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Microbial genetic diversity in soils along saline-alkaline gradients at the coast of Laizhou Bay

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Summary

The soils along the coast of Laizhou Bay in China have suffered serious salinization due to natural reasons and anthropogenic effects, and thus represent a salt-affected environment. Using denaturing gradient gel electrophoresis (DGGE) and gene sequencing, we investigated the genetic diversity of microorganisms in relation to environments, in particular soil salinity (0.1-19 psu) and pH (7.9-10) at five sites in the coastal soils of the Changyi National Marine Specific Protection Area and an adjacent idle salt field. There were obvious shifts of both the microeukaryotic and prokaryotic communities along the environmental gradients. The sequencing of microeukaryotic bands uncovered highly diverse groups which were primarily dominated by Cercozoa. The sequences of Cercomonadida were detected for the first time from highly saline soils (about 19 psu), while the upper limit of Cercomonadida in saline soil and aquatic environments detected by previous studies was 5 psu. The sequencing of bacterial bands revealed the presence of Proteobacteria at all sites, whereas Acidobacteria were only detected at sites with low salinity and high pH. Cluster analysis of both microeukaryotic and prokaryotic DGGE profiles classified the five sites into three main groups, which was consistent with the analysis based on both the environmental factors and the abundance of diatoms, ciliates, testate amoebae and nematodes. Statistical analyses indicated that soil salinity was the primary factor in regulating the microbial genetic diversity, while the combination of pH and phosphorous content was the most important factor in explaining the changes of microeukaryotic and prokaryotic communities along the environmental gradients in the coastal soils.

Key words: Cercozoa, coastal soils, microeukaryotes, genetic diversity, proteobacteria, saline-alkaline stress

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Introduction

Single-celled microorganisms include mainly bacteria, protozoa, lower fungi and microalgae and as essential components in natural environments play key roles in energy flows and several soil biochemical processes (Barrios, 2007). Bacteria have been confirmed to directly participate in the carbon and nitrogen cycling, nitrous oxide and methane emissions and plant diseases (Hirsch et al., 2010). Protists promote the mineralization through feeding bacteria then eaten by carnivores of higher order (Hunt et al., 1987). Although the importance of microorganisms has been recognized in natural environments, the knowledge about their diversity and distribution is far from complete (Strong et al., 2015).

Molecular methods based on rRNA genes have been applied successfully for investigating prokaryotic and microeukaryotic community changes in aquatic and soil environments (Lara and Acosta-Mercado, 2012; Urich et al., 2014). Among these techniques, denaturing gradient gel electrophoresis (DGGE) is one of most commonly used methods, which can quickly estimate the shifts in microbial communities in response to the changes of environmental factors and obtain their species composition by combination with sequencing (Liu et al., 2013; Aydin et al., 2015). DGGE was used to survey the shifts of microbial community caused by environmental stress in solar salterns and hypersaline-alkaline lakes (Benlloch et al., 2002; Wu et al., 2009). In comparison with aquatic environments, our knowledge about the prokaryotic and microeukaryotic diversity and their response to environmental stress in coastal soils is very scant.

Coastal zones represent transitional ecosystems between the marine and soil environments; they are especially sensitive to the natural and man-made changes. Laizhou Bay is one of the three major bays in Bohai Sea and the important spawning and breeding grounds for many marine organisms in China. Due to the abundant seawater resources and underground brine resources, Laizhou Bay is the major origin of the crude salt in China and thus has suffered serious salinization due to natural and anthropogenic influences. Therefore the coast of Laizhou Bay has provided an appropriate study site for investigating the response of microbial community to environmental stress and determining the main factors that regulate the microbial community in the salt-affected soil environments.

The aim of the study is to uncover (i) the genetic diversity and distribution of soil microeukaryotes and prokaryotes along the saline-alkaline gradients, and (ii) the major factors regulating their distribution in the coastal soils of Laizhou Bay. For this purpose, we selected five sites with different saline-alkaline gradients for soil sample collection in the Changyi National Marine Specific Protection Area (CNMSPA) and an adjacent idle salt field on the coast of Laizhou Bay, where the soil salinity ranged from 0.1 to 19 psu and pH varied from 7.9 to 10. The soil samples were investigated by the micro-eukaryotic and prokaryotic rRNA gene DGGE as well as gene sequencing and their genetic patterns in the saline-alkaline soil were analyzed along with the soil environmental variables and abundance of microorganisms.

Material and methods

Costal soil sample collections

The Changyi National Marine Specific Protection Area (CNMSPA) is located on the coast of Laizhou Bay. It was formally approved by the State Ocean Administration of China in 2007, aiming to protect the largest natural tamarisk shrub in the northern China. Coastal soil samples with different salinity and alkalinity levels were collected from five sites in the CNMSPA and an adjacent idle salt field (37°4′-7′N, 119°22′-23′E) on March 2010 (Fig. 1). Sites I, II, III and IV were located in the CNMSPA, where the impact of human disturbance was the minimal. Site I was mainly covered with the Chinese tamarisk (*Tamarix chinensis*), and site II was covered with a dense canopy of *Setaria viridis* and sparsely with *T. chinensis*. Sites III and IV, which are near the seacoast, were mainly covered with seepweed *Suaeda glauca* and reeds. Site V was located in an idle salt field without any plants and was adjacent to a living area for salt farmers (Fig. 1).

At each site, three replicate samples were randomly collected from the surface layer (0-5 cm) of soils. Each replicate sample was treated in the field as follows: 1) 5 g of soil were preserved by ice-cold glutaraldehyde (4%) and used for investigating the abundance of ciliates, testate amoebae, diatoms and nematodes; and 2) the remaining soils were taken back to the laboratory using an icebox, and there ca. 10 g of soil which were prepared for DNA extraction were stored at -20° C; the other soils were used for determining the soil salinity, pH, water content, total organic carbon (TOC), total phosphorus (P), potassium (K) and total nitrogen (N) content.

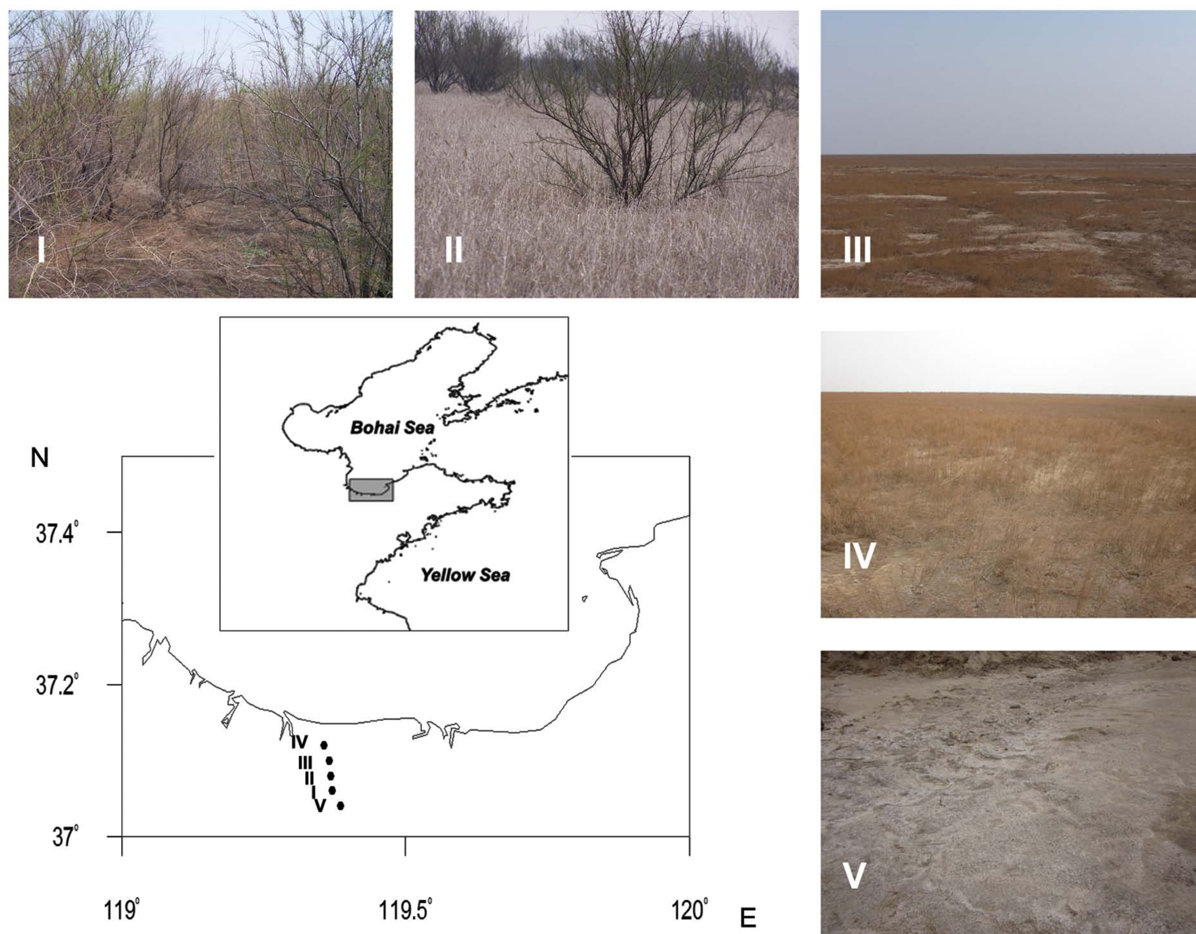

Fig. 1. Location of the study area and the habitats of sampling sites I-V in the coastal zone of Laizhou Bay, China.

