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Illumina-Based Analysis of Bulk and Rhizosphere Soil Bacterial Communities in Paddy Fields Under Mixed Heavy Metal Contamination

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ABSTRACT

There is an increasing concern about rice (*Oryza sativa* L.) soil microbiomes under the influence of mixed heavy metal contamination. We used the high-throughput Illumina MiSeq sequencing approach to explore the bacterial diversity and community composition of soils in four paddy fields, exhibiting four degrees of mixed heavy metal (Cd, Pb and Zn) pollution, and examined the effects of these metals on the bacterial communities. Our results showed that up to 2 104 to 4 359 bacterial operational taxonomic units (OTUs) were found in the bulk and rhizosphere soils of the paddy fields, with the dominant bacterial phyla (greater than 1% of the overall community) including Proteobacteria, Actinobacteria, Firmicutes, Acidobacteria, Gemmatimonadetes, Chloroflexi, Bacteroidetes and Nitrospirae. A number of rare and candidate bacterial groups were also detected, and Saprospirales, HOC36, SC-I-84 and *Anaerospora* were rarely detected in rice paddy soils. Venn diagram analysis showed that 174 bacterial OTUs were shared among the bulk soils with four pollution degrees. Rice rhizosphere soils displayed higher bacterial diversity indices (ACE and Chao 1) and more unique OTUs than bulk soils. Total Cd and Zn in the soils were significantly negatively correlated with ACE and Chao 1, respectively, and the Mantel test suggested that total Pb, total Zn, pH, total nitrogen and total phosphorus significantly affected the community structure. Overall, these results provided baseline data for the bacterial communities in bulk and rhizosphere soils of paddy fields contaminated with mixed heavy metals.

Key Words: bacterial diversity, community structure, Illumina MiSeq sequencing approach, long-term contamination, paddy soil

INTRODUCTION

Heavy metal contamination in agricultural soils due to anthropogenic mining activities can result in adverse environmental effects, including soil quality degeneration, inhibition of crop growth and potential risks to human health by food chain transfer (Li Z *et al.*, 2014). Paddy fields, a unique agro-ecosystem, are of vital importance for cereal production in Asia, specifically in China (Kögel-Knabner *et al.*, 2010). Thus, heavy metal contamination of paddy soils in the vicinity of mining areas in China has become a growing concern in recent years (Li *et al.*, 2012; Liu *et al.*, 2012; Chen *et al.*, 2014).

Paddy fields consist of diverse habitats for microorganisms, which play significant roles in the maintenance of soil quality and rice (*Oryza sativa* L.) fitness (Liesack *et al.*, 2000). In particular, as the most abundant group of soil microorganisms, bacteria are actively involved in various biogeochemical processes of bulk and rhizosphere soils (Buée *et al.*, 2009). Compared to bulk soil bacteria, rhizosphere bacteria can be more directly beneficial to the rooting patterns and supply of nutrient elements to plants (Buée *et al.*, 2009). Given the confirmed importance of soil microbes, it is crucial to understand whether heavy metal contamination can affect soil microbial diversity and community structure. Khan *et al.* (2010) and Pan and Yu (2011) reported that the addition of Cd or Pb had a significant effect on microbial community structure in greenhouse experiments. However, short-term studies under greenhouse or laboratory conditions using soils spiked with heavy metals cannot be used to infer the long-term effects of heavy metals on soil microorganisms under field conditions (Giller *et al.*, 2009). Tang *et al.* (2014) found no apparent correlations between

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heavy metals and the microbial community in contaminated soils. Moreover, among the environmental factors, soil pH (Chodak *et al.*, 2013) and organic matter concentrations (Hirsch *et al.*, 2010) are considered more important factors than the presence of toxic metals with regard to the effects on soil bacterial communities. Thus, the current findings are inconsistent with reference to heavy metal effects on soil bacterial communities, and such studies have often focused on a single toxic metal. To date, few studies have explored the effects of long-term multi-metal contamination on paddy soil bacterial diversity and community structure under field conditions. In addition, because the diversity and composition of rice rhizosphere bacterial communities are highly complex and likely region-specific, the differences in bacterial communities between rice bulk and rhizosphere soils with different pollution degrees have not been well studied.

