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ORIGINAL ARTICLE

Bacterial community in cold and alkaline environments of Hoh Xil basin in Qinghai–Tibet Plateau and isolation of potential sources of microbiota

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Abstract

The Hoh Xil basin is the largest Cenozoic sedimentary basin in the Qinghai–Tibet Plateau (QTP) with an average altitude of above 5000 m. It is also the coldest region in the QTP. However, due to the difficulty of sample collection caused by the harsh natural environment, a limited number of studies have been conducted on soil microorganisms in this region. We used culture-dependent and independent methods to investigate the bacterial communities in desert soil (n1), saline–alkali land (n2), saline–alkali wetland (n3), and soda lake sediment (n4). The results showed distinct bacterial communities between different environmental types. We found that the Chao1 and Shannon diversity indices of n1 were significantly lower than those of n4 ($P < 0.05$). At the phylum level, all samples were dominated by representatives of Proteobacteria, Bacteroidetes, and Actinobacteria, which were similar to the findings of previous studies on the desert soil in the same region. Moreover, we identified 10 strains of bacteria from 109 isolates, most of which belonged to *Pseudomonas* (90.8%). The growth of the isolate *k9* was optimal at a high pH value (pH 10.0) and a low temperature (5 °C), and this stain could produce extracellular enzyme (alkaline phosphatase, acid phosphatase, and naphthol-AS-BI-phosphohydrolase) under alkaline (pH 10) and cold (5 °C) condition. These results demonstrate the diversity of bacteria in the Hoh Xil basin and identify potential psychrophilic and alkaliphilic bacteria with multiple types of extracellular enzyme activity.

Keywords Qinghai–Tibet Plateau · Alkaliphiles bacteria · Psychrophilic bacteria · Psychrotolerant alkaliphile · Microbial community

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Introduction

The Qinghai–Tibet Plateau (QTP) is the highest and most extensive plateau on our planet. It is well known as the "roof" of the world and the "third pole" of the Earth due to its high elevation and cold environment (Tang and Shen 1996). The Hoh Xil basin, located on the platform of the QTP, has been listed among the World Heritage Sites as "the largest and highest plateau in the world," with an area of 101,000 km^2 and an average elevation of over 5000 m. It is the largest Cenozoic sedimentary basin in the hinterland of the Tibetan Plateau with a large area of saline–alkali land (Wen et al. 2007). This area is one of the coldest regions on the QTP, with an average temperature in January of below − 30 °C and average temperatures only in July and August of above 0 °C (Liu et al. 2014). This region contains a wide variety of algae and animals, many of which are unique. Nevertheless, it has received much

less attention than it deserves because of its harsh conditions for fieldwork.

The extreme alkaline, acidic, and extremely cold environments represent a great microbial resource. A growing body of evidence has shown the advantages of using enzymes derived from psychrophilic microorganisms which live in cold environments, such as the depths of oceans, the high-altitude regions, and the polar regions (Feller 2013; Struvay and Feller 2012). The cold-active biocatalyst compounds produced by these microorganisms have a more flexible structure, which enables their high specificity of action at low temperatures. Certain industrial applications use such enzymes to save energy, improve hygiene, and reduce the risk of contamination (Cavicchioli et al. (2011). Meanwhile, the enzymes active at high pH that are produced by alkaliphiles are also of industrial interest, focused in fields, such as waste management and the textile and detergent industries. In the detergent industry, alkaline proteases, amylases, cellulases, and lipases are all widely used in detergent formulations (Fujinami and Fujisawa 2010; Horikoshi 1999). Thus, the isolation of psychrophilic and alkaliphile bacteria from cold and alkaline environments is required for the discovery and isolation of cold- and alkaline-active enzymes with industrial potential. How even, most of the natural, stable alkaline environments, such as soda lakes and soda deserts, are located mainly in the temperate areas of Africa and Central Asia. Thus, the alkaliphilic bacteria isolated from soils of these regions are predominantly meso- or thermophilic. Nevertheless, isolation of psychrophilic and alkaliphilic bacteria from natural environments has been rarely reported (Duckworth et al. 1996; Zhilina and Zavarzin 1994).

Due to the difficulty in collecting samples in the harsh natural environment of Hoh Xil, only a few studies have been performed on soil microorganisms in this region. For example, Sun et al. (2011) isolated five strains of bacteria from the saline–alkali soil in Hoh Xil and found that the *Geomicrobium halophilum* strain CPCC100153 had an optimal growth at a temperature of 28 °C and pH of 8.0–9.5. Extracellular enzymes produced by this strain, such as phosphatase and esterase, were identified to possess industrial potential (Sun et al. 2011). In addition, Su et al. (2011) used culture-dependent and culture-independent (PCR-DGGE) methods to investigate the bacterial communities in a soil sample from Hoh Xil and found that β-Proteobacteria was the dominant phylum (75.0%) in the sample studied (Su et al. 2011). In another investigation, Wang et al. (2017) isolated 66 strains of the *Bacillus* species in soil samples from Hoh Xil and discovered two potential new species of alkaliphilic *Bacillus* with 16S rRNA gene similarities of 97.00% and 98.65% (Wang et al. 2017).

It is considered that only a small number of bacteria can be cultured in the laboratory. Previous work on soil bacteria mainly focused on isolate cultures. Therefore, to comprehensively investigate the structure, diversity, and abundance of soil bacteria in soil samples from the soda desert, saline–alkali land, saline–alkali wetland, and soda lake sediment in the Hoh Xil basin of the QTP, we employed a combination of culture-dependent and culture-independent (Illumina sequencing) methods to identify and characterize psychrophilic and alkali-tolerant bacteria with industrial potential from this unique environment.

