Environment International 65 (2014) 9–15

Contents lists available at ScienceDirect

Environment International

journal homepage: www.elsevier.com/locate/envint

Functional metagenomic characterization of antibiotic resistance genes in agricultural soils from China

Jian Qiang Su [a], Bei Wei [a], Chun Yan Xu [a], Min Qiao [b], Yong Guan Zhu [a,b,*]

[a] Key Lab of Urban Environment and Health, Institute of Urban Environment, Chinese Academy of Sciences, Xiamen 361021, China
[b] State Key Lab of Urban and Regional Ecology, Research Center for Eco-environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China

ARTICLE INFO

Article history:
Received 4 June 2013
Accepted 13 December 2013
Available online 8 January 2014

Keywords:
Antibiotic resistance
Functional metagenomics
Agricultural soils

ABSTRACT

Soil has been regarded as a rich source of antibiotic resistance genes (ARGs) due to the complex microbial community and diverse antibiotic-producing microbes in soil, however, little is known about the ARGs in unculturable bacteria. To investigate the diversity and distribution of ARGs in soil and assess the impact of agricultural practice on the ARGs, we screened soil metagenomic library constructed using DNA from four different agricultural soil for ARGs. We identified 45 clones conferring resistance to minocycline, tetracycline, streptomycin, gentamicin, kanamycin, amikacin, chloramphenicol and rifampicin. The similarity of identified ARGs with the closest protein in GenBank ranged from 26% to 92%, with more than 60% of identified ARGs had low similarity less than 60% at amino acid level. The identified ARGs include aminoglycoside acetyltransferase, aminoglycoside 6-adenyltransferase, ADP-ribosyl transferase, ribosome protection protein, transporters and other antibiotic resistant determinants. The identified ARGs from the soil with manure application account for approximately 70% of the total ARGs in this study, implying that manure amendment may increase the diversity of antibiotic resistance genes in soil bacteria. These results suggest that antibiotic resistance in soil remains unexplored and functional metagenomic approach is powerful in discovering novel ARGs and resistant mechanisms.

© 2013 Elsevier Ltd. All rights reserved.

1. Introduction

Environmental contamination with antibiotics and antibiotic resistance genes (ARGs) is becoming a global health problem (Bush et al., 2011). The successful treatment for infections using antibiotics is being threatened by antibiotic resistance, which has developed from resistance to one class of antibiotics to increasingly prevalent multidrug resistance and extreme drug resistance. Antibiotic resistance has been one of the most important sustained driving forces for antibiotic discovery. Most of the research on the risk of ARGs to human health focused on the clinical setting, however, ARGs from pathogens comprise only a tiny fraction of the resistome (Wright, 2007). Soil has been regarded as a rich source of ARGs, deriving from both natural and anthropogenic processes. Although high frequency of antibiotic resistance was revealed in soils by culture-dependent approaches, it represents only a fraction of soil-dwelling bacteria (D'Costa et al., 2006), and uncultured soil bacteria represent a reservoir of new ARGs, which can be transferred to clinical pathogens via mobile genetic elements (Riesenfeld et al., 2004).

Culture-independent molecular approaches have provided powerful means to explore ARGs in nature and address the dissemination of ARGs in different environments. Quantitative PCR (qPCR) based approaches, including high-throughput qPCR (Looft et al., 2012), has been applied to the quantification of ARGs for comparison of their distribution in soil, water, sediment and sewage, and also for the assessment of impacts of anthropogenic activity on the ecology of ARGs (Gaze et al., 2011; Luo et al., 2010; Popowska et al., 2012; Zhang and Zhang, 2011). Although qPCR is sensitive in detection of ARGs, it relies on specific primers designed from known target sequences, thus it cannot detect unknown ARGs. Metagenomics approach has been used to characterize ARGs in humans and environmental microbial communities and has shed new insights into the prevalence and diversity of ARGs (Monier et al., 2011). High throughput sequencing of metagenomic DNA using next generation sequencing technology has led to impressively accelerated accumulation of environmental metagenomic sequences, which provide information of the prevalence of species of interest, ARGs and mobile genetic elements in various environments (Monier et al., 2011), and enable discovery of novel ARGs. However, activity-based functional screening of metagenomic libraries, which provide direct evidence for antