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Metagenomic assembly unravel microbial response to redox fluctuation in acid sulfate soil

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ABSTRACT

Acid sulfate soil (ASS) is sensitive to redox fluctuations induced by climate change and human activities. Oxidation of sulfur and sulfide in ASS leads to the release of acid and consequently metals, posing severe hazards to coastal environment, while microbial contribution and response to oxidation is poorly understood. Here we used metagenomic sequencing to delineate the shift in microbial community structures and functional genes in ASS upon exposure to aerobic conditions. Aerobic incubation resulted in significant shifts in microbial communities in both topsoil and parent material. Archaea decreased significantly in the parent material after aerobic incubation. The relative abundance of sulfur cycling genes in the parent material layer was significantly higher than those in the topsoil, and multiple sulfide oxidation genes increased after aerobic oxidation. Metagenomic assembly enabled construction of eight key draft genomes from ASS. Three of them (GS3, GS6 and GT3) are novel strains of *Thermoplasmatales*, *Acidothermales* (*Acidothermus*) and *Acidimicrobiales*, respectively. Functional gene annotation of these population genomes revealed a dominance of sulfur cycling genes and acid tolerant genes. These findings highlight the microbial response to environmental change and identify the ecological adaptation and survival strategies of microbial communities in acid sulfate soils.

1. Introduction

Acid sulfate soils (ASS) occupy about 1 million Km^2 worldwide (White et al., 2007), containing iron sulfides (predominately as pyrite) and the products of sulfide oxidation (White and Melville, 1996), and are mainly located in the delta plain and coastal lowlands in tropical and subtropical regions (Dent, 1992). The potential environmental hazard associated with ASS often results from the acid released by oxidation of sulfide minerals. These sulfide minerals are generally stable when kept submerged. However, due to global climate change (e.g. drought) and human activities (agricultural cultivation), these soils are frequently exposed to air, resulting in the oxidation of reduced sulfur compounds. This can negatively impact agricultural productivity, acidify groundwater and water bodies, poison aquatic organisms and degrade concrete and steel structures. Redox fluctuation will also affect ionic composition including Fe and other mineral elements that coupled with sulfur reduction and oxidation. In coastal regions, climate change and soil management may subject the ASS to frequent alternate draining/wetting cycles. Rewetting of ASS can release both acid and metals to surface water and to coastal environments (Stroud et al., 2014).

A variety of redox reactions involving inorganic and organic compounds of sulfur, mediated by microorganisms, can drive sulfur cycling in ASS (Biderre-Petit et al., 2011). Microbial processes,

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including sulfur reduction and oxidation, are considered to promote the formation of acid soil (Bronswijk et al., 1993; Ward et al., 2002). Oxygen (O_2) and ferric ion (Fe^{3+}) are the main pyrite oxidizing agents. Diverse soil microbes, including sulfur-oxidizing bacteria and iron-oxidizing bacteria, are key contributors in sulfur transformation. However, little is known about the role of structural and functional microbial communities in sulfur transformation and soil acidification. Few studies have characterized soil microbial communities relevant to sulfur cycling targeting functional genes that code for key enzymes, such as dissimilatory sulfate reductase and sulfur dehydrogenase (Wu et al., 2013). Thus our understanding of microbial-mediated processes in ASS remains extremely limited.

Dominant microbes are considered to play significant roles in the maintenance and regulation of key processes because they are adapted to particular soil conditions. Given the vast majority of microbes are uncultivable (Singh et al., 2009), genome-centric metagenomics and draft genome assembly of uncultivated microbes are needed to understand their diverse role in ecology, ecosystem functions and adaptation to environmental conditions (Albertsen et al., 2013; Prosser, 2015). A variety of microbial genomes were obtained using single-cell genomics (Rinke et al., 2013), and binned genomes have been retrieved from different environments (Narasingarao et al., 2012; McLean et al., 2013; Yelton et al., 2013; Hug et al., 2016), including soil (Mondav et al., 2014; Hultman et al., 2015; Lin et al., 2015). However, the application of metagenomic sequencing to construct genomes of uncultivable microbes from soil is still challenging due to the higher diversity of soil microbial communities and the high relative abundance of key species is rare (Howe et al., 2014). Here we used deep metagenomic sequencing to determine the effect of oxidation on microbial phylogenetic composition and functional genes, and relate these data to the dynamics of sulfur transformation and soil acidification in ASS. Further, we constructed draft genomes of eight key microbial species from these samples and determined potential metabolic pathways involved in sulfur cycling.

