Front. Biol. China 2009, 4(2): 187–191
DOI 10.1007/s11515-009-0002-0

RESEARCH ARTICLE

Seasonal dynamic changes in bacterial compositions in the Inner Mongolia desert steppe

Ping LU[1], Wanli MA (✉)[1,3], Hao LI[1], Yongsheng WU[1], Guifen LV[1], Jizheng HE[2]

1 College of Life Science and Technology, Inner Mongolia Normal University, Huhhot 010022, China
2 Research Center of Ecology and Environment, Chinese Academy of Sciences, Beijing 100083, China
3 Planning and Design Academy of Forestry Products Industry, State Forestry Administration, Beijing 100714, China

Abstract Polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) technique was employed to examine the seasonal dynamic changes in bacterial community composition in the Inner Mongolia desert steppe using specific primers F954 and R1369. Bright and reproducible bands were sequenced, and the phylogenic tree was constructed. The results show that the bacterial community composition changed between different seasons. The specific bands were different between the sampling sites with light and heavy levels of degraded grassland. Three main types of bacteria constituting the microbial community in the Inner Mongolia desert steppe belonged to the α, γ and δ-sub phyla of Proteobacteria, Bacteroidetes and Acidobacteria. The unculturable bacteria accounted for 69% of the whole bacterial community of the Inner Mongolia desert steppe.

Keywords Polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE), Inner Mongolia desert steppe, community composition, seasonal dynamic change

1 Introduction

The desert steppe is a highly xeric type of grassland specific to middle Asia. The coverage of the area includes the middle and western regions of Inner Mongolia, China. It constitutes an important part of the Inner Mongolia steppe. As a consequence of global climate change and excessive grazing pressure, the desertification of the desert steppe is accelerating. In extreme circumstances, the vegetation in some regions is vanishing. In addition, the desertification of the steppe is also accountable for deterioration of the grassland ecosystem and its limited ability for self regulation and natural repair. As decomposers, soil microbes participate in almost all of the biochemical metabolism in soil, such as the cycles of C, N, P and S, nutrient conversion from soil to plant and depollution of soil (Chen et al., 2006).

Investigations on soil microbes in grasslands have been carried out mainly on typical steppes and deserts, and soil microbes in desert steppes have not been studied so far, to the best of our knowledge. Traditional techniques have been used to assess the soil microbes, including analysis of the physical and chemical characteristics, nutritional components, microbial quantities, and microbial flora of the soil, and so on. In recent years, the evaluation of the microbial community in soil has employed molecular biological approaches. Denaturation gradient gel electrophoresis (DGGE), for example, has been widely applied to the analysis of microbial community structures since it was first used in the detection of environmental microbes (Muyzer et al., 1993; Xing et al., 2005; Song et al., 2005; Fu et al., 2005; Liu et al., 2005; She et al., 2005; Gao et al., 2005; Yuan et al., 2005; Chu et al., 2003; Yin et al., 2004; Ye et al., 2006).

In this paper, we employed DGGE in the investigation of seasonal dynamics of microbial community in the Inner Mongolia desert steppes. This research provides a basis for in-depth understanding of the effect of soil microbes on the stability and health situation of the desert steppe soil ecosystem, and their influences on and interactions with vegetation growth and development.

2 Materials and methods

2.1 Sampling sites and soil sample collection

Two sampling sites, DM and SH, were chosen. DM is located in Damao Banner, Baotou, Inner Mongolia, with a

Received September 15, 2008; accepted October 27, 2008

E-mail: luping@imnu.edu.cn, wanlima518@126.com

latitude of 41°50′ N, longitude of 110°11'E, and an altitude of 1425 m. The assessment showed slight degradation of the steppe. The steppe type was *Stipa klemenzil* Roshev., *Cleistogenes songorica* Ohwi. and *Convolvlus ammannii* Desr. desert steppe. The vegetation had low grasses with an average height of 10 cm, and a few species. SH was in Suniteyou Banner, Xilingol, Inner Mongolia, with a latitude of 42°25′ N, longitude of 112°26′ E and an altitude of 1202 m. The steppe type was *Caragana* spp. and *Cleistogenes songorica* Ohwi. desert steppe. *C. microphylla, C. stenophylla* and *C. intermedia* usually grow in the steppe. The latter two were 25–30 cm in height and were mingled with other species.

