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The effect of heavy metal contamination on the bacterial community structure at Jiaozhou Bay, China

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ABSTRACT

In this study, determination of heavy metal parameters and microbiological characterization of marine sediments obtained from two heavily polluted sites and one low-grade contaminated reference station at Jiaozhou Bay in China were carried out. The microbial communities found in the sampled marine sediments were studied using PCR-DGGE (denaturing gradient gel electrophoresis) fingerprinting profiles in combination with multivariate analysis. Clustering analysis of DGGE and matrix of heavy metals displayed similar occurrence patterns. On this basis, 17 samples were classified into two clusters depending on the presence or absence of the high level contamination. Moreover, the cluster of highly contaminated samples was further classified into two sub-groups based on the stations of their origin. These results showed that the composition of the bacterial community is strongly influenced by heavy metal variables present in the sediments found in the Jiaozhou Bay. This study also suggested that metagenomic techniques such as PCR-DGGE fingerprinting in combination with multivariate analysis is an efficient method to examine the effect of metal contamination on the bacterial community structure.

Introduction

Pollution of coastal zones caused by heavy metals, such as Cd, Pb, Hg, and Ni, is one of the important environmental problems faced in many parts of the world.[1] Heavy metal pollution can lead to severe changes in the composition of microbial communities that inhabit these zones.[2,3] Microbial communities present in marine sediments primarily decompose organic matter derived from plant litter but also play a vital role in the transformation of pollutants.[4,5] They can also influence the availability of heavy metals and are associated

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with other areas of the ecosystem.[6,7] The influence of heavy metals on the decomposer subsystem and several other experimental systems has been studied in detail, such as, there are numbers of studies on the community structure of marine sediments from the continental shelf area is limited. Previous ecological and biological studies are mainly focused on the specific groups of bacterial communities that drive the biogeochemical cycling, e.g., ammonia-oxidizing bacteria.[8–10] However, only a little is known on the dynamics of indigenous microbial populations that inhabit heavy metal contaminated coastal marine sediments. Although these studies provide vital information on the effects of heavy metals on bacteria found in marine sediments,[11] they lack important ecological information, such as that offered by polymerase chain reaction (PCR) fingerprinting of the microbial community DNA extracted in these environments. It has been widely accepted that metagenomic techniques, such as PCR-DGGE (denaturing gradient gel electrophoresis) fingerprinting, are some of the widely used methods for examining the diversity of prokaryotic communities in environmental habitats.[12–15] The PCR-DGGE profiles were used to construct a binary matrix for a quantitative comparison between different communities.[16,17] These data were obtained either by visual scoring of gels or by commercially available software programs (for example, Bionumerics, Applied Maths, Belgium). These data can be presented as cluster analysis, e.g., an unweighted pair group method with arithmetic means,[18] in which dendrograms are used to illustrate the relationship between different communities.[19] Alternatively, PCR-DGGE profiles can be combined with multidimensional scaling, which is widely used to study the relationships between microbial diversity and various measured environmental parameters.[20–22] Multivariate analysis has been utilized to determine the effect of metal contamination on bacterial community structure.[23]

Jiaozhou Bay is the largest semi-enclosed water body in the Yellow Sea (Fig. 1). It is located on the Chinese coast in the western Pacific Ocean. The environmental quality of this bay has deteriorated dramatically in the past three decades due to rapid increase in agriculture, industry, urbanization, and mariculture in the surrounding areas.[24] This region contains very high levels of heavy metals in the sediments, due to the discharge of considerable amounts of heavy metals from the industrial plants located at the head of the Bay.[25] The heavy metals levels in these regions were far exceed than their crustal average background values in the sediments at Jiaozhou Bay. The concentrations of the heavy metals in the sediments show a remarkable gradient ranging from high concentrations at the inner bay (Licunhe estuary and Haibohe estuary stations) to background levels at the outside of the bay (Shilaoren Beach station). The representative areas chosen for this study included the Lou Hill estuary, Licunhe estuary, Haibohe estuary, and Dagong Island that are located outside the area under study. The highest level of concentration of heavy metals was reported in Haibohe estuary wherein the concentrations of five heavy metals were 2.6–23.4 folds higher than their corresponding background values. In addition, the concentrations of Zn and Cu were much higher than that observed for other metals; the highest concentration of Zn was 1005.40×10^{-6}, and that of Cu was 394.71×10^{-6}. The concentrations of metals such as Zn, As, Pb, Cu, and Cd were higher than the corresponding background values in Licunhe estuary.[26,27]

The objectives of the present study were: (1) to use molecular techniques in combination with multivariate analysis to identify the sediment-associated microbial communities from both pristine and heavy metal-contaminated marine sediments in Jiaozhou Bay; (2) to assess the changes in the microbial community structure caused by heavy metal stress. In this study, the microbial communities inhabiting the Jiaozhou Bay were studied using PCR-DGGE fingerprinting profiles in combination with multivariate analysis. The concentrations of individual heavy metals present in the sediments were also determined.

Materials and methods

Site description

The study location was Jiaozhou Bay, which is a semi-enclosed bay located on the south bank of the Shandong Peninsula, China. This bay is linked to the Yellow Sea by a very narrow entrance measuring only 3.1 km across. It extends from 120°16′-120°17′ E to 36°00′-36°02′ N (Fig. 1). The average depth of this bay is 7 m with a maximum of 64 m. It covers an area of 362 km^2 of seawater and has a population of 7.2 million. The long-term annual rainfall ranges from 340 to 1243 mm with an average of 775.6 mm, 58% of that in summer and 23% in winter. More than ten small seasonal streams empty into the bay with varying water and sediment loads, notably the Yanghe, Daguhe, Moshuihe, Baishahe, Haibohe, and Licunhe estuaries.[27]

Sample collection and environmental factor measurements

Sediment samples were collected from two stations in Jiaozhou Bay and one station outside of the bay on December 30, 2011 using a 0.05 m^2 stainless steel box corer (Fig. 1). Seven samples were obtained from different points located at distances of 0.03, 0.06, 0.09, 0.12, 0.15, 0.18, and 0.21 km from the Licunhe estuary, which has a waste input, and were numbered as LC1, LC2, LC3, LC4, LC5, LC6, and LC7, respectively. Similarly, five samples were obtained from different points located at a distance of 0.03, 0.06, 0.09, 0.12, and 0.15 km from the Haibohe estuary, which has a waste input, and were numbered as HB0, HB1, HB2, HB3, and HB4, respectively. Another five samples were obtained from different points located at distances of 0.03, 0.06, 0.09, 0.12, and 0.15 km from Shilaoren Beach and were treated as the low-grade contaminated control station and numbered as SLR1, SLR2, SLR3, SLR4, and SLR5, respectively.

The concentrations of heavy metals (V, Ni, U, Mo, Zn, Se, Sb, Co, Cr, Cd, Pb, As, Cu, and Hg) in the sediments were determined by graphite furnace atomic absorption spectrophotometry (GFAAS) using an AAnalyst 800 graphite furnace atomic absorption spectrometer (Perkin-Elmer, CT, USA).

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