DNA extraction and PCR amplification

One gram of soil from each replicate was taken to extract the total DNA with the Ultraclean soil DNA isolation kit (MolBio, USA). Three DNA samples from each site were mixed for the following study. The primers of Euk1A and Euk516r were used to amplify the eukaryotic 18S rRNA gene (Díez et al., 2001). The PCR amplification mixture contained: 0.3 μM of each primer, 2μl template DNA, 12.5 μl 2× PCR TaqMIX (100 mM KCl; 20 mM Tris-HCl; 3mM $MgCl_2$; 400 μM dNTP mix; 0.1 U $μl^{-1}$ Taq DNA polymerase) (TransGene, China), and deionized water in a reaction volume of 25 μl. 18S rRNA gene was amplified with the following program: an initial denaturation at 94 °C for 130 s, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 56 °C for 45 s and extension at 72 °C for 130 s, a final extension at 72 °C for 10 min. The primers of F357-GC and R518 were used to amplify the prokaryotic 16S rRNA gene (Muyzer et al., 1993). The PCR program included: an initial denaturation at 94 °C for 5 min, then 10 touchdown cycles of 94 °C for 30 s, 67-58 °C for 30 s and 72 °C for 60 s, followed by 20 cycles of 94 °C for 30 s, 57 °C for 30 s and 72 °C for 60 s, a final extension at 72 °C for 10 min.

Denaturing gradient gel electrophorsis and gene sequencing

Denaturing gradient gel electrophoresis was performed with the DCode universal mutation detection system (Bio-Rad laboratories, USA). The 6% polyacrylamide gels with denaturing gradient from 20% to 50% were applied for estimating the diversity of eukaryotic 18S rRNA gene. The 8% polyacrylamide gels prepared with denaturing gradient ranging from 40% to 60% were used for evaluating the diversity of prokaryotic 16S rDNA gene. Electrophoresis was run under 100 V for about 16 h at 60 °C. After the electrophoresis, the gels were

stained and visualized with Gene Finder (Bio-v, China) for 30 min.

The sterile pipette tips were used to dip the DNA fragments from DGGE bands, then the pipette tips were transferred into 20 µl of sterile deionized water, and incubated 24 h at 4 °C. After the incubation, a 2 µl of water was reamplified by the 16S and 18S primers, and then PCR products were used to construct the clone library. The clones which were migrated to the same position in the gel as the sampled band were subsequently sequenced with the M13 universal forward primer. The 18S rDNA sequences were deposited in GenBank with accession numbers of KC851725-KC851757 and the 16S rDNA sequences were deposited in the European Bioinformatics Institute (EBI) under submission ID of ERP002427.

Abundance of diatoms, ciliates, testate amoebae and nematodes

The abundance of diatoms, ciliates, testate amoebae and nematodes was evaluated by the Ludox-QPS method (Xu et al., 2010). The procedures of Ludox-QPS method mainly include soil sample fixation in the field, extraction of organisms by density gradient centrifugation, and quantitative protargol staining (Zhao et al., 2012). Nematodes, testate amoebae, ciliates, and diatoms were enumerated under light microscope at a magnification of 200×; the abundances of each group were recorded.

Statistical analyses

The binary (0/1) matrix which was constructed basing on the presence or absence of DGGE bands was used for the following statistical analysis. Cluster analysis was used to investigate the similarity among the five sites. UPGMA cluster analysis of DGGE profiles was done using the Dice similarity coefficient and that of biotic and abiotic factors was based on Euclidean distance similarity matrices. BIOENV analysis was aimed to find a group of environmental factors which could best explain the shifts in both the microeukaryotic and prokaryotic DGGE profiles. The cluster and BIOENV analysis were performed by the PRIMER v6 (Plymouth Marine Laboratory, UK).

