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Investigation into the Biodiversity of Low-Temperature Microorganisms in Frozen Soil of the Tianshan Mountain in China

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Studying microbial diversity in the frozen soil is crucial to screen and utilize the cold-adapted microorganisms. In this paper, selected the Tianshan permafrost in the unique geographical environment in Xinjiang of China as study material, then diversity of the frozen soil microorganism were determined by pure culture and sequencing. The results indicated that, among 68 isolated strains, 65.2% of them belong to Gram-positive bacteria, and the colony colors were mainly creamy white, lemon yellow, white. The optimum growth temperature of the isolated microorganisms were 20-24°C, the majority of them are cold-adaptable bacteria. after the 16S rDNA sequence analysis, the selected representative strains were subjected to the phylogenetic analyses, these strains cultivable at low temperature belong to 6 phylogenetic groups of α-*proteobacteria* subclass, β-*proteobacteria* subclass, γ-*proteobacteria* subclass, Gram-positive bacteria, positive bacteria and CFB, among them, α-, β-, γ-*proteobacteria* was the dominant flora. *Pseudomonas* spp. is the dominant genus, which accounts for the highest percentage in the grains. We can conclude that permafrost of the Tianshan Mountain can nurture rich diversity of microorganisms at low temperature, and may be viewed as a good source for screening low-temperature microorganism.

Key words: Tianshan permafrost, Cold-adapted microorganism, Phylogenetic diversity.

Many studies have been focused on investigating into the characteristics of organisms living in extreme conditions at present [1]. The investigation into the biodiversity and physiological and ecological characteristics of microorganisms living in extreme conditions can provide new basis for revealing the history of biological evolution and the mysteries about the origin of life. The investigation also promotes the knowledge of men about the resources of microorganisms on the earth [2]. The frozen soil of the Tianshan Mountain belongs to the high-altitude, high-mountain permafrost of our country, which distributes in the Tianshan Mountain Glacier Observation Station and the ice-free cirque area at the headwaters of Urumqi River with the altitude of over 3250 m [3]. Because the frozen soil of the Tianshan Mountain is located in the cold area characterized by low temperature, strong radiation and freezing-and-thawing the low-temperature microorganisms there have the corresponding characteristics for adapting to low temperature, strong radiation, freezing-and-thawing and oligotrophic conditions [4]. Frozen soil is called the treasury of the resources of low-temperature microorganisms, therefore separating and purifying low-temperature microorganisms from frozen soil of the Tianshan Mountain will give a hefty boost to the efforts of the scientific community to screen and utilize the resources of psychrophilic and psychrotrophic microorganisms.

Studies abroad had got an early start on the microorganisms in the frozen soil. In 1911, Russian scientist Omelyansky first reported there were biologically active microorganisms in the

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frozen soil when he was investigating into the frozen soil of Siberia [5]. In the 1930s and the 1940s, scientists of former Soviet Union also found biologically active microorganisms in the frozen soil of Lake Baikal, North Ural, Central Yakutsk and Arctic islands [6]. Between 1950 and 1970, after extensive investigations into the microorganism colonies in the soil of the North Pole, and the frozen soil of Canada and North Alaska, scientists found that there were many kinds of microorganisms and many physiological groups in the soil of the North Pole [7-10]. In 1974, Cameron et al first reported microorganisms which had lived at below -14°C before Late Pleistocene in the frozen soil of the South Pole [11]. In recent years, many new species of bacteria from frozen soil have been separated and identified [12-14]. Psychrotrophic microorganisms from the frozen soil of the peninsula at the shore of Laptev Sea to the Northeast of Siberia have been separated [15]; the strains of microorganism from the frozen soil and ice core of the arctic high-altitude area of Canada have been separated, but the diversity was low [16]; microorganisms which produce enzymes from the glacier and frozen soil of Rahul and Spiti of India in the West Himalayan region have been separated [17]. Preliminary collection, investigation and research on resources of low-temperature microorganisms (mainly microorganisms of the South Pole and in deep sea) have been started in our country since the early 1990s. Researches on the separation and cultivation of permafrost of the Qinghai-Tibet Plateau have shown that there are microorganisms in the permafrost and they have special mechanisms of physiological activity [18]. It is also shown that strains of psychrotrophic microorganism can be separated from the permafrost at the headwaters of Urumqi River in Tianshan Mountain [3].