Material and methods

Soil sampling

We collected a total of 20 soil samples from the desert (n1), saline–alkali land (n2), saline–alkali wetland (n3), and soda lake sediment (n4) (50 cm depth) in the Hoh Xil from July to August 2015 (Fig. S1; Table S1). The samples were collected from five sites in each of the environments examined. More specifically, the upper 15-cm layer of soil was collected from five random locations within a given plot of 100 m^2 and composited into a single soil sample. All soil samples were placed for storage at 4 °C and immediately transported to the Key Laboratory of Adaptation and Evolution of Plateau Biota, Chinese Academy of Sciences, Qinghai, China, where they were stored at − 20 °C until processing. Soil analyses were performed for determination of total C (TC), total N (TN), and C:N ratio. Initially, the soil was air-dried, then sieved (using a 2-mm mesh), followed by removal of fine roots, ground. Further, the samples were subjected to combustion analysis using the LECO CNS-2000 elemental analyzer (LECO, St. Joseph, MI, USA). Soil total phosphorus was extracted with double acid (0.025 NH_2SO_4 and 0.05 N HCl) and analyzed using an inductively coupled spectrophotometer Shimadzu RF-5301PC (Shimadzu Institute, University of Texas at Arlington, Arlington, TX, USA). Nitrate-N (NO^{-3}-N) and ammonium-N (NH^{+4}-N) were extracted in 2 M KCl, and their concentrations were measured using Scalar SANplus segmented flow analyzer (Scalar SANplus segmented flow analyzer, *Scalar SANplus*, Skalar, the Netherlands). Total soil pH was determined in a fresh soil to water ratio of 1:5 by Leici PHS-3c pH meter (Shanghai Leici Corporation, Shanghai, China), and the soil conductivity was measured using Leici DDS-307A conductivity meter (Shanghai Leici Corporation, Shanghai, China). Mean annual temperature (MAT) was compiled from the WorldClim database (www.worldclim.org) at 30 arc sec resolution.

DNA extraction and HiSeq sequencing

Total DNA from each sample was extracted under sterile conditions from 0.5 g of soil by using a FastDNA® Spin kit (MoBio, Carlsbad, CA, USA) according to the manufacturer's

instructions and stored at − 20 °C. The DNA samples were then frozen-transported to the laboratory of Novogene Bioinformatics Technology Co., Ltd., Beijing, China, and analyzed using the Illumina HiSeq platform and the primers 515F (5′- GTG CCA GCM GCC GCG GTA A)/806R (5′- GGA CTA CHV GGG TWT CTA AT) (Caporaso et al. 2011). The thermal cycling conditions consisted of initial denaturation at 95 °C for 3 min, followed by 30 cycles of denaturation at 95 °C for 30 s, annealing at 50 °C for 30 s, and elongation at 72 °C for 60 s, and finally, the cycling was completed at 72 °C for 7 min. A sequencing library was generated using the KAPA Library Preparation Kit (Kapa, MA, USA) following the manufacturer's instructions and quantified by an Agilent Bioanalyzer 2100 system (Agilent Technologies, San Diego, CA, USA). Finally, the quantified library was sequenced on a HiSeq2500 platform (Illumina, CA, USA), and 2 × 250 base pairs (PE250) were generated.

Data analysis

Paired-end reads from the original DNA fragments were merged using FLASH V1.2.7 (Mago and Salzberg 2011). Raw sequence data were processed and analyzed using the quantitative insight into microbial ecology (QIIME) pipeline (http://qiime.sourceforge.net/) (Caporaso et al. 2010). Reads with a length of < 200 bp or with an average quality score of < 25 were removed (Huse et al. 2007). Clustering of quality sequences into operational taxonomic units (OTUs) was performed through UCLUST at a similarity level of 97% (Edgar 2010). The taxonomy of the generated OTUs was analyzed by RDP Classifier (Wang et al. 2013) against the Silva rRNA gene database (https://www.arb-silva.de/) with a confidence threshold of 80%. All samples were normalized at the same sequence depth, and the OTUs were used to calculate alpha-diversity indices (Chao1, Shannon, and Simpson) by using in-house Perl Scripts. Beta-diversity indices between samples were determined based on weighted and unweighted UniFrac distance matrices (Lozupone and Knight 2005), which were also applied for principal coordinates analysis (PCoA). A two-dimensional plane determined by PCoA was used to determine whether communities with similar characteristics tended to cluster together. The microbial community data were subjected to non-metric multidimensional scaling analyses (NMDS) using the Bray–Curtis dissimilarity matrix in the vegan package (Dixon 2003) R364 3.0.1(http://cran.stat.sfu.ca/). The comparison of relative abundances of microbes between different zones was performed in STAMP software (Parks et al. 2014), using the data generated by QIIME's taxonomy assignment. STAMP calculated diversity differences between samples based on the analysis of variances (ANOVA) with a significant level of $P < 0.05$, and significant differences between groups of samples were investigated using Tukey–Kramer's post hoc test. Canonical correspondence analysis (CCA) was performed to evaluate the chemical properties that had the most significant influence on the microbial community structure. The significant correlations of the physiochemical parameters were examined by the vegan package (Dixon 2003) R364 3.0.1 (http://cran.stat.sfu.ca/).

Sequences generated in this study have been deposited in the National Center for Biotechnology information (NCBI) database under accession number PRJNA480132 (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA480132).