CANOCO 4.5 software packages were used to further investigate the relationship between the microbial genetic diversity and the measured biotic and abiotic factors (Ter Braak and Šmilauer, 2002). The biotic and abiotic data were log(x+1) transformed to eliminate the influence of extreme values on the ordination scores. Detrended correspondence analysis on the microeukaryotic and prokaryotic data indicated that the longest gradient lengths were longer than 4.5, revealing that the DGGE profiles exhibited unimodal responses to the soil environmental variations, and thus canonical correspondence analysis (CCA) was used. The abiotic and biotic factors with variance inflation factors > 20 were eliminated from the CCA. Four most important factors (abundance of testate amoebae, salinity, P content and water content) were selected basing on the automatic forward selection.

Results

Soil environmental variables

The salinity levels of soil samples collected from the five sites increased from 0.1 psu at sites I and II to 19 psu at site V. In contrast, the soil pH decreased almost linearly from about 10 at site I to 7.9 at site V (Table 1). The values of TOC, N content, P content and K content were the highest at site I. The lowest value of TOC existed at site IV and that of N, P and K content were lowest at site V.

Abundance of diatoms, ciliates, testate amoebae and nematodes

The abundance of testate amoebae was higher at sites I & II than at the others, and diatoms were most abundant at site IV. The abundance of ciliates was the highest at the moderate-salinity site III, whereas it was relatively lower than for the other groups. Almost no organisms except for a few individuals of nematodes and diatoms were examined at the very high-salinity site V, meanwhile, the alkalinity at this site was the lowest (Table 1).

The UPGMA clustering of the environmental factors and abundance of diatoms, ciliates, testate amoebae and nematodes revealed that sites I and II formed a cluster first, and then grouped with the sites III and IV, and site V was separated from the other sites (Fig. 2A). Based on the cluster analysis and the variables measured, sites I and II could be classified as low-salinity but strongly alkaline soil, site III as moderate-salinity and strongly alkaline soil, site IV as high-salinity and alkaline soil, and site V as very high-salinity and alkaline soil.

Table 1. The environmental factors and abundance of diatoms, ciliates, testate amoebae and nematodes tested at the sampling stations.

	I	II	III	IV	V
Salinity (psu)	0.10	0.10	1.40	6.50	19.00
pH	10.00	9.84	9.09	8.45	7.91
Water content (%)	11.75	9.55	14.4	7.39	10.23
TOC (%)	0.84	0.33	0.14	0.14	0.21
N (%)	0.09	0.04	0.03	0.03	0.01
P (mg/Kg)	346.40	304.70	291.20	273.60	160.30
K (mg/Kg)	1509.10	1050.30	1000.50	1261.50	752.40
Nematodes (ind/g dry soil)	9.40	3.70	22.20	4.70	0.40
Testate amoebae (ind/g dry soil)	101.20	18.40	1.90	1.40	0.00
Ciliates (ind/g dry soil)	3.00	0.00	13.60	2.20	0.00
Diatoms (ind/g dry soil)	19.60	193.50	469.20	35296.00	3.30

Microbial genetic diversity in relation to environmental variables

A total of 37 bands and 23 bands were detected by the microeukaryotic 18S rRNA gene and the prokaryotic 16S rRNA gene DGGE profiles, respectively (Fig. 3). The prokaryotic DGGE detected 8 bands at site I, 6 bands at site II, 14 bands at site III, 13 bands at site IV and 2 bands at site V. The microeukaryotic DGGE detected 12 bands at site I, 13 bands at site II, 17 bands at site III, 13 bands at site IV and 2 bands at site V. Both the 18S and 16S rRNA gene DGGE profiles yielded the highest number of bands at site III with moderate salinity and alkalinity and the lowest number at site V with the highest salinity and lowest alkalinity. Moderate numbers of bands were detected from the lowest-salinity but highest-alkalinity sites I and II. Almost all the bands were unique to one or two sites except for the band E-9 (detected at sites I, II, III and IV) and bands B-13 and E-11 (detected at sites II, III and IV).

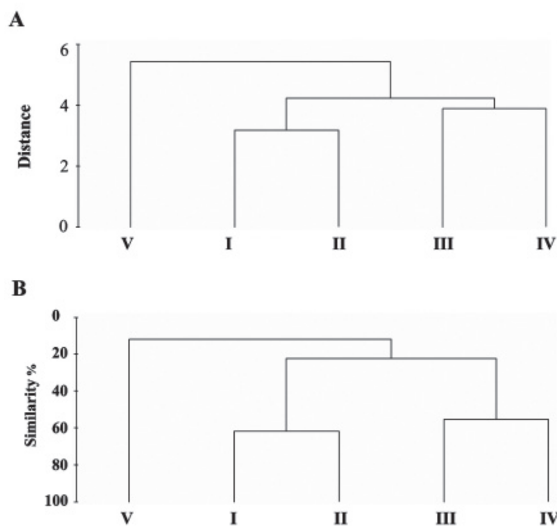

Fig. 2. UPGMA clustering analysis of five sites on the basis of the measured abiotic and biotic factors (A) and the microeukaryotic and prokaryotic DGGE profiles (B).

