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Diversity of Cultivable Bacteria of Frozen Soil in Lake Issyk-Kul Basin, Kyrgyzstan

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Microbial ecology studied on frozen soils in Lake Issyk-Kul basin, Kyrgyzstan, has not yet been reported. With the aim of identifying the pure culture of bacteria in this less known earth, five culture media, PYGV, R2A, 1/4TSB, TSB, and 1/4NA, were employed to isolate the soil bacteria from four samples in this region. The numbers of the cultivable bacterial community were $1.17 \times 10^4 \sim 1.38 \times 10^5$ CFU/g, which decreased with the reduction of the environmental biomass. According to colonial morphology, 173 bacterial strains were isolated. On the basis of 16S rRNA gene sequences, the bacterial strains were belonged to 4 phyla, including Actinobacteria (52.60%), Firmicutes (18.50%), Proteobacteria (22.54%), and Bacteroidetes (6.36%). As the dominant genera, *Arthrobacter*, *Planococcus,* and *Pseudomonas* were found in all the frozen soil samples. The similarities of the 16S rRNA gene sequence with the corresponding type species were lower than 97.00% for 5 strains (N-2, N-3, N-13,R-19 and P-11). The presence of potentially new species reinforces the need for new studies in the permafrost environments of the Lake Issyk-Kul basin.

Key words: Cultivable bacteria, Frozen soil, 16S rRNA gene, Diversity.

Frozen soil refers to the various rock and soils containing ice at or below 0°C. According to the duration of the frozen state, the frozen soil can be divided into short-term frozen soil, seasonal frozen soil, and permafrost in general. Frozen soil on the earth collectively accounts for about 50% of the continental area[14,28]. Due to the wide range of distribution and special hydrothermal characteristics, frozen soil is one of the important factors in the circulation process of the land surface ecosystem[23].

Traced back to the late nineteenth century and early twentieth century, scientists first found the cultivable microorganisms in frozen soils when they researched a mammoth fossil in Siberia and the Far East[15]. However, there had been no significant breakthrough in subsequent one hundred years. Since the 1990s, studies on the vital signs and adaptation mechanisms of the microorganisms in frozen soil have attracted worldwide attention[36]. As the population structure and genetic evolution of microorganisms are very sensitive to environmental conditions, studies on the microbial diversity of frozen soils in different regions have become the focuses[25].

The Republic of Kyrgyzstan is located in the center of Central Asia. One-third of the country's altitudes are 3000–4000 m. Thus, the Republic of Kyrgyzstan is known as a "mountainous country." Lake Issyk-Kul is the largest alpine lake in Kyrgyzstan, and the ecological types include forest, grasslands, meadow, desert,

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and semi-desert. Currently, the studies on Kyrgyzstan mainly refer to the perspectives of climate, water resources, topography, and ecological environment, etc. As to the microbial diversity of frozen soil in the specific habitats has not yet been reported[2,24].

In this study, bacteria colonies were isolated from the frozen soil samples of Lake Issyk-Kul basin by pure culture method. The aims of this study were the isolation of microorganisms, description of new species, understanding physiological aspects and showing the phylogenetic identification of the survival microorganisms from frozen soil samples collected on Lake Issyk-Kul basin.

MATERIALS AND METHODS

Study site and soil samples

The soil samples were collected from four points in the Lake Issyk-Kul region in September 2012. Lake Issyk-Kul basin is an arid zone with continental alpine climate characterized by cool summer and cold winter. The annual average temperature is 4.3°C, and the monthly average temperature from October to March is constantly below 0°C. The annual average rainfall is about 290 mm, and more than 80% precipitation concentrated from April to September, which the monthly average temperature is higher than 0°C [27].

In the sampling process, the litter on the ground was removed, the soil samples were collected at the depth of 0–20 cm by using a sterilized sampling shovel and sub-packed in 50ml sterile centrifuge tube. The tube was stored at 4°C until to further use. The locations of the sampling site are shown in Table 1.

The basic physicochemical indices of soil were determined according to the conventional method. The organic carbon content was detected by spectrophotometry. The total nitrogen content was evaluated by using the Kjeldahl method. X-ray fluorescent spectrometry (XRF) was used to calculate the total phosphorus content. The soil pH value was measured by ion selective electrode method and the moisture content was determined by using drying method[18,21].

Strain isolation and culture conditions

Five types of culture media were used for the enrichment of isolates. PYGV regeneration medium[11] and R2A improved medium[13], with the addition of 0.25 g casein L^{-1} and 0.1 g $MgSO_4 \cdot 7H_2O$ L^{-1}, instead of peptone. The other components and contents were the same as those given in the reference. 1/4 strength and 1 strength TyPtone soya broth (TSB) concentration was prepared according to the corresponding ratios[16] and 1/4 strength NA culture medium contained 2.5 g peptone, 0.75 g beef powder, 1.25 g NaCl, 15 g agar, and 1 L distilled water. The different types of culture media (PYGV and R2A were oligotrophic media, whereas 1/4 strength TSB, TSB, and 1/4 strength NA were eutrophic media) were used to improve the culturability of bacteria in the soil.

For the isolation of microorganisms, samples were serially and decimally diluted from 10^{-1} to 10^{-3} with double-distilled water (ddH_2O) as diluent. Then, the plates were incubated at 15°C for 21 days. The bacterial colonies with different shapes, sizes, and colors were selected and transferred to new agar plates several times, for their isolation and purification.

PCR amplification and sequencing of 16S rRNA gene

The fresh cultured colonies were picked with toothpicks, suspended in 1 ml of ddH_2O, and mixed thoroughly. Then, 1 µl of the suspension was taken as the PCR template, and the PCR amplification was performed by using universal primers of bacterial 16S rRNA gene (27F: 5'-AGAGTTTGATCCTGGCTCAG-3', 1492R: 5'-AAGGATGGTGATGCCGCA-3')[21]. The PCR reaction conditions were as follows: 95ºC for 5 min, followed by 30 cycles of denaturation at 95ºC for 45 s, annealing at 57ºC for 30 s, and extension at 72ºC for 1.5 min, before a final extension at 72ºC for 7 min. The PCR-amplified products were detected by employing 0.8% agarose gel electrophoresis. Then, the amplified products were purified and sequenced by Sangon Biotech (Shanghai) Service Co., Ltd.

Phylogenetic analysis

Identification of the isolates was carried out online with EzTaxon server 2.0[5]. Subsequently, the sequences were arranged with ClustalX according to the principle of maximum homology [8]. The differences in the values of nucleotide were calculated by using Kimura-2. Construction of phylogenetic tree and diversity analysis were accomplished with MEGA 5.0 software package

by using Neighbor-Joining method[9]. The number of bootstraps was 1000.

Analysis of bacterial diversity

The strains of the same species were defined to be within the same operational taxonomic unit (OTU) if more than 97.00% of the 16S rRNA gene sequence homology was observed. The diversity of the bacteria was calculated by using the Simpson's diversity index (D), Shannon-Wiener diversity index (H), and Shannon-Wiener evenness index (E)[26].

The 16S rRNA gene sequences obtained have been submitted to the GenBank database under the accession number KF318377-KF318414.

RESULTS

Basic physicochemical properties of the soil samples

The main soil types were marsh soil and meadow soil in sample 1 where the vegetation was the most abundant in all samples. The dominant plants included Carex, Platycodon, and Compositae. Tundra was alpine meadow soil in sample 2, with more than 70% of vegetation coverage. Desert soil was observed, which the surface covered with gravel and some plants such as Caryophyllaceae and Gramineae in sample 3. The vegetation coverage was over 20% in this point. The soil layer of sample 4 was gravel covered with snow. Residues of Gramineae were distributed in the rock and stone gap.

The physicochemical properties of the frozen soil at high altitude in these four different habitats in Lake Issyk-Kul were analyzed, and the results are shown in Table 1. The moisture content was 8.49–40.68%, organic carbon content was 4.78–26.19 g/kg, total nitrogen content was 109–1118 mg/kg, total phosphorus content was 361–739 mg/kg, and the pH variation was in the range of 6.40–7.32. The sampling points covered by vegetation had higher nitrogen content, and the acidity or alkalinity of the soil had no correlation with the nutrients. However, on the whole, the pH was weakly acidic or neutral.

Isolation and counting of bacteria in frozen soil region

Colony counts of the samples, as determined in five culture media PYGV, R2A, 1/4TSB, TSB, and 1/4NA , varied between 1.17 and 13.8× 10^4 CFU/g of soil. PYGV and 1/4 strength NA culture media were the optimum media from which the maximum counts were isolated. The number of isolated strains per sample varied between 14 (sample 4) and 69 (sample 2) (Table 1). According to the phenotypic characteristics, including color, size, surface smoothness and so on, 173 microorganisms were isolated from the five culture media employed. Overall, the tendencies were observed in these counts, in relation to the biomass

Table 1. Location information, physicochemical characteristics, and culturable bacterial counts of the frozen soil samples