Isolation and characteristics of psychrophilic and alkaliphilic bacteria

The soil samples (1 g fresh weight) were suspended in 9 mL sterile water under shaking for 15 min. Bacterial suspension (1 mL) was transferred into a tube with 9 mL of sterile water. Serial dilutions were made, and 0.1 mL aliquots (10^{-2}–10^{-6}) of homogenized suspensions were spread on a Petri dish containing R2A agar (pH 7.0) and cultured at 5 °C. 16S rDNA sequencing was used for identification of all the isolates. The 16S rDNA amplification was carried out by colony PCR reaction, using 27F (AGA GTT TGA TCM TGG CTC AG) and 1492R (TAC GGY TAC CTT GTT ACG AC TT) primers (Stackebrandt and Goodfellow 1991). PCR was performed in 15 μL of a solution containing 20 ng of genomic DNA, 200 mM 10 × PCR buffer, 1.5 mM MgCl2, 0.2 mM dNTPs, 200 nM of each primer, and 1 U of Taq DNA polymerase (Takara, Dalian, China). The following PCR program was used: initial denaturation for 5 min at 95 °C, 35 cycles of 30 s each at 95 °C, 57 °C for 90 s, 40 s at 72 °C, and a final extension for 10 min at 72 °C. The PCR products were detected using 1% agarose gels run at 100 V for 20 min and visualized under UV light. After purification by the PCR product purification kit (Qiagen), following the recommended protocol, the PCR products were sequenced using an ABI 3730 automated sequencer (Beijing Sunbiotech, Biotech Co., Ltd.). The sequences were then compared to available bacterial sequences in GenBank using the BLAST program in the National Center for Biotechnology Information (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Determination of temperature, pH optimum, and extracellular enzymatic activity

The R2A solid medium was adjusted to various pH values, pH 10.0 (addition of 100 mL of 1 M Na_2CO_3), pH 9.0 (addition of 100 mL 1 M $NaHCO_3$), pH 8.0 (addition of 100 mL of 1.0 M $NaHPO_4$), pH 7.0 (addition of 100 mL of 1.0 M sodium phosphate buffer), and pH 6.0 (adjusted with HCl), and the signal isolates were incubated at 15 °C on each medium. The pH was also measured at the end of the incubation. The growth performance of the individual colonies was interpreted

on a scale from "–" (no growth) to "++" (good growth). The pH was measured at the end of the incubation. To determine the optimum temperature of the individual strains, the R2A medium was adjusted to the optimal pH and incubated at 5 °C, 10 °C, 15 °C, 20 °C, 30 °C, and 37 °C. The growth was measured using a scale from "–" (no growth) to "+ +" (good growth) as described above. Extracellular enzyme activity (alkaline and acid phosphatases, butyrate esterase, caprylate esterase lipase, myristate lipase, leucine, valine and cystine aminopeptidases, trypsin, chymotrypsin, phosphoamidase, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, β-glucosaminidase, α-mannosidase, and α-fucosidase) was assessed using the API ZYM kit (bioMérieux) according to the manufacturer's instructions. In addition, the extracellular enzyme activity of selected psychrotolerant alkaliphiles was also detected under 5 °C and pH 10.

Phylogenetic analysis

The phylogenetic relationships of the strains were deduced from their 16S rRNA gene sequences. The sequences showing

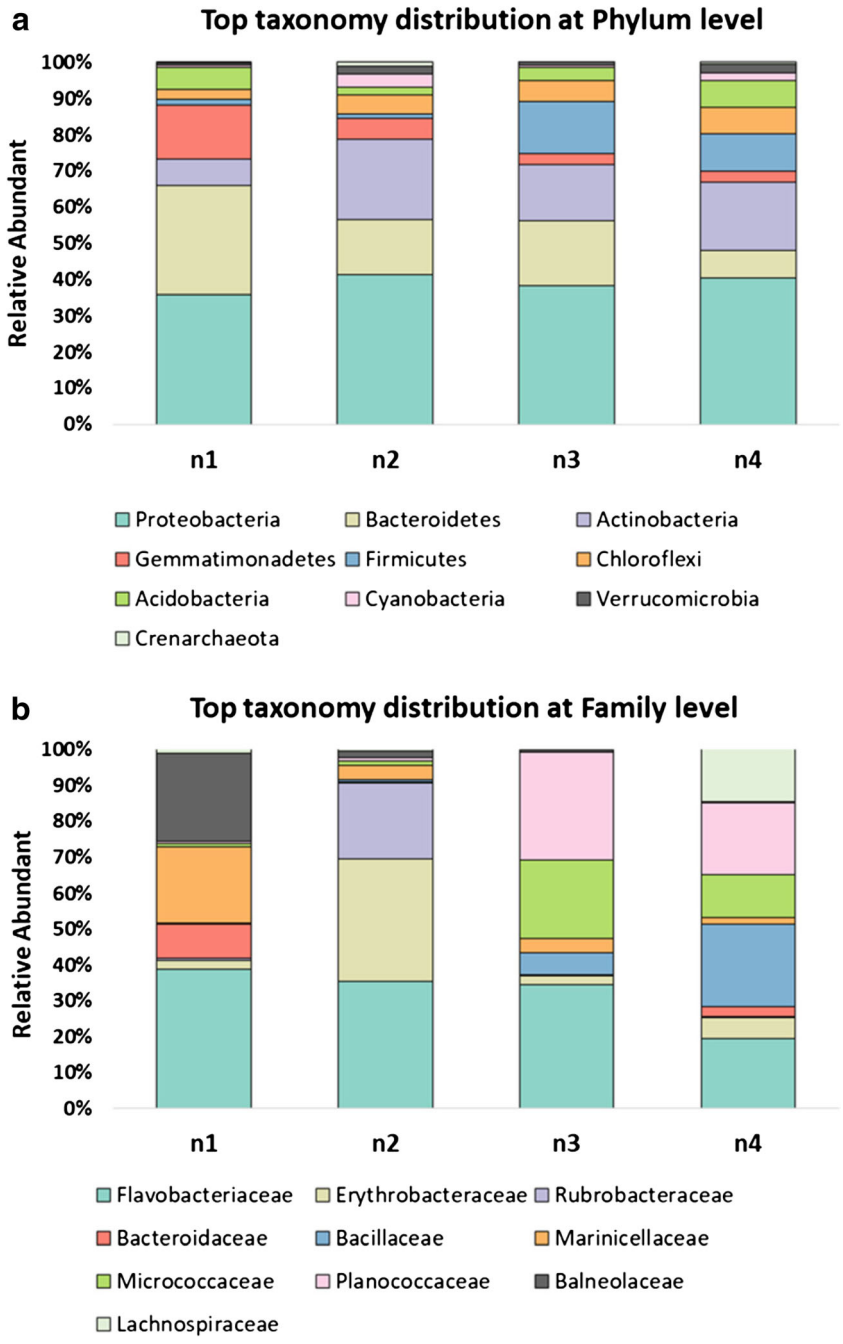

Fig. 1 Taxonomic classification (phylum (**a**) and family (**b**)) of bacterial reads retrieved from four environment types in Hoh Xil by 16S rRNA Illumina sequencing. n1 desert soil, n2 saline–alkali land, n3 saline–alkali wetland, n4 soda lake sediment

