Acta Ecologica Sinica 37 (2017) 213–218

Contents lists available at ScienceDirect

Acta Ecologica Sinica

journal homepage: www.elsevier.com/locate/chnaes

Community structure and elevational distribution pattern of soil Actinobacteria in alpine grasslands

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ARTICLE INFO

Article history:
Received 19 December 2015
Received in revised form 1 April 2016
Accepted 31 May 2016

Keywords:
Actinobacteria
Distribution pattern
Alpine grassland
Environmental factors
Community structure

ABSTRACT

Actinobacteria are widely distributed in various ecological environments and play essential roles in the carbon and nitrogen cycles in natural ecosystems. Understanding the community composition, structure and distribution of Actinobacteria is not only fundamentally important for studying their functions to ecosystem, but also conducive to protecting Actinobacteria resources. Furthermore, studies regarding the distribution of Actinobacteria in specific areas would also help us understand the impact of global environmental changes on the world's microbial communities. In this study, six alpine grassland sites, from 3220 m and 4790 m in elevation, were selected in Sanjiangyuan National Natural Reserve, which is located in the center of Qinghai-Tibetan Plateau. Then, using 16S rDNA high-throughput sequencing, the composition and elevational distribution patterns of soil Actinobacterial communities were analyzed. A total of 5052 OTUs were detected, which could attributed to 230 genera, 52 families, 9 orders and 5 subclasses of Actinobacteria, and the Actinobacterial communities were significantly ($P < 0.05$) different among the six sites, according to detrended correspondence analysis (DCA) and several dissimilarity tests (MRPP, Anosim and Adonis). The subclasses of Actinobacteridae and Rubrobacteridae were dominant in all six sites, and accounted for >80% of relative abundances, indicating that Actinobacteridae and Rubrobacteridae were the most important subclasses in the alpine grasslands. Through analysis of Actinobacterial α-diversity of the six samples, the number of OTUs ranged from 447.23 ± 52.83 to 675.00 ± 114.69, and the Shannon index ranged from 5.35 ± 0.15 to 5.78 ± 0.11. Both of the measures exhibited strong negative correlations ($P < 0.01$) with the elevation, indicating that the environmental conditions of higher elevation may not benefit to the survival of Actinobacterial species. Mantel test was implemented to identify the major environmental factors that impact Actinobacterial communities, and the result indicated that elevation and soil temperature, moisture, organic carbon and nitrogen contents were all significantly ($P < 0.05$) correlated with Actinobacterial community structure and they could be the key factors in determining the distribution of soil Actinobacteria in the alpine grassland in the Tibetan Plateau.

1. Introduction

As important microbial communities, Actinobacteria are widely distributed in various ecological environments [1]. Actinobacteria are playing an irreplaceable role in maintaining the ecological function of soil, marine and freshwater [2,3]. For example, Actinobacteria could effectively regulate the decomposition and synthesis of soil organic matters [2], and they also have significant impact on protecting the environment or improving soil fertility [4]. In addition, Actinobacteria could produce all kinds of medicine for human, such as antibiotics, enzymes, immunomodulator, and so on [5]. Strengthening the research to Actinobacteria would be helpful for people to find more beneficial Actinobacterial strains, and would be conducive to improve the sustainable utilization to Actinobacteria resource.

In the past, soil Actinobacteria were mainly studied through conventional separating techniques and identification methods [5,6]. With the rapid development of molecular technology, metagenomics technologies by directly extracting DNA from environment samples had been widely applied to study microorganism [7,8]. Currently, the high-throughput sequencing could be used to analyze the diversity, composition, structure and dynamics of microbial communities in different habitats [9,10,11]. For example, He et al. [12] studied the phylogenetic composition and structure divergence of soil microbial communities at elevated CO_2 for 10 years based on metagenomics technologies, and discovered that the abundance and taxonomic structure were significantly altered at eCO_2. Chu et al. [13] once studied microbial community structure of Arctic region based on metagenomics technologies, and found

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http://dx.doi.org/10.1016/j.chnaes.2017.02.010
1872-2032/

that microbial community structure in extreme environments was similar with other region. With further development of high-throughput sequencing technology, it will provide strong technical support for studying Actinobacterial structure and diversity.

Sanjiangyuan, with the average elevation of 4400 m, located in the center of Qinghai-Tibetan Plateau. It was the resource of Yangtze, Yellow River and Lantsang River, and known as "Chinese water tower" [14]. The unique natural geography environment and special climate condition have bred special ecosystems and abundant species resources [15]. Ma et al. [5] had studied the impact of grassland degradation to soil Actinobacteria, and indicated that there was abundant Actinobacterial resource in Sanjiangyuan. Li et al. [16] once studied soil microbial flora in different ecological system of Sanjiangyuan, and indicated there were significant difference in quantity of soil Actinobacteria among three ecosystems. Compared with forest and desert ecosystem, the number of Actinobacteria in grassland system was much larger [16]. At present, the reports on Actinobacteria in alpine grassland were still fewer.

In this study, we adopted Illumina high-throughput sequencing technology to analyze the characteristics of soil Actinobacteria in Sanjiangyuan Natural Reserve. The aims of our study were to determine: (i)the community structure and distribution pattern of soil Actinobacterial community along the elevation in alpine grassland; (ii) the major environmental factors in impacting the distribution of soil Actinobacterial community.