Culture-independent molecular approaches based on extraction, amplification and sequencing of 16S rRNA genes provide feasibility and a new perspective to characterize soil microbial communities (Hirsch *et al.*, 2010). Recent high-throughput sequencing (*e.g.*, Illumina) has been described as a promising and rapid approach to analyse soil microbial diversity and community structure (Rincon-Florez *et al.*, 2013; Myrold *et al.*, 2014). Thus, the bacterial communities in soils were analysed using the Illumina MiSeq sequencing approach in the present work. Soil samples were collected from four selected paddy fields in the vicinity of several traditional mines in southern China. The aims of this study were: 1) to characterize the bacterial diversity and community composition of bulk and rhizosphere soils in paddy fields with different degrees of mixed heavy metal (Cd, Pb and Zn) contamination and 2) to determine the role of environmental factors, specifically heavy metals, on the bacterial diversity and community structure in paddy soils.

MATERIALS AND METHODS

Study sites and soil sampling

In southern China, many paddy fields have been subjected to the contamination of mixed heavy metals due to mining activities (Liu *et al.*, 2012). Our study was performed at different paddy fields around traditional mining areas in Guangdong Province, southern China (Table I). The selected paddy field sites included an unpolluted site, LT, from Liantang Village, Qingyuan City and three sites with long-term heavy metal pollution, SB, from Shangba Village around Dabaoshan Mine, Shaoguan City, TX, from Tongxi Village around Tongxi Mine, Qingyuan City, and FK, from Fankou Town around Fankou Mine, Shaoguan City. Bulk soils, LB, SB, TB and FB, from the four sites, LT, SB, TX and FK, respectively, were sampled from the top 10 cm of the profile, and rhizosphere soils, LR and FR, from the two sites, LT and FK, respectively, were separated from the roots of the rice (*Oryza sativa* L.) cultivar Tianyou 122 (pure line seeds from the Rice Research Institute of Guangdong Academy of Agricultural Sciences, China) (Table I). The soil that remained adhered to the root hairs after gentle shaking was sampled as rhizosphere soils according to an operational definition (Lynch, 1990; Chen *et al.*, 2006). For each plot (bulk or rhizosphere soil), multiple random soil cores were collected and immediately mixed thoroughly in June 2013 immediately after drainage. The soil samples were then transported to the laboratory on dry ice. A fraction of the soil sample was immediately stored at −20 °C for molecular analyses. Another fraction was air-dried for soil chemical characterization.

Chemical analysis

Soil pH was measured using a portable pH meter (pH 510, Eutech Instruments, Singapore) in 1:2.5 (weight:volume) soil-deionized water ratio, and total

TABLE I

Description of soil samples from four paddy fields in Guangdong Province, southern China

Site	Location	Pollution source and history	Sample(s)
LT	Liantang Village, Qingyuan City (23°52′27″ N, 113°35′59″ E)	No direct heavy metal pollution	Bulk soil (LB), rhizosphere soil (LR)
SB	Shangba Village, Shaoguan City (24°27′83″ N, 113°48′16″ E)	Waste water irrigation from a lead and zinc mine since the 1960s	Bulk soil (SB)
TX	Tongxi Village, Qingyuan City (23°51′49″ N, 113°39′54″ E)	Waste water irrigation from a limonite mine since the 1960s	Bulk soil (TB)
FK	Fankou Town, Shaoguan City (25°07′06″ N, 113°39′12″ E)	Waste water irrigation from a lead and zinc mine since the 1950s	Bulk soil (FB), rhizosphere soil (FR)