	Sample	1	2	3	4
Site information	Latitude	41°54'53"	41°53'42"	41°52'19"	41°52'08"
	Longitude	78°10'27"	78°10'40"	78°10'21"	78°12'03"
	Altitude	3688	3677	3913	4150
	Soil type	Wet meadow soil	Alpine meadow soil	Desert soil	Desert soil
Physicochemical characteristics	Moisture (%)	40.68	23.35	8.49	33.75
	Organic C (g/kg)	26.19	13.23	4.78	2.32
	Total N (mg/kg)	1118	600	217	109
	Total P (mg/kg)	739	846	361	371
	Total K (g/kg)	21.80	18.55	18.42	12.86
	pH	6.76	7.32	6.89	6.40
Colony numbers (CFU/g)	PYGV	2.16×10^5	3.04×10^5	2.03×10^4	4.51×10^3
	R2A	6.68×10^4	6.13×10^4	1.83×10^4	8.66×10^3
	1/4TSB	1.44×10^4	7.20×10^4	9.17×10^3	1.89×10^4
	TSB	3.18×10^4	8.00×10^4	1.81×10^4	1.32×10^4
	1/4NA	2.07×10^5	1.72×10^5	2.22×10^4	1.93×10^4
	Average	1.07×10^5	1.38×10^5	1.76×10^4	1.17×10^4

of the sampling locations (Table 1). The diversities of cultivable bacteria in different sampling stations were assessed by using the three diversity indices. The variation range of the Simpson's diversity index, Shannon-Wiener's diversity index, and Shannon-Wiener's evenness index was 0.096–0.164, 2.320–2.649, and 1.783–2.249, respectively. There was no obvious difference in the trend of bacterial diversity with respect to the altitude (Table 3).

Diversity and phylogenetic analysis of cultivable bacteria

According to the partial sequencing of the 16S rRNA gene, the 173 isolated microorganisms were identified as belonging to four phylogenetic groups, Actinobacteria, Firmicutes, Proteobacteria, and Bacteroidetes. Most of the strains belonged to Actinobacteria (91 strains), Proteobacteria(39 strains) and Firmicutes(32 strains). Only eleven strains

Table 2. Number of cultivable strains distributed in each sample

phylum	Strain No.	Reference taxa	Sample			
			1	2	3	4
Actinobacteria	T-7	*Oerskovia enterophila*	2	0	0	0
	P-5	*Streptomyces coeruleorubidus*	1	0	0	0
	R-7	*Arthrobacter scleromae*	0	0	2	1
	T-21	*Agrococcus lahaulensis*	4	0	0	1
	P-1	*Arthrobacter pascens*	7	21	3	0
	P-8	*Arthrobacter nicotianae*	13	1	2	2
	P-3	*Arthrobacter antarcticus*	2	2	7	1
	T-22	*Micromonospora echinospora*	0	0	2	0
	R-9	*Micromonospora saelicesensis*	0	0	0	1
	B-5	*Nocardia soli*	5	1	0	0
	B-8	*Arthrobacter agilis*	0	2	0	0
	N-8	*Amycolatopsis lurida*	0	0	0	1
	T-9	*Lentzea violacea*	1	6	0	0
Firmicutes	N-2	*Bacillus anthracis*	2	0	0	0
	N-3	*Paenibacillus lentimorbus*	0	0	1	0
	N-13	*Paenibacillus nanensis*	1	0	0	0
	N-25	*Exiguobacterium mexicanum*	0	2	3	0
	N-19	*Bacillus safensis*	2	1	0	0
	R-19	*Planomicrobium koreense*	0	1	0	0
	B-9	*Planococcus antarcticus*	3	1	6	2
	N-23	*Planococcus maritimus*	0	3	1	1
	R-10	*Enterococcus faecium*	2	0	0	0
Proteobacteria	P-21	*Stenotrophomonas rhizophila*	0	1	0	0
	P-10	*Pseudomonas thivervalensis*	2	0	0	0
	R-15	*Pseudomonas brassicacearum*	4	2	2	0
	B-1	*Pseudomonas grimontii*	0	16	0	1
	R-2	*Ensifer meliloti*	0	1	0	0
	R-4	*Ensifer arboris*	0	0	1	0
	N-12	*Rhizobium radiobacter*	2	0	0	0
	P-16	*Mesorhizobium huakuii*	0	0	1	0
	B-11	*Rhizobium vignae*	2	0	0	0
	N-14	*Variovorax paradoxus*	0	2	0	0
	P-20	*Janthinobacterium lividum*	1	1	0	0
Bacteroidetes	N-4	*Olivibacter soli*	0	2	0	0
	P-27	*Chryseobacterium balustinum*	0	0	1	2
	P-11	*Empedobacter brevis*	0	1	0	0
	B-6	*Flavobacterium chilense*	2	2	0	0
	N-17	*Myroides profundi*	0	0	0	1

belonged to Bacteroidetes. After comparison with the known sequences in GenBank, the isolated strains could be divided into the following 27 genera: *Arthrobacter, Oerskovia, Streptomyces, Agrococcus, Micromonospora, Nocardia, Amycolatopsis, Lentzea, Bacillus, Paenibacillus, Exiguobacterium, Planomicrobium, Planococcus, Enterococcus, Stenotrophomonas, Acinetobacter, Pseudomonas, Ensifer, Rhizobium, Mesorhizobium, Variovorax, Janthinobacterium, Olivibacter, Chryseobacterium, Empedobacter, Flavobacterium,* and *Myroides* (Fig.1,Table 2).

