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Short communication

Molecular analysis of intestinal bacterial communities in *Cipangopaludina chinensis* used in aquatic ecological restorations

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ABSTRACT

High pollutant concentrations have led to the breakout of planktonic bloom and the breakdown of the ecosystem in several bodies of water in China. Some restoration projects using constructed wetlands have been enacted to increase the water clarity and rebuild the aquatic ecosystem in these bodies of water. *Cipangopaludina chinensis* were usually placed to manage the particles and microbes adhering to the surface of the aquatic plants in the wetlands.

In the current study, the intestinal bacteria in *C. chinensis* collected from three restoration projects in Shanghai, China were investigated using denaturing gradient gel electrophoresis and 16S rRNA gene clone library analyses. The species affiliated to *Firmicutes* were proven the dominant species in the intestinal bacterial population in *C. chinensis*. The most dominant phylotypes are closely related to *Pseudobutyrivibrio ruminis* and *Faecalibacterium prausnitzii*. Furthermore, significant differences between the intestinal bacterial community constructs and different structures were found in *C. chinensis* collected from different restoration projects. This information on the intestinal bacteria of *C. chinensis* is helpful in further understanding the function of these mollusks and in screening for useful bacterial strains.

1. Introduction

In China and many other countries, water in several bodies of water has a high concentration of organic and inorganic pollutants. Hence, these water bodies are often dominated by planktonic algae. The dominant algae cause other aquatic plants and animals to wither away, which leads to the breakdown of the ecosystem (Duan et al., 2009; Yin and Yin, 2009). To increase water clarity and rebuild the aquatic ecosystem in these bodies of water, some restoration projects have been enacted. In most of these projects, merged and/or submerged plants were planted and expected to absorb the suspended particles to improve water clarity. The growth and harvesting of these plants can remove nitrogen and phosphorus out of the aquatic ecosystem (Susarla et al., 2002; Richardson et al., 2011). On the other hand, microbes adhering to the surface of the plants can degrade and consume the organic pollutants (Faulwetter et al., 2009). To manage the organics and microbes excessively adhering to the plants, some animals such as mudsnails were usually introduced into these water bodies to live on them.

Cipangopaludina chinensis is the most frequently used mudsnail in aquatic ecosystem restoration projects and is widespread in China and many other countries. This species resides in pools, lakes, streams, paddy fields, and other water bodies and lives on organic particles and microbes. Understanding the intestinal bacterial community of *C. chinensis* would be helpful for aquatic ecosystem restorations. However, to our knowledge, no research on this topic has been reported. In the current study, the intestinal bacteria in *C. chinensis* collected from three restoration projects in Shanghai, China were investigated using denaturing gradient gel electrophoresis (DGGE) and 16S rRNA gene clone library analyses. The objective of the present study is to determine the dominant intestinal bacterial phylotypes in *C. chinensis* used in aquatic ecosystem restoration projects. The knowledge gained from this work will be the basis of further studies on the dynamics and functions of these intestinal bacteria.

2. Materials and methods

2.1. Sample sources and DNA extraction from intestinal bacteria

The intestinal tracts of *C. chinensis* samples were collected from three aquatic ecosystem restoration projects carried out in Shanghai, China in the spring of 2009. These bodies of water had high levels of planktonic algae and suspended solids prior to ecosystem restoration. About one month after the introduction of *C. chinensis* and submerged plants, mainly *Vallisneria natans* and *Elodea canadensis*, to the muddy bottoms of the water bodies, their clarity of water improved about 50 cm. Approximately 15–20 tracts from each sampling site were pooled as one sample. Samples 1 and

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2 were collected from urban rivers and sample 3 was obtained from a landscape lake.

All intestinal tracts were preserved in a refrigerator at −70 °C to prevent sample decay until the analysis. Each sample was then aseptically ground to the homogenate using sterile mortars. Total bacterial DNA was isolated according to the instructions of the QIAamp® DNA Stool Mini Kit (Qiagen, Hilden, Germany) and was then eluted with 100 μL H_2O.

2.2. DGGE analysis of the 16S rRNA gene

The samples were analyzed using the polymerase chain reaction (PCR)-DGGE fingerprinting technology.

