



Diversity of actinobacteria in sediments of Qaidam Lake and Qinghai Lake, China

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Abstract

Using 16S rRNA gene analysis and high-throughput, the diversity and community structure of actinobacteria in the sediments of Qaidam Lake and Qinghai Lake with different salinity and alkalinity in Qinghai-Xizang Plateau were studied, and the differences of actinobacteria community structure and their relationship with environmental factors were discussed. A total of 77 genera belonging to actinobacteria were found in the samples, of which 31 genera were found in the sediment samples of Qaidam Lake with 19 genera being dominant genera, such as *Actinomycetes*, *Corynebacterium*, *Morella*, *Bifidobacterium*, and 69 genera were found in the sediment samples of Qinghai Lake with 17 genera becoming dominant, such as *Ilumattalaer*, *Actinotalea*, *Aquihaans* and so on. The correlation analysis of environmental factors and community showed that the community structure of the two salt lakes was mainly affected by total salinity, total organic carbon (TOC) and CO_3^{2-} , among which TOC was the most influential factor. The functional differences of metabolic pathway enrichment analysis (KEGG) showed that there was a high abundance of metabolic-related functions in the two salt lakes. There were significant differences in the biosynthesis of energy metabolism and other secondary metabolites between the two salt lakes, which may be the main reason for the difference of actinomycete community. The results show that the actinobacteria diversity was rich in the plateau salt lakes, and affected by a variety of physicochemical factors. In addition, there were a large number of unculturable actinobacteria in the sediment, which provides a theoretical basis for the excavation and utilization of actinobacteria resources in salt lakes.

Keywords Saline lakes · High-throughput sequencing · Actinobacteria · Diversity · Environmental factors

Introduction

The phylum actinobacteria is one of the largest taxonomic units among the major lineages currently recognized within the bacteria domain, and has a broad application prospect in the process of industrial production, (Ludwig et al. 2012). It has the ability to degrade complex organic compounds and produce antibiotics, and 70% of the world's natural antibiotics are produced by actinobacteria (Barka et al. 2016), which has aroused great concern in the scientific community (Al-Zarban et al. 2002). Actinobacteria widely exist in ocean (Sharma et al. 2019), soil (Sapkota et al. 2020) and

freshwater ecosystem (Zothanpuia et al. 2018), and many new species have been found (Lam 2006; Fu et al. 2011; Xu and Li 2012; Tian et al. 2013). With the discovery of new species of actinobacteria, the exploration of actinobacteria in various environments has been booming (Tian et al. 2013). However, in recent years, the proportion of new actinobacteria found in conventional environments has been greatly reduced (Zotchev 2012). Fortunately, actinobacteria with new evolution patterns can be found in some unique undeveloped environments, which often have unique physical–chemical conditions (such as high concentration of salt, high pH, etc.). It is of great significance to explore the effects of these physical–chemical conditions on the actinobacteria community for the development and utilization of actinobacteria resources with different environmental evolution patterns.

Saline lake is an interesting model system for studying microbial diversity and ecosystem function in extreme environment, which has high productivity (Jones et al. 1998).

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It is rich in microbial resources and has become a valuable source of new microorganisms, providing natural experimental materials for scientific research (Hou et al. 2019). Generally speaking, the composition of microbial community in high-salinity environment is usually related to salinity levels (Çınar and Mutlu 2016), or more specifically, microbial community diversity decreased with the increase of salinity (Foti et al. 2008). Of course, not all microbial communities have observed this pattern (Herlemann et al. 2011; Wang et al. 2011). At present, many microbial community ecologists want to better understand the composition and diversity of microbial communities in Saline lakes and the environmental mechanisms that affect the community structure (Westoby 2006). Studies of hypersaline ecosystems have mainly focused on solar salterns and saline lakes. At present, there are many studies on the effect of salinity on microbial community structure and diversity in salt lakes (Foti et al. 2008; Wu et al. 2006), however, the understanding of actinobacteria community composition and diversity across salinity gradient and other physical–chemical parameters in plateau salt lakes is very limited. In fact, some progress has been made in isolating actinobacteria from saline–alkali environments, most of which have shown potential in biotechnology applications (Gulder and Moore 2010; Vijayakumar et al. 2012; Hamed et al. 2013). Although some findings relating to actinobacteria in common environments have been reported, the understanding of the diversity of actinobacteria in salt environment is not deep enough. The difference of unique environmental actinobacteria, and their adaptability to environmental factors, actinobacteria are worthy of in-depth exploration, due to their promising prospect in providing new materials for the development and utilization of new resources of actinobacteria in the ocean, low-altitude salt lakes, saline–alkali land and other ecological environment.

Qaidam Basin, located in the northeast of Qinghai Tibet Plateau, is surrounded by Qilian, Kunlun and Altun mountains. It is famous for its rich salt lakes, with the largest saline area in the world. It is composed of thousands of lakes, which ranged from fresh water lakes to high-salt lakes with altitudes increasing from 2700 to 3200 m. (Liu et al. 2014; Zhong et al. 2016). These lakes are characterized by low nutrient content, low temperature, low productivity and high exposure to ultraviolet radiation (Guan et al. 2013). In this study, the sediments of Qaidam Lake with high salinity and low pH and Qinghai Lake sediments with low salinity and high pH were used as sample models. The 16S rRNA genes of actinobacteria from Qaidam Lake and Qinghai Lake were sequenced by Illumina MiSeq high-throughput technology, and corresponding environmental factors were also analyzed. These actinobacteria provide theoretical support for the species diversity and distribution characteristics of actinobacteria in saline lakes, promoting the development

and utilization of actinobacteria resources in the follow-up extreme environment.