the highest degree of similarity were imported into the ClustalX V2.0 software (Jeanmougin et al. 1998) and then corrected manually using the Bioedit software V5.0 (Hall 1999). A phylogenetic dendrogram was then generated based on the 16S rRNA gene sequences using the neighbor-joining method in MEGA X software package (Kumar et al. 2018).

Results

The electrical conductivity and pH of the soil samples used in the present study were 81.1–902 μs/cm and 7.02–8.91, respectively. The values of TC, TN, and TP ranged within 7.74–23.70 g/kg, 0.22–1.70 g/kg, and 0.19–0.65 g/kg, correspondingly. The mean values of the content of NO^{-3}-N and NH^{+4}-N were 3.26 g/kg and 12.65 g/kg in n1; 3.36 g/kg and 4.09 g/kg in n2; 3.03 g/kg and 6.31 g/kg in n3; and 3.49 g/kg and 1050 g/kg in n4 (Table S2).

Twenty samples obtained from four different environmental types of Hoh Xil basin were analyzed. A total of 1,110,373 reads (55,518 per sample) were obtained from the 16S rRNA sequencing. From these data, we annotated 8510 operational taxonomic units (OTUs: 97% identity) which were assigned to a total of 23 bacterial phyla, including Proteobacteria (36.9%), Bacteroidetes (16.9%), Actinobacteria (15.1%), and Firmicutes (6.4%). A total of 59.9% of the sequences were assigned to 238 known bacterial families and 88.57% to 412 known bacterial genera.

The different soil samples had distinct bacterial community compositions. At the phylum level, all samples were dominated by Proteobacteria and Bacteroidetes (Fig. 1a), and the abundance of Actinobacteria in n1 was significantly lower than that in n2 and n4 (Figs. S2 and S3). Alphaproteobacteria and Gammaproteobacteria were the most abundant classes in all the samples, but the bacterial community structures at the class level in n3 and n4 showed heterogeneity. A total of 162 microbial orders and 190 families were detected in all the samples. Gammaproteobacteria was the predominant bacterial order in the n1 and n3, whereas Alphaproteobacteria was the predominant bacterial order in n2 and n4 (data not shown). The abundance of Actinomycetales in n1 was significantly lower than that in the other soil samples (Figs. S4, S5, and S6). The most abundant family in n1 (9.14%), n2 (6.64%), and n3 (8.11%) was Flavobacteriaceae (Fig. 1b). In addition, heterogeneous bacterial community structure was observed in n4 at the family level. At the genus level, *Bacillus* and *Psychrobacter* were the most abundant in the n3 and n4 samples. The core microbiota (family) accounted for 84.8, 86.8, 76.2, and 77.5% of the total sequences in the n1, n2, n3, and n4 samples, respectively (Fig. 2).

The average taxonomic richness (Chao1) estimated for the 16S rRNA libraries of n1, n2, n3, and n4 was 2322.63, 2547.56, 3394.214, and 3617.96, respectively; the bacterial richness of n1 was significantly lower than that of n4 ($P < 0.05$) (Fig. 3a). The Shannon diversity index (H) ranged from 7.75 (n1) to 9.16 (n4); this index in n1 was also significantly lower than that in n4 ($P < 0.05$) (Fig. 3b). Microbial community β-diversity of n1 clustered separately from the other sample locations (except for one sample of n3) based on the weighted UniFrac distances, and an overlap in the distribution was observed among n2, n3, and n4 (Fig. 4a). Meanwhile, the non-metric multidimensional scaling (NMDS) showed a clear differentiation in bacterial communities among all the soil samples (Fig. 4b).

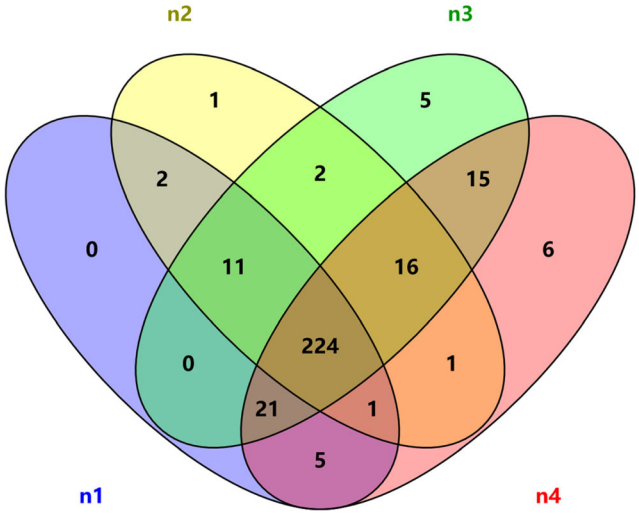

Fig. 2 Sharing of core microbiota families of four environment types. n1 desert soil, n2 saline–alkali land, n3 saline–alkali wetland, n4 soda lake sediment

