Acta Ecologica Sinica 31 (2011) 205–211

Contents lists available at ScienceDirect

Acta Ecologica Sinica

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

Investigation of bacterial community diversity in water of Zoige Alpine Wetland

Chen Xi [a,*], Feng Shugeng [a], Zhang Hongxun [a], Wang Yanfen [a], Bai Zhihui [b], Gu Likun [b]

[a] Graduate University of Chinese Academy of Sciences, Beijing 100049, China
[b] Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China

ARTICLE INFO

Article history:
Received 18 March 2010
Revised 7 March 2011
Accepted 12 April 2011

Keywords:
Zoige Alpine Wetland
Bacterial diversity
PCR-DGGE
16S rDNA clone library
ARDRA

ABSTRACT

Spatial isolation is currently thought to represent one of the major factors resulting in bacteria genetic variation and population abundance. The bacterial diversity in a distinct environment Zoige Alpine Wetland located in the northeast of the Qinghai-Tibetan Plateau with the altitude 3400 m on average aroused our great attention. This area belongs to Qinghai-Tibetan cold climate zone with the mean annual temperature about 1 °C. Although several studies on bacterial diversity in Qinghai-Tibetan Plateau had been reported, there is no report on wetland water in this area. In this work, six water samples were collected and the water qualities including COD_{Cr}, NH_4^+-N, NO_3^--N, NO_2^--N, TN, TP, TOC were investigated, of which results indicated that more than 80% samples sorted as II–V class of surface water sources according to the National Water Quality Standard of China (GB3838-2002). Comparison of bacterial communities among the six samples was analyzed by DGGE of PCR-amplified 16S rDNA with universal bacterial primer sets. The profiles demonstrated that samples from the Flower Lake had more DNA bands than the Conservatory Station inferring higher diversity. In addition, the samples from the same environment shared similar compositions of bacterial communities. Bacterial community composition and predominant bacteria were analyzed by 16S rDNA clone library. The dominant group was *Proteobacteria* (51.6% of the total clones, which contained 24.2% alpha *proteobacteria*, 14.5% beta *proteobacteria* and 12.9% gamma *proteobacteria*). And the *Bacteroidetes* added to 17.7%, *Verrucomicrobia* to 4.8%. More than 24.2% of the total clones showed high similarity to uncultured bacteria. The above work provides some information on bacterial diversity for special site of spatial isolation.

1. Introduction

Zoige Alpine Wetland, at the northwest part of Sichuan Province of China, is located in the northeastern margin of the Qinghai-Tibetan Plateau (32°10′–34°10′N; 101°45′–103°25′E), with an altitude of 3400 m [1]. Air pressure is low, about 668.8 hPa, due to its high altitude [2]. This region belongs to Qinghai-Tibetan cold climate zone and it is strongly affected by the southwest monsoon from the Indian Ocean. The site is affected by quite complex patterns of atmospheric circulation and therefore particularly sensitive to climatic variation. The climate of the area is cool and moist. Both temperature and precipitation exhibit a remarkable seasonality. The mean annual temperature is 1 °C with an overall range of −10 to 11 °C. Annual precipitation ranges between 560 and 860 mm, and 80% of the moisture falls in June–August [3]. The cool and moist climate has led to the development of marshy grassland with peaty soils [4]. The biodiversity in this region is rich in wetland plants such as *Kobresia kansuensis*, *Carex muliensis* and *Carex lasiocarpa* [5]. One species of sedge, *Carex muliensis*, has been a major source of cellulose to the peat throughout the history of the bog [3]. Zoige Alpine Wetland is bearing the biggest alpine peat marsh zone in China and well-known to be the classical alpine wetland in the world [6].

In aquatic systems, it is important to evaluate changes in the microbial community structure, because the microbial community is the foundation of biogeochemical cycles. The Zoige Alpine Wetland is special for its location, climate and geological features, which include low latitude, high altitude, low air pressure, strong radiation, low temperature, high moisture, developed peatland and so on. It is these features that make the wetland to be a reservoir of unique microbial resources. Existing studies on Zoige Alpine Wetland mainly focused on the macro features, such as climate changes [4], landscape pattern [5] and ecosystem evaluation [7]. However, there were few studies concerning the microorganism communities. In this study, bacterial community structure in water of Zoige Alpine Wetland was studied using Denaturing Gradient Gel Electrophoresis (DGGE) and clone library analysis of Polymerase Chain Reaction (PCR) – amplified 16S rDNA with universal bacterial primer sets. The data collected from our research would not

* Corresponding author.
E-mail address: chenxi20080808@gmail.com (C. Xi).

1872-2032/$ - see front matter © 2011 Ecological Society of China. Published by Elsevier B.V. All rights reserved.
doi:10.1016/j.chnaes.2011.04.002

only facilitate the study on variation of ecological environment of Qinghai-Tibetan Plateau, but also lay a foundation to uncover the unique microbial resources of this region.

2. Materials and methods

2.1. Site description and sampling

Surveys were carried out in Zoige Alpine Wetland in August 2007. Two sites were selected, one was in the Flower Lake, and the other was in the bog down the slope of the Conservatory Station which was used to observe the ecological changes in the wetland. The two sites were away from approximately 20 km.

Water samples were collected at four points surrounding the Flower Lake (Nos. 1–4) and at two points in the bog near the Conservatory Station (Nos. 5 and 6). The geographical and environmental characteristics were detected by GPS and HI 9828 Autoanalyzer (Table 1). Samples used for analysis of water qualities (TN, NH_4^+-N, NO_3^--N, TP and TOC) were fixed with sulfuric acid. Samples used for analysis of bacterial community structure were filled into sterile glass bottles sealed with butyl rubber stopper, and then kept in an ice box during transportation.