The phylogenetic analysis indicated that Actinobacteria (high G+C mol% gram-positive bacteria) was the most dominant phylum with a total of 13 OTUs, and the genus *Arthrobacter* was the phylogenetic group detected in all samples. The highest numbers of strain was observed in *Arthrobacter pascens* (n=31). According to the 16S rRNA gene sequence similarity, the nearest phylogenetic neighbors of strain T-21 were "*Agrococcus lahaulensis* K22-21$^{(T)}$", with 97.85% similarity, while all other standard strains showed less than 97.00% similarities to sequences in the EzTaxon published sequences.

32 isolated strains were defined among the phylum Firmicutes (low G+C mol% gram-positive bacteria) with 9 OTUs. The representative strains B-9 and N-23, belonged to the genus *Planococcus,* were founded in four sampling points. Phylogenetic analysis based on 16S rRNA gene sequences indicated the novel OTU, strain N-2, belonged to the genus Bacillus, but differed from its closest relative, *Bacillus anthracis* ATCC 14578$^{(T)}$, at the species level with 96.97% similarity. the sequence similarities between N-3 and *Paenibacillus lentimorbus* ATCC 14707$^{(T)}$,N-13 and *Paenibacillus nanensis* MX2-3$^{(T)}$, R-19 and *Planomicrobium koreense* JG07$^{(T)}$ were 96.36%, 95.07% and 95.91%, respectively. These four strains may represent new species, since their sequences similarities showed less than 97.00%.

Proteobacteria was the second dominant phylum inferior to the gram-positive bacteria among the cultivable bacteria of the frozen soil in Lake Issyk-Kul basin. The 39 strains of this phylum were clustered into α-Proteobacteria, β-Proteobacteria and γ-Proteobacteria. The bacteria of the genus *Pseudomonas* were common in all sampling points, accounting for 26.09% at sample 2. In the species level, two strains had high similarity with *Pseudomonas thivervalensis*, whereas 8 and 17 strains exhibited high similarity with *Pseudomonas brassicacearum* and *Pseudomonas grimontii*, respectively.

Five OTUs were identified in Cytophaga/Flexibacter/Bacteroides (CFB) phylum, accounting for 6.36% of the total isolated strains. Two strains belonged to Olivibacter, three to Chryseobacterium and one to Empedobacter. The other OTUs were related to the genera Flavobacterium and Myroides. P-11 isolated from wet meadow soil may represent a different OTU possibly being a new species. In addition, N-17 was closely related to *Myroides profundi* D25$^{(T)}$, with a species level similarity of 97.09%.

Table 3. Culturable bacterial diversity indices in the frozen soil samples

Sample	Diversity indices		
	Simpson's diversity index (D)	Shannon-Wiener's diversity index (H)	Shannon-Wiener's evenness index (E)
1	0.096	2.649	2.071
2	0.164	2.320	1.783
3	0.121	2.325	2.087
4	0.102	2.342	2.249

DISCUSSION

In this study, five culture media were selected to isolate bacteria from four typical types of frozen soil in Lake Issyk-Kul basin, Kyrgyzstan. The results indicated that not only the number of bacterial colonies varied, but the diversity of cultivable bacteria and community structure also changed with the ecological environment. The highest numbers of OTUs were detected in samples 1, 2, collected at alpine and wet meadow, where also had the highest number of colonies. These observations may be associated with enrichments of the soils with organic matter and nutrients. In the natural environment, factors such as vegetation, soil type, geographical location, and climatic condition may all associated with the exportation of carbon to the samples necessary for microbial growth, which further affect the composition of microbial community[4,17,30]. The

enrichment of nutrients may result in a higher diversity of cultivable bacteria since these microorganisms, which are typically adapted to environments richer in organic matter in comparison to non-cultivable species, may become dominant members of the community. Meanwhile, the number of colonies in sample 1 was less than that of 2, although the nutrient contents were more abundant in this sample (Table 1), may be related to the fact that most of the bacteria from this phylogenetic