2. Methods and materials

2.1. Site and sampling

The climate type of Sanjiangyuan belongs to the typical plateau continental climate, with the characteristics of cold, dry, and strong radiation condition [17]. The annual mean air temperature is −5.6-3.8 °C, and the average precipitation is 262.2–772.8 mm [18]. Alpine grassland usually distributes in the regions of 3000 m above the sea level, and is considered to be one of the most important and representative vegetation types [17].

The study sites were situated in the counties of Maduo and Xinghai, which located in the center of Sanjiangyuan National Nature Reserve. In August 2012, six sampling sites were set with less human disturbance along the elevation (3220–4790 m). When setting sampling sites, the slope and aspect should keep in the same direction as far as possible. At each site, we set up a grid with 200 × 200 firstly, and then, a corner of the grid was selected as the original point. According to "L" sampling method, we placed two transect belts which along the horizontal and vertical direction respectively. Sampling locations were placed at 5-, 10-, 20-, 50-, 100-, and 200-m distance from the original point along transects in two directions. A total of 13 sample plots of 1 × 1 m were set up at each site. Soil samples were collected at the depth of 0–10 cm according to diagonal method at each plot [19]. All soil samples in the same plot were mixed together. Then, these soil samples were divided into two parts. One part was stored in −80 °C and used for DNA extraction. Another part was stored in 4 °C and used to analyze soil physical and chemical properties. When sampling, we also investigated some basic information of the sampling sites, such as the longitude and latitude, elevation, terrain, etc. (Table 1).

2.2. Vegetation diversity and soil property measurement

The properties of plant species number, height, abundance, coverage, were measured according to the common protocols [19]. Vegetation diversity was characterized by Shannon-Wiener index, which was calculated by above measured data [20]. Plant biomass was determined through mowing. The plant was cut off at ground level in 1 × 1 m plot, and cleaned up. All obtained plants should be air-dried and then weighted [19].

All soil samples were air-dried and then sieved by 2 mm sifter. Soil moisture was measured by drying method at 105 °C for 10 h. Soil pH was measured by pH meter according to the ratio of 2.5 H_2O:1 soil. Soil temperature of 10 cm was determined by the Hobo Temperature instrument. Besides, total organic carbon, total nitrogen, total phosphorus, total potassium, total sulfur, available potassium, available phosphorus, available nitrogen, nitrate nitrogen (NO_3^--N) and ammonia nitrogen (NH_4^+-N) were measured according to previously described [21].

2.3. DNA extraction, purification, and quantitation

Soil DNA extraction was obtained by using fast DNA spin kit for soil (MP Biomedical, Carlsbad, CA), following the protocol instructions [22]. Soil DNA, which was extracted in the previous step, needed to be further purified twice by using 0.5% low melting point agarose gels. Then, the purity of soil DNA was determined by analyzing the ratios of 260 nm/280 nm and 260 nm/230 nm using ND-1000 spectrophotometer (Nanodrop Inc.), and the ratio of 260 nm/280 nm and 260 nm/230 nm should be around of 1.80 and 1.70, respectively [23]. Finally, DNA exact concentration was quantified according to a PicoGreen method using a FlUOstar Optima (BMG Labtechm Jena, Germany) and then saved in −80 °C [24].

2.4. Illumina sequencing and data processing

Purified DNA from soil samples were used as a template and the primer was designed for amplification according to V4 hypervariable region of bacterial 16S rDNA. The sequence of forward primer was 5′-GTGCCAGCMGCCGCGGTAA-3′ (515F), and the reverse primer was 5′-GGACTACHVGGGTW TCTAAT-3′ (806R) [25,26]. PCR reaction system was used following the previous reports [27,28].

Raw data were separated to samples according to same barcode sequence. Adapters, low quality and ambiguous reads were trimmed by Btrim [29], and the forward and reverse reads were integrated into a whole sequence by FLASH. Sequences were divided into groups according to the similarity of each other, and operational taxonomic units (OTUs) were formed at 97% similarity level by using UCLUST [30]. Ribosomal database project (RDP) classifier was used to determine the taxonomic identity of each phylotype [31]. Random resampling was processed with 15,000 sequences per soil sample.

2.5. Data analyses

All data were preliminary processed and analyzed by Microsoft excel. Then, detrended correspondence analysis (DCA) was used to assess the distribution of Actinobacterial community according to high-throughput sequencing data. Dissimilarity tests (MRPP, Anosim and

Table 1
The basic data of all plots.

Sample number	Elevation	Latitude	Longitude	Slope	Aspect	Vegetation type
SJY1	3220 m	35°56′6″N	100°05′27″E	5°	N	*Elymus nutans*
SJY2	3490 m	35°40′10″N	99°55′13″E	5°	N	*Poa annua-Stipa capillata*
SJY3	3880 m	35°41′26″N	99°33′1″E	15°	NW30°	*Kobresia pygmaea*
SJY4	4140 m	35°24′28″N	99°21′6″E	5°	NW20°	*Kobresia humilis*
SJY5	4480 m	34°22′15″N	97°56′57″E	18°	NW30°	*Kobresia tibetica*
SJY6	4790 m	34°08′16″N	97°40′22″E	15°	N	*Kobresia tibetica*