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Assessment of abundance and diversity of exoelectrogenic bacteria in soil under different land use types

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ABSTRACT

Soil exoelectrogenic bacteria have been found to perform various functions. However, little is known about whether exoelectrogenic bacteria are widely distributed in soil and which physico-chemical properties of soil significantly affect their distribution? To address the research questions, 37 soil samples covering six land use types, including arid farmland soils, woodland soils, grassland soils, paddy soils, lakeshore soils, and coastal soils were collected. DNA was extracted from all the samples for quantitative PCR and high throughput sequencing of the 16S rRNA gene. The sequencing result showed a total of 16 exoelectrogenic bacteria-associated genera from different soils. The total abundance of these genera varied from 1.14×10^8 copies g^{-1} in a farmland soil to 7.89×10^9 copies g^{-1} in a coastal soil, accounting for 0.26% to 7.70% of the total bacteria. Among the 16 genera, *Bacillus* was dominant in the arid land soils, including the farmland, woodland, and grassland soils. The coastal soils were dominated by *Desulfobulbus* whereas the paddy and lakeshore soils were characterized by abundant *Geobacter*. Principal component analysis showed a distinct separation of the exoelectrogenic bacterial community between the arid land (farmland, woodland, and grassland soils), freshwater wetland (paddy and lakeshore soils), and coastal soils. Redundancy analysis revealed that the water content, electrical conductivity and concentrations of total sulphur and amorphous Fe in the soil were significant factors driving the community structure and abundance of exoelectrogenic bacteria-associated genera. Our results should help in better understanding of the exoelectrogenic bacteria-mediated functions in soil, which might contribute to the biogeochemical cycling of a large number of organic and inorganic materials.

1. Introduction

Soil contains exoelectrogenic bacteria, which are capable of transferring electrons outside of the cells to extracellular acceptors (Logan, 2009). Evidence for the presence of exoelectrogenic bacteria in soil includes their isolation from rice paddy soil (Zhong et al., 2017), vegetated arid soil (Jiang et al., 2016; Deng et al., 2017), and the detection of exoelectrogenic bacteria associated families or genera in soil using sequencing methods. The native exoelectrogenic bacteria of soil have attracted increased attention because of their crucial role in a variety of biotechnological and biogeochemical processes. For example, they convert chemical energy of organic substrates present in the soil into electrical power in microbial fuel cells (MFCs), which have been successfully deployed to power sensors (Knight et al., 2013; Zhang et al., 2016b). The native exoelectrogenic bacteria could also accelerate the removal of a variety of pollutants in soil when they are generating electricity in MFCs, which is a potentially novel biotechnological strategy for remediation (Vijay et al., 2016; Wang et al., 2016; Abbas et al., 2017; Zhao et al., 2018). Additionally, the direct interspecies electron transfer (DIET) between exoelectrogenic bacteria and electrotrophic methanogens (e.g. *Methanosaeta harundinacea*, *Methanosarcina barkeri*) was reported to contribute to a substantial portion of global methane production (Rotaru et al., 2015; Shen et al., 2016). Moreover, exoelectrogenic bacteria can reduce a large number of electron acceptors, such as Mn(IV), Fe(III), Cr(VI), U(VI), sulphate, selenite, and nitrate, being a crucial enhancer of biogeochemical processes (Bond and Lovley, 2003; Li et al., 2014; Yokoyama et al., 2016).

Unfortunately, although the significance of soil exoelectrogenic

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bacteria in biotechnological and biogeochemical processes is increasingly understood, our knowledge about the abundance and diversity of exoelectrogenic bacteria in soil is quite limited. Therefore, investigations on the abundance and diversity of exoelectrogenic bacteria in different soils with varied physico-chemical properties would provide information about the distribution of exoelectrogenic bacteria, and help in predicting exoelectrogenic bacteria-mediated biotechnological and biogeochemical processes. However, because of the lack of universal functional gene markers for exoelectrogenic bacteria (Jiang et al., 2016), it is difficult to identify and quantify exoelectrogenic bacteria by using the molecular biology-based methods. Nevertheless, several alternative strategies have been reported for the study of the exoelectrogenic bacterial community in soil and sediments. Three such strategies are as follows: 1) quantification of the model exoelectrogenic bacteria, at the genera (e.g. *Geobacter* and *Shewanella*) or family (e.g. *Geobacteraceae*) level, as representatives of the total exoelectrogenic bacteria (Schilirò et al., 2016). This strategy provides little information about the abundance and diversity of exoelectrogenic bacteria; 2) study of the diversity of bacteria enriched on the anode of MFCs by sequencing their 16S RNA gene (Deng et al., 2014; Dolch et al., 2016; Hamdan et al., 2017), denaturing gradient gel electrophoresis (DGGE) analysis (Deng et al., 2015; Jiang et al., 2015), or culture dependent most probable number (MPN) analysis (Heidrich et al., 2016); 3) study of the diversity of enriched iron-reducing bacteria, which generally possess exoelectrogenic activity (Jiang et al., 2016; Peng et al., 2016). However, the enrichment process favours the fast-growing bacteria and masks the original diversity and abundance of exoelectrogenic bacteria in the soil and sediments. Additionally, bacteria enriched on the anode might include fermentative bacteria that do not have exoelectrogenic activity (Lay et al., 2015). As a result, little is known about the diversity and abundance of exoelectrogenic bacteria in different soils under a wide range of physico-chemical conditions as well as about the effect of the physico-chemical properties of soil on them.