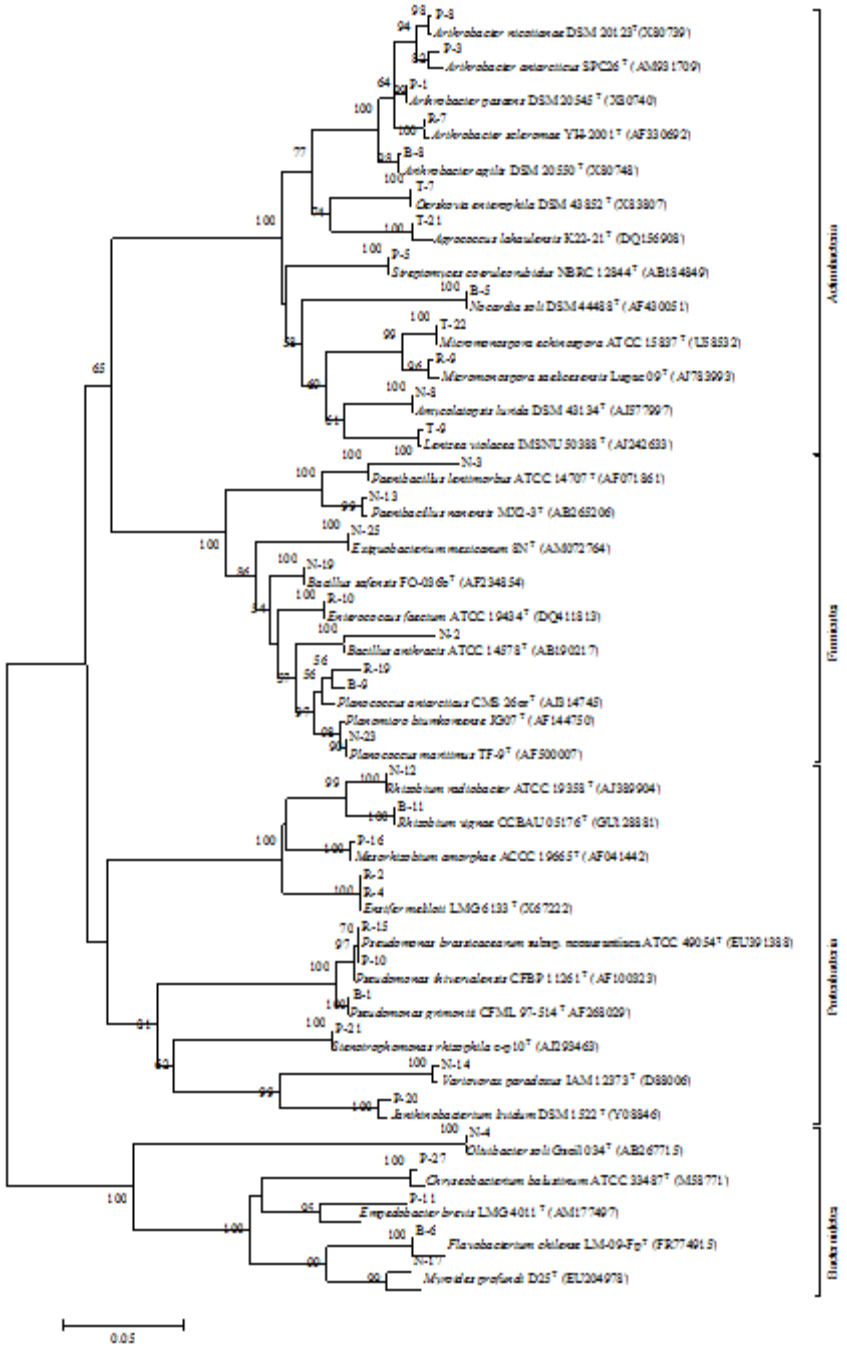

Fig.1. Phylogenetic trees of cultivable bacteria based on the 16S rRNA gene sequences

group are aerobic or facultative anaerobic, and their occurrence is favored on oxygen, while the higher moisture content may lead to a drop in oxygen levels[37].

Most of the research on microbial diversity conducted on frozen soils are based on cultivation-independent methods, which are considered more appropriate, since they allow the detection of the non-cultivable microorganism that are generally constitute the largest fraction of the total microbial community[12,20,35]. However, cultivation-based studies may provide information on the physiological characteristics of the organisms living in the frozen soils, and allow the description of new species and the prospecting for microorganisms of biotechnological interest[32,34]. The microbial diversity in North Pole soil were ranged from 10^3 to 10^8 cells/g, while the number of cultivable microbial were 10^3~10^6 CFU/g, and the major bacterial genera were *Cellulomonas* and *Arthrobacter*[7]. In Hinsa-Leasure's study, Colony counts of the northeast Siberian seacoast permafrost samples, varied between 10^3 and 10^5 CFU/g. Most of the strains belonged to Acidobacteria, Firmicutes, and γ-Proteobacteria, and the dominant genera were *Arthrobacter* and *Planococcus*[10]. Most of the isolated strains were capable of growing under conditions similar to those prevailing at the source of the samples, relating to temperature and nutrient concentrations.