The purpose of this study was to (1) compare the composition and diversity of actinobacteria in the two lakes using Illumina Miseq sequencing method amplified by 16S rRNA gene, and (2) evaluate the distribution pattern of actinobacteria diversity affected by salinity and other physical–chemical parameters in the saline lakes.

Materials and methods

Sample collection and physico-chemical analysis

Sampling location: Qaidam Lake, Haixi Prefecture, Qinghai Province and Qinghai Lake, Xining City, Qinghai Province. The sampling time was August 3, 2019, and the sample collection depth was 1–20 cm. The sediment samples of salt lake were collected every 5 km along Qaidam Lake (95°02′–95°22′E, 37°46′–37°55′N) and Qinghai Lake (99°36′–100°16′E, 36°32′–37°15′N). There were three sampling sites in each lake, which were recorded as CDM1, CDM2, CDM3; QH01, QH02 and QH03, respectively. The three samples of each part were mixed and put into a sterile centrifuge tube. The remaining samples were used for the determination of physical–chemical indexes. The collected sediment samples were stored on dry ice and transported to the laboratory as soon as possible. The sediment samples used to extract DNA were immediately stored at –80 °C for further analysis.

The determination of physical–chemical parameters (Total salinity, TOC, TN, pH) of each sample was completed by Shenggong Bioengineering (Shanghai) Co., Ltd. The total salinity was determined by gravimetric method, the pH was measured by PHS-25 acidity meter (soil: water is 1: 2.5), Total organic carbon and total nitrogen were determined by combustion methods using a Shimadzu TOC (total organic carbon)/TN (total nitrogen) analyser (Hawkes et al. 2018).

Extraction and high-throughput sequencing of DNA

Microorganisms in sediments DNA were extracted from each sample using E.Z.N.A.TM Mag-Bind Soil DNA Kit (Omega Bio-Tek, Norcross, GA, USA) in accordance with the manufacturer's protocols. The quantity and integrity of extracted DNA were assayed by QubitTM ssDNA Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA) and 0.8% agarose gel electrophoresis, respectively. The V3–V4 hypervariable region of the bacterial 16S rRNA gene was amplified two rounds using the universal primer set 341F/805R (5'-barcode-CCTACGGGNGGCWGCAG-3'/5'-GACTAC HVGGGTATCTAATCC-3').

For bacterial 16S rRNA gene, the first PCR reaction mixture contained 15 µl of 2×Taq master Mix (Vazyme, Nanjin, China), 1 µL of forward primer and reverse primer both at 10 µM, and about 10–20 ng of genomic DNA as template, then made up to a final volume of 30 µL with ddH₂O. The second PCR reaction mixture was identical to the first one except replacing the template DNA with the previous round of PCR products. The first PCR program of bacterial 16S rRNA gene was operated in triplicate using the following procedures: initial denaturation for 3 min at 94 °C, and 5 cycles of 30 s at 94 °C, 20 s at 45 °C, 30 s at 65 °C, then 20 cycles of 20 s at 94 °C, 20 s at 55 °C, 30 s at 72 °C, followed by a final extension for 5 min at 72 °C and cooling at 10 °C. Subsequently, the second PCR program of bacterial 16S rRNA gene was operated in triplicate using following thermal cycling conditions changed: initial denaturation for 3 min at 95 °C, and 5 cycles of 20 s at 94 °C, 20 s at 55 °C, 30 s at 72 °C, followed by a final extension for 5 min at 72 °C and cooling at 10 °C. The final PCR amplicons were checked on a 2% agarose gel and purified with MagicPure™ Size Selection DNA Beads (Trans gen biotech, Beijing, China). The purified amplicons from three technical replicates were pooled together in equimolar concentrations after quantifying using Qubit 2.0 DNA Kit. Specifically, the purified amplicons of bacteria are pooled individually to be sequenced in their runs, respectively (Wang et al. 2017). Finally, the paired-end sequencing was implemented on an Illumina MiSeq platform (Sangon biotech, Shanghai, China) with a final sequencing concentration of 20 pM. Sequence data were submitted to the Sequence Read Archive (SRA) of the National Center for Biotechnology Information (NCBI; Bethesda, MD, USA) under the Accession Number: PRJNA591718 for actinobacteria.

Sequencing data processing

The raw sequence data were processed by QIIME pipeline (version 1.8.0) (Caporaso et al. 2010). The samples were identified and distinguished according to barcode tag sequence, and the quality of each sample data was filtered to get the effective data. The remaining satisfactory sequences were clustered into operational taxonomic units (OTUs) to create an OTU table at 97% sequence similarity level (Edgar et al. 2011). The most abundant sequences for each OTU were designated as the representative sequences and taxonomically annotated using ribosomal database project (RDP) classifier (version 2.12) (Wang et al. 2007). Alpha diversity was assessed with MOTHUR (version 1.30.1) (Schloss et al. 2009), based on the following two metrics: Chao1 estimators representing community richness, and Simpson indices (D) reflecting community diversity. Statistical analysis of all data was completed by SPSS software (version 21, IBM, Armonk, NY, USA). Based on the results of PICRUST

functional secondary classification, the abundance differences between groups were compared, and the functional classification in which there were significant differences in abundance between samples or groups is found, the default filter condition is $P \leq 0.05$.