Five sampling sites were chosen in a quincuncial pattern. The soil samples were taken by soil auger from two layers 0–10 cm and 10–20 cm from the ground surface and then mixed. They were put in polyethylene bags in an ice box, and were stored at −20°C until use. The samples were respectively collected on November 28 of 2005, July 10 of 2006, October 9 of 2006, January 6, April 3, and July 9 of 2007.

2.2 Soil microbial DNA extraction and PCR amplification of 16S rDNA

Soil microbial total DNA was extracted using Ultra-Clean™ Ultraclean Soil DNA isolation kit (Mo Bio Laboratories, Solana Beach, CA, USA) with modifications (Lu et al., 2008). The concentration and quality of DNA samples were determined by UV spectrometry (NanoDrop ND-1000; NanoDrop Technologies). In addition, 5 μL of each DNA sample was also assessed on 0.8% agarose gel to check for its integrity. The DNA samples were stored at −20°C until required for use.

BACT-954-F-GC (cgc ccg ccg cgc gcg gcg ggc ggg gcg ggg gca cgg ggg ggc aca agc ggt gga gca tgt gg) and BACT-1369-R (gcc cgg gaa cgt att cac cg), commonly used as DGGE-PCR primers for different bacteria groups (Tzeneva et al., 2008), were used. PCR amplification was performed on a Peltier Thermal Cycler (PTC-100) in a total volume of 50 μL. The reaction mixture contained 5 μL of 10×buffer, 4 μL of 2.5 mmol/L dNTPs, 0.5 μL of 10 mmol/L each primer, 0.4 μL of ExTaq (5 U/μL), 5 ng of soil microbial total DNA, and 0.6 μL of BSA (25 mg/mL).

The amplification was performed using a touchdown PCR protocol: first, denaturation at 94°C for 5 min, then 35 cycles of denaturation at 94°C for 30 s, annealing for 30 s at 61°C for the first cycle, and thereafter at a 0.5°C decrease gradient each for 10 cycles, extension for 60 s at 72°C, and a final extension at 72°C for 7 min. PCR products were separated using 1% agarose gel and stained with SYBR green to examine the amplification results.

2.3 DGGE specific bands cloning and sequencing

Thirty-five μL of amplified PCR products was analyzed by DGGE on a Bio-RAD DCode™ Universal Mutation Detection System using 6% polyacrylamide gel with a 35%–60% denaturing gradient (100% gradient gel contained 7 mol/L of urea and 40% of deionized formamide). The DGGE was performed at 85 V for 10 h and the gels were stained with SYBR green solution for 30 min. The images were photographed with a GelDoc system (GelDoc 2000, BioRad) and analyzed by Quantity One software (Bio-Rad Laboratories Inc., Hercules, CA, USA).

Each bright specific band was excised under UV light from the gel and the DNA was eluted by 0.5×TE at 4°C overnight. One μL of the elute was PCR-amplified following the same protocol described above, and the PCR products were purified using TaKaRa Agarose Gel DNA Purification Kit Ver.2.0 and then ligased with the pGEM-T Easy vector (Promega) following the instruction in the product manual.

Five μL of ligation solution was used for transformation of the ligased plasmid to DH5α competence cells by incubation of the mixture on ice for 20 min. The cells were cultured in 500 μL SOC Medium (containing: 2% tryptone, 0.5% yeast extract, 0.05% NaCl, 0.0186% KCl, 20 mmol/L glucose, 10 mmol/L $MgCl_2$) with shaking for 1 h at 37°C. One hundred μL of the culture was spread on an LB plate containing isopropyl-β-D-thiogalactopyranoside (IPTG), X-gal and ampicillin, and incubated at 37°C overnight.