Thus it can be seen that there are more studies overseas about microorganisms in frozen soil, whereas some achievements have been made in relevant studies in China, which are mainly studies on microorganisms in frozen soil of Qinghai-Tibet Plateau [18,19]. There are a small number of reports on microorganisms in frozen soil of the Tianshan Mountain, which are mainly based on studies on genetic diversity and phylogenetic relationship. But there is a lack of accuracy with regard to identification of microbial population. No extensive investigation into the biodiversity of microorganisms in frozen soil of the Tianshan Mountain by means of combining morphological characteristic, physiological and biochemical reactions molecular genetics and phylogenetic relationship has been reported.

Microorganisms in frozen soil are unique biological resources and precious materials for scientific study, which are conferred to men by the nature. This study on microorganisms in high-mountain frozen soil has been based on the unique geographical environment of Xinjiang province of China. It selected frozen soil of the Tianshan Mountain as the medium and combined the technologies and means home and abroad for the study of the microorganisms in high-mountain frozen soil. Through the pure cultivation method to separate low-temperature microorganisms in frozen soil and determine their diversity by sequencing, which would promote to establish a library of microorganisms in frozen soil of the Tianshan Mountain by means of metagenomic technologies.

The study investigated the morphological characteristic, physiological and biochemical characteristics and genetic diversity to reveal the phylogenetic relationship of frozen soil of the Tianshan Mountain. It aims to improve the level of knowledge about low-temperature microorganisms and provide scientific basis for their screening and utilization by finding new characteristics of life from microorganisms in frozen soil of the Tianshan Mountain.

MATERIALS AND METHODS

Collection of sample

The sample of frozen soil was taken from the permafrost in the ice-free cirque near the Tianshan Mountain Glacier Observation Station at the altitude of 3671 m. The geographic coordinates were 43°6.78´N, 86°50.517´E. Sections of frozen soil at the depth of 3 m were dug out, with the interval at 15 cm. The samples were collected in a sterile way and put into sealed preservation boxes pretreated with high temperature sterilization. The samples were then transferred to laboratory under low temperature and kept at -20°C.

Isolation and culture of microorganisms

The sample of frozen soil was taken out from the refrigerator (-20°C) and put under the

temperature of 4°C to unfreeze. 2 g of sample was taken in super clean bench and put into a sterilized triangular flask containing 18 ml of saline (0.85%) and glass beads. Then the sample was shaken at 250 r•min^{-1} for 15 min and the suspension diluted with gradient dilution. 200 ¼l of soil suspension with the dilution concentration of 10^{-3} and 10^{-4} was taken and spread over the oligotrophic PYGV (peptone, yeast extract, glucose and varied vitamin) medium plate respectively and cultivate away from light at 25°C. Studies were undertaken at each gradient concentration and the samples were observed under Olimpus fluorescence microscope 7 days after cultivation, repeated 3 times. The colonies were purified and cultivated in suspension with starkly different forms as manifested by the size, color, glossiness and shape of the edge of the colonies in fresh PYGV medium and LB medium (20%), and they were kept in fresh sterile LB medium (-70°C) containing 20% glycerol or 7% dimethyl sulfoxide [21].

Morphological, physiological and biochemical characteristics of microorganisms

The morphological characteristics were examined under optical microscope with cover slip-buried method, the identification of physiological and biochemical characteristics were done with reference to the methods in the 9th edition of *Bergey's Manual of Systematic Bacteriology* [22].

Construction of phylogenetic tree

The extraction of genomic DNA was done with reference to the methods for the preparation of bacterial genome in the 3rd edition of *Current Protocols in Molecular Biology* [22], and the genome was amplified with primers 16SF (5'-AGA GTT TGA TCC TGG CT CAG-3') and 16SR (5'-AAG GAG GTG ATC CAG CC GCA-3'). The conditions for PCR were: initial denaturation at 94°C for 2 min, denaturation at 94°C for 1 min, annealing at 54°C for 1 min, extension at 72°C for 1.5 min, with a total of 30 cycles; extension at 72°C for 10 min. The PCR products were separated by agarose gel electrophoresis and purified with cutting gel. The purified products were cloned into sequencing vector pMD18-T and transformed into competent Escherichia coli cells DH5±. Then positive clones were screened for sequencing.