In the present study, a total of 109 bacterial strains were isolated and identified to belong to 10 species. *Pseudomonas* included 99 strains, accounting for 90.8% of the total isolates. *Psychrobacter* was identified in 16 isolates (14.7%), and there were only two isolates with *Acinetobacter*, one with *Hafnia*, and one with *Exiguobacterium* (Table 1). The pH values of n2, n3, and n4 ranged from 7.02 to 8.91; thus, alkaliphilic bacteria with a growth optimum at pH 8–9 were expected to be isolated. The isolates *k7* and *k9* displayed optimal growth at high pH (pH 9–10), six isolates (*k1*, *k4*, *k5*, *k6*, *k8*, and *k10*) had their pH optimum within 7.0–9.0, and the isolates *k2* and *k3* exhibited their optimal growth at pH 8.0 and 9.0, respectively (Table 2). To determine the temperature optimum of the cultured isolates, the bacteria were plated on R2A medium buffered to the pH where their growth was optimal and cultured under different temperatures (ranging within 5–37 °C). We found that all isolates were able to grow at low temperatures (5 °C). However, optimal growth temperatures for most isolates were between 10 and 20 °C. Moreover, the isolates *k2* and *k9* did not grow at 37 °C (Table 2). The isolates and related reference strains were used to construct a phylogenetic tree (Fig. 5), which was composed mainly of four clusters.

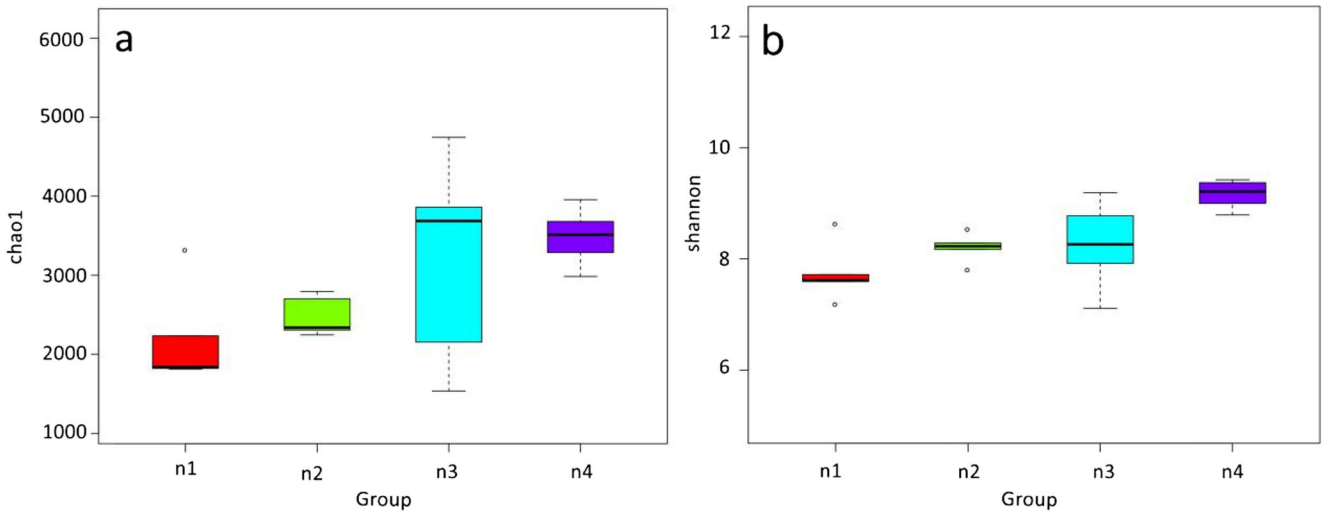

Fig. 3 Bacterial alpha diversity among different environmental types. **a** chao1. **b** Shannon index. n1 desert soil, n2 saline–alkali land, n3 saline–alkali wetland, n4 soda lake sediment

Cluster I represented the *Pseudomonas* group, which was composed of *k2–4*, *k7*–8, and *k10*. Cluster II included *Psychrobacter* (*k5*) and *Acinetobacter* (*k9*), whereas *Hafnia* (*k1*) was represented in cluster III, and the *Exiguobacterium* group (*k6*) was present in cluster IV. The extracellular enzyme activities of all 10 isolates were also investigated. All the isolates had alkaline phosphatase and naphthol-AS-BI-phosphohydrolase activities. Moreover, almost all the strains (except for *k5*) showed acid phosphatase activity. In addition, multiple types of extracellular enzyme activity were detected in *k4* and *k5*, including that of leucine arylamidase, valine arylamidase, cystine arylamidase, and trypsin in both *k4* and *k5* and naphthol-AS-BI-phosphohydrolase and N-acetyl-β-glucosaminidase activities in *k5* (Table S3). Additional test on *K7* and *K9* has shown that those two strains could produce extracellular enzyme under alkaline (pH 10) and cold (5 °C) condition (Table S4).

Discussion

In the present study, the most abundant phyla in all samples were Proteobacteria (33.6–39.3%), Bacteroidetes (7.3–28.2%), and Actinobacteria (6.5–21.1%). This structure was similar to that established in previous studies conducted on the desert soil in the same region, in which Proteobacteria (91%) was the most abundant phylum, followed by Actinobacteria (7%) and Firmicutes (2%) (Su et al. 2011). Similarities were found also concerning the bacterial communities of permafrost layers in Qinghai–Tibet Plateau (Proteobacteria 48.5%