Each white clone was PCR-amplified using T7 (5′-taatacgactcactataggg-3′), and SP6 (5′-atttaggtgacactatagaata-3′) primers, and the products were sequenced using an ABI 3700 sequencing platform and BigDye Terminator Version 3.1. The sequences were submitted to the GenBank database (ID: EU328310-EU328325). The sequences were blasted against the non-redundant sequence database (nr) in NCBI, and the homology was assessed by alignment of the sequences using DNAMAN software. The UPGMA phylogenetic tree of 16S rRNA was established by using CLUSTAL 1.83 and Mega 3.1. The tree was produced by parsimony analysis for sequence comparisons, closest step-wise addition and branch swapping by tree bisection-reconnection, and distance analysis by neighbor-joining with kimura 2-parameter distances. For each analysis, 1000 bootstrap replicates were performed to assess statistical support for the resulting phylogenetic tree topology.

3 Results and discussion

3.1 Extraction of soil microbial genomic DNA and PCR amplification of bacterial 16S rDNA

The successful extraction of soil microbial DNA is a prerequisite for the analysis of microbial communities in the soil using molecular approaches. However, the extraction from a desert steppe is difficult due to the humus in soil. With modification of the extraction protocol

Table 1 OD values and concentrations of soil microbial DNA samples

		Nov 2005	Jul 2006	Oct 2006	Jan 2007	Apr 2007	Jul 2007
DM	ng/μL	27.65±1.25	33.72±2.04	24.47±0.98	21.81±1.08	23.79±1.10	22.35±0.94
	OD_{260}/OD_{280}	1.67±0.12	1.52±0.09	1.60±0.08	1.63±0.09	1.65±0.08	1.65±0.09
SH	ng/μL	30.98±1.05	29.05±1.09	28.32±0.95	32.16±1.10	32.40±0.86	27.69±0.69
	OD_{260}/OD_{280}	1.67±0.08	1.56±0.03	1.70±0.09	1.65±0.08	1.57±0.05	1.67±0.08

DM and SH: the two sampling sites chosen.

of the kit, good quantity and quality of the microbial DNA from soil has been successfully obtained (Lu et al., 2008). The molecular weight of integrate total genomic DNA extracted from the soil microbial community was about 23 kb (Fig. 1). The concentrations of the DNA were in the range of 20–30 ng/μL, and the DNA was considered to be of good quality when the ratio of 260/280 nm absorbance was above 1.5 (Table 1).

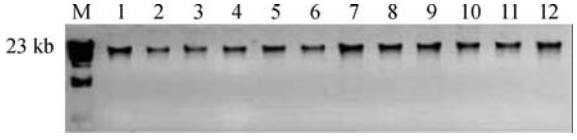

Fig. 1 Agarose gel electrophoresis of soil microbial genome. 1–6: soil microbial DNA samples from DM in Nov 2005, Jul 2006, Oct 2006, Jan 2007, Apr 2007, and Jul 2007; 7–12: soil microbial DNA samples from SH in Nov 2005, Jul 2006, Oct 2006, Jan 2007, Apr 2007, and Jul 2007. M: λDNA *Hin*d III marker, DM and SH are the two sampling sites chosen.

The DNA samples were diluted 10-fold and PCR-amplified using the aforementioned 16S rDNA primers. The 16S rDNA PCR product was about 500 bp (Fig. 2). As expected, the touchdown PCR program reduced non-specific amplification effectively.

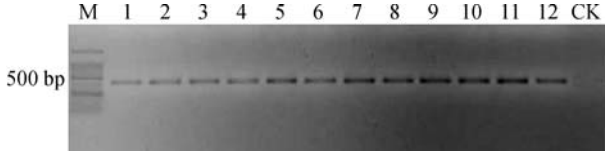

Fig. 2 PCR amplification of bacterial 16S rDNA. 1–6: soil microbial DNA samples from DM on Nov 2005, Jul 2006, Oct 2006, Jan 2007, Apr 2007, and Jul 2007; 7–12: soil microbial DNA samples from SH on Nov 2005, Jul 2006, Oct 2006, Jan 2007, Apr 2007, and Jul 2007; M: 100 bp DNA ladder; CK: PCR negative control. DM and SH are the two sampling sites chosen.

3.2 PCR-DGGE detection of seasonal dynamic changes in bacterial community constitutions in Inner Mongolia desert steppe

The bacterial community structures of both DM with light degradation and SH with heavy degradation changed seasonally among 16 bright DNA bands (Fig. 3).