The 16S rDNA fragments representing different restrictive enzymatic maps were sequenced with universal primers 27F (5c-AGA GTT TGA TCC TGG CT CAG-3c), 517F (5c-CCA GCA GCC GCG GT AAT-3c) and 907F (5c-AAA CTC AAA TGA ATT GAC GGG-3c) with ABI 370 DNA Sequencer (Applied Biosystems, USA) [23]. Sequencing was completed by Beijing Aolaibo Biotech Co., Ltd. The spliced sequencing results were compared to the data in NCBI (http://www.ncbi.nlm.nih.gov/) and RDP II (http://rdp.cme.msu.edu/) and the most similar sequences were downloaded. Comparison of sequences and treatment to equal length were conducted with ClustalW (Version 1.81, available from http://www.ebi.ac.uk/clustalw) embedded in MEGA3. Calculation of distance was done and phylogenetic tree was constructed with NJ (Neighbor joining) method.

Results and analysis

Morphological characteristics of microorganisms in frozen soil of the Tianshan Mountain

Preliminary Examination of the characteristics of colonies of the purified strains such as colony form, color, cellular form, Gram stain and liquid culture found that of the 68 cultivable strains, 65.2% were Gram-positive bacteria, 34.8% were Gram-negative bacteria, and the number of Gram-positive bacteria was twice that of Gram-negative bacteria. Of the cultivable bacteria in frozen soil of the Tianshan Mountain, 68.4% of the colonies presented with the colors such as lemon yellow, creamy white, and orange. Of the colorful colonies, over 50% presented as being lemon yellow. The cells of the strains presented mainly with the characteristic shape such as circular protuberance, wet protuberance, wet and flat, with irregular edge and with snowflake-liked edge. Characteristics of the representative strain are as shown in Table 1.

Adaptability of isolated strains to growth temperature

Biochemical reaction processes are determined by temperature, and relatively low temperature is the best condition for storage of microbial cells. The maximum growth temperature for the cultivable microorganisms in frozen soil of the Tianshan Mountain was 37°C, while the optimal growth temperature was 18-30°C. And they could even grow and multiply at 4°C. Most of the bacteria (91.4%) were psychrotrophs, 2 strains of psychrophiles b16 and c4 were also isolated. The strains could grow at 4°C, with the optimal growth