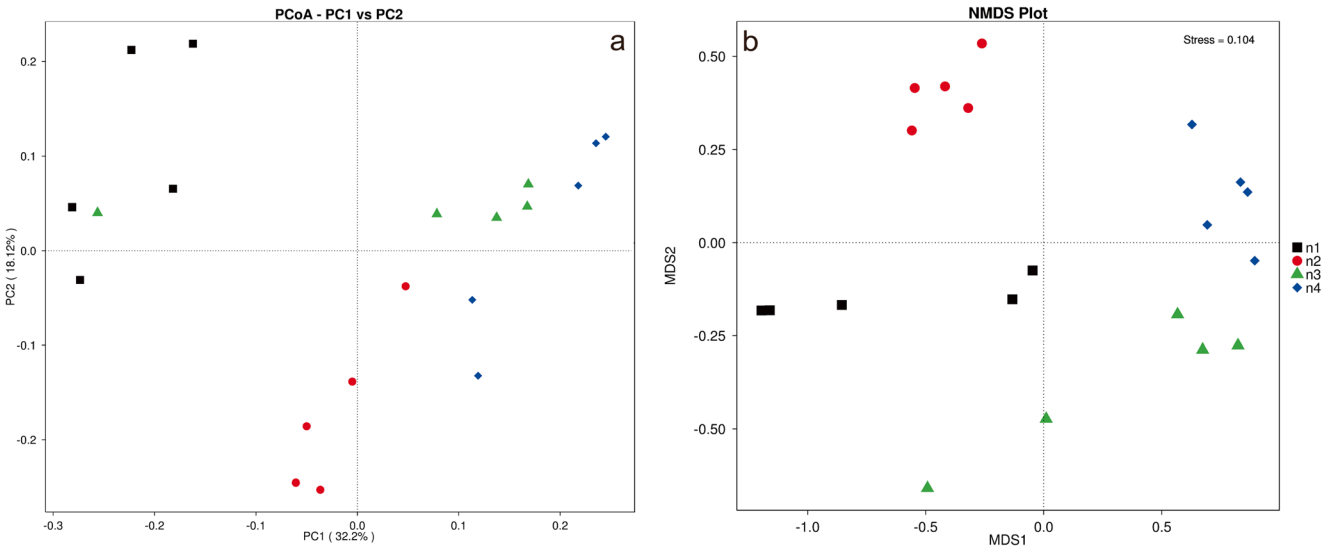

Fig. 4 Principal coordinate analysis (PCoA) based on weighted uniFrac distances (**a**) and non-metric multidimensional scaling analyses (NMDS) (**b**) showed a clear differentiation in bacterial communities among the environmental types. n1 desert soil, n2 saline–alkali land, n3 saline–alkali wetland, n4 soda lake sediment

Table 1 The culturable bacteria isolated from Hoh Xil

Isolate no.	Closest related in database	Identity to closest related %	Base pairs sequenced	Accession no.
K 1	*Hafnia psychrotolerans*	99.78	1382	MH125153
K 2	*Pseudomonas frederiksbergensis*	99.00	1382	MH125154
K 3	*Pseudomonas mandelii*	99.64	1379	MH125155
K 4	*Pseudomonas syringae*	98.86	1228	MH125156
K 5	*Psychrobacter aquaticus*	99.93	1382	MH125157
K 6	*Exiguobacterium antarcticum*	99.72	1404	MH125158
K 7	*Pseudomonas helmanticensis*	99.35	1380	MH125159
K 8	*Pseudomonas ficuserectae*	99.94	1384	MH125160
K 9	*Acinetobacter lwoffii*	98.70	1388	MH125161
K 10	*Pseudomonas jessenii*	99.00	1384	MH125162

and Actinobacteria 25.4%) (Su et al. 2011); however, they were different from the bacterial community present in the surface soil (dominated by Actinobacteria) in Ngari (the highest and driest region in the western Tibetan Plateau) (Chu et al. 2016). Moreover, we found that the bacterial community structure identified in our study was exceedingly similar to that of ikaite columns (one of the few permanently cold and alkaline environments on Earth) (Stougaard et al. 2002; Vester et al. 2014). The structures of samples n3 and n4 were similar to those of samples from the Mono Lake (an alkaline lake in the USA) at a depth of 10 m (Valenzuela-Encinas et al. 2009). We found a set of OTUs (1224) that were present in all samples in the Hoh Xil basin, and the relative abundance of these OTUs did not vary significantly among the samples. Our study reinforces the hypothesis by Baas Becking (1934): "Everything is everywhere, but the environment selects" and was similar with many large-scale soil bacterial community studies that bacteria can disperse globally (Green et al. 2008). Gammaproteobacteria was included, containing several known psychrophiles, such as *Psychrobacter* sp. and members of the genera *Halomonas* and *Pseudomonas*. This finding reveals that the extremely cold environmental on the Qinghai–Tibet Plateau provides ideal conditions for the growth of psychrophilic microorganisms. Meanwhile, at the family level, the bacterial diversity of n1 differed considerably from those of the other samples. For example, the abundance of *Bacillus* and *Micrococcus*, which include many alkaliphilic microorganisms in n1, was significantly lower than that in n3 and n4, and there was almost no (only one) *Streptomyces* OTUs available in n1, whereas 9, 19, and 22 *Streptomyces* OTUs were found in n2, n3, and n4, respectively. The result of PCoA and NMDS also showed a distinct distribution of n1. To identify the factors that caused the divergence among the bacterial communities in the samples, canonical correspondence analysis (CCA) was performed to evaluate their chemical properties. The pH, TC, and NH^{+4}-N ($P < 0.05$) had the most significant influence on the microbial

Table 2 Growth physiology of culturable bacteria from Hoh Xil

Isolate no.	Relative growth[a]										
	pH 6.0	pH 7.0	pH 8.0	pH 9.0	pH 10.0	5 °C	10 °C	15 °C	20 °C	30 °C	37 °C
K 1	+	++	++	++	+	+	+	++	++	++	+
K 2	+	+	++	+	+	++	++	++	++	+	–
K 3	+	+	+	++	+	+	+	++	++	++	+
K 4	+	++	++	++	+	+	+	+	++	++	+
K 5	+	++	++	++	+	+	++	++	++	++	+
K 6	+	++	++	++	+	+	++	++	++	++	+
K 7	+	+	+	++	++	+	+	++	++	++	+
K 8	+	++	++	++	+	+	++	++	++	+	+
K 9	+	+	+	++	++	++	++	++	++	+	–
K 10	+	++	++	++	+	+	+	+	++	++	+