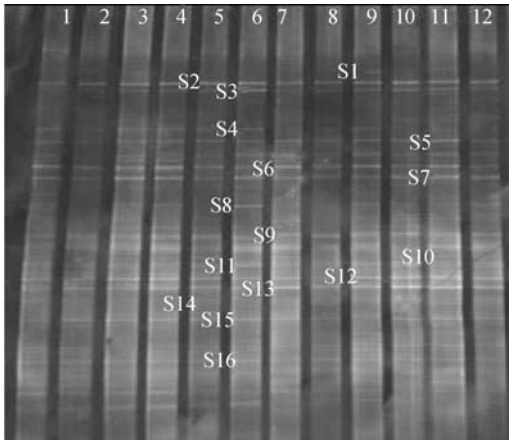

Fig. 3 DGGE profiles of seasonal dynamic changes in bacterial composition in the Inner Mongolia desert steppe. 1–6: soil microbial DNA samples from DM in Nov 2005, Jul 2006, Oct 2006, Jan 2007, Apr 2007, Jul 2007; 7–12: Soil microbial DNA samples from SH in Nov 2005, Jul 2006, Oct 2006, Jan 2007, Apr 2007, and Jul 2007. DM and SH are the two sampling sites chosen.

In the sampling site DM, the dominant microbes differed seasonally. The bands of S2, S3, S4, S5, S6, S7, S9, S12, S13, S15 and S16 appeared in four seasons, and these bands only showed brightness differences, which reflected the changes in microbial quantities. The presence of band S8 and absence of band S14 appeared to be the exclusive feature of the microbial community of site DM in spring, while the presence of band S10 and absence of band S11 was the exclusive feature of the microbial community of site DM in winter (Fig. 3).

In the sampling site SH with heavy degradation, the dominant microbes showed slight changes between different seasons. For example, in autumn, the bands S1 and S4 were present and band S16 was absent, and in winter and spring, S10 was present. All the other bands were present in all the seasons with the changes in brightness.

Respective specific bands were identified for the sites SH and DM. Band S1 was unique to the site SH while band S4 was common in the site DM in all seasons, and it was only present in autumn in the site SH. Band S10 appeared in winter in the two sites, and in the spring samples of site SH. While band S14 characterized the bacterial community in site DM soil in spring, it was found in the soil of site SH

in all seasons. This suggested that the apparent variations in temperatures and precipitations in different seasons led to the dynamic changes in soil microbes.

3.3 Phylogenetic analysis of bacterial groups in the Inner Mongolia Desert Steppe

The phylogenetic relationship is shown in Fig. 4. Among 16 scorable bands, the sequence of band S12 was closely aligned with Acidobacteria 16S rDNA sequences, as indicated by the blast against the non-redundant sequence database (nr) in NCBI. The sequences of bands S3, S5, S13, S14 and S15 were similar to Proteobacteria 16S rDNA sequences. The genotypes of the Proteobacteria type accounted for 31% of the genotypes among all the bacterial communities shown in the DGGE profile. This frequency was lower than that estimated of the Proteobacteria genotypes in the upland grass pastures in the UK (McCaig et al., 1999), but was higher than that in the typical steppe in Inner Mongolia (Zhou et al., 2007). The sequences of bands S2, S6, S7, S9 and S11 were close to Bacteroidetes 16S rDNA sequences, and the sequences of bands S1, S8, S10 and S16 showed similarities to Sphingobacteria 16S rDNA sequences. Many Bacteroidetes bacteria are components of the major microbial populations in the intestinal tracts of animals. Over-grazing may lead to accumulation of substantial amount of animal intestinal bacteria in the soil through their feces in the Inner Mongolia desert steppe.