The UPGMA clustering based on the combination of the microeukaryotic and prokaryotic DGGE profiles clustered all the sites into three groups, and this was consistent with the results of analysis based on the measurements of the biotic and abiotic variables (Fig. 2B). The two replicates from each site had high similarity and always clustered together. BIOENV analysis indicated that the DGGE patterns were related quite well to the measurements of environmental variables. The highest correlation ($R = 0.952$) between the microbial DGGE profiles and environmental variables was found for a combination of soil pH and P content ($p = 0.004$). When analysis was restricted to the single best variable, the soil salinity was the most influential factor with a high correlation of 0.936 ($p = 0.017$), and pH was the second significant correlation with the microbial DGGE profiles ($R = 0.794$, $p = 0.012$).

In CCA, 64.1% of cumulative variance of the species-environment relationship was explained by the first two axes (37.6% for axis 1 and 26.5% for axis 2) (Fig. 4). The axis 1 was positively correlated with the abundance of testate amoebae and P content showing correlation coefficients of

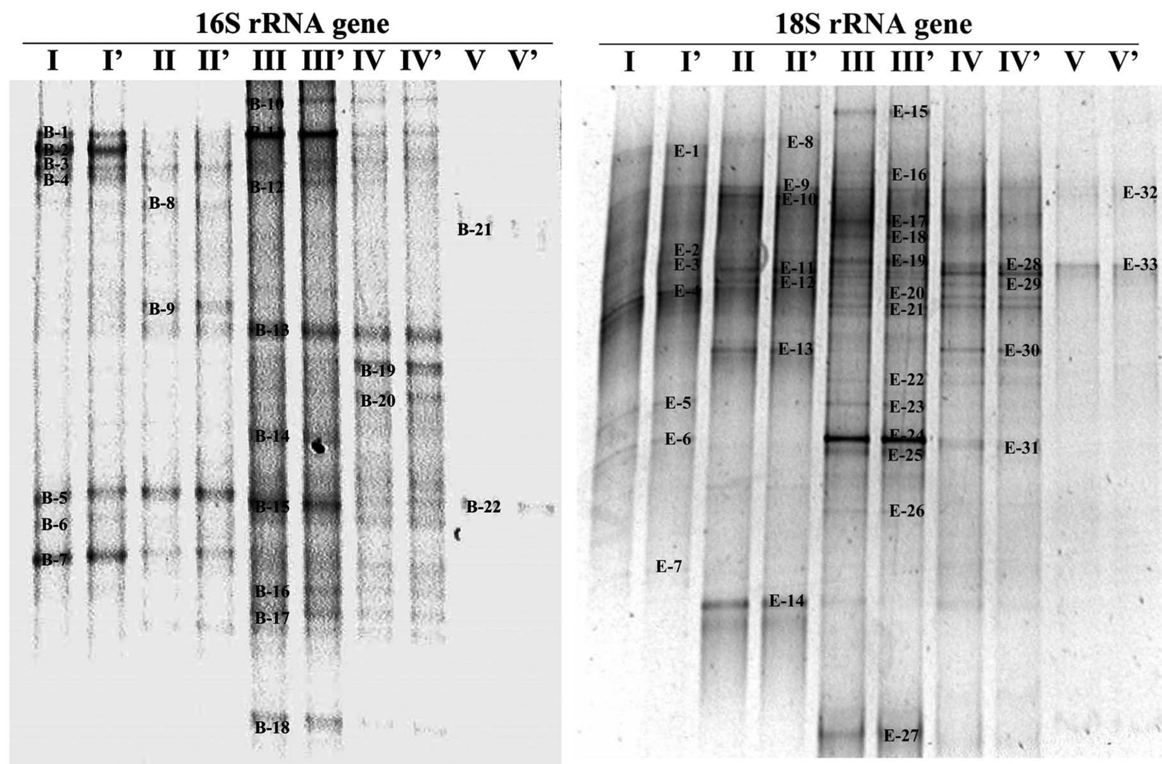

Fig. 3. DGGE patterns showing the prokaryotic 16S rRNA gene diversity (A) and the microeukaryotic 18S rRNA gene diversity (B) at the five sites. The *B/E-digital* (B: bacteria; E: microeukaryote) within the gel refers to the excised and sequenced bands as shown in tables 2 and 3.

0.9110 and 0.6103, respectively, and negatively correlated with salinity (R = -0.6960). The CCA revealed that the abundance of testate amoebae was also an important factor for shaping the microbial community. Besides, CCA classified the five sites into three major groups, which coincided well with the result from the cluster analyses (Fig. 2B; Fig. 4).

