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European Journal of Protistology 49 (2013) 516–525

European Journal of PROTISTOLOGY

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Microbial genetic diversity and ciliate community structure along an environmental gradient in coastal soil

Feng Zhao[a,b], Kuidong Xu[a,*]

[a] *Institute of Oceanology, Chinese Academy of Sciences, 266071 Qingdao, China*
[b] *University of Chinese Academy of Sciences, 100049 Beijing, China*

Received 26 September 2012; received in revised form 13 December 2012; accepted 24 January 2013
Available online 30 April 2013

Abstract

We investigated the microbial genetic diversity and ciliate community in coastal soil from five sites with an environmental gradient using denaturing gradient gel electrophoresis (DGGE), gene sequencing and the Ludox–QPS method. The analyses of both the 16S ribosomal RNA (rRNA) gene and 18S rRNA gene DGGE resulted in equal or even a higher number of bands found in the samples taken from the high-salinity sites IV and V than in those taken from the low-to-moderate-salinity sites I–III. Cluster analysis of both DGGE profiles classified the five sites into three main groups (sites I, II and III and IV and V), which corresponded well to the analysis of environmental factors. There were 13 species observed at site I, three species at site II and nine species at site III, while no active ciliates were observed at the high-salinity sites IV and V. By contrast, the ciliate-specific DGGE revealed a higher number of bands in the samples taken from the high-salinity soil. Furthermore, gene sequencing suggested that the ciliates in the high-salinity soil comprised forms originating not only from soil but also from marine environments. The data indicate that saline soil may maintain a high diversity of ciliates and soil salinity might be the most influential factor regulating the community structure of ciliates.

Keywords: Ciliate community; Coastal soil; Molecular diversity; Salinisation

Introduction

Coastal soils are generally salt-affected mainly due to hydrological and geographical reasons and thus represent a special and extreme environment. Soil salinisation is a serious ecological problem yielding a range of adverse effects on the physical and chemical properties of soil and resulting in the degradation of land productivity (Sardinha et al. 2003). Underground microorganisms are essential components of the soil environment and play key roles in energy flow and soil processes (Zahran 1997). Sardinha et al. (2003) indicated that soil salinity was one of the most important environmental conditions for soil microorganisms and significantly affected the microbial biomass in terms of C and N, fungal ergosterol and adenosine triphosphate. However, the knowledge regarding microbial community composition in salt-affected soil and its response to salinisation is far from complete.

Molecular techniques based on ribosomal RNA (rRNA) genes have provided appropriate tools for monitoring microbial community shifts in natural environments (Kleinsteuber et al. 2006; Lara et al. 2011). The denaturing gradient gel electrophoresis (DGGE) technique can quickly evaluate the response of a microbial community to various environmental conditions and obtain taxonomic information in combination with gene sequencing (Moon-van der Staay et al. 2006).

*Corresponding author. Tel.: +86 532 82898776; fax: +86 532 82898776.
E-mail address: kxu@qdio.ac.cn (K. Xu).

0932-4739/$ – see front matter © 2013 Elsevier GmbH. All rights reserved.
http://dx.doi.org/10.1016/j.ejop.2013.01.002

It has been used to survey microbial community shifts along a salinity gradient (Casamayor et al. 2002). In comparison with those in aquatic environments, our knowledge about the response of microbial genetic diversity to soil salinisation is very scant.

Ciliates are a major group of protozoa with high diversity and wide distribution in the soil environment. Soil ciliates could quickly respond to soil environmental changes (Bamforth 1995). Foissner et al. (2002) indicated that there was an obvious correlation between soil salinity and the number of species in samples from Namibia. The total number of species decreased in extremely saline soil. However, the data are very scant and our understanding of the diversity and distribution of ciliates in soil is hampered by the shortcoming of integrating quantitative and qualitative techniques. Xu et al. (2010) developed the Ludox–QPS method that combines the Ludox density gradient centrifugation and the quantitative protargol stain (QPS) initially described by Montagnes and Lynn (1987) to estimate the role of ciliates in marine sediments. Zhao et al. (2012) used the Ludox–QPS method to assess the diversity of active ciliates in soil and confirmed that the method fulfils the requisite of enumeration of soil ciliates with good taxonomic resolution. So far knowledge of the relationship between ciliate diversity and soil environment is sparse.