– no growth, + moderate growth, ++ good growth

[a] Relative growth was scored as relative colony size on agar plates

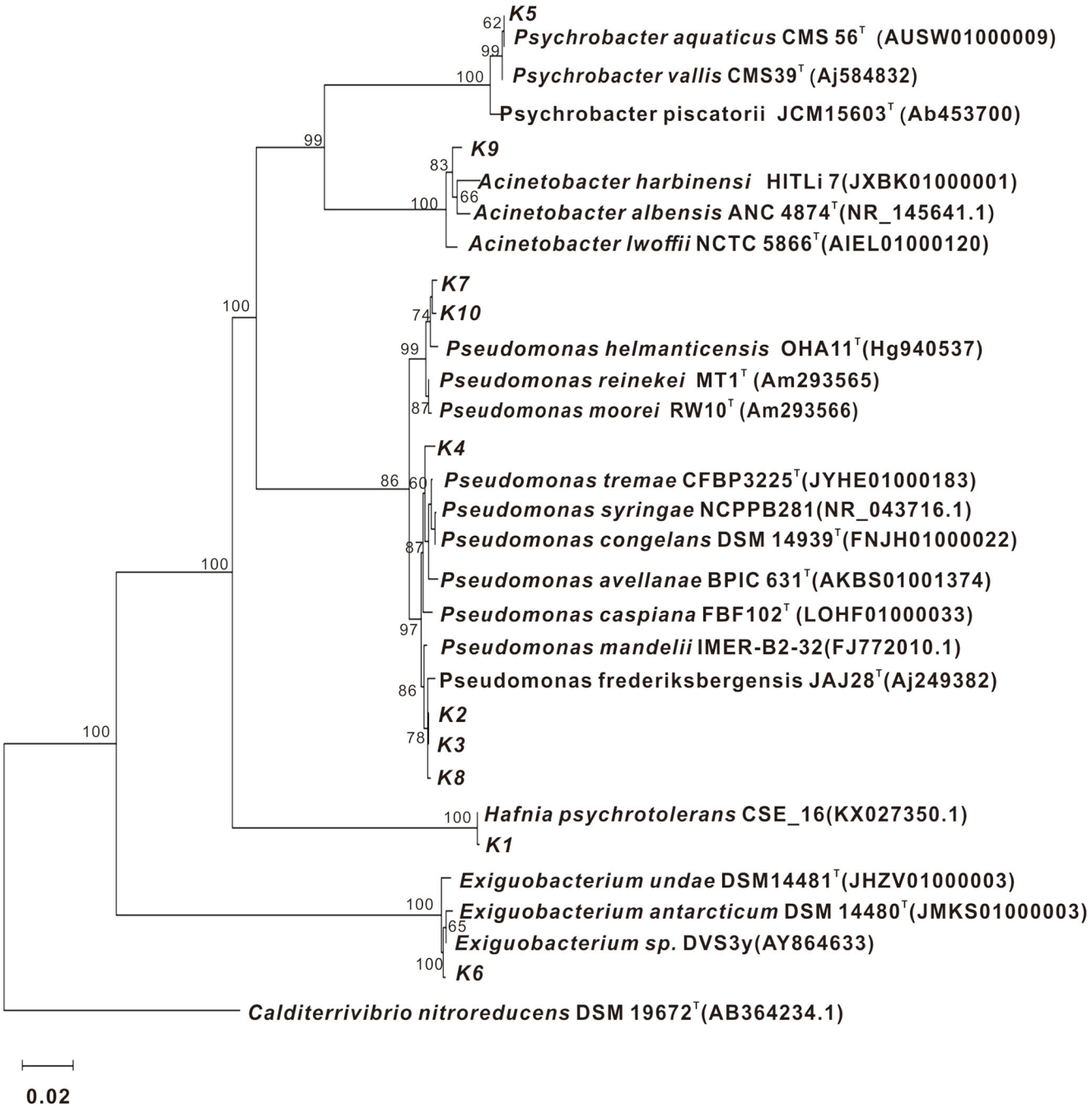

Fig. 5 Phylogenetic analysis based on 16S rDNA sequence. Distances and clustering with the neighbor-joining method were performed by using the software MEGA X. Bootstrap values based on 1000 replications (bootstrap values of < 50%, not shown) are listed as percentages at the branching points. Bar, 0.02 substitutions per site

community structure (Fig. 6), which could explain 36.8% and 35.5% and 14.3% of the total variations.

In the present study, we identified 10 strains of bacteria from 109 isolates, most of which were representatives of *Pseudomonas* (90.8%). Our results were considerably different from the findings of former studies, which reported a higher diversity of culturable bacteria, including *Bacillus*, *Gracilibacillus*, *Halobacillus*, *Jeotgalibacillus*, *Paenibacillus*, and *Psychrobacillus* (Su et al. 2011; Sun et al. 2011; Wang et al. 2017). One important reason for that discrepancy is that we cultured the suspended soil samples at an initial temperature of 5 °C for the isolation of psychrophiles, while most of the bacteria mentioned above could not grow at such a low temperature. Moreover, our sample was collected from cold and alkaline environments, whereas those in the former studies were collected from the desert. Only Proteobacteria and Firmicutes were detected using culture-dependent methods in all the samples. Another