Identification of microbial populations

A total of 55 DGGE bands were sequenced, including 22 bands of prokaryotes and 33 bands of microeukaryotes. The identities of the prokaryotic bands were shown in table 2 and those of the microeukaryotic bands were shown in table 3. The original environments of sequences were identified according to the information of their closely related sequences in NCBI.

Most of the bacterial sequences were closely related to the sequences from the described organisms or environmental samples with a similarity > 98%, except for the band B-13 with similarity of 93%. The sequences closely related to Proteobacteria, in particular Alphaproteobacteria, were commonly found at all sites. The sequences affiliated to Acidobacteria and Actinobacteria were only detected at sites I and II with high alkalinity and low salinity.

The sequences affiliated to Bacteroidetes in coastal soils studied herein were various in classes level along an environmental gradient. Flavobacteria were only detected in moderate-salinity soils, and none was detected in highly alkaline or highly saline soils. In contrast, Sphingobacteria were only detected in highly alkaline soils.

The sequencing of microeukaryotic bands revealed the presence of the Cercozoa, Fungi, Ciliophora, Dinoflagellata, Apicomplexa, Streptophyta, stramenopiles and uncultured groups. Most of the microeukaryotic sequences were closely related to the sequences deposited in GenBank with a similarity > 95%, except for the bands E-5, 6, 21 and 22 with similarity < 94%. In contrast to more than 98% similarity of prokaryotic 16S rRNA gene sequences with the known ones, many more 18S rRNA gene sequences had low similarity with known ones, especially those of E-5 and E-21, which had the similarity of only 88 % and 90 %, respectively.

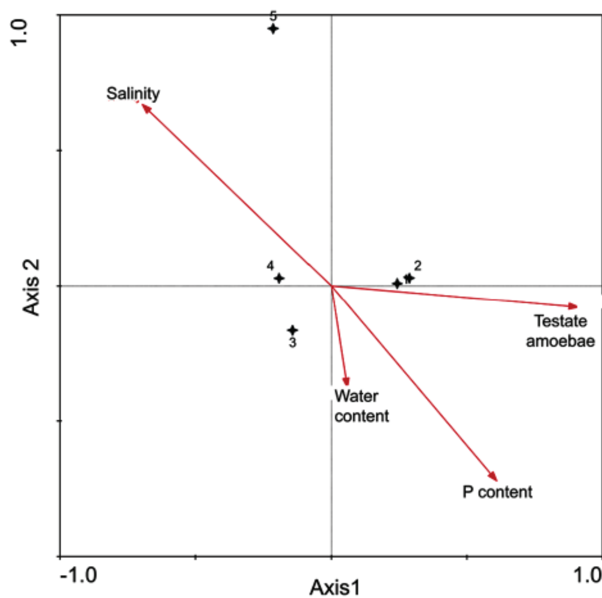

Fig. 4. Canonical correspondence analysis (CCA) based on the microbial genetic diversity and the measured abiotic and biotic variables (abundance of testate amoebae, salinity, P content and water content) selected by automatic forward selection.

The microeukaryotic sequences affiliated to Cercozoa were predominant, and were frequently detected in all the five sites. It is noteworthy that only the sequences affiliated to Cercozoa were detected at site V. Most sequences affiliated with Cercozoa belonged to Cercomonadida and Silicofilosea. With increase of soil salinity, the proportion of Silicofilosea increased. Fungi were the second dominant group commonly detected from sites I through IV.

Most sequences of the 18S and 16S DGGE bands were closely related to those originated from soil environments, while some sequences obtained from the near-shore sites III and IV were affiliated with organisms of marine origin (Table 2, Table 3).

Discussion

Microbial genetic diversity in relation to soil environmetns

Salinity has been confirmed to be a major environmental determinant for bacterial community along the environmental gradients of marsh-mangrove-seagrass (Ikenaga et al., 2010). Zhao et al. (2013) indicated that soil salinity was also an important factor for shaping the microeukaryotic community in the coastal soils from the Yellow River Delta in China. Our study further demonstrated that soil salinity was the primary factor in regulating the microbial genetic diversity, while the combination of soil pH and P content was the most important factor in explaining the changes of microeukaryotic and prokaryotic communities along the environmental gradients in the coastal saline-alkaline soils.