Table 1. Features of the strains isolated from the Tianshan mountains permafrost

Strain	Morphological characteristics
a1	Yellowish, humid, irregular edge, 2-3 mm
a2	White, neurite, round, < 1 mm
a3	Lemon yellow, neurite, round, 1-3 mm
a4	Lemon yellow, neurite, humid, 0.5-2 mm
a5	Lemon yellow, flat humid, 0.5-2 mm
a6	Lemon yellow, neurite, humid, 0.5-3 mm
a7	White, flat irregular edge, 2-3 mm
a8	Creamy white, neurite, regular edge, 0.5-2 mm
a9	Orange, few colony, 0.5-1 mm
a10	White, few colony, 0.5-1 mm
a11	Lemon yellow, irregular edge, 0.5-2 mm
a12	Lemon yellow, neurite, regular edge, 0.5-1 mm
a13	White, snow shaped edge, 0.5-1 mm
a14	Lemon yellow, neurite, regular edge, 0.5-2 mm
a15	Lemon yellow, humid, < 1 mm
a16	Lemon yellow, oily, 0.5-1 mm
a17	Creamy white, oily, 0.5-1 mm
a18	Creamy white, neurite, 0.5-2 mm
a19	Lemon yellow, neurite, humid, 0.5-1 mm
b1	Creamy white, neurite, humid, < 1 mm
b2	Lemon yellow, neurite, humid, < 1 mm
b3	Lemon yellow, oily, 0.5-3 mm
b4	White, eminent, < 0.5 mm
b5	Lemon yellow, flat, 0.5-2 mm
b6	Creamy white, neurite, 1 mm
b7	Yellow neurite, humid, 0.5-1 mm
b8	Creamy white, eminent, 0.5-2 mm
b9	Lemon yellow, flat, 0.5-2 mm
b10	Lemon yellow, flat, 0.5-1 mm
b11	Lemon yellow, flat, 0.5-1 mm
b12	Lemon yellow, neurite, 0.5-2 mm
b13	Orange, round regular edge, < 1 mm
b14	Lemon yellow, eminent, 0.5-1 mm
b15	Lemon yellow, flat, 0.5-2 mm
b16	Creamy white, clear, neurite, 1 mm
b17	Orange, round regular edge, < 1 mm
b18	Lemon yellow, neurite, regular edge, 0.5-1 mm
b19	Red, neurite, < 1 mm
c1	White, clear, 0.5-1 mm
c2	Orange, neurite, 1-2 mm
c3	White, < 0.5 mm
c4	Lemon yellow, neurite, humid, 0.5-2 mm
c5	Brick, neurite, regular edge, 0.5-1 mm
c6	Maize yellow, neurite, humid, 0.5-2 mm
c7	White, neurite, 0.5-1 mm
c8	Creamy white, humid, 1-2 mm
c9	Creamy white, neurite, humid, 0.5-1 mm
c10	Lemon yellow, flat humid, 1-3 mm
c11	Orange, snow shaped edge, neurite, 0.5-1 mm
c12	Orange, neurite, < 1 mm
c13	Pink, neurite, humid, < 1 mm
c14	Creamy white, neurite, 0.5-1 mm
c15	White, neurite, humid, 0.5-1 mm
d1	Orange, oily, 1-3 mm
d2	Lemon yellow, neurite, humid, 0.5-2 mm
d3	Brick, neurite, < 1 mm
d4	Orange, neurite, humid, 0.5-2 mm
d5	Creamy white, neurite, humid, < 1 mm
d6	Lemon yellow, neurite, humid, < 1 mm
d7	Creamy white, neurite, regular edge, 0.5-2 mm
d8	Pink, neurite regular edge, 0.5-2 mm
d9	Lemon yellow, flat, humid, 1-3 mm
d10	Brick, neurite, 0.5-1 mm
d11	Creamy white, neurite, humid, < 1 mm
d12	White, snow shaped edge, 0.5-1 mm
d13	Lemon yellow, neurite, humid, < 1 mm
e1	White, neurite, humid, 0.5-1 mm
e2	Lemon yellow, irregular edge, 0.5-1 mm

temperature at about 12°C, and the maximum growth temperature at lower than 20°C. Other strains could grow in a wider range of temperature. For example, the range of growth temperature for strains a9, c3, b2 and c9 was 4-30°C; a11 could even grow in the temperature range of 4-37°C (Table 2).

Diversity analysis of microorganisms

Amplification of the 16S rDNA of isolated strains

The strains were verified by PCR on 16S rDNA and agarose gel electrophoresis on amplification products. The product of PAR reaction was amplified by PCR to form a characteristic band, and then the PCR product was sequenced. The size of the amplification products was consistent with the size that is expected from the 16S rDNA theoretically, which was about 1.5 kb. The results show the fragment obtained from amplification is the target 16S rDNA fragment.

Phylogenetic analysis of isolated strains

68 low-temperature strains were obtained from the sample of frozen soil of the No. 1 Glacier of the Tianshan Mountain. Figure 1 shows the phylogenetic tree analysis of the representative strains. It can be known from the phylogenetic tree that cultivable microorganisms in frozen soil of the Tianshan Mountain belong to the following 6 phylogenetic groups respectively: α-*proteobacteria*, β-*proteobacteria*, γ-*proteobacteria*, high G+C Gram positives (*Firmicute*), low G+C Gram positives and CFB (*Cytophaga-Flavobacterium-Bacteroides*).

