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The diversity of iron reducing bacteria communities in subtropical paddy soils of China

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ABSTRACT

Iron(III) reducing bacteria (FeRB), involved in C and N cycle, are vital to regulating environmental biogeochemical processes. Although many important FeRB have been isolated and identified, the diversity of FeRB communities in paddy soils remains largely unknown. Four soils (rice–rapeseed rotation and rice–fallow/flooded rotation collected from Qianjiang, QR and QF soil, respectively, and rice–rapeseed rotation and rice–fallow/flooded rotation collected from Xianning XR and XF soil, respectively) that varied with respect to crop rotation and soil properties, were used in the current study. Incubation experiments were conducted under flooding at 25 °C for the evaluation of FeRB and Fe^{2+} production. The diversity of FeRB community in each soil was evaluated. The composition of the FeRB community of each soil (QR, QF, XR and XF soil) was determined using enrichment culturing techniques under anaerobic conditions and high-resolution bar-coded reversible terminators. The dominant groups (≥5% of all sequences) were Proteobacteria and Firmicutes, and some rare phyla were also identified. At the genus level, the dominant composition of the clone libraries suggested that known FeRB genera were well represented (e.g. *Brevundimonas*,*Pseudomonas*, *Burkholderia*, *Stenotrophomonas* and *Sporomusa*). The analysis also identified novel enrichment culture bacteria genera, such as *Sphingomonas*, *Pandoraea* and *Azospira*, which might be involved in Fe^{3+} reduction in paddy soils. The diversity of FeRB communities were greatly affected by crop rotation, as well as soil properties such as parent material, pH and C/N ratio. Additionally, the Fe^{2+}production in four soils were significantly different on 40-day incubation. These results indicate that the variation in soil properties and crop rotation have significant effects on the diversity of FeRB community which regulated soil Fe^{+3} reducing processes.

1. Introduction

Paddy soils are the largest type of anthropogenic wetlands and are known to be an important source of greenhouse gases (GHGs) in terrestrial ecosystems, especially CH_4 and N_2O under flooding conditions with heavy nitrogen (N) fertilizer applications (Ding et al., 2014a; Ferre et al., 2012). Various studies have demonstrated that paddy soil microbial communities play a vital role in driving soil organic matter (SOM) accumulation, transformation, and mineralization, as well as N leaching (Ding et al., 2014b; Straub et al., 1996; Zhang et al., 2014). Additionally, many microbial functions involved in C and N cycling are coupled to other minerals (e.g., Fe). However, these biogeochemical processes are still not clearly understood (Bongoua-Devisme et al., 2012; Dubinsky et al., 2010; Yang et al., 2012; Zhang et al., 2014).

Iron(III) reducing bacteria (FeRB) are regarded as crucial mediators of C and N processes in paddy soils (Bongoua-Devisme et al., 2013; Ding et al., 2014b; Tan et al., 2006; Wang et al., 2009). Ferric iron can be reduced through respiration (as the electron acceptor) as well as fermentation (as an electron sink) by FeRB under anaerobic conditions (Bongoua-Devisme et al., 2012; Lin, 2006). Previous studies have demonstrated that short-chain fatty acids (e.g., acetate, formate and propionate) produced during anaerobic conditions are oxidized by FeRB and other anaerobes (e.g., methanogens), thereby affecting C mineralization (Bongoua-Devisme et al., 2012). Additionally, the Feammox pathway, whereby anaerobic NH_4^+ oxidation is coupled to Fe^{3+} reduction with either N_2, nitrite or nitrate as the end-product, is a potentially important pathway for nitrogen loss in paddy soils (Ding et al., 2014b). Based on these key FeRB-mediated biogeochemical

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processes, investigating FeRB community composition is essential to understanding C and N cycles in paddy soils.

In recent decades, various FeRB belonging to different phylogenetic groups have been described by both culture-dependent and culture-independent methods. Isolating FeRB using culture-dependent approaches was advocated in the early years (Shaaban et al., 2014). It is more common for studies to report the abundance and diversity of FeRB using Most Probable Number (MPN) analysis (Petrie et al., 2003), MPN–PCR (Petrie et al., 2003), denaturing gradient gel electrophoresis (DGGE) analysis (Cahyani et al., 2008) or terminal restriction fragment length polymorphism (T–RFLP) technologies (Wang et al., 2009). Although many studies have been conducted on FeRB, including bacteria, fungi and archaea in various environments (Lin, 2006), the lack of a universal functional gene marker makes it difficult to track FeRB (North et al., 2004), especially those of rare taxa. Despite the use of these conventional molecular biology approaches, FeRB remain to be fully described. Therefore, to evaluate the diversity of FeRB in paddy soils we utilized a high resolution technique.

High-throughput microbial community analysis on the Illumina MiSeq platform (Illumina, San Diego, CA, USA) is an effective method for studying environmental microbial communities (Caporaso et al., 2012). This platform is able to analyze V_2 or V_4 variable regions from microbial 16S rRNA (Baker et al., 2003; Broadhurst et al., 2012; Roden et al., 2012). We hypothesized that soils undergone different crop rotations with distinctive properties contain unique FeRB communities which have significant influence on Fe^{3+} reduction processes. Therefore, using high-resolution high-throughput technique, we evaluated whether the diversity of FeRB communities for anaerobic enrichment coincides with (i) crop rotation and/or (ii) soil properties. To assess FeRB community diversity, an exploratory comparative study based on analysis of the 16S rRNA metagenome of four paddy soils was conducted using Illumina MiSeq reversible terminators.

2. Materials and methods

2.1. Sample collection and preparation

Soils were collected from cultivated paddy fields in the cities of Qianjiang and Xianning, Hubei Province, China. Both cities have a typical subtropical monsoon climate. In March 2012, two soils from Qianjiang city, a rice–rapeseed (*Brassica napus*) crop rotation (QR) and a rice–fallow/flooded rotation (QF), were sampled at flowering stage of rapeseed and fallow/flooded period, respectively. Both soils are classified as calcareous alluvial soil and cambisol in FAO system (FAO, 1974). Similarly, two soils from Xianning city, a rice–rapeseed rotation (XR) and a rice–fallow/flooded rotation (XF), were also sampled at flowering stage of rapeseed and fallow/flooded period, respectively. These two soils are classified as Quaternary red clay (Peng et al., 2015), and Ferralsols in FAO system (FAO, 1974).

Soil samples (0–15 cm) at 10 subsamples were sampled with an auger and the samples were thoroughly mixed to obtain a single homogeneous sample for soil analysis. After manual removal of visible plant residues and roots, the sample was placed in airtight plastic bags that had been purged with N_2. A subsample from each replication fresh soil sample (total 10 subsamples) was stored at 4 °C for high-throughput sequencing analysis, and the remainder was air-dried and ground to pass through a 2 mm sieve for incubation experiments. Soil physical and chemical properties were analyzed as described previously (Peng et al., 2015; Shaaban et al., 2014). The main characteristics of these soils are given in Table 1.

Table 1
The properties of the fresh soils.

Soils	Fe(II+III)	pH	BD	TC	TN	C/N
QR	0.53 ^{c}	7.14 ^{a}	1.29 ^{b}	1.88 ^{b}	0.20 ^{a}	9.40
QF	0.86 ^{c}	7.39 ^{a}	1.10 ^{b}	1.91 ^{a}	0.16 ^{b}	11.94
XR	2.97 ^{b}	5.54 ^{b}	1.40 ^{a}	1.41 ^{c}	0.15 ^{b}	9.40
XF	7.18 ^{a}	5.63 ^{b}	1.20 ^{b}	1.89 ^{ab}	0.20 ^{a}	9.45

Notes: Different letters in a column indicate significant differences among soils (Tukey's test, $p < 0.05$); BD, Bulk density (g cm^{-3}); TC (%), soil total carbon; TN (%), soil total nitrogen; Fe(II) and Fe(III) in fresh soil, mmol kg^{-1}, extracted by 0.5 mol L^{-1} HCl. QR and QF: rice–rapeseed rotation and rice–fallow/flooded rotation soils were collected from Qianjiang; XR and XF: rice–rapeseed rotation and rice–fallow/flooded rotation soils were collected from Xianning

2.2. FeRB enrichment and reversible terminators

Fresh soil of four different locations (QR, QF, XR, and XF soils) was used for anaerobic enrichment with three replicates. First, 90 mL anoxic water was added to 10.0 g fresh soil followed by the addition of 3 mmol $Fe(OH)_3$ and the cultures were incubated at 25 °C in the dark. After 1 week, a 20 mL suspension was added to 180 mL enrichment medium in a 250 mL serum bottle (Wang et al., 2009), and incubated at 25 °C in the dark. Next the culture was added to mineral medium containing 10 mmol L^{-1} acetate and 30 mmol L^{-1} ferrihydrite and incubated under anaerobic conditions (90:10 N_2:CO_2, v/v). The synthetic ferrihydrite method was performed as described by Straub et al. (2005), and confirmed using Bruker X-ray diffraction (data not shown).

Extraction, purification, and sequencing of genomic DNA from anaerobic enrichment were conducted at the Shanghai Personal Biotechnology Co., Ltd. (Shanghai, China). Partial sequences of 16S rRNA, including the V_4 hypervariable region, were amplified by PCR using the forward primers V_4 F(5′-AYTGGGYDTAAAGNG-3′) and reverse primer V_4 R(5′-TACNVGGGTATCTAATCC-3′) (Garcia-Pichel et al., 2013). Sequencing was performed on an Illumina MiSeq sequencer (Illumina). Sequences with an average phred score lower than 25, containing ambiguous bases, max homopolymer run exceeding 6, having mismatched primers, or sequence lengths shorter than 200 bp were discarded. For V_4 pair-end reads, only sequences with overlaps longer than 10 bp and without any mismatches were assembled according to their overlap sequence.

2.3. FeRB diversity and richness analysis

The Quantitative Insights Into Microbial Ecology (QIIME) suite of analysis tools was used to filter and analyze the sequence data (Broadhurst et al., 2012). Sequences were assigned to operational taxonomic units (OTUs) with a threshold of 97% pair-wise identity and then classified taxonomically using Ribosomal Database Project (RDP) classifiers (Broadhurst et al., 2012). The RDP classification assignments were randomly confirmed using BLASTN by comparing sequences of strains reported in the NCBI 16S rRNA database. Rarefaction curves were plotted for each sample to ensure adequate coverage. To further characterize FeRB community diversity and relative abundance, 16S rRNA was classified taxonomically at the genus level.

Mothur was also used to estimate FeRB diversity and richness (Schloss et al., 2009). The Shannon and Simpson indices were used to calculate community diversity for the chosen OTUs (Bowman et al., 2012). The Chao1 and ACE estimators were used to calculate community richness, and heat map was utilized to community structure (Dong et al., 2014). BioEnv and canonical correspondence analysis (CCA) were used to identify the abiotic factors that were most important to bacterial community composition (Liu et al., 2014).