The importance of pH in regulating microbial diversity in soils has already been recognized. Fierer and Jackson (2006) indicated that any significant deviation in environmental pH should impose stress on single-celled organisms. The bacterial diversity was highest in soil with pH close to 7, and their diversity would be decreased when the value of soil pH was higher or lower than 7 (Fierer and Jackson, 2006). Our investigation of the microbial diversity in alkaline soils indicated that a lower number of 16S bands was obtained from the high-pH soils (strongly alkaline sites I and II) compared to those from the moderate-pH soils (alkaline site IV). In contrast, the number of 18S DGGE bands in the high-pH soils could be maintained at a similar level as those in the moderate-pH soils. The results indicate that pH might be more important in shaping the prokaryotic than microeukaryotic community in alkaline soils.

However, we could not explain how the combination of soil pH and P content influences the microbial diversity. Future research should focus on the interaction of multiple environmental factors and their impacts on the microbial community in nature.

Moreover, our study showed that the abundance of testate amoebae was closely correlated with the microbial community in coastal soils. Protozoans represent a major trophic pathway in the food web through grazing the bacteria, cyanobacteria and algae, and then being eaten by other metazoan (Azam et al., 1983; Huws et al., 2005). Among protozoans, testate amoebae have larger volume and higher abundance, and thus higher production of biomass than others in soil environments (Foissner, 1999). Therefore, testate amoebae could be an important factor in influencing the dynamics and development of microbial communities in soils.

Microbial genetic diversity along saline-alkaline gradients in coastal soils

The species composition of microbial organisms changed along the saline-alkaline gradients in costal soils. The sequences closely to Acidobacteria were only inspected in low salinity but high alkalinity soils. Acidobacteria phylum was firstly defined in

Table 2. Phylogenetic affiliation and BLAST hit of sequenced DGGE bands of prokaryotic 16S rRNA gene.

Band	Band match	Similarity	Taxonomic affiliation	Environment
B-1	Uncultred Oxalobacteriaceae GU472925	100%	Betaproteobacteria	Soil
B-2	Uncultured Acidobacteria HM193101	99%	Acidobacteria	Unknown
B-3	Uncultured Acidobacteria JF521700	99%	Acidobacteria	Soil
B-4	Uncultured Actinobacterium EU299760	100%	Actinobacteria	Soil
B-5	*Acinetobacter* sp. HM352317	98%	Gammaproteobaceria	Unknown
B-6	*Nocardioides* sp. FN386752	98%	Actinobacteria	Volcanic ash
B-7	Uncultured Firmicutes EF664505	98%	Firmicutes	Soil
B-8	Uncultured Sphingobacteria EU299209	100%	Bacteridetes Sphingobacteria	Soil
B-9	*Sphingomonas* sp. (GQ484921)	100%	Alphaproteobacteria	Unknown
B-10	*Flavobacterium arcticum* DQ658240	98%	Bacteroidetes Flavobacterium	Freshwater
B-11	Alphaproteobacterium GQ274095	98%	Alphaproteobacteria	Marine
B-12	*Pseudomonas* sp. FJ889644	100%	Gammaproteobaceria	Marine
B-13	Gammaproteobacterium HQ857705	93%	Gammaproteobaceria	Saline soil
B-14	Uncultured Salegentibacter JF421169	99%	Bacteroidetes Flavobacteria	Saline soil
B-15	*Roseobacter* sp. AY697914	99%	Alphaproteobacteria	Seawater
B-16	*Halomonas* sp. GU254014	100%	Gammaproteobaceria	Salt lake
B-17	*Salinimicrobium* sp. HQ857678	98%	Bacteroidetes Flavobacteria	Saline soil
B-18	Uncultured Flavobacteria JF421210	100%	Bacteroidetes Flavobacteria	Saline soil
B-19	*Pseudoalteromonas* sp. JN107745	100%	Gammaproteobaceria	Unknown
B-20	Uncultured Flavobacteria FN433421	99%	Bacteroidetes Flavobacteria	Ocean
B-21	*Brevundimonas* sp. JF742954	98%	Alphaproteobacteria	Rock
B-22	*Sphingomonas aurantiaca* HE587994	100%	Alphaproteobacteria	Soil

1997, and the name of this phylum was according to the species *Acidobacterium capsulatum*, which was isolated and cultured only in the acidic environments with pH values between 3 and 6 (Kishimoto et al., 1991; Ludwig et al., 1997). The phylum name is somewhat misleading because Acidobacteria are not restricted to acidic environments and have been detected from seawaters, sediments, peat bogs, and soils (Kielak et al., 2010). In particular, George et al. (2011) ever recovered 18 Acidobacteria strains from slightly alkaline soils with pH of 7.4. We detected two sequences affiliated to Acidobacteria in soils with high alkalinity (pH of 10) herein. The data suggest that Acidobacteria could exist in a wide range of pH from acidic to alkaline soils.

