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Phylogenetic analysis of bacterial community composition in sediment contaminated with multiple heavy metals from the Xiangjiang River in China

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ABSTRACT

Understanding the ecology of sediments that are contaminated with heavy metals is critical for bioremediating these sediments, which has become a public concern over the course of the development of modern industry. To investigate the bacterial community composition of sediments that are contaminated with heavy metals in the Xiangjiang River, a total of four sediment samples contaminated with multiple heavy metals were obtained, and a culture-independent molecular analysis, polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP), was performed. The results revealed that heavy metal pollution affected the sediment microbial community diversity, and the greatest species diversity appeared in the moderately polluted sediment X sample. The dominant family in these sediments includes α-*Proteobacteria*, β-*Proteobacteria* and *Firmicutes*. Moreover, α-*Proteobacteria* was significantly increased with increases in heavy metal. A redundancy analysis (RDA) also confirmed this phenomenon.

1. Introduction

Heavy metal contamination is a global environmental problem that has been caused by the development of metal processing, tanneries and electroplating industries (Jiang et al., 2010). The accumulation of multiple heavy metals in the environment may significantly influence public health and damage ecosystems through different pathways (Guo et al., 2009; Sun et al., 2010; Yoon et al., 2006). Microorganisms, as an essential component of ecosystems, are involved in organic material degradation, the release of nutrients, biogeochemical cycling and the maintenance of soil structure (Brandt et al., 2010; Goberna et al., 2005; Tian et al., 2008). However, the environmental microbial community composition, structure and diversity were unclear until molecular approaches were invented and applied to characterizing microbial species and assemblages. These techniques significantly increased our understanding of environmental microbial communities and the relationships between plants, animals and other factors (Green et al., 2008).

Previous studies have reported that microorganisms are far more sensitive to heavy metal stress than plants growing in the same area, and they have different sensitivities to metal toxicity (Giller et al., 1998, 2009; Sun et al., 2012). Heavy metals may change microbial biomass, activities and structure (Epelde et al., 2010; Giller et al., 1998; Jiang et al., 2010; Stephen et al., 1999; Wang et al., 2010; Yang et al., 2006). Bacterial communities can be sensitive indicators for contaminant stress, particularly metal contamination (Sun et al., 2012). However, little attention has been devoted to the diversity and structure of indigenous microbial populations within the contaminated sediments of the Xiangjiang River. The Xiangjiang watershed is the most developed and urbanized region in the province (Chai et al., 2010). In the basin, mining and the metallurgical industry are well-developed, and the Xiangjiang River is being subjected to serious pollution due to emissions of chromium, cadmium, lead, zinc and mercury in the Hunan Province (Chai et al., 2010; Guo et al., 2010). The pollution threatens human health and the balance of aquatic ecosystems, economic development and social prosperity.

This study describes the bacterial diversity in the Xiangjiang River's contaminated sediments by sequencing 16S rDNA genes in clone libraries. We focused on 16S rDNA because of its universal distribution among all prokaryotes, the presence of diverse species-specific domains, and its reliability in inferring phylogenetic relationships. (Pace, 1997). Four sediment samples that were contaminated with different degrees of multiple heavy metals were studied, and the main objectives of this study were threefold: (1) document the predominant bacterial community structure and diversity differences in the contaminated sediment from the Xiangjiang River in Zhuzhou City using PCR–RFLP analysis from the total bacterial DNA extracted from the sediment; (2) provide

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a valuable basis for further investigation on heavy metal-resistant and metal-sensitive bacteria to assess the usefulness of microbial indicators for evaluating the health and function of sediment, and (3) potentially assist in situ bioremediation of metal contaminated sediment and optimize the process of bioleaching to support river restoration decisions with the knowledge of accurate natural populations.

2. Materials and methods

2.1. Site description and sampling

The Xiangjiang River, one of the tributaries of the Yangtze River in Hunan province (where there is a typically hot, humid, tropical climate with an annual mean precipitation of 1400 mm), originates on Guangxi Haiyang Mountain. It drains an area of approximately 94,600 km^2 and has a total length of approximately 856 km. Contaminated sediments for the present study were collected from Zhuzhou, an industrial town in Hunan Province, in September, 2010. Large numbers of non-ferrous metal and chemical enterprises have located in this area over a period of approximately 50 years, contaminating the Xiangjiang River with their pollutants. Four different sediments (0–10 cm depth) were sampled for this study (Fig. 1): H and X samples were taken at the Xiawan Port (the most polluted tributary of the Xiangjiang River); the F sample was collected at the confluence of the Xiangjiang River and Xiawan Port; the S sample (a control site) was collected 6 km upriver of F site in the Xiangjiang River. The sample at each site was homogenized and divided into two parts. One part was temporarily stored at 4 °C for microbial community analysis, and the other was air-dried and then processed with a 2 mm sieve to remove root fragments and large particles.