Results

Physico-chemical characteristics

There were significant differences in physical–chemical indexes between Qaidam Lake and Qinghai Lake (Table 1). The salinity of the three samples taken from Qaidam Lake ranges from 236.71 to 247.66 g/L, which was a typical high-salinity lake. However, the salinity range of the three samples of Qinghai Lake was only 8.64–10.78 g/L, which was a low-saline lake, and the difference between the two samples was statistically significant. The pH range of Qaidam Lake sample was 7.13–7.61, which was slightly alkaline, while the pH range of Qinghai Lake sample was 8.68–9.12, which was alkaline, and the difference between them was significant. The total organic carbon content (TOC) of the two salt lakes was similar, ranging from 3.3 to 5.1%, with no significant difference in statistics, while the total nitrogen (TN) content of Qaidam Lake (0.31–0.38%) was significantly higher than that of Qinghai Lake (0.16–0.21%).

There was also a significant difference in ion concentration between Qaidam Lake and Qinghai Lake (Table 2). There were significant differences in ion concentrations among the three samples in Qaidam Lake. In CDM1, it has the highest concentration of Cl⁻, and in CDM2, the concentration of K⁺, Mg²⁺, SO₄²⁻ and CO₃²⁻ was the highest. In CDM3, Na⁺ and Ca²⁺ have the highest concentration. Except for Ca²⁺, the concentrations of other ions in Qinghai Lake were also significantly different, and except for CO₃²⁻, the concentrations of other ions in Qinghai Lake were lower

Table 1 physical–chemical parameters of Qaidam lake and Qinghai Lake

Treatment	Total salinity (g/L)	TOC (%)	TN (%)	pH
CDM1	236.71 ± 9.8a	4.1 ± 0.3bc	0.31 ± 0.07a	7.13 ± 0.18d
CDM2	245.46 ± 10.2a	3.3 ± 0.5d	0.38 ± 0.03a	7.61 ± 0.02c
CDM3	247.66 ± 9.6a	3.7 ± 0.5 cd	0.31 ± 0.08a	7.22 ± 0.21d
QH01	10.78 ± 0.8b	4.7 ± 0.2ab	0.18 ± 0.03b	8.68 ± 0.17b
QH02	8.64 ± 0.21b	4.3 ± 0.3bc	0.16 ± 0.04b	9.12 ± 0.03a
QH03	10.21 ± 0.18b	5.1 ± 0.1a	0.21 ± 0.02b	8.93 ± 0.05a

The data are expressed as means ± standard deviation ($n=3$). Different lowercase letters indicate significant difference among different treatments at 0.05 level

Table 2 Ion parameters of Qaidam lake

Samples	Ion parameters (mg/Kg)						
	Na ⁺	K ⁺	Mg ²⁺	Ca ²⁺	Cl ⁻	SO ₄ ²⁻	CO ₃ ²⁻
CDM1	103,647 ± 109b	3678 ± 86b	10,366 ± 196c	376 ± 32b	153,233 ± 125a	18,644 ± 128c	117 ± 3c
CDM2	98,728 ± 122c	4125 ± 59a	11,634 ± 185a	263 ± 17c	129,872 ± 142c	22,625 ± 112a	175 ± 5a
CDM3	111,323 ± 165a	3211 ± 47c	11,441 ± 156b	418 ± 26a	150,394 ± 160b	20,837 ± 131b	131 ± 3b
QH01	3012 ± 62d	1645 ± 21f	7312 ± 156d	11 ± 1d	4322 ± 5f	2315 ± 56d	412 ± 6f
QH02	2863 ± 56d	1874 ± 46e	6821 ± 123e	10 ± 1d	4875 ± 11e	2644 ± 11e	461 ± 8e
QH03	2839 ± 54d	1536 ± 74d	6634 ± 146e	10 ± 1d	4633 ± 14d	2436 ± 62d	375 ± 11d

The data are expressed as means ± standard deviation ($n=3$). Different lowercase letters indicate significant difference among different treatments at 0.05 level

than those in Qaidam Lake, and there were significant differences between the two saline lake samples.

In terms of physical–chemical factors, Qaidam Lake belongs to magnesium sulfate subtype saline lake, while Qinghai Lake belongs to typical saline lake. Except for TOC, the physical–chemical factors of the two lakes were significantly different.

Alpha diversity analysis of actinobacteria in Qaidam Lake and Qinghai Lake

The microbial diversities of three sediment samples from Qaidam Lake and Qinghai Lake were detected and analyzed by Illumina MiSeq sequencing platform. The effective sequence number of actinobacteria in Qaidam Lake was 2498 and the average base length was 418 bp. The ratio of the number of actinomycete sequences to the total number of bacteria in each sampling point was 0.7%, 1%, 0.8%. The effective sequence number of actinobacteria in Qinghai Lake was 10,702 and the average base length was 416 bp. The ratio of the number of actinomycete sequences to the total number of bacteria in each sampling point was 7.1%, 6.1%, 7.1%.

The Alpha diversity of actinobacteria was expressed by OTU count, ACE index, Chao1 richness estimation, Shannon index and Simpson index (Table 3). It can be found that the coverage rate of six samples was between 0.93 and 0.98, which is close to 1, indicating that the sequencing depth used

in this study can capture the diversity of actinobacteria community in the sediment of Qaidam Lake. There were some differences in species abundance and diversity among different samples. According to the OTU count, the OTU number of three samples from Qaidam Lake was 118 and the OTU number of three samples from Qinghai Lake was 775. From the diversity index and richness index, the average Shannon index of actinobacteria community in the sediments of Qaidam Lake (2.35) was lower than that of Qinghai Lake (4.3), and the ACE index of three samples of Qinghai Lake was higher than that of Qaidam Lake. This showed that the diversity and richness of actinobacteria in Qinghai Lake are higher than that in Qaidam Lake.