The aim of this study was to estimate the responses of microbial genetic diversity and ciliate community structure to coastal soil salinisation. Based on the coastal soil samples collected from five sites along an environmental gradient, we evaluated: (i) the prokaryotic and microeukaryotic genetic diversity using the DGGE technique, (ii) the ciliate community structure using the Ludox–QPS method and (iii) the molecular diversity of soil ciliates using ciliate-specific DGGE and gene sequencing.

Material and Methods

Soil sample collection

Soil samples with different salinity levels were collected from five sites of the Yellow River delta in the town Dongying, China (37°40′–46′ N, 118°44′–119°02′ E) on 19 September 2009 (Fig. 1). The Yellow River delta has suffered serious salinisation due to drying up of the Yellow River, rising sea level and seashore erosion (Xu et al. 2004). Sites I, II and III were distant from the coastal wetland and were about 25 km away from sites IV and V. Site I was near a farmland and was mainly covered with perennial trees as well as grasses, site II was covered with the common reed *Phragmites australis* and site III with the seagrass *Suaeda glauca*. Sites IV and V were located in a wetland near the sea and were mostly covered with *S. glauca* and *P. australis*, respectively (Fig. 1).

At each site, three replicate samples about 10 m apart from each other were randomly taken from the surface 0–5 cm of soil using an auger (inner diameter 3.2 cm). Each replicate sample was first sieved with a 2-mm-sized mesh and then treated as follows: (1) 5 g of soil were fixed with ice-cold glutaraldehyde (2% f.c.) in the field for evaluating the ciliate community by the Ludox–QPS method and (2) the remaining soil was quickly brought back to the laboratory in an icebox, from which about 10 g of soil were stored at −20 °C for DNA extraction. The remaining soil was used for measuring the environmental factors.

The soil water contents were determined by drying at 105 °C for 48 h. Soil salinity and pH were determined in a soil/water slurry (5 parts and 2 parts distilled water with 1 part soil, respectively) (Bao 2000). Total organic carbon (TOC) was examined in Vario TOC cube (Elementar, Hanau, Germany). The phosphorus (P) content and the potassium (K) content were measured using the inductively coupled plasma optical emission spectrometry (Thermo Fisher Scientific Inc., Rockford, IL, USA). The nitrogen (N) content was measured by the Kjeldahl method (Bremner and Mulvaney 1982).

DNA extraction and polymerase chain reaction amplification

To evaluate the prokaryotic and microeukaryotic genetic diversity, 1 g of soil of each replicate was taken to extract the total DNA with the modified sodium dodecyl sulphate (SDS)-based method (Zhou et al. 1996). Three DNA samples of each site were mixed and purified using the TIANquick maxi purification kit (Tiangen Biotech, Beijing, China). The eukaryotic 18S rRNA gene was amplified using primers Euk1A (5′-CTGGTTGATCCTGCCAG-3′) and Euk516r with a GC-clamp (5′-CGCCCGGGGCGCGC-CCCGGGCGGGGCGGGGGCACGGGGGGACCAGAC-TTGCCCTCC-3′), as suggested by Díez et al. (2001). The polymerase chain reaction (PCR) amplification mixture contained: 0.3 μM of each primer, 12.5 μl 2 × PCR TaqMIX (100 mM KCl; 20 mM Tris–HCl; 3 mM $MgCl_2$; 400 μM deoxyribonucleotidetriphosphate (dNTP) mix; 0.1 U μl^{-1} Taq DNA polymerase) (Dongsheng Biotech, Guangdong, China), template DNA and deionised water in a final reaction volume of 25 μl. Soil DNA was amplified using GeneAmp® PCR System 9700 (PE Applied Biosystems, Foster City, CA, USA) with the following programme: 94 °C for 130 s; 35 cycles consisting of 94 °C for 30 s, 56 °C for 45 s and 72 °C for 130 s; followed by 72 °C for 10 min. The prokaryotic 16S rRNA gene was amplified using primers F357-GC (5′-CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCA-CGGGGGGCCTACGGGAGGCAGCAG-3′) and R518 (5′-ATTACCGCGGCTGCTGG-3′) (Muyzer et al. 1993). The reaction conditions were the same as those for the eukaryotic 18S rRNA gene. The PCR programme included: 94 °C for 5 min; 10 touchdown cycles consisting of 94 °C for 30 s, 67–58 °C for 30 s and 72 °C for 60 s; then 20 cycles consisting of 94 °C for 30 s, 57 °C for 30 s and 72 °C for 60 s; followed by 72 °C for 10 min.

To investigate the molecular diversity of soil ciliates from all sites, 0.6 g of soil was taken from each replicate to