Microorganisms belonging to Actinobacteria were dominant in the samples studied. This group and Firmicutes comprise the Gram-positive bacteria. This is not unexpected considering that bacteria from this phylogenetic group are among the most known and readily cultivable microorganisms from the permofrost environment[1,6]. In the culture–independent study of Susanne et al. (2008) conducted at Lena Delta, Siberia, Actinobacteria was also the dominant group, although most of the phylotypes identified belonged to not yet cultivated species[29]. Bacteroidetes was the group isolated from all the samples in this work. According to Shannon's report this group yielding many cultivates that are cold-adapted, psychrophiles, or psychrotrophs, and the strains of this phylum are able to utilize a large number of carbohydrates. Also, relative to other strains, cold-adapted Bacteroidetes are often enriched in either anteiso- or unsaturated cellular fatty acids, compounds known to enhance the ability of microbial cells to adapt to low temperatures[19]. So 11 strains belonged to the Bacteroidetes are capable of surviving under extreme conditions such as those permafrost soils in Issyk-Kul basin. The physiological and chemical properties of these bacteria may allow them to successfully compete with other soil bacterial groups. However, Acidobacteria, which was the dominant population isolated from the frozen soil regions in the South Pole and Siberia, was not isolated from this region[22,29].

The 16S rRNA gene sequences revealed that our isolates were predominantly *Arthrobacter*, *Planococcus,* and *Pseudomonas*. Other works also separated these genera from Canada, Siberia, Antarctica, and China in culture-dependent and -independent studies[3,20,28,31,33]. This work further supports that the genera *Arthrobacter*, *Planococcus,* and *Pseudomonas* are well adapted for cold environments and both genera are relatively easy to culture. Unlike a majority of the previous permafrost studies, we did not identify *Rhodococcus* and *Cellulomonas* isolated from our sample, which were the dominant genera in the North Pole, this could be due to our limited isolate sample size or these microorganisms are not dominant in these particular permafrost samples[24].

On the species level, the bacterial community in permafrost soils was found to be highly diverse. This observation is supported by related studies on high Arctic soils from Norway and Canada, in which bacterial diversities that partly even exceeded those of boreal forest soils were reported[30]. Our results also point to species-level diversities higher than those of boreal forests and tundra, habitats that are similar to that studied in this work (Shannon indices between 2.3 and 2.6). The ability to produce endospores and the great metabolic and physiological diversity are two characteristics of the endospore-forming bacteria (EFB) that allow their distribution in all environments of our planet[19], *B.anthracis* and *B. safensis* were isolated in Issyk-Kul basin, and this may explain the detection of these bacteria in samples analyzed in our study.

Five strains (N-2, N-3, N-13, R-19 and P-11), the similarities of 16S rRNA gene sequences compared with the corresponding type species

were lower than 97.00%, might be potential new species, which also implies that this fraction of the microbial community is unique, and that might contain some other undiscovered microorganisms in this region. N-25, B-9 and N-2 were classified as *Exiguobacterium* and *Planococcus*, which have been widely found in many low-temperature environments. These strains have the ability to utilize substrates at low temperature, which is important for bioremediation, and might have broad application in low-temperature region. Furthermore, the similarity between P-16 and *Mesorhizobium amorphae* ACCC19665^T that could fix nitrogen element was 99.72%. Maybe this species has the ability to transform atmospheric nitrogen and increase the nitrogen content in the soil at the primary bare land where deficient nutrition.

In summary, a preliminary exploration of microflora at four high altitude soil samples in Lake Issyk-Kul basin, Kyrgyzstan was carried out in this study. A total of 173 strains were isolated, and belonged to 27 genera, suggesting that the microbial resources are abundant in this region. In future studies, the relationship between the microbial diversity of frozen soil and external factors such as plant types, climate, and geographical environment will be demonstrated by combining pure culture and molecular biological methods. It is expected that a higher number of strains and metabolites could be isolated.