2.2. Physicochemical and microbiological parameters

2.2.1. Physicochemical parameters

The selected physicochemical properties of the sediments were analyzed using standard methods. The pH of the sediments was measured using a 1:2.5 (sediment:distilled water) sediment slurry. The total organic carbon (% TOC) content was determined by loss-on-ignition, combusting at 450 °C for 4 h in a Muffle Furnace and at 105 °C for several hours. The total concentrations of metals in the sediment were analyzed with an Inductively Coupled Plasma Mass Spectrometer (ICP-MS, Agilent 7500 series, USA). The extractable fraction of metals was obtained using 1 M $MgCl_2$ (pH = 7.0) (Akcay et al., 2003) and then analyzed with ICP-MS.

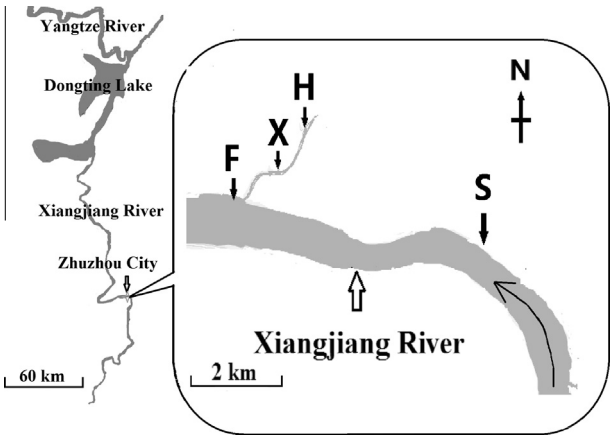

Fig. 1. Map showing the location of the sample sites in the Xiangjiang River.

2.2.2. DNA extraction and 16S rDNA gene amplification

Bacterial DNA was extracted from the sediments using a protocol described by Zhou et al. (1996). 16S rDNA was then amplified by PCR with a thermocycler using an initial denaturing step of 4 min at 95 °C followed by 32 cycles of 1 min at 94 °C, 1 min of annealing at 55 °C, a 90 s extension at 72 °C, and a final polymerization step of 72 °C for 10 min. Each reaction mixture consisted of 5 μl of 10 × reaction Buffer (Mg^{2+} plus), 1 μl of dNTP (10 mmol l^{-1}), 1 μl of each primer: forward primer (27f, 5′-AGAGTTTGATCCTGGC TCAG-3′, 5 μmol l^{-1}) and reverse primer (1492r, 5′- GGTTACCT TGTTACGACTT-3′, 5 μmol l^{-1}) (Konstantinidis et al., 2003), 0.25 μl of Taq polymerase (Fermentas, MBI) and 2 μl of DNA extraction products. Double-distilled water was added until its final volume reached 50 μl. The resulting PCR products were run on a 1.0% low-melting-point agarose gel in tris–acetate-buffer and analyzed by staining with ethidium bromide (EB) under UV light. The bands of the expected size (approximately 1500 bp) were cut off and purified with a E.Z.N.A.™ Gel Extraction Kit (OMEGA, USA).

2.2.3. Cloning and RFLP analysis

The purified PCR products were ligated into pGM-T plasmid vectors (TIANGEN) and then transformed into *Escherichia coli* TOP10 cells. Ampicillin-resistant transformants were selected on blue–white screening and grown overnight in the plates with ampicillin (80 mg ml^{-1}), IPTG (50 mg ml^{-1}) and X-gal (15 mg ml^{-1}). In total, 180 white colonies were randomly selected. Then, white colonies were randomly selected and re-amplified by PCR with vector primers M13 forward (−20) (5′-GTA AAAC-GACGGCCA G-3′) and M13 reverse (5′-CAGGAAACAGCTATGAC-3′). Next, 180 of the positive, reamplified products from each library were digested by the restriction endonuclease *Hin*61 and *Msp*I (Fermentas, MBI) at 37 °C overnight. The system for the reaction of *Msp*I and *Hin*61 digestion consists of 1 μl buffer, 0.1 μl of each enzyme, and 6 μl of purified clone PCR products. Double-distilled water was added until its final volume reached 10 μl. The digestion products were separated by gel electrophoresis (3 h, 80 V) in 3.0% low-melting-point agarose in tris–acetate-buffer and analyzed by staining with EB under UV illumination. The banding patterns were grouped into an operational taxonomic unit (OTU) based on the DNA banding pattern of individual clones. Each banding pattern found on the agarose gel constitutes one distinct OTU based on how the restriction enzyme cuts the PCR product. The representative OTUs were submitted for sequence analysis.

2.2.4. Sequencing and phylogenetic analysis

In total, 105 unique clones were sequenced by the Beijing Genomics Institute (BGI), and 96 sequences were initially estimated using the BLASTN facility at the National Center for Biotechnology Information (NCBI Taxonomy ID: 48184). The closest 16S rDNA gene sequences were aligned with CLUSTALX 1.83. A phylogenetic tree was constructed by the neighbor joining method, using MEGA3.1, and the robustness of the phylogeny was tested with a bootstrap analysis with 1000 iterations.

2.2.5. Nucleotide sequence accession numbers

All of the sequences described in this study have been submitted to GenBank under accession numbers HQ132379–HQ132474.

2.3. Statistical analysis

We used the geochemical characteristics of the samples presented in Table 1, except for pH and TOC, as environmental data for ordination by principal component analysis (PCA) to examine the correlations between sample and site variables (Canoco, version 4.5, biometric – Plant Research International, the Netherlands, for Windows XP). The geochemical variables were *z* transformed to