Community structure of actinobacteria in Qaidam Lake and Qinghai Lake

At the subordinate classification level, a total of 77 genera of actinobacteria were found in the two salt lakes, including 31 genera in Qaidam Lake (Fig. 1). There were 19 dominant genera (relative abundance ≥ 1%), such as *Actinotalea*, *Corynebacterium*, *Tsukamurella*, *Gaiella*, *Rhodococcus*, *Tetrasphaera*, the number of unclassified was high, accounting for 7.93% of the relative abundance. There were 69 genera in Qinghai Lake (Fig. 2), of which 17 genera, such as *Ilumatobacter*, *Actinotalea*, *Aquihabitans*, *Marmoricola*, *Arthrobacter* and *Demequina*, were dominant genera, and, unclassified (32.48%) occupied the highest abundance.

Table 3 The microbial diversity in the Qaidam lake and Qinghai Lake

Samples	number	OTUs	Shannon index	ACE index	Chao1 index	Coverage	Simpson index
CDM1	633	42	2.52	242.93	84	0.97	0.16
CDM2	989	52	2.82	422.54	328	0.98	0.14
CDM3	872	38	1.72	1919.5	200.5	0.97	0.40
QH01	4373	418	4.32	1479.8	1105.1	0.95	0.04
QH02	2905	335	4.25	1228.4	947.62	0.94	0.05
QH03	3172	394	4.32	1644.8	981.57	0.93	0.04