It can be seen from Figure 1 that the

Table 2. Gram staining and optimum temperature of the isolated strains

Strain	Gram stain	Optimal temperature (°C)
a1	G-	24
a2	G-	18
a3	G+	18
a4	G-	30
a5	G+	24
a6	G-	24
a7	G-	18
a8	G+	30
a9	G-	24
a10	G+	24
a11	G+	18
a12	G-	18
a13	G-	24
a14	G+	24
a15	G-	18
a16	G-	30
a17	G-	18
a18	G-	18
a19	G+	24
b1	G+	24
b2	G+	18
b3	G+	18
b4	G-	30
b5	G+	24
b6	G-	24
b7	G-	24
b8	G-	18
b9	G+	18
b10	G-	18
b11	G+	24
b12	G+	24
b13	G-	24
b14	G-	18
b15	G-	24
b16	G+	12
b17	G+	18
b18	G+	18
b19	G-	24
c1	G-	24
c2	G+	18
c3	G-	18
c4	G-	12
c5	G-	24
c6	G-	18
c7	G+	18
c8	G+	24
c9	G+	24
c10	G+	18
c11	G-	24
c12	G+	30
c13	G-	18
c14	G+	18
c15	G+	18
d1	G+	18
d2	G-	24
d3	G+	24
d4	G+	24
d5	G+	24
d6	G+	18
d7	G-	18
d8	G+	24
d9	G+	18
d10	G+	18
d11	G+	24
d12	G+	24
d13	G-	30
e1	G+	18
e2	G+	18

strains b3 and b9 have the closest phylogenetic relationship with the known species *ArthrobaAter*, and the two strains show 100% sequence identity compared with *Arthrobacter oxydans*Z1369. The strains of this genus do not form spore and they grow the best in the neutral and slightly alkaline conditions. They are strictly aerobic and almost exist in all cold environments. The sequence similarity of a4 to that of *Kocuria rosea* BCT-6 reaches over 99%. The strains b2 and d4 show over 98% identity compared with the known bacteria *Cryobacterium* sp. ZS1-15 and *Streptomyces cavourensis* xsd08096 respectively. The strains c9, c13 and d6 all belong to the *Brevundimonas* genus, a genus which degrades dibenzofuran and some strains of which can degrade hydrocarbons and phenol according to study. The low-temperature strains described above all belong to high G+C Gram positives. Strains c4, a19, b15, a5, b5, a7, c15, c7 all belong to the *Brevundimonas* genus, which constitute the biggest branch of the phylogenetic tree of the microflora in frozen soil of the Tianshan Mountain together with the reference sequences. d1 is assorted with the *Flavobacterium* genus in the phylogenetic tree. In addition, the findings show that there are 10 pseudomonas, 3 CFB strains, 2 low G+C Gram positive strains and 4 grains belonging to β-*proteobacteria*, all contribute greatly to the diversity of psychrophilic microorganisms in frozen soil of the Tianshan Mountain.

Biodiversity analysis of cultivable microorganisms in frozen soil of the Tianshan Mountain

The isolated cultivable microorganisms in frozen soil of the Tianshan Mountain distributed in 6 phylogenetic groups. Among them *Alphaproteobacteria* and *Firmicute* each contained 2 genera; β-*proteobacteria* and γ-*proteobacteria* each contains 1 genus; *Actinobacteria* contained 4 genera, which is the greatest number; what followed was CFB, which contained 3 genera. The information above shows that the frozen soil of the Tianshan Mountain has a rich reserve of various resources of microorganism. It can be known from Table 3 that *Pseudomonas* spp. is the dominant genus, which accounts for the highest percentage in the grains producing cold-active enzymes and thus contributes the most to the biodiversity of cultivable psychrotrophic microorganisms producing cold-active enzymes in frozen soil. What follow are *Rhodococcus* and *Brevundimonas*. Of the 6 major floras producing cold-active enzymes of α-proteobacteria, β-*proteobacteria* and γ-*proteobacteria* is the dominant flora which account for the highest percentage in the 6 major phylogenetic groups where the grains distribute.