Thirty one percent of bacterial types including S1, S8, S10, S16 and S13 were culturable, while 69% including S2, S3, S4, S5, S6, S7, S9, S11, S12, S14 and S15 were

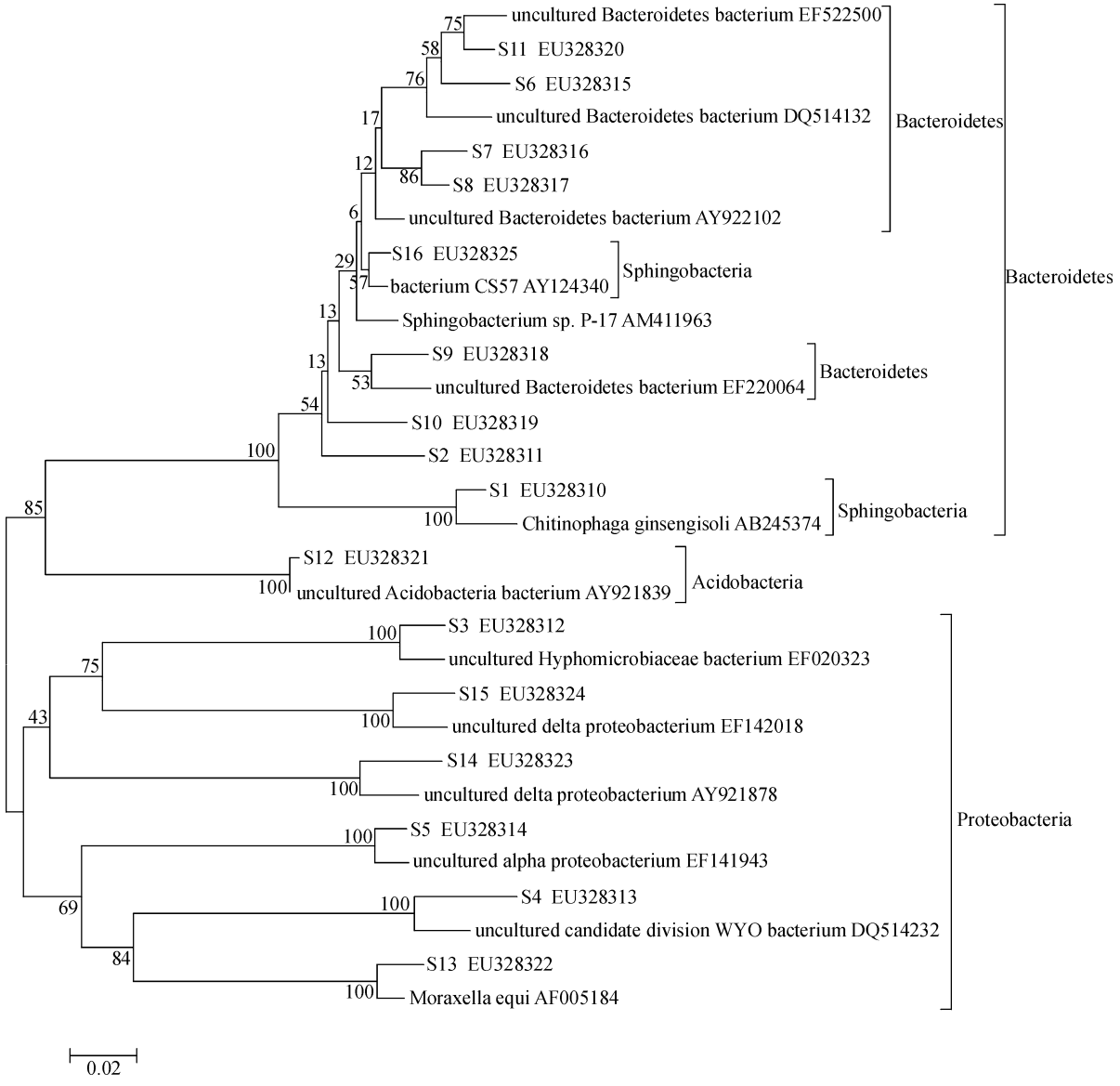

Fig. 4 Neighbor-joining tree showing the relationship of Inner Mongolia desert grassland clones based on analysis of 16 bases of aligned 16S rRNA sequences clones

unculturable. The present results provide valuable information on soil bacterial diversity in the Inner Mongolia desert steppe and illustrate the necessity of using molecular techniques such as PCR-DGGE to examine the seasonal dynamic changes in bacterial community composition.

In conclusion, the bacterial community structure of the Inner Mongolia desert steppe, with severe ecological situations and poor soil nutrients, changed seasonally in both type and quantity, and the key soil bacterial groups also changed with different degradation gradients. Useful information on dynamic spatial and temporal changes has been obtained, which provides theoretical evidence for the management of the Inner Mongolia desert steppe.

