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Diversity and distribution patterns of acidobacterial communities in the black soil zone of northeast China

Junjie Liu [a], Yueyu Sui [a], Zhenhua Yu [a], Qin Yao [a], Yu Shi [b], Haiyan Chu [b], Jian Jin [a], Xiaobing Liu [a], Guanghua Wang [a, *]

[a] Key Laboratory of Mollisols Agroecology, Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences, Harbin 150081, China
[b] State Key Laboratory of Soil and Sustainable Agriculture, Institute of Soil Science, Chinese Academy of Sciences, Nanjing 210008, China

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ABSTRACT

Although *Acidobacteria* are ubiquitous and are commonly one of the most abundant bacterial phyla in soils, knowledge regarding their diversity and distribution is still limited. Our previous studies discovered the biogeographical distribution patterns of bacterial and fungal communities in the black soil zone of northeast China. In this study, we further investigated the diversity and composition of acidobacterial communities generated with the Acidobacteria-specific primers ACIDO/342r in the same soil samples using quantitative PCR and Illumina MiSeq sequencing methods. A total of 412,203 acidobacterial 16S rRNA gene sequences were obtained from 26 soil samples that were collected from arable lands across the black soil zone. These sequences belonged to 21 subgroups, and GP1, GP3, GP4 and GP6 were the most abundant subgroups, accounting for 22.63%, 17.17%, 23.82% and 27.47% of acidobacterial sequences across all soils, respectively. The abundance of *Acidobacteria* displayed a more significant positive correlation with soil carbon content than with soil pH, and the relative abundance of certain subgroups was significantly positive or negative related with soil pH. The OTU richness, phylogenetic diversity and community composition of *Acidobacteria* were significantly correlated with soil pH. A variance partitioning analysis showed that the soil pH contributed 25% of the community variation, while the geographic distance explained only approximately 5% of the variation. These results indicated that soil pH was a main factor structuring acidobacterial communities in the black soil zone of northeast China. Our results also suggested that the Acidobacteria-specific primers could be better used for studying the distribution of acidobacterial communities in soils.

1. Introduction

Acidobacteria play an important role in soil ecological processes, and this phylum is one of the most widely distributed and diverse bacterial phyla in various natural environments (Hugenholtz et al., 1998; Tringe et al., 2005; Janssen, 2006). Based on data analysis of the 16S rRNA gene, members of this phylum commonly represent 10–50% of total soil bacterial communities (Barns et al., 1999; Dunbar et al., 1999; Branco et al., 2005; Fracchia et al., 2006; Penn et al., 2006; Lee et al., 2008). However, because bacteria from this phylum are rarely cultured, our understanding of their putative ecological roles (such as turnover of soil organic carbon etc.) is hampered (Jones et al., 2009). Currently, using culture-independent molecular biological techniques, the phylogenetic diversity within the *Acidobacteria* phylum has been revealed to be comparable to that of *Proteobacteria* (Hugenholtz et al., 1998; Barns et al., 1999), and the number of subdivisions or subgroups within *Acidobacteria* increased from 4 to 5 (Ludwig et al., 1997) to 26 (Barns et al., 2007). Because different acidobacterial subgroups are known to respond differently to particular environmental factors (Naether et al., 2012), the research at the subgroup level or, even more, at the taxonomic levels will promote understanding of the ecology, physiology and life history characteristics of this bacterial phylum.

Recently, the biogeographical distribution of bacterial (Lauber et al., 2009; Chu et al., 2010; Rousk et al., 2010; Shen et al., 2013) and fungal communities (Polme et al., 2013; Wu et al., 2013; Schmidt et al., 2014) in various environments had been extensively studied using high-throughput sequencing (HTS) methods. However, similar in-depth studies of *Acidobacteria* are limited

* Corresponding author. Tel.: +86 451 86602745; fax: +86 451 86603736.
E-mail addresses: wanggh@iga.ac.cn, guanghuawang@hotmail.com (G. Wang).

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(Fierer et al., 2007; Jones et al., 2009; Naether et al., 2012; Zhang et al., 2014). Quantitative PCR results showed that the relative abundance of *Acidobacteria* was negatively related with soil carbon availability (Fierer et al., 2007). Other research showed that the soil pH was the major edaphic factor in determining the relative abundance of *Acidobacteria*, with the highest abundances present in the lowest pH soils (Fierer et al., 2007; Lauber et al., 2009; Chu et al., 2010; Shen et al., 2013). Further analysis indicated that the response of the different subgroups of *Acidobacteria* to soil pH was varied; the relative abundance of subgroups 1, 2, 3, 12, 13 and 15 were negatively correlated with soil pH, whereas, subgroups 4, 6, 7, 10, 11, 16, 17, 18, 22 and 25 were positively correlated with soil pH (Jones et al., 2009). However, Zhang et al. (2014) found that the relative abundances of subgroups 1, 2 and 3 were significantly correlated positively with soil pH, while other subgroups presented opposite patterns in the Shennongjia Mountain. Some work also revealed that other soil parameters, such as mineral element contents (Navarrete et al., 2013), soil enzyme activity (Zhang et al., 2014), even soil nanofauna and vascular plant diversity (Naether et al., 2012) were closely related to the relative abundance of certain subgroups of *Acidobacteria*. Therefore, soil pH often observed as the dominating factors in shifting acidobacterial communities (Barns et al., 1999; Eichorst et al., 2007; Jones et al., 2009; Naether et al., 2012). Beside of soil pH, other environmental variables were also detected to influence the acidobacterial community composition. For example, a broad-scale survey of acidobacterial communities across 87 soils throughout North and South America indicated that the mean annual precipitation, the organic carbon content, and the C/N ratio etc. also influenced the compositions (Jones et al., 2009). Another investigation of 57 soils collected from grassland and forest in Germany also showed that the soil C/N ratio, ammonium, and P concentration exerted an additional effect on the acidobacterial community composition (Naether et al., 2012). Thus, we conclude that the distributions of soil *Acidobacteria* are varied among studies and according to the sampling regions, and more work is needed to discover the biogeographical distribution patterns of acidobacterial communities in soils (Jones et al., 2009).