To date, approximately 76 species of exoelectrogenic bacteria have been isolated and identified (Niessen et al., 2004; Slobodkina et al., 2007; Fedorovich et al., 2009; Marshall et al., 2009; Yang et al., 2012; Nandy et al., 2013; Wang et al., 2014a; Koch and Harnisch, 2016; Deng et al., 2017; Venkidusamy et al., 2018). They belong to 43 genera, such as *Clostridium*, *Geobacter*, *Bacillus*, and *Pseudomonas* (Table S1). These species allow the assessment of the abundance and diversity of exoelectrogenic bacteria in soil. In the present study, we collected 37 soil samples from different land use types, including arid farmlands, woodlands, grasslands, paddy fields, lakeshore, and coastal areas across China. A high throughput sequencing approach and quantitative PCR (*q*PCR) targeting the V1–V3 region of bacterial 16S rRNA gene was employed to identify the bacteria present in the soil samples. The genera that contained the exoelectrogenic bacterial species were selected to evaluate their abundance and diversity at the genus level. The aim of this study was to evaluate the abundance and diversity of exoelectrogenic bacteria by targeting exoelectrogenic bacteria-associated genera in different soil samples and to determine the physico-chemical properties that might be responsible for their distribution.

2. Material and methods

2.1. Sample collection

Soil samples were collected in July 2017 at 37 sites across China (Fig. 1), including 11 sites in arid farmlands (Farm-1 to Farm-11), nine sites in arid woodlands (Wood-1 to Wood-9), three sites in the grasslands (Gra-1 to Gra-3), eight sites in paddy fields (Pad-1 to Pad-8), three sites in lakeshore areas (Lake-1 to Lake-3), and three sites in intertidal coastal areas (Coast-1 to Coast-3). The soil in the lakeshore and coastal areas was waterlogged with fresh water and seawater, respectively. The detailed site description, including climate and dominant vegetation type, is provided in Table S2. In each site, three soil samples (0–20 cm depth) were collected from three randomly selected plots (0.5 m × 0.5 m). The distance between plots was approximately 5 m. After sampling, the three soil samples were thoroughly mixed to form a homogeneous soil sample, then sealed in plastic bags and taken to the laboratory within 48 h (Zhang et al., 2016a). Three aliquots of 5.00 g fresh soil as triplicates from each sample were weighed to determine the water content in the soil. The remaining portion of the samples was passed through a sieve with a 2-mm diameter mesh size. Thereafter, a 2-g aliquot of soil was stored at −20 °C for subsequent DNA extraction. The sieved soil samples were further air dried for determining their physico-chemical properties.

2.2. Determination of the physico-chemical properties of soils

The physico-chemical properties of soils were determined using the routine methods. Briefly, the soil water content (WC) was determined by oven-drying method (105 °C for 24 h) (De Sosa et al., 2018), soil pH was measured at 1:2.5 (soil:water), and soil electrical conductivity (EC) was determined at 1:5 (soil:water). The soil total carbon (TC), total nitrogen (TN), and total sulphur (TS) were measured using an elemental analyser (Vario EL III, Elementar, Hanau, Germany). Soil sulphate was extracted by acidic hydrolysis (HF–HNO_3–HCl, 1:3:2) and measured using ion chromatography (Schellenberger et al., 2011), and the soil amorphous Fe (aFe) was extracted with 0.2 M $H_2C_2O_4$-$(NH_4)_2C_2O_4$ (McKeague and Day, 1966) and measured using a flame atomic absorption spectrometer (AA240, Agilent Technologies, Santa Clara, US). The content of dissolved organic carbon (DOC; extracted with 0.5 M K_2SO_4) and humic carbon (HC; extracted with 0.1 M $Na_4P_2O_7$ and 0.1 M NaOH) was measured using a TOC analyser (TOC-L, Shimadzu, Kyoto, Japan) (Cui et al., 2017a).

2.3. DNA extraction

The genomic DNA of all the 37 soil samples was extracted using the Fast DNA SPIN kit for soil (BIO101, MP Biomedicals, US), following the manufacturer's instructions. The purity and the quantity of the extracted DNA were determined using a nanodrop ND-1000 UV–Vis spectrophotometer (NanoDrop Technologies, Wilmington, US) at 230, 260, and 280 nm.

2.4. qPCR assay

The bacterial 16S rRNA gene was quantified by qPCR with a real-time PCR detection system (CFX96, Bio-Rad Laboratories, Hercules, US) using the primer set 27F (5′-AGA GTT TGA TCC TGG CTC AG-3′) and 533R (5′-TTA CCG CGG CTG CTG GCA C-3′), for amplification of the V1–V3 region of this gene (Franke-Whittle et al., 2015). The 20-μL reaction mixture contained 10 μL of SYBR® Premix EX Taq™ II, 0.4 μL of each primer (20 μM), 2 μL of template DNA, and 7.2 μL of ddH_2O. The thermal program consisted of an initial denaturation step at 95 °C for 3 min, followed by 40 cycles of denaturation at 95 °C for 10 s, annealing at 55 °C for 20 s, and extension at 72 °C for 20 s. Negative control reactions without matrix DNA were performed in parallel to ensure purity of the qPCR reagents. The calibration curve was constructed using serially diluted (10^3–10^9 copies μL^{-1}) plasmid DNA containing 16S rRNA gene fragments. The efficiency of amplification ranged from 96.3% to 103.9%, with R^2 values > 0.99. The abundance of gene was decided in triplicate for each soil sample based on the standard curve and was expressed as copies per g dry soil (Leloup et al., 2018).

2.5. Sequencing of the V1–V3 region of 16S rRNA gene

The V1–V3 region of bacterial 16S rRNA gene was amplified using the universal primer set 27F and 533R. The final volume of the reaction mix (25 μL) comprised of 2.5 U of Taq DNA polymerase (Sangon Biotech, Shanghai, China), 1 μL of each primer (10 μM), 2.5 μL of 10 ×