Discussion and conclusion

A total of 68 cultivable strains were isolated from the sample of frozen soil of the Tianshan Mountain. 65.2% of the strains were Gram positive, 34.8% were Gram negative. Of the microorganisms in the frozen soil of Siberia in the study by Shi et al, 55% were Gram negative. Balkwill found 86% of the strains of deeply-underground microorganisms which had been reported were Gram negative[24]. Whereas the microorganisms isolated from the frozen soil of the Qinghai-Tibet Plateau in the study by Feng Huyuan et al were mainly Gram positive [19]. Therefore it can be seen that the difference in the constitution of microbial community is to a great degree determined by their environment for living. The thicker cells of Gram positive bacteria can adapt better to the low-temperature environment of the Tianshan Mountain, which may be associated to their adaptability to cold. 68.4% of the cultivable bacteria in the frozen soil of the Tianshan Mountain presented as being lemon yellow, creamy white and orange, which may be because in low-

Table 3. Comparison of frozen soil cultivable microbial flora

Group	Genus	Typical stains	Frequencies	Nearest strain	Similarity(%)	Accession No.
Actinobacteria	*Rhodococcus*	c9	7	*Rhodococcus kroppenstedtii* K22-05	99	GQ153645
	Arthrobacter	b3	3	*Arthrobacter oxydans* strain Z1369	98	EU086823
	Kocuria	a4	4	*Kocuria rosea* BCT-6	99	DQ015980
	Flavobacterium	d1	2	*Flavobacterium xinjiangensis* 1.2748	99	AF433172
CFB	*Hymenobacter*	c7	3	*Hymenobacter roseosalivarius* AA718	96	NR029359
	Chryseobaeterium	d5	4	*Chryseobacterium sp.* TM3_8	98	DQ279360
	Flavobacterium	d1	6	*Flavobacterium xinjiangensis* 1.2748	99	AF433172
Firmicute	*Paenibaillus*	a1	4	*Paenibacillus polymyxa* EBL2071	99	EF545556
	Enterococcus	d11	2	*Enterococcus faecalis* JCM 20313	98	AB507170
Alphaproteobacteria	*Brevundimona*	c4	9	*Brevundimonas sp.* V4.BP.05	99	AJ244710
	Methylobacterium	d7	6	*Methylobacterium sp.* GW2	99	FP103042
Betaproteobacteria	*Duganella*	d10	5	*Duganella zoogloeoides* IAM 12670	99	NR025833
Gammaproteobacteria	*Pseudomona*	b8	11	*Pseudomonas reactans* strain CAI-4	99	DQ257418

temperature environment, colorful organisms can better absorb radiation from sun and use it to raise their temperature and improve cold adaptability; some pigments also have the effect of shielding ultraviolet so as to protect microorganisms in high-altitude glacier.

Temperature is an important environmental factor influencing the growth and function of microorganisms, and it is also one of the key factors in the regulation of geochemical process of many organisms in the terrestrial ecosystem. According to the minimum, optimal and maximum growth temperatures, the microorganisms isolated from the frozen soil can be divided into the 2 categories, namely psychrophilic microorganism (with the 3 values < 0, < 15 and < 20) and psychrotrophic microorganism (with the 3 values < 0, > 15 and > 20)[25]. For the cultivable microorganisms in the frozen soil of the Tianshan Mountain, the maximum growth temperature was lower than 37°C, some strains could grow and multiply at a temperature as low as 4°C; for the vast majority of the strains, the optimal growth temperature was 18-24°C, so they belong to psychrotrophic bacteria. In previous studies in the North Pole and Siberia with the temperature of - 9~