Acknowledgements The authors are very grateful to Dr. Shubiao Wu from the University of New England, Australia for his kind suggestions to this paper. This work was supported by the National Natural Science Foundation of China (Grant No. 30560030), and the University Scientific Research Foundation of Inner Mongolia (No. NJ05099).

References

Chen C L, Liao M, Zeng L S (2006). Methods to measure the microbial composition and functional diversity in polluted soils. Acta Ecologica Sinic, 10: 3404–3412 (in Chinese)

Chu L, Wang Y B, Liu D Y (2003). Investigation on the vegetation condition of copper tail mining wasteland in Tongling (Wugongli), Anhui J Biol, 2: 13–15 (in Chinese)

Fu Y G, Wang F, He P S, Xia S Q, Zhao J F (2005). Analysis of microbial composition with DGGE sludge compost technology. China Environ Sci, 25: 98–101 (in Chinese)

Gao H W, Lv X, Dong M S (2005). Application of PCR-DGGE finger printing in food microbiology. Food Sci, 26: 465–468 (in Chinese)

Liu X C, Wu C Q, Zhang Y, Yang M, Li H Y (2005). Application of polymerase chain reaction-denaturing gradient gel electro-phoresis (PCR-DGGE) to the analysis of changes of microbial ecological communities in activated sludge systems. Acta Eco Sinic, 4: 842–846 (in Chinese)

Lu P, Li H, Wu Y S, Lv G F, Ma W L (2008). Extraction methods of microbial DNA from desert grassland soil in Inner Mongolia. J Inner Mongolia Univ, 39(4): 430–434 (in Chinese)

McCaig A E, Glover L A, Prosser J I (1999). Molecular analysis of bacterial composition and diversity in unimproved upland grass pasture. Appl Environ Microbiol, 65: 1721–1730

Muyzer G, de Waal E C, Uitterlinden A G (1993). Profiling of complex microbial population by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Appl Environ Microbiol, 59: 695–700

She Y H, Zhang F, Xiang T S, Liu B B, Zhao L P, Zhou L G, Shu F C (2005). Microbial diversity in petroleum reservoirs analyzed by PCR-DGGE. Acta Ecol Sinic, 2: 238–242 (in Chinese)

Song T Y, Lin Z M, Zheng W W (2005). Analysis on the species diversity of cyanobacteria in paddy field by comparison between normal culture and DGGE method. Chin J Eco-Agricul, 7: 140–143 (in Chinese)

Tzeneva V A, Heilig H G, van Vliet W A, Akkermans A D, de Vos W M, Smidt H (2008). 16S rDNA targeted DGGE fingerprinting of microbial communities. Methods Mol Biol, 410: 335–349

Xing D F, Ren N Q, Song Y Y, Li Q B, Zhao L H, Xu X L (2005). Application of DG-DGGE to analyze microbial community diversity and population dynamics in fermentative hydrogen-producing system. Acta Ecol Sinic, 7: 1818–1823 (in Chinese)

Ye Y F, Min H, Lu Z M (2006). Monitoring impact of mefenacet treatment on soil microbial communities by PCR-DGGE fingerprinting and conventional testing procedures. J Environ Sci, 2: 373–378

Yin J, Chen Y X, Liu H, Wang Y P (2004). Preliminary application of PCR-DGGE to analyzing microbial diversity in biofilters treating air loaded with ammonia. Environ Sci, 11: 11–15 (in Chinese)

Yuan F, Ran W, Hu J, Shen Q R (2005). Ammonia-oxidizing bacteria communities and their influence on the nitrification potential of Chinese soils measured by denaturing gradient gel electrophoresis (DGGE). Acta Ecol Sinic, 6: 1318–1324 (in Chinese)

Zhou X Q, Wang Y F, Cai Y, Huang X Z, Hao Y B, Tian J P, Chai T (2007). PCR-DGGE detection bacterial community structure in the Inner Mongolia steppe with two different DNA extraction methods. Acta Ecol Sinic, 5: 1684–1689 (in Chinese)