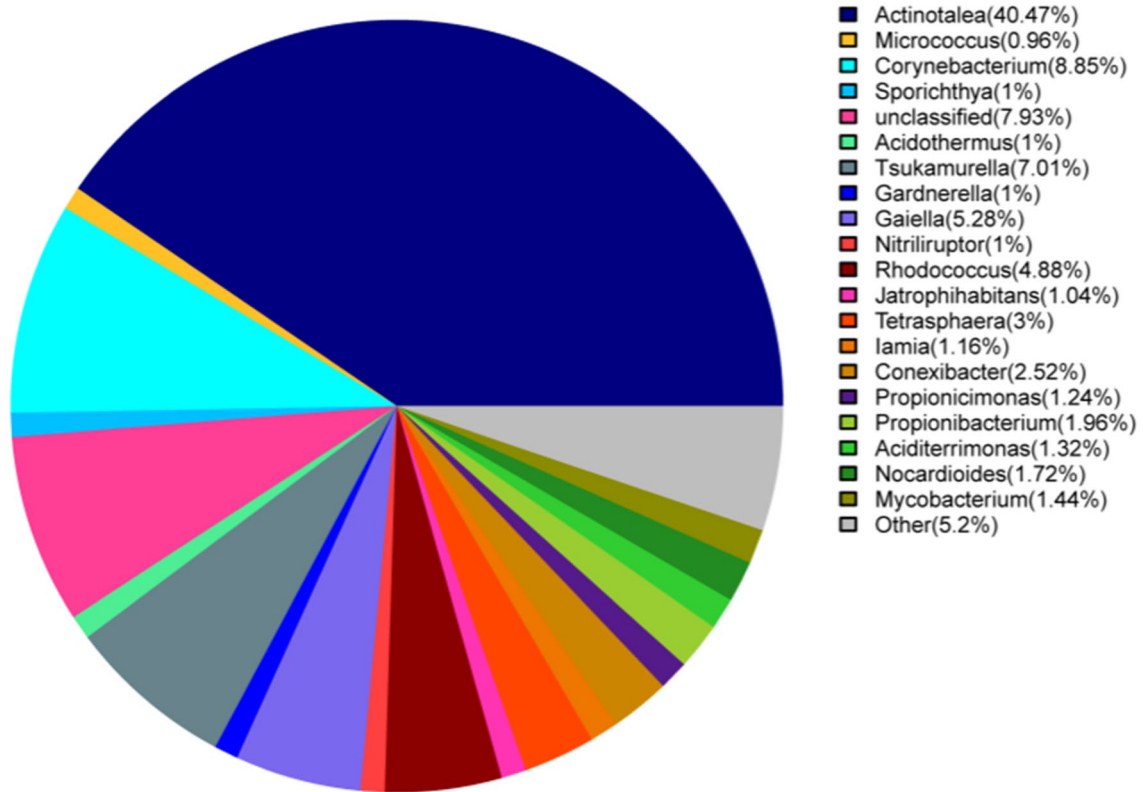


Fig. 1 The relative abundance at the genus level of Qaidam lake

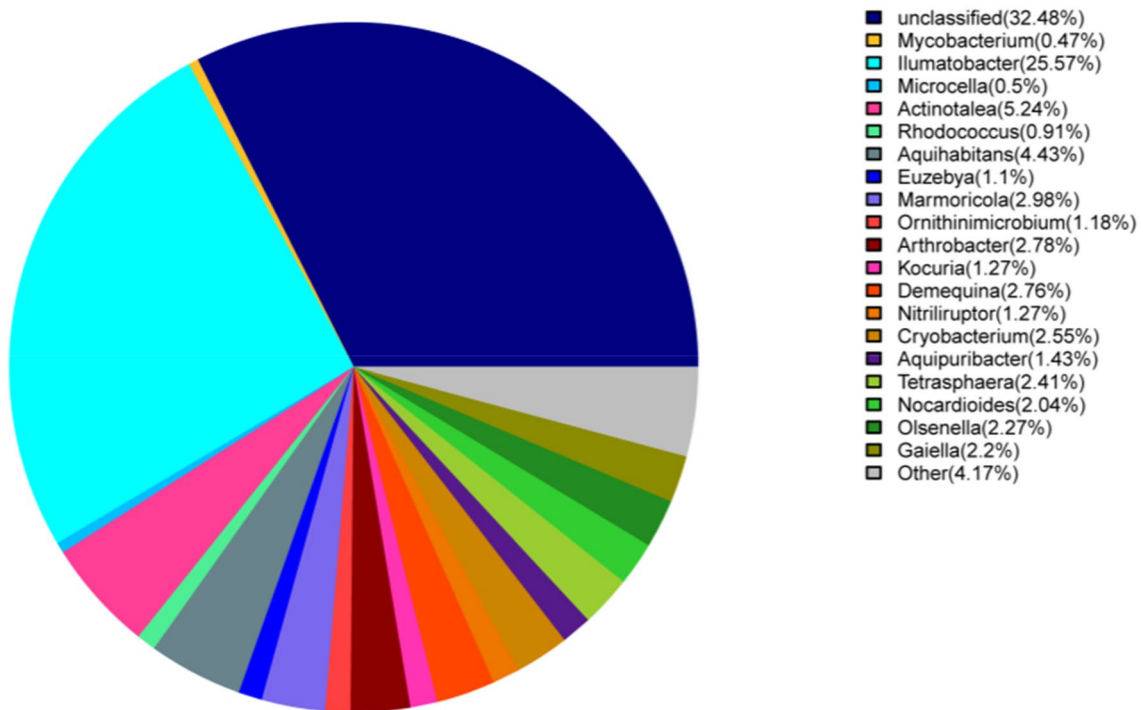


Fig. 2 The relative abundance at the genus level of Qinghai Lake

The Heatmap was drawn according to the abundance ratio of each effective sequence OTU analyzed by Usearch software (Fig. 3). Comparing the similarity of species abundance among the samples, there were 2 clades of actinobacteria in Qaidam Lake and Qinghai Lake, in which CDM2 and CDM3, QH01 clustered parallelly with QH02, while CDM1 and QH03 were separate branches.

The heatmap at genus level showed that among the three samples of Qaidam Lake, the main genus groups with higher abundance and distribution of the three samples

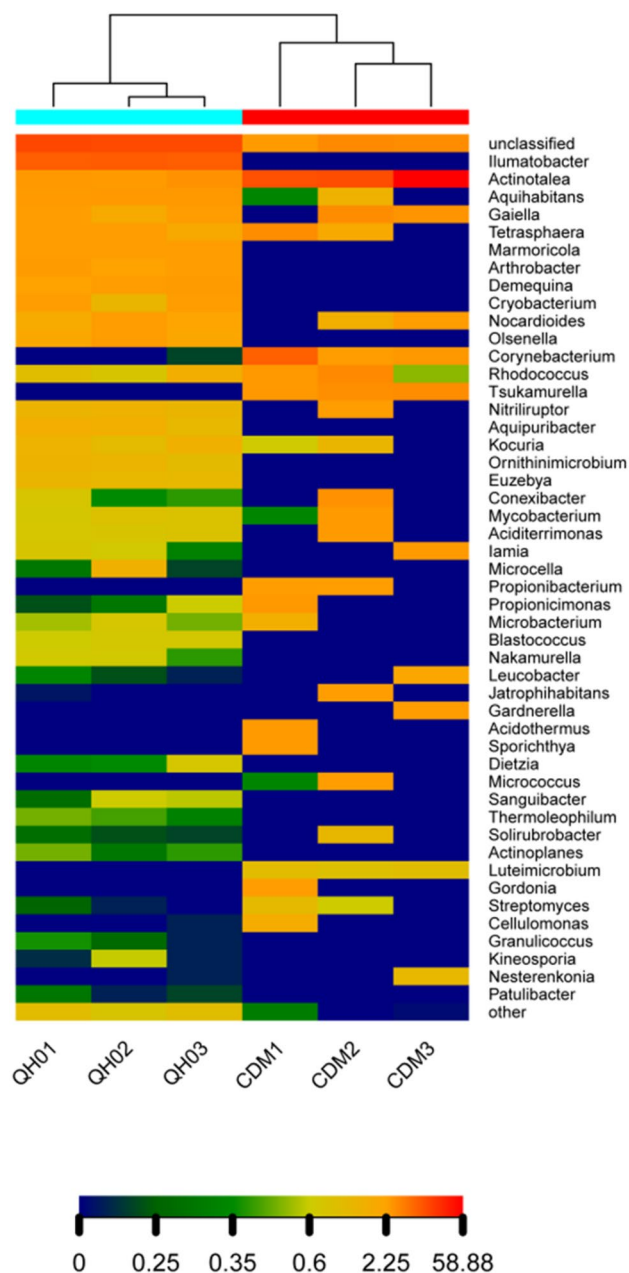


Fig. 3 Microbial community heatmap analysis at the genus level in the Qaidam Lake and Qinghai Lake

were *Actinotalea*, *Corynebacterium*, *Rhodococcus* and *Tsukamurella*. Among them, *Actinotalea* was the most dominant population in CDM1, CDM2 and CDM3. Some groups only appeared in specific samples, such as CDM1-specific *Propionicimonas*, *Microbacterium*, *Acidothermus*, *Sporichthya*, *Gordonia*, CDM2-specific microbiota *Nitriliruptor*, *Conexibacter*, *Aciditerrimonas*, *Jatrophihabitans*, *Solirubrobacter* and CDM3-specific *Iamia*, *Leucobacter*, *Gardnerella*, which had high relative abundance and were dominant microbiota.