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REFERENCES

1. Aislabie JM, Chhour KL, Saul DJ, Miyauchi S, Ayton J, Paetzold RF, Balks MR. Dominant bacteria in soils of Marble Point and Wright valley, Victoria Land, Antarctica. *Soil Biology Biochemistry*, 2006; **38**:3041-3056.
2. Alamanov A, Mikkola H. Is biodiversity friendly fisheries management possible on Issyk-Kul Lake in the Kyrgyz Republic? *Ambio.*, 2011;**40**: 479-495.
3. Bai Y, Yang D, Wang J, Xu S, Wang X, An LÿMicrobiol R. Phylogenetic diversity of culturable bacteria from alpine permafrost in the Tianshan Mountains, northwestern China. *Res Microbiol.*, 2006; **157**:741-751.
4. Bowers RM, McLetchie S, Knight R, Fierer N. Spatial variability in airborne bacterial communities across land-use types and their relationship to the bacterial communities of potential source environments. *ISME J*., 2011; **5**:601-612.
5. Chun J, Lee JH, Jung Y, Kim M, Kim S, Kim BK, Lim YW. EzTaxon: a web-based tool for the identification of prokaryotes based on 16S ribosomal RNA gene sequences. *Int. J. Syst. Evol. Microbiol.* , 2007; **57**: 2259-2261.
6. Ganzert L, Lipsk A, Hubberten HW, Wagner D. The impact of different soil parameters on the community structure of dominant bacteria from nine different soils lacated on Livingston island, South Shetland Archipelago, Antarctica. *Microbiology Ecology*, 2011;**76**: 476-491.
7. Hansen AA, Herbert RA, Mikkelsen K, Jensen LL, Kristoffersen T, Tiedje JM, Lomstein BA, Finster KW. Viability, diversity and composition of the bacterial community in a high Arctic permafrost soil from Spitsbergen, Northern Norway. *Environ. Microbiol.*, 2007; **9**: 2870-2884.
8. Heringa J. Two strategies for sequence comparison: profile-preprocessed and secondary structure-induced multiple alignment. *Comput Chem.*, 1999; **23**: 341-364.
9. Higgins J, Camp P, Farrell D, Bravo D, Pate M, Robbe-Austerman S. Identification of *Mycobacterium* spp. of veterinary importance using rpoB gene sequencing. *BMC Vet Res.*, 2011;**7**: 77.
10. Hinsa-Leasure SM, Bhavaraju L, Rodrigues JL, Bakermans C, Gilichinsky DA, Tiedje JM. Characterization of a bacterial community from a Northeast Siberian seacoast permafrost sample. *FEMS Microbiol Ecol.*, 2010; **74**: 103-113.
11. Hirsch P, Gallikowski CA, Siebert J, Peissl K, Kroppenstedt R, Schumann P, Stackebrandt E, Anderson R. *Deinococcus frigens* sp. nov., *Deinococcus saxicola* sp. nov., and *Deinococcus marmoris* sp. nov., low temperature and draught-tolerating, UV-resistant bacteria from continental Antarctica. *Syst Appl Microbiol.*, 2004; **27**: 636-645.
12. Humbert S, Tarnawski S, Fromin N, Mallet MP,

Aragno M, Zopfi J. Molecular detection of anammox bacteria in terrestrial ecosystems: distribution and diversity. *ISME J*., 2010; **4**: 450-454.