2.2. Water qualities detection

COD_{cr}, NH_4^+-N, NO_3^--N and NO_2^--N were measured with a WTW Water Quality Tester according to the manufacturer's instructions. TN, TP and TOC were measured by the Environmental Monitoring Central Station of Chengdu, Sichuan Province, China.

2.3. DNA extraction

Bacteria total DNA was extracted from 500 ml of each water sample by using the modified procedures described previously. In brief, water was placed in a screw-cap tube and the bacterial cells were collected by centrifugation, cracked by glass beads and SDS. Then the protein was removed with phenol: chloroform: isoamyl alcohol (25:24:1). Subsequently, the nucleic acids were precipitated and washed twice in 75% (v/v) ethanol. The final DNA was re-suspended in 100 μl TE buffer. The size of the bacteria total DNA was verified on a 0.7% agarose gel.

2.4. PCR-DGGE

The V3 region of bacterial 16S rDNA fragments were amplified by using primers F357-GC (5′-CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG GCC TAC GGG AGG CAG CAG-3′) and R518 (5′-ATT ACC GCG GCT GCT GG-3′) [8]. The final concentration of different components in the mixture (50 μl) were as follows: 5 μl 10× Reaction Buffer, 1.5 mM $MgCl_2$, 0.2 μM of each deoxynucleoside triphosphate, 0.4 μM of each primer, 0.8 mg/ml bovine serum albumin, 1.25 U *Taq* DNA polymerase, and DNase and RNase free filter sterilized water. About 50 ng of bacteria total DNA was added to 50 μl of PCR mixture. The bovine serum albumin and *Taq* DNA polymerase were bought from Takara, Japan.

A hot start and touchdown program was implemented as follows: after initial denaturation at 94 °C for 5 min, bovine serum albumin and *Taq* DNA polymerase were added; followed by 30 cycles of 94 °C for 1 min, the annealing temperature for 1 min, and 72 °C for 45 s were performed, and then the reaction mixture was kept at 72 °C for 10 min. During the reaction cycle, the annealing temperature was decreased by 0.5 °C from 65 °C to 55.5 °C every cycle in the first 20 cycles. The annealing temperature was 55 °C in the last 10 cycles. All reactions were performed in a MyCycler Thermal Cycler™ System (Bio-Rad, USA). The size of the amplification product was verified on a 1.5% agarose gel.

DGGE was performed by using a Dcode™ System (Bio-Rad, USA). For the generation of bacterial community profiles, 10% polyacrylamide gels (acrylamide:bisacrylamide = 37.5:1) were prepared and electrophoresed with 1 × TAE buffer [50 × TAE buffer is 2 M Trisbase, 1 M sodium acetate and 50 mM EDTA (pH8.0)]. The DGGE gel contained a 30–48% gradient of urea and formamide in the direction of electrophoresis as a denaturant [100% denaturant was defined as 7 M urea with 40% (v/v) formamide]. DGGE was conducted at 60 °C, firstly at a constant voltage of 20 V for 30 min and subsequently at 150 V for 5.5 h. The gel was stained with EtBr and photographed on a UV transilluminator Gel Doc™ System (Bio-Rad, USA). The processing of the DGGE gel was done with the Bio-Rad software Quantity One 4.3.0.

2.5. Construction of 16S rDNA clone library and ARDRA analysis

Samples 1–5 were selected to construct the 16S rDNA clone library. The PCR primers used to amplify the 16S rDNA of bacteria were primers F27(5′-GAG AGT TTG ATC CTG GCT CAG-3′) and R1492(5′-CTA CGG CTA CCT TGT TAC GA-3′) [9]. The amplification reaction mixture used was the same as that used for amplification of DNA for DGGE, as described above. The reaction conditions were as follows: after initial denaturation at 95 °C for 1 min 30 s, 5 cycles of 95 °C for 30 s, 60 °C for 30 s, and 72 °C for 1 min; 5 cycles of 95 °C for 30 s, 55 °C for 30 s and 72 °C for 1 min; 15 cycles of 95 °C for 30 s, 50 °C for 30 s and 72 °C for 1 min were performed, and then the reaction mixture was kept at 72 °C for 10 min, lastly at 60 °C for 10 min. The amplicons were visualized on a 1.5% agarose gel.

The PCR products were cloned into the pGEM-T Easy Vector Systeml (Promega, Japan) according to the manufacturer's instructions. Ligations were transformed into competent cells of *Escherichia coli* DH5α. White colonies were randomly picked and

Table 1
The geographical and environmental characteristics of the sampling points.

Sample no.	1	2	3	4	5	6
Location	33°54′998N, 102°49′032E	33°54′945N, 102°48′941E	33°55′003N, 102°48′774E	33°54′974N, 102°48′549E	33°55′683N, 102°52′230E	33°55′683N, 102°52′230E
Altitude (m)	3437	3434	3433	3429	3440	3440
Sampling time	2007.8.19 12:30:00	2007.8.19 14:00	2007.8.20 12:30	2007.8.19 16:00	2007.8.20 10:00	2007.8.20 10:45
Depth (cm)	20	30	10	100	20	30
Main vegetation populations	*Hipperis vulgaris*	*Carex muliensis*	*Hipperis vulgaris, Polygonum amphibium*	–	*Carex muliensis, Eleocharis valleculosa*	*Carex muliensis*
Air temperature (°C)	28	23	19	23	23	23
Water temperature (°C)	19	23	20	23	16	17

Note: "–" means there was no plant in Location No. 4.