The species richness of the three samples in Qinghai Lake was similar. The main genera and groups with high abundance and distribution in all three samples accounted for the majority and the abundance was high. Among them, *Ilumatobacter* was the most dominant population in QH01/02/03. *Actinotalea*, *Aquihabitans*, *Aquipuribacter*, *Arthrobacter* and *Tetrasphaera* were common microbiota with high abundance. However, the relative abundance of the endemic microbiota of QH01, the endemic microbiota of *Iamia*, QH02, the endemic flora of *Microcella*, QH02, the endemic microbiota of *Aciditerrimonas*, etc., and the relative abundance were low. In addition, there was high abundance of unclassified in QH01/02/03.

The diversity analysis showed that the relative abundance of actinobacterial taxa varied in different samples (Figs. 1, 2 and 3). PCA analysis further confirmed obvious variations in the actinobacterial community among the samples (Figure S1 Electronic supplementary material). The community composition of actinobacteria in Qaidam Lake and Qinghai Lake was quite different, and they were clustered separately, a result in line with that of physicochemical factors (Tables 1 and 2).

Pearson correlation analysis of environmental factors in Qaidam Lake and Qinghai Lake

Based on the parameters of physicochemical factors and Alpha diversity index of Qaidam Lake and Qinghai Lake, the relationship between community diversity and environmental factors in Qaidam Lake was analyzed by Pearson correlation.

Pearson analysis showed that the Alpha diversity index of actinobacteria community in the sediments of Qaidam Lake and Qinghai Lake had a certain correlation with physical–chemical factors and chemical ions (Table S1, Table S2, Table S3 and Table S4, Electronic supplementary material). In Qaidam Lake, the richness index of actinobacteria community Chao1 index and ACE index were negatively correlated with TOC, and Chao1 index was significantly negatively correlated with TOC, positively correlated with total salt, and had no correlation with TN and pH. It was positively correlated with Mg^{2+} and SO_4^{2-} , but not with other ions. The Shannon index has a strong positive correlation with TN, pH, Na^+ and K^+ , a negative correlation with total

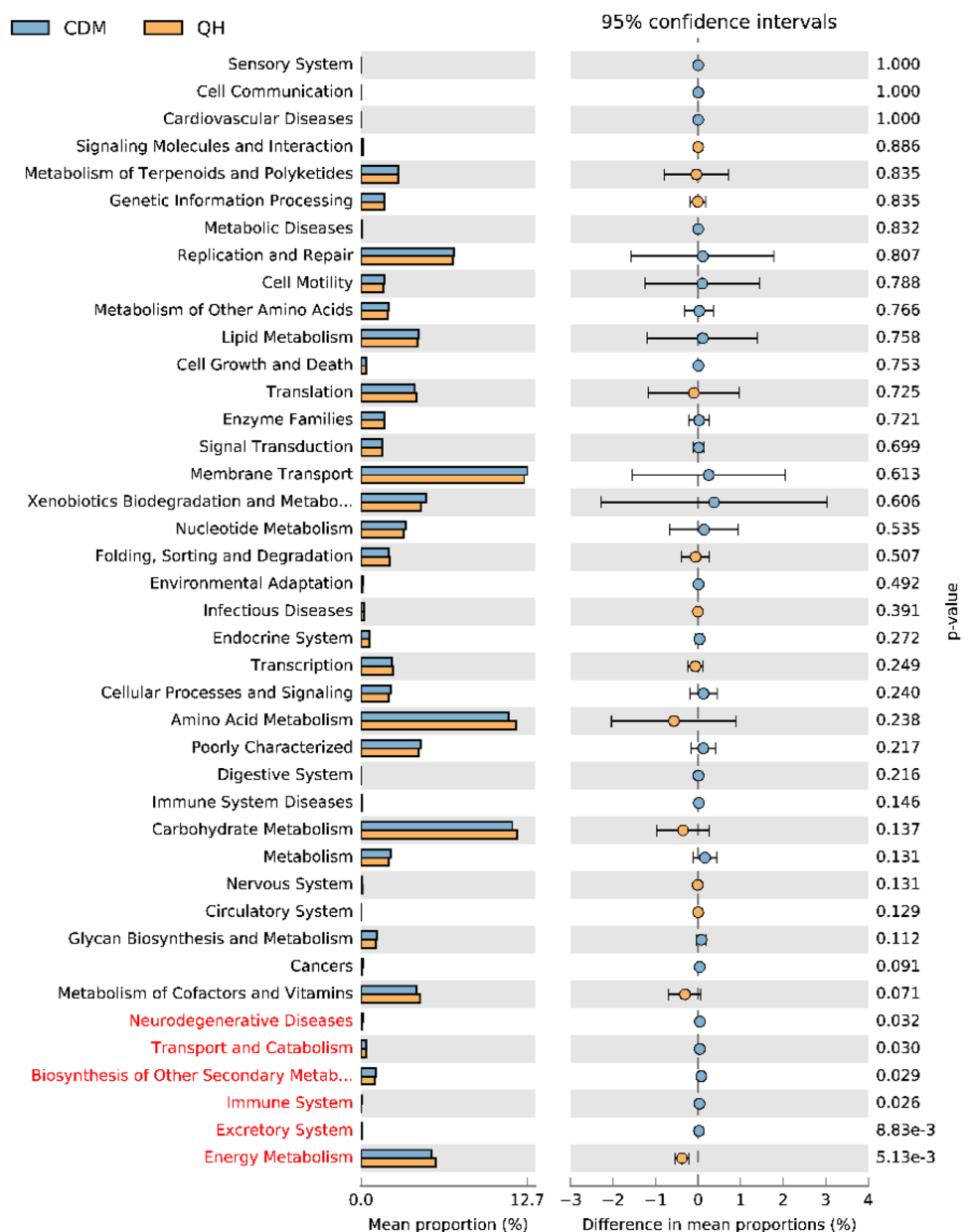
salt and TOC, Ca^{2+} and Cl^- , and a weak correlation with other ions. The results showed that the community richness of actinobacteria in Qaidam Lake was affected by total salt, TOC, Mg^{2+} and SO_4^{2-} , and TOC was the main influencing factor, while the community diversity of actinobacteria was mainly affected by TN, pH, Na^+ , K^+ , Ca^{2+} and Cl^- , but the effect was not significant. In Qinghai Lake, the Chao1 index and ACE index of actinobacteria community were positively correlated with total salt and Ca^{2+} , and negatively correlated with pH, K^+ , Cl^- , SO_4^{2-} and CO_3^{2-} . Among them, there was a significant negative correlation with CO_3^{2-} . There was no significant correlation with TOC, TN, Na^+ and Mg^{2+} . Shannon index was positively correlated with total salt, TOC, TN, Na^+ , Mg^{2+} and Ca^{2+} , and negatively correlated with pH, K^+ ,