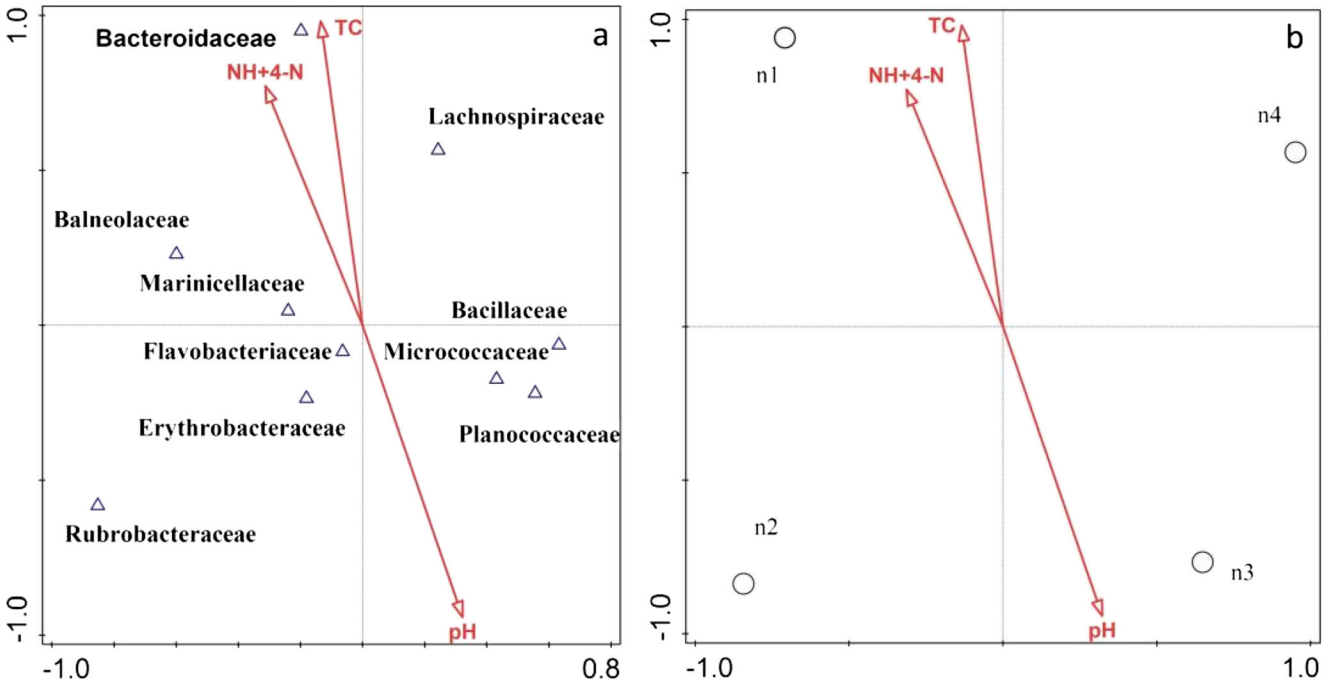

Fig. 6 Canonical correspondence analysis (CCA) of 16S gene data and geochemical parameters. Arrows indicate the direction and magnitude of geochemical parameters associated with bacterial community structures (**a**) and sample types (**b**). n1 desert soil, n2 saline–alkali land, n3 saline–alkali wetland, n4 soda lake sediment

possible reason is that the culture medium used in our study is the relative eutrophic one; this does not allow to isolate, possibly, many oligotrophic bacteria, as culture-dependent techniques can only disclose some cultivable aerobic and facultative anaerobic bacteria. To contrast, there were six bacterial phyla that have been detected that thought culture-independent method and Proteobacteria, Bacteroidetes, and Actinobacteria were the dominant phyla in all the samples. It was shown that culture-independent methods might provide a powerful strategy to investigate microbes. But, unculturable does not mean can never be cultured; recent studies have shown that previously uncultivable microorganisms could be grown in pure culture if provided with the chemical components from their natural environment (Puspita et al. 2012); thus, a combination of culture-dependent and culture-independent methods might be necessary for bacterial community studies.

Nevertheless, all the isolates were able to grow at a low temperature (5 °C), but the optimal growth for the majority occurred at a slightly higher temperature (10–30 °C). They belong to the psychrotolerant microorganisms having a wide temperature range for growth. Only the isolates k2 and k9 had a good growth at 5 °C, both of which did not grow at 37 °C. Our findings corroborate the former studies that even in the permanent cold environments, the majority of the microflora is psychrotolerant, but not psychrophilic (Simankova et al. 2003). Only the isolates k2 and k9 had a good growth at 5 °C, both of which did not grow at 37 °C. The psychrophile *k2* was able to grow well at pH 8.0 and 5 °C and had a high degree of similarity to a psychrotolerant bacterium (KY405891) isolated from Antarctica. On the other hand, the isolate *k8* had a good growth at pH 9.0 and 10 °C, which was similar to the optimal conditions for growth of a bacterium (KU958692) isolated from a soda lake in North America. The isolate *k9* grew extremely well under a high value of pH (pH 10.0) and a low temperature (5 °C), which was similar to the best growth conditions of HF562449, isolated from high-mountain lakes in Russia, and JX949550, identified in a glacier in China. In addition, all isolates were able to grow within a wide range of pH values (pH 6.0–10.0), and almost all of them (except for *k2*) grew exceedingly well at pH 9.0. Nonetheless, only the isolates *k7* and *k9* had a good growth at pH 10.0. All of the abovementioned isolates are representatives of aerobic bacteria, and the long-term storage in a refrigerator in the presence of oxygen is the most possible reason for the absence of culturable anaerobic bacteria.

Conclusion

Bacterial communities in cold and alkaline environments of Hoh Xil basin were revealed through Illumina HiSeq 2500, and distinct bacterial communities between different environmental types were found. The bacterial assemblage shifts could be triggered by total carbon, NH^{+4}-N, and pH value. The growth of the isolate *k9* was optimal at a high pH value (pH 10.0) and a low temperature (5 °C) which is a potential psychrophilic and alkaliphilic bacteria with multiple types of extracellular enzyme activity.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Research involving human participants and/or animals This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent Informed consent is not required in this study.

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