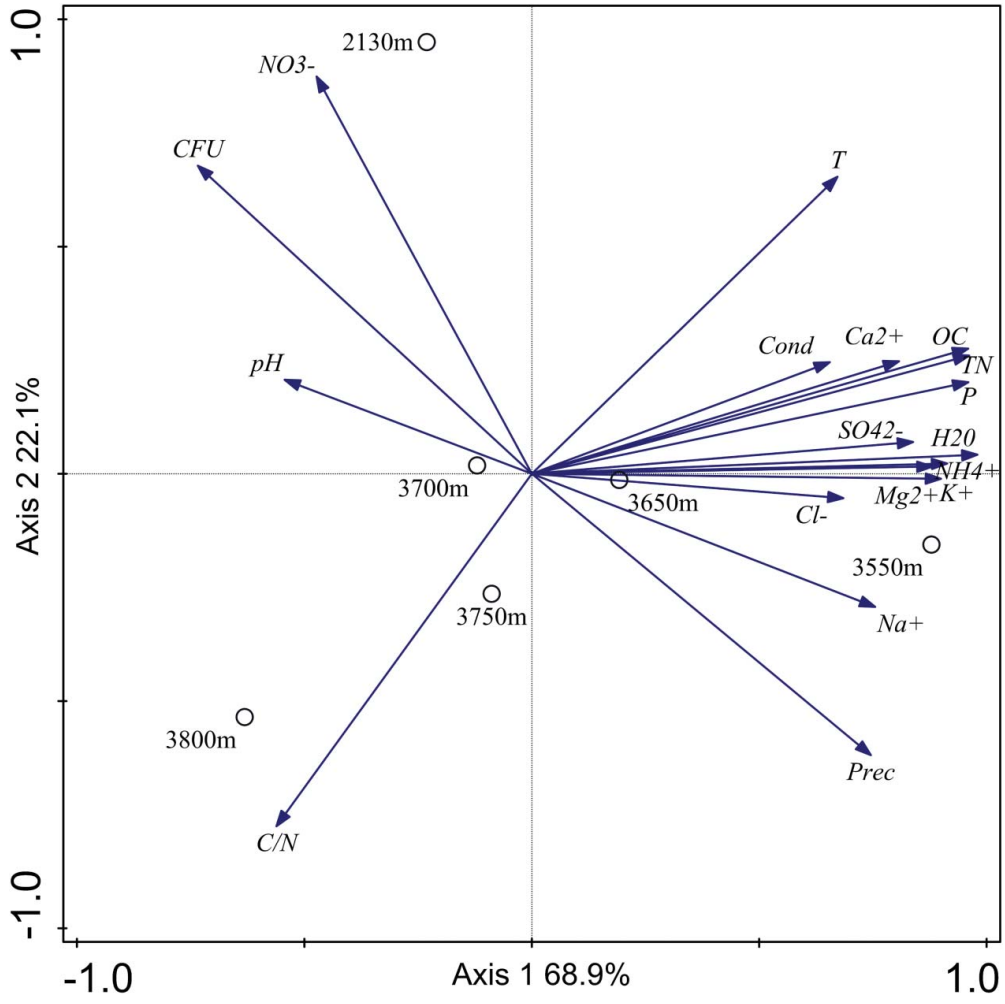

Figure 4. Principal component analysis (PCA) showing analysis of environmental factors and numbers of bacteria.

the altitudinal change in the soil community of culturable bacteria.

Conclusions

This study cast light on the abundance and diversity of culturable bacterial communities in the source area of Urumqi River in the Tianshan Mountains, and illustrated the structure and distribution of bacterial communities among different elevations. The analysis of the relationship between the soil bacterial communities and environmental factors indicated that the NO_3^- content played the largest role in the abundance of culturable bacterial communities along the altitudinal gradient. These results are essential for understanding the microbial diversity and the ecological roles of bacteria in alpine soil. We have actually obtained an isolate library, in which the strains are especially good candidates for revealing microbial cold adaptation mechanisms and further studies into the interaction relationship between plants and microorganisms.

Disclosure statement

No potential conflict of interest was reported by the authors.

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