Cl^- , SO_4^{2-} and CO_3^{2-} , but had no significant effect. The results showed that the community richness of actinobacteria in Qinghai Lake was mainly affected by total salt, Ca^{2+} , pH, K^+ , Cl^- , SO_4^{2-} and CO_3^{2-} . Among them, CO_3^{2-} is the main influencing factor. Diversity is affected by many physical–chemical factors, but had no significant effect.

Difference of functional abundance between Qaidam Lake and Qinghai Lake

As shown in Fig. 4, 25 functions with great difference between the two salt lakes were selected in the figure, among them, Replication and Repair, Membrane Transport, Amino Acid Metabolism, Carbohydrate Metabolism,

Fig. 4 Error chart of difference comparison. The left figure shows the abundance ratio of different functional abundances in two samples (groups), and the middle shows the difference proportion of functional abundance within 95% confidence interval. The rightmost value is p value, P value < 0.05, indicating significant difference, and functional abundance is marked with red



Energy Metabolism had a high abundance in both salt lakes, especially the functions related to metabolism, significantly higher than in other functions. These functions may be necessary to maintain the growth and propagation of actinobacteria in salt lakes. There were significant differences in energy metabolism, excretion system, immune system, biosynthesis of other secondary metabolism, transport and catabolism, and functional abundance of neurodegenerative diseases between the two salt lakes. Among these significantly different functions, the biosynthesis of other secondary metabolism and energy metabolism had the highest abundance. And the functional abundance of energy metabolism of Qaidam Lake was higher than that of Qinghai Lake, and the biosynthesis of other secondary metabolism was lower than that of Qinghai Lake.

Discussion

The purpose of this study is to explore the diversity and community structure of actinobacteria in the sediments of two salt lakes with different physical–chemical properties. It is generally believed that the taxonomic diversity of microbial populations in high-salt environment is relatively low (DasSarma and DasSarma 2002; Oren 2001). In fact, saline lake ecology shows different microbial community results. In this study, it was found that there were abundant actinobacteria communities in two salt lakes, and 77 genera of actinobacteria were obtained. Among them, *Actinotalea* was the dominant population in Qaidam Lake, *Ilumatobacter* was absolutely dominant in Qinghai Lake. And the strains of *Actinotalea*, *Tetrasphaera*, *Aquipuribacter*, *Euzebya* and *Olsenella* were found in saline lake for the first time, among them, *Tetracoccal* bacterium is a kind of microorganism with a wide range of uses, which is suitable to live in a highly dynamic system. They can use oxygen and nitrate/nitrite as electron acceptors to ferment glucose, glycine and other possible compounds; they can absorb and release phosphate and secrete extracellular enzymes. It can also increase the intracellular storage capacity of several soluble anaerobic substrates (Nguyen et al. 2015). But it is not clear what role they play in the plateau saline lake ecosystem. In addition, *Streptomyces*, as the dominant actinobacteria in common environment (Kieser et al. 2000), has very low abundance in Qaidam Lake and Qinghai Lake, it is possible that its growth in the plateau salt environment is limited. It also shows that the special ecology of plateau salt lakes affects the composition of actinobacteria community in the environment. The results showed that the actinobacteria communities in the sediments of Qaidam Lake and Qinghai Lake were more diverse than those in marine and freshwater ecosystems. Previous studies have shown that actinobacteria communities in marine and freshwater ecosystems are mainly composed of

actinobacteria and uncultured marine actinobacteria (Stach et al. 2003a, b; Warnecke et al. 2004, 2005; Maldonado et al. 2005). In this study, the microbial sequences detected in the sediments of Qaidam Lake and Qinghai Lake included not only actinobacteria, but also high abundance of Acidimicrobiales. Acidimicrobiales is an important group in the saline sediments investigated, but it usually does not exist in marine and freshwater ecosystems (Stach et al. 2003a, b). Most of its members are uncultured, and grow well around a pH value of 2, and are isolated only from hot spots and acidic mine water (Goodfellow and Fiedler. 2010). And there are a large number of unclassified actinobacteria, with the highest abundance present in Qinghai Lake. These unknown taxonomic groups are new resource of actinobacteria, indicating that the saline lake environment is a natural place to mine new species of actinobacteria.