13. Kim JK, He D, Liu QM, Park HY, Jung MS, Yoon MH, Kim SC, Im WT. *Novosphingobium ginsenosidimutans* sp. nov., with the ability to convert ginsenoside. *J. Microbiol Biotechnol.* , 2013; **23**: 444-450.
14. Kobabe S, Wagner D, Pfeiffer EM. Characterisation of microbial community composition of a Siberian tundra soil by fluorescence in situ hybridisation. *FEMS Microbiol Ecol*., 2004; **50**:13-23.
15. Li M, Feng H, Yang Z, Liu C, Xia X, Wang C, Jiang L, Jiang H. Diversity of culturable bacteria in the typical frozen soil areas in China. *Wei Sheng Wu Xue Bao*, 2011; **51**: 1595-1604.
16. Li Q, Peng Z, Chen X, Sun X, Pan Y, Zhao Y. Selection of reference genes for virulence gene expression in Vibrio parahaemolyticus. *Wei Sheng Wu Xue Bao* , 2013; **53**: 306-312.
17. Liebner S, Harder J, Wagner D. Bacterial diversity and community structure in polygonal tundra soils from Samoylov Island, Lena Delta, Siberia. *Int Microbiol.* , 2008; **11**: 195-202.
18. Malicka E, Sitko R, Zawisza B, Heimann J, Kajewski D, Kita A. Nondestructive analysis of single crystals of selenide spinels by X-ray spectrometry techniques. *Anal Bioanal Chem* , 2011; **399**: 3285-92.
19. Marcus ACdS, Angélica C, Ananda S, Daniele CR. Phylogenetic identification of marine bacteria isolated from deep-sea sediments of the eastern South Atlantic Ocean. *SpringerPlus* , 2013; **2**: 127.
20. Martineau C, Whyte LG, Greer CW. Stable isotope probing analysis of the diversity and activity of methanotrophic bacteria in soils from the Canadian high Arctic. *Appl Environ Microbiol* , 2010; **76**: 5773-5784.
21. Nosrati K. Assessing soil quality indicator under different land use and soil erosion using multivariate statistical techniques. *Environ Monit Assess.*, 2013; **185**: 2895-2907.
22. Ochsenreiter T, Selezi D, Quaiser A, Bonch-Osmolovskaya L, Schleper C. Diversity and abundance of Crenarchaeota in terrestrial habitats studied by 16S RNA surveys and real time PCR. *Environ Microbiol.*, 2003; **5**: 787-797.
23. Olefeldt D, Turetsky MR, Crill PM, McGuire AD. Environmental and physical controls on northern terrestrial methane emissions across permafrost zones. *Glob Chang Biol.*, 2013; **19**: 589-603.
24. Onishchenko A, Zhukovsky M, Veselinovic N, Zunic ZS. Radium-226 concentration in spring water sampled in high radon regions. *Appl Radiat Isot.*, 2010; **68**: 825-827.
25. Pascale DD, De Santi CD, Fu J, Landfald B. The microbial diversity of Polar environments is a fertile ground for bioprospecting. *Mar Genomics*, 2012; **8**: 15-22.
26. Ren J, Yan B, Hong K. Comparison of bacterial and archaeal community of mangrove soil under different vegetation in Dongzhaigang, Hainan Island. *Wei Sheng Wu Xue Bao*, 2012; **52**: 736-743.
27. Ricketts RD, Johnson TC, Brown ET, Rasmussen KA, Romanovsky VV. The Holocene paleolimnology of Lake Issyk-Kul, Kyrgyzstan: trace element and stable isotope composition of ostracodes. *Palaeogeography, Palaeoclimatology, Palaeoecology*, 2001; **176**: 207–227.
28. Stomeo F, Makhalanyane TP, Valverde A, Pointing SB, Stevens MI, Cary CS, Tuffin MI, Cowan DA. Abiotic factors influence microbial diversity in permanently cold soil horizons of a maritime-associated Antarctic Dry Valley. *FEMS Microbiol Ecol.*, 2012; **82**: 326-340.
29. Susanne Liebner, Jens Harder, Dirk Wagner. Bacterial diversity and community structure in polygonal tundra soils from Samoylov Island, Lena Delta, Siberia. *International Microbiology*, 2008; **11**:195-202.
30. Vishnivetskaya TA, Petrova MA, Urbance J, Ponder M, Moyer CL, Gilichinsky DA, Tiedje JM. Bacterial community in ancient Siberian permafrost as characterized by culture and culture–independent methods. *Astrobiology*, 2006; **6**: 400-414.
31. Wagner D, Kobabe S, Liebner S. Bacterial community structure and carbon turnover in permafrost-affected soils of the Lena Delta, northeastern Siberia. *Can J. Microbiol* , 2009;**55**: 73-83.
32. Wilson SL, Walker VK. Selection of low-temperature resistance in bacteria and potential applications., 2010; **31**: 943-956.
33. Xu YL, Wang DW, Shi XW, Zheng XJ, Zhou H, Liu Y, Ni YQ. Selective isolation and diversity of cold-adapted lipase-producing strains from permafrost soil at the terminus of a glacier in the Tianshan Mountains. *Wei Sheng Wu Xue Bao*, 2011; **51**: 233-240.
34. Yang S, Wen X, Jin H, Wu Q. Pyrosequencing investigation into the bacterial community in permafrost soils along the China-Russia Crude Oil Pipeline (CRCOP). *PLoS One* , 2012;**7**: e52730.

35. Zhang G, Ma X, Niu F, Dong M, Feng H, An L, Cheng G. Diversity and distribution of alkaliphilic psychrotolerant bacteria in the Qinghai-Tibet Plateau permafrost region. *Extremophiles* , 2007;11:415-424.
36. Zhang M, Gu YL, Xu YL, Shi XW, Zheng XJ, Zhou H, Ni YQ. Phylogenetic and physiological diversity of cold-adapted bacteria producing beta-galactosidase from permafrost sediments of the bottom layer of the Glacier No. 1 in the Tianshan Mountains. *Wei Sheng Wu Xue Bao*, 2011; **51**: 1605-1615.
37. Zhang XF, Zhao L, Xu SJ, Liu YZ, Liu HY, Cheng GD. Soil moisture effect on bacterial and fungal community in Beilu River (Tibetan Plateau) permafrost soils with different vegetation types. *J. Appl Microbiol*, 2013; **114**: 1054-1065.