The V3 region of the 16S rRNA gene was amplified. The primer pairs, PCR mixture, and program used were as previously reported (Li et al., 2007), except that the PCRs were cycled in a T1 thermocycler PCR system (Biometra, Germany) at an annealing temperature of 56 °C. DGGE was performed using a Bio-Rad DCode™ Mutation Detection System (Bio-Rad, Hercules, CA, USA) according to the manufacturer's instructions. Approximately 500 ng of PCR products were deposited in each well of the polyacrylamide gels containing a linear 30–60% denaturing gradient. Details of the electrophoresis are as previously reported (Li et al., 2007). Gels were photographed using a Molecular Imager® Gel Doc™ XR system (Bio-Rad, USA).

2.3. 16S rRNA gene library construct and analysis

The 16S rRNA gene was amplified from each sample as previously reported (Li et al., 2007), except for the use of the T1 thermocycler PCR system. The PCR products were purified from gels using the Wizard® SV Gel and PCR Clean-Up System, and cloned into the pGEM-T vector (Promega, USA) according to the manufacturer's instructions. Competent *Escherichia coli* JM109 cells (Promega, USA) were transformed and screened for plasmid insertions according to the manufacturer's instructions.

Approximately 100 clones from each clone library were randomly chosen for further analysis. The coverage index (*C*) (Good, 1953) was calculated. The representative clones for each phylotype were produced via one-reaction-sequencing (BGI LifeTech, Peking, China) and the distances analysis using ClustalX version 2.1 (Thompson et al., 1997) and MEGA version 4.0 (Tamura et al., 2007). In this study, 99% gene sequence similarity was used as a criterion to define a phylotype. The sequences within a phylotype defined in this manner may have originated from different species (Stach et al., 2003). Bidirectional sequencing of representative clones of the dominant phylotype (with more than one clone) was performed (BGI LifeTech) to obtain partial sequences, as previously reported (Li et al., 2007). All sequences were compared with current accessible sequences, and the closest known neighbor in the GenBank reference RNA sequence (RefSeq Rna) database was determined. These sequences were aligned using ClustalX version 2.1. An unrooted tree was constructed using the neighbor-joining method of MEGA version 4.0.

2.4. Nucleotide sequence accession numbers

The bacterial 16S rRNA gene sequences from the intestinal tract of *C. chinensis* were submitted to the GenBank under accession numbers JN157650–JN157662.

3. Results and discussion

The purpose of the present study was to describe the natural intestinal bacterial diversity and elucidate the composition of the bacterial communities in *C. chinensis*. Furthermore, the data will

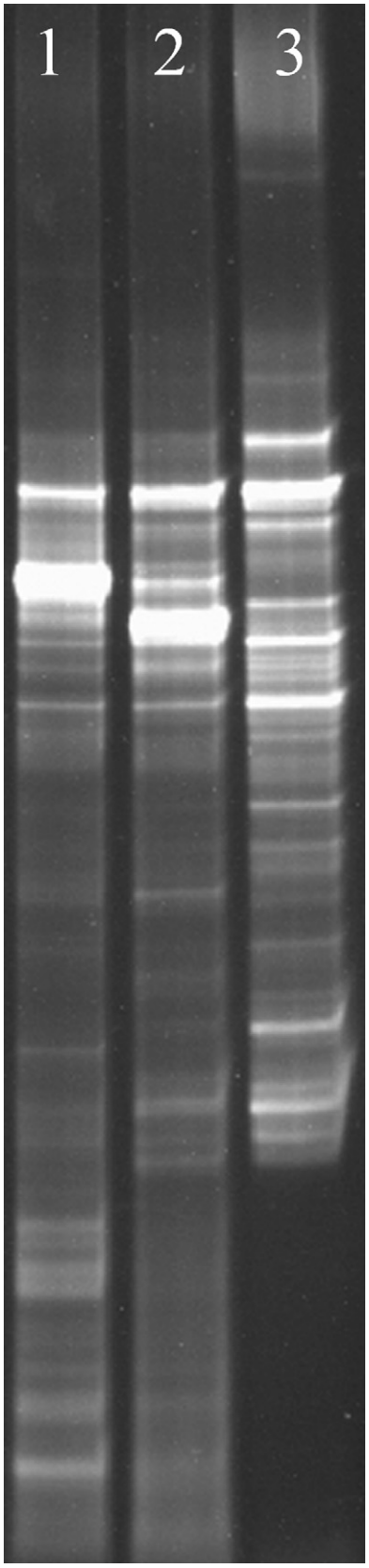

Fig. 1. DGGE band profiles of the V3 regions produced from the intestinal bacterial community DNA extracted from *C. chinensis*, which were collected from three aquatic ecological restoration structures.