Most studies have shown that environmental factors are vital for affecting the microbial community in the ecosystem (Bryanskaya et al. 2016). There is a close relationship between the species abundance and community structure of microorganisms in saline lake and environmental factors (total salinity, TOC, TN and various ion concentrations, etc.) (Sorokin et al. 2014). When Chen Ping (Chen et al. 2016) and others studied the diversity of actinobacteria in deep-sea sediments along the southwest Indian Ridge, they found that pH had a significant effect on actinobacteria in the marine environment, and TOC, TP and TN, which represent nutrients, were also significantly related to the community structure of actinobacteria in the deep sea. Jiang Hongchen (Jiang et al. 2010) found that the diversity of actinobacteria in lakes on the Qinghai-Xizang Plateau was positively correlated with salinity, TN and TOC, but not with pH. In this study, actinobacteria community diversity in Qaidam Lake was positively correlated with TN and pH, but negatively correlated with total salt and TOC, while actinobacteria community diversity in Qinghai Lake was positively correlated with total salt, TOC, TN, Na⁺, Mg²⁺ and Ca²⁺, and negatively correlated with pH. It shows that the actinobacteria derived from same plateau saline lake still present differences in the community composition, our data further confirmed these findings, in which actinobacteria may imply that the influence of microenvironment on the composition of microbial community cannot be underestimated. Other studies have shown that lake salinity and pH have significant effects on the structure and composition of microbial communities in water (Salgaonkar et al. 2016). There was a negative correlation between actinobacteria diversity and salinity in Qaidam Lake, while there was a negative correlation between actinobacteria community and salinity in Qinghai Lake, that is, actinobacteria diversity increased with the increase of salinity, which is inconsistent with the general ecological principle that diversity decreases due to the increase of extreme environment (Hocine et al. 2003).

In general, microbial diversity decreases with the increase of salinity (Oren 2002; Simachew et al. 2016). The possible reason for this inconsistency is that actinobacteria may tolerate a wide range of salinity, or the difference of sampling points. In terms of pH, the pH of Qaidam Lake was slightly alkaline, while that of Qinghai Lake was higher than that of Qaidam Lake, but the diversity of actinobacteria in the two salt lakes was positively correlated with pH, that is, the higher the pH, the higher the diversity of actinobacteria, indicating that some actinobacteria in Qaidam Lake and Qinghai Lake have wider alkali tolerance.

Sediment ion detection showed that the concentration of Na^+ , Mg^{2+} , Cl^- , SO_4^{2-} in Qaidam Lake and Qinghai Lake was very high, but 77 actinobacteria were still found in the sediments of the two salt lakes, indicating that the actinobacteria in the saline lake may have extensive adaptability to Na^+ and Mg^{2+} . In this study, Pearson correlation analysis revealed that there was a certain correlation between Alpha diversity index and ion concentration (such as Mg^{2+} , SO_4^{2-} , K^+ , Ca^{2+} , Cl^- , etc.) of actinobacteria communities in the sediments of Qaidam Lake and Qinghai Lake, but the correlation was not significant.

After analyzing the composition of the gene function of the existing sequenced microbial genome, the species composition obtained by 16 s sequencing inferred the composition of functional genes in the sample, and the functional differences between different groups were analyzed. In this study, we found that the two salt lakes are abundant in amino acid metabolism, carbohydrate metabolism and other metabolic functions. Related studies have shown that their potential for refractory organic degradation in terms of carbon metabolism (Yilmaz et al. 2016). This characteristic implied the potential roles of actinobacteria in the recycling of organic matter in the salt environments, which might enhance their competitive ability in situ. In addition, there were significant differences in the functions of energy metabolism, excess system, immune system, biosynthesis of other secondary metabolism, transport and taxonomy, neurodegenerative diseases between the two salt lakes, and the biosynthesis of other secondary metabolism and energy metabolism have the highest abundance. Actinobacteria are the most important sources of bioactive natural compounds (Jose and Jebakumar. 2014), the biosynthesis of secondary metabolism is the key to the production of bioactive compounds. The high abundance of actinobacteria in Qaidam Lake indicates that actinobacteria in salt lake environment had the potential to produce new antibiotics. At the same time, in view of the high-abundance metabolic function of the two salt lakes, the ability to utilize refractory organic matter may be very important for the survival of actinobacteria in salt environment.

There were still some limitations to be solved in our research. The small sample size may limit the accurate

understanding of microbial compositions. Our analysis on the microbial communities and correlations to physico-chemical factors are mainly based on the OTUs sequencing data and statistical analysis. Whether the physicochemical properties of salt lake are a function of microbiota or adaptation of microbiota should be further explored. Future study may expand samples and employ tools of metagenomics and metabolomics analyses, accompanied by culture-dependent methods to further identify the key microorganisms and explore the function of these strains related to the succession of physical and chemical changes.

China is rich in saline lake resources. In-depth and systematic study of actinobacteria resources in plateau environmental salt lakes is of great significance for the protection, development and utilization of extreme environmental microbial resources in China.

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