In the microeukaryotic community, the sequences affiliated to Cercozoa have been the most predominant ones; they were commonly detected from all sites. Cercozoa with high global biodiversity could exist in different habitats (Bass and Cavalier-Smith, 2004), but have been found only in soil and freshwater environments with salinity less than 5 psu (Mylnikov and Karpov, 2004; Bass et al., 2009). Thus, the discovery of the sequences closely related to Cercomonadida from highly saline soils with salinity 19 psu expands our knowledge on the distribution of Cercomonadida. A further study is necessary to investigate the taxonomy and phylogeny of Cercomonadida in saline soils by combining the culture-dependence method and morphological method.

In both the prokaryotic and microeukaryotic communities, the sequences closely related or even 100% matching those from marine or salt environments were found. Those sequences were possibly originated by the erosion of seawater, which brought marine active cells or cysts into the coastal soils (Zhao et al., 2013). These results indicated that coastal soils in the transitional zone might play an important role in microbial geographical

Table 3. Phylogenetic affiliation and BLAST hits of sequenced bands of microeukaryotic 18S rRNA gene DGGE.

Band	Band match	Similarity	Taxonomic affiliation	Environment
E-1	Uncultured Eukaryote HQ999847	99%	Unknown	Freshwater
E-2	Oxytrichidae EF024639	99%	Ciliophora	Soil
E-3	Chytridiomycota EU162636	95%	Fungi	Freshwater
E-4	Uncultured Fungus HQ190252	99%	Fungi	Oil field
E-5	Heterocapsaceae EF024723	88%	Dinophyceae	Soil
E-6	*Cercozoa* sp. HQ121439	98%	Cercozoa	Unknown
E-7	Uncultured Eukaryote FN396431	98%	Unknown	Soil
E-8	*Eocercomonas* sp. AY884324	98%	Cercozoa Cercomonadida	Unknown
E-9	Uncultured Chytridiomycota GQ995315	98%	Fungi	Soil
E-10	Phaseoleae EF024891	98%	Streptophyta	Soil
E-11	Uncultured Chytridiomycota EU162636	94%	Fungi	Freshwater
E-12	Oxytrichidae EF024702	99%	Ciliophora	Soil
E-13	Heteromitidae EF024641	98%	Cercozoa Cercomonadida	Soil
E-14	Eimeriidae EF024986	99%	Apicomplexa	Soil
E-15	*Euplotes rariseta* FJ423449	99%	Ciliophora	Marine
E-16	*Allas* sp. AY268040	99%	Cercozoa Silicofilosea	Unknown
E-17	*Protaspis* sp. FJ824125	99%	Cercozoa Silicofilosea	Marine
E-18	*Alternaria alternate* AF218791	98%	Fungi Dikarya	Antarctic
E-19	*Spongomonas minima* AF411281	97%	Cercozoa Cercomonadida	Unknown
E-20	*Hemiurosoma terricola* AY498651	98%	Ciliophora	Soil
E-21	Uncultured Eukaryote FN690483	90%	Unknown	Baltic Sea
E-22	Uncultured Cercozoa GQ844488	93%	Cercozoa	Lake
E-23	*Euglypha rotunda* AJ418784	98%	Cercozoa Silicofilosea	Unknown
E-24	*Colpodella pontica* AY078092	99%	Apicomplexa	Unknown
E-25	Uncultured Eukaryote GU908472	98%	Unknown	Soil
E-26	*Pulchromyces fimicola* EF442001	98%	Fungi Dikarya	Unknown
E-27	Diplolaimelloides EF659924	98%	Nematoda	Hypersaline mat
E-28	*Cercozaoa* sp. FJ824128	95%	Cercozoa	Marine
E-29	*Sporobolomyces bischofiae* AB035721	98%	Fungi Dikarya	Plants
E-30	*Isaria farinose* JF429859	95%	Fungi Dikarya	Unknown
E-31	Uncultured Labyrinthulid FJ800596	98%	Stramenopiles	Marine
E-32	Uncultured Cercomonadida EU647174	96%	Cercozoa Cercomonadida	Freshwater
E-33	Uncultured Cercozoan AY620271	98%	Cercozoa	Unknown

dispersal. Nonetheless, only the predominant groups of microeukaryotes could be identified by DGGE and sequencing due to relatively low resolution of these methods which therefore provide limited information on the contribution of these organisms to overall biodiversity (Gafan and Spratt, 2005). Thus, in the future the details of genetic diversity should be investigated by the high-throughput sequencing which can obtain hundreds of thousands of sequences simultaneously (Hirsch et al., 2010).

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