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Biodiversity patterns of soil ciliates along salinity gradients

Feng Zhao, Kuidong Xu*

Institute of Oceanology, Chinese Academy of Sciences, 266071 Qingdao, China

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Abstract

We evaluated ciliate diversity in saline soils with a salinity range from 6.5 to 65 psu by the morphological method of the Ludox-quantitative protargol stain (QPS) and the molecular techniques of ciliate-specific clone library and denaturing gradient gel electrophoresis. No active ciliates could be detected with the Ludox-QPS method, while high molecular diversity of ciliates was found. The highest ciliate molecular diversity was obtained from the soil at salinity of 8.9 psu, moderate diversity was found at salinity of 6.5 psu, and the diversity sharply decreased at salinity of 50.5 psu. By contrast, the number of ciliate classes clearly decreased with increasing soil salinity: six, five, four and two classes from sites with salinity of 6.5 psu, 8.9 psu, 29.5 psu and 50.5 psu, respectively. Ciliate diversity pattern is different from that of bacteria, whose diversity is also high in extremely saline environments. Meanwhile, the composition of ciliate community was significantly different along salinity gradient. Colpodea and Oligohymenophorea were diverse in soils at salinity less than 29.5 psu, while absent in soils with salinity above 50.5 psu. BIOENV analysis indicated soil salinity and water content were the main factors regulating the distribution of ciliates in saline soils.

Keywords: Ciliate community; Culture-independent method; Molecular diversity; Saline soils

Introduction

Ciliates are a major group of protozoa with high diversity and wide distribution in soil environments. They are known to contribute to nutrient cycling through on feeding bacteria, diatoms and fungi, and then eaten by higher order predators (Vargas and Hattori 1990). Although the importance of ciliates has been recognized in natural environments, the knowledge on their diversity and distribution is far from complete (Lara and Acosta-Mercado 2012). Saline soils are one of the biotopes, in which inhabited diverse ciliates but are insufficiently investigated (Foissner et al. 2008).

Salinity has been confirmed to be a major environmental determinant for bacteria and archaea in different soil environments (Ikenaga et al. 2010; Lozupone and Knight 2007). Previous studies indicated that the molecular diversity of bacteria do not decrease along the gradient of increasing salinity, and could maintain a high level in extremely saline environments (Wang et al. 2011; Wu et al. 2006). By contrast, Foissner et al. (2002) indicated that the total number of ciliate species decreased with extremely increasing salinity in the Namibian saline soils. Foissner et al. (2002) adopted the classic non-flooded Petri dish (NFPD) method which was used to reactivate ciliates from the air-dried samples. However, this method causes under-sampling of species which are rare and have special demands to leave the resting cysts and

*Corresponding author at: Institute of Oceanology, Chinese Academy of Sciences, 7 Nanhai Road, Qingdao 266071, PR China.
Tel.: +86 532 82898776; fax: +86 532 82898776.
E-mail address: kxu@qdio.ac.cn (K. Xu).

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to reproduce to detectable numbers (Foissner 1987; Foissner et al. 2002). Therefore, whether different diversity patterns of bacteria and ciliate in saline environments are true or artifact are caused by methodological limitations. This is still unclear.

Molecular methods are useful to assess the total diversity of ciliates which include active and hidden ciliate diversities and thus to provide a more deeply and comprehensively approach to evaluate the ciliate diversity (Lara and Acosta-Mercado 2012). Molecular techniques based on 18S rRNA gene used for revealing protozoan diversity have been applied successfully in marine and freshwater environments (Epstein and López-García 2008). Recently, high molecular diversity of ciliates is revealed by the 18S rRNA gene clone library and denaturing gradient gel electrophoresis (DGGE) in soils (Jousset et al. 2010; Lara and Acosta-Mercado 2012).

Meanwhile, Xu et al. (2010) developed the Ludox-QPS method which combines Ludox density gradient centrifugation and the QPS to estimate the role of active ciliates in marine sediments. Zhao et al. (2012) utilized the Ludox-QPS method to assess the diversity of active ciliates in soil, and obtained significantly higher number of ciliates than the direct count as well as revealed higher diversity than the DGGE in the non-saline soils. Zhao et al. (2012) confirmed the Ludox-QPS method fulfills the enumeration of active ciliates with good taxonomic resolution.