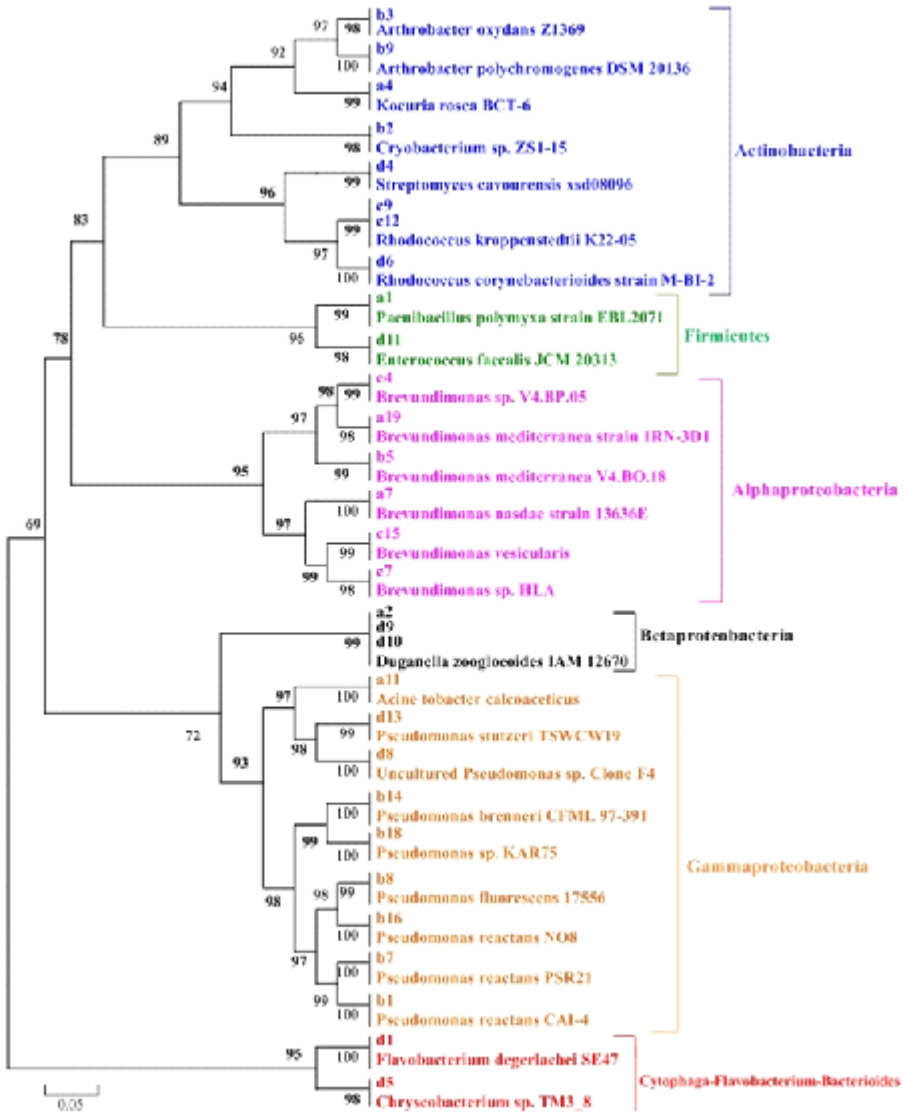

Fig. 1. Phylogenetic tree of the representative strains

-12°C and the South Pole with the temperature of -25 ~ -30°C, cultivable microorganisms were obtained in the permafrost[15, 26, 27]. The growth temperature of cultivable microorganisms obtained in the frozen soil of the Tianshan Mountain was obviously higher than that of the areas mentioned above. Microorganisms in the frozen soil of the Tianshan Mountain could adapt to environment with wider range of temperature, whether it is due to the direct or indirect influence of lasting global warming still needs further confirmation. The long-lasting stable environment of low-temperature frozen soil not only inhibited the growth and metabolism of microorganisms, it was also an important factor for the long-term preservation of their biochemical properties. The cultivable low-temperature strains isolated from the frozen soil of the Tianshan Mountain in this study mostly belonged to psychrotrophic bacteria, which may be the result of long-term evolution of thermophilic microorganisms in the low-temperature frozen environment.

Cultivable low-temperature microorganisms in the frozen soil of the Tianshan Mountain belong to 2 genera of α-*proteobacteria*, 1 genus of β-*proteobacteria*, 1 genus of γ-*proteobacteria*, 5 genera of high G+C Gram positives, 2 genera of low G+C Gram positives and 3 genera of CFB. They have rich phylogenetic diversity. Among them Pseudomonas is the dominant genus which contributes the most to the biodiversity of cultivable low-temperature microorganisms in frozen soil. What follow are *Rhodococcus* and *Brevundimonas*. Studies have shown that *Flavobacterium* isolated from oligotrophic environment was better adapted to grow in low-temperature and oligotrophic conditions, and they could degrade persistent substances more effectively [28, 29]. Many studies have shown that the two groups Arthro*bacter* and *Planococcus* were ubiquitous in frozen soil of the North Pole, Siberia, the South Pole and Qinghai-Tibet Plateau, which shows after long time of low-temperature induction, the groups have developed physiological properties more adaptable to these conditions [30, 31,32].

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