Through a 454 pyrosequencing technique, we examined the biogeographical distribution of bacterial and fungal communities across the black soil (classified as Mollisol) zone (Liu et al., 2014, 2015), which is one of the most important soil resources for China's food security (Liu et al., 2012). Our work revealed that *Acidobacteria* was the second most abundant bacterial phylum in the black soils (Liu et al., 2014). However, in our previous study, the closely significant negative relationship between relative abundances of *Acidobacteria* and soil pH was not discovered (Liu et al., 2014). Given the knowledge of soil *Acidobacteria* in most studies were retrieved from pyrosequencing data using universal bacterial primers (Jones et al., 2009; Lauber et al., 2009; Liu et al., 2014; Zhang et al., 2014), we worried that some of the valuable information about *Acidobacteria* in soils had not been well described.

The precondition of using the HTS method to analyze the acidobacterial diversity and community composition is to design or select the suitable specific primers (Fierer et al., 2007; Jones et al., 2009; Lee and Cho, 2011; Zhang et al., 2014). Primer 31F combined with a universal bacterial primer such as 1492r was previously designed as a primer set for the selective amplification of acidobacterial members (Barns et al., 1999). However, this primer had been demonstrated to be unable to cover many acidobacterial subgroups (Sait et al., 2006; Barns et al., 2007; Jones et al., 2009), and the methods of using this primer to investigate *Acidobacteria* in soil were mainly dependent on constructing a clone library and T-RFLP (Tarlera et al., 2008; Jones et al., 2009; Kielak et al., 2009; Wang et al., 2010; Naether et al., 2012), none of which use the HTS method. Recently, Lee and Cho (2011) designed a new acidobacterial specific primer ACIDO, and they demonstrated that this primer had larger coverage for *Acidobacteria* than primer 31F (83.0% vs. 45.5% *in silico* evaluation). Therefore, in this study, we chose this specific primer and adopted the HTS method to investigate the acidobacterial community diversity and distribution in the black soil zone of northeast China. Additionally, to strengthen the findings of this study are reliability; the data were also compared with pyrosequencing data obtained previously using universal bacterial primers (Liu et al., 2014). Through this study, we wanted to address the following questions: (1) What are the distribution patterns of acidobacterial communities across the black soil zone? (2) How does the diversity of soil acidobacterial communities change in the black soils? (3) What factors drive acidobacterial community distribution in the black soils?

2. Materials and methods

2.1. Soil sampling and DNA extraction

The methods of soil sampling and the determinations of soil physicochemical properties and microbial biomass carbon (MBC) were previously described (Liu et al., 2014). Briefly, 26 soil samplings were collected from arable lands across the black soil zone of northeast China in September 2012. Soil DNA was extracted from soil samples (0.5 g wet weight) with an E.Z.N.A Soil DNA Kit (OMEGA, USA), according to the manufacturer's instruction. The extracted DNA was diluted in TE buffer (10 mM Tris–HCl, 1 mM EDTA, pH 8.0) and stored at −20 °C until use. The locations of the sampling sites and some soil physiochemical properties are shown in Fig. S1 and Table S1, respectively.

2.2. Quantitative PCR analysis

In this study, we conducted four rounds of real-time PCR to determine the abundances of different taxonomic levels of bacteria by using different specific primer sets. That is, the primer set 357f/517r was used for assessing the total bacterial abundance. For determining the abundance of *Acidobacteria*, we used two acidobacterial specific forward primers, ACIDO and 31F, combined with a universal bacterial reverse primer 342r, individually. For determining the abundance of acidobacterial subgroup GP2, the primers S2/1100r were used. The standard curves of different bacterial levels were generated using 10-fold serial dilutions of a plasmid containing the targeted gene inserts. Each PCR reaction contained 10 μl of SYBR Premix Ex Taq™ (Takara, Dalian, China), 0.4 μl of 10 μM forward and reverse primers (each), 7.2 μl of sterilized MilliQ water, and 2 μl of standard or extracted soil DNA. The PCR was performed in a LightCycler® 480 (Roche Applied Science) using a program of initial denaturation at 95 °C for 30 s (ramp rate of 4.4 °C/s), followed by 30 cycles of 95 °C for 5 s for denaturation, 60 °C for 30 s for annealing and elongation, and one final cycle at 50 °C for 30 s for cooling. The copy number of different bacterial 16S rRNA genes was calculated using a regression equation for converting the cycle threshold (C_t) value to the known number of copies in the standards. All of the real-time PCR reactions were run in triplicate with the DNA extracted from each soil sample. The sequences of primers used for quantitative real-time PCR are shown in Table S2.

2.3. Acidobacterial 16S rRNA gene amplification and Illumine MiSeq sequencing

The acidobacterial community was analyzed using the primers ACIDO/342r to amplify the acidobacterial specific phylum (Lee and Cho, 2011), with the forward primer modified to contain a unique