In this study, we combined the Ludox-QPS method and the molecular techniques of ciliate-specific clone library and DGGE to investigate the ciliate diversity in saline soils collected from six sites of inland and coastal areas across a broad salinity. We aim to (i) investigate both the active and hidden ciliate diversities along salinity gradients, and (ii) to reveal the main factors that regulate the community structure and distribution of soil ciliates.

Material and Methods

Soil sample collection and environmental measurements

Soil samples were collected from one site in the coastal region of Changyi county, Shandong Province, China (CHY, 37°6′ N, 119°22′ E) and five sites in the inland areas of Lujinshui county (LJS, 36°27′–28′ N, 103°38′–39′ E) and Jingtai county (JT, 37°16′ N, 104°07′ E) in Gansu Province, China in March 2011 (Fig. 1). The soils in our study wetted only in raining season. LJS and JT are about 100 km distant, and about 1400 km apart from CHY. Site I was located in CHY, which was covered with the seepweed *Suaeda glauca*. Sites II, III and IV were about 1 km apart from each other in the LJS and covered with the seepweed *Suaeda glauca* and reed *Phragmites australis* in an inland wetland. Sites V and VI were located in an inland farm in JT about 500 m apart from each other and were sparsely covered with medlar trees (Fig. 1).

At each site, three replicate samples about 5 m apart from each other were randomly taken from the surface 0–2 cm of soil using an auger (i.d. 3.2 cm). For each replicate, a 5 g of soil was fixed with ice-cold glutaraldehyde (2% f.c.) in the field for evaluating the ciliate community by the Ludox-QPS method described by Xu et al. (2010). The rest of soil was put in an icebox and quickly brought back to the laboratory, where about 10 g of soil was stored at −20 °C for DNA extraction and the remaining soil was determined for environmental variables following Zhao and Xu (2013). Briefly, the soil water contents were examined by drying at 105 °C for 48 h. Soil salinity was measured in a soil/water slurry of 1:5 (w:w), and pH was determined in a soil/water slurry of 1:2 (w:w) (Bao 2000). The nitrogen content was measured

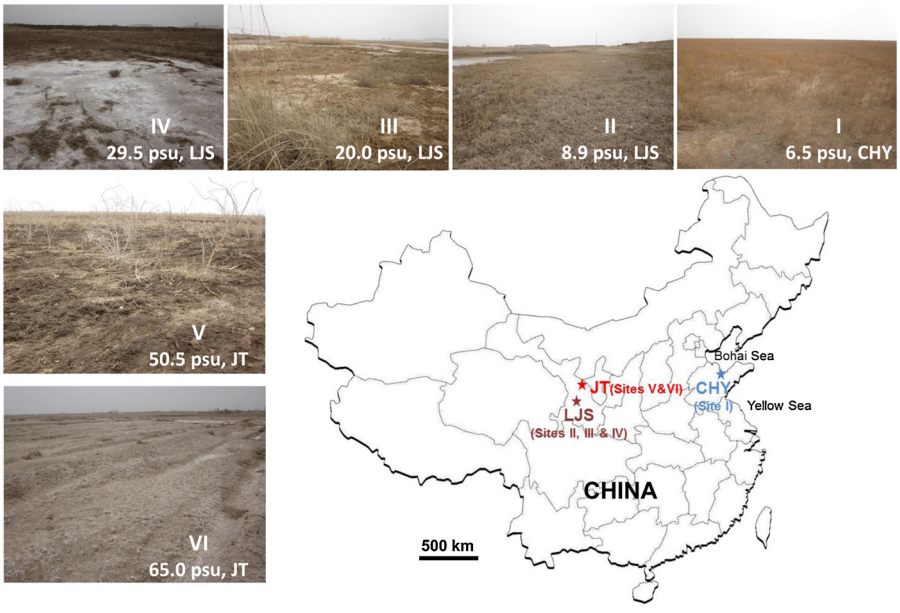

Fig. 1. Location of the study area and the habitats of sampling sites I–VI.