2. Materials and methods

2.1. Soil sampling and incubation

Soil samples were collected from a typical area with ASS in Taishan county, Guangdong Province, China (22.11N, 112.77E). This area is located in the western part of the Pearl River Delta and faced South China Sea. Subtropical monsoon climates prevail here with an average temperature 21.8 °C and annual rainfall 2350 mm. Double rice cropping has been practiced in this site since 2003, and soil samples were collected at November 2012, during the ripening stage of rice plants. Soil samples with four replicates from four plots, were taken from depth of 0–15 cm (topsoil) and 80–100 cm (parent material layer), respectively. To evaluate the effect of land use on genetic diversity, neighboring native wasteland (topsoil) was also chosen as a study site for DNA extraction and metagenomic analysis. The samples from parent material layer were collected using a gouge auger, and the outer layer of each core was removed to prevent contamination. The parent material layer samples were placed into anaerobic plastic bags and flushed with nitrogen, thoroughly mixed, and then put into car-refrigerator for transport. The samples from the top layer (T) and parent material layer (S) were separated into three portions. The first portion (approximately 100 g of fresh soil) was passed through a 2.0 mm sieve, and then incubated at 25 °C and 90% relative air humidity for two weeks in aerobic condition. The soil samples were adjusted to a moisture content of 60% water holding capacity and distilled water was added to maintain initial moisture during the incubation. The second portion was stored at 4 °C for chemical analyses, and the third portion was stored at −80 °C for DNA extraction. Soil pH, water-soluble sulfur, total oxidizable sulfur and other chemical characteristics (Table S1) were measured, soil DNA were extracted and sequenced before and after incubation, respectively.

2.2. Soil chemical analysis

Soil pH was measured using a glass electrode (soil: water, 1:2.5). The inorganic N was extracted with 1 M KCl (soil: solution, 1:10), and determined by colorimetrically using a micro-plate reader (Shand et al., 2008). The total C, N, and S content in soil were determined by macro elemental analyzer (Vario MAX). Soil available phosphorus and potassium were determined by $NaHCO_3$ and NH_4OAc extraction methods, respectively (Bao, 1999). Water-soluble sulfate ions were extracted by adding 50 mL of distilled water purged with helium to 2.5 g of soil. All the samples were shaken for 1 h on a reciprocal shaker. The suspensions were filtered through a 0.22 μm membrane, and then immediately analyzed using an ion-exchange chromatograph. Total oxidizable sulfur (TOS) were estimated and calculated following the peroxide oxidation-combined acidity and sulfate method (Ahern et al., 1998). According the change of water-soluble sulfate concentrations, the proton production was calculated using the software ECOSAT (Keizer and Riemsdijk, 1998).

2.3. Soil DNA extraction, sequencing and quality control

We tried DNA extraction by using commercial kits with 0.25–0.5 g soil, however, we did not obtain sufficient DNA for metagenomic sequencing due to low biomass in our samples, particularly in parent material. Therefore, we performed DNA extraction from a large volume of soil samples (5 g for topsoil and 50 g for parent material) by the freeze-grinding, SDS-based methods. Extracted DNA was purified using a low melting agarose gel followed by phenol extraction (Zhou et al., 1996). All DNA samples were stored under −20 °C before Illumina sequencing. Soil DNA was sequenced on Illumina Hiseq2000 platform with PE100 strategy at Personal Biotechnology Co., Ltd. (Shanghai, China). The derived raw reads were quality filtered with Prinseq (Schmieder and Edwards, 2011). First, reads with more than one ambiguous character “N” or with average quality score lower than 20 were filtered out. Next, reads showed more than 10 exact duplications were removed as artifacts.

2.4. Phylogenetic and functional annotation

To resolve the phylogenetic structure of the soil community, rRNA reads were identified from the dataset by searching against Silva SSU database version 11.5 (Quast et al., 2013) using BLASTN (Camacho et al., 2009) with e-value cut-off of 1E-20. The rRNA BlASTN result was parsed in Megan 5 (Huson et al., 2011) with default lowest common ancestor (LCA) algorithm to assign taxonomy. To facilitate the annotation speed, nucleotide sequences of targeted KEGG Orthologies (KO) involve in sulfur, nitrogen, methane metabolic pathways were extracted from the KEGG database and used as the subject database for BLASTN search. The pass-QC reads were searched against the extracted database with e-value cut-off of 1E-5, after that, in order to prevent false positive hit resulted from searching against subset database, strict similarity cutoff allowing only three mismatches over >80% of the read length were used to filter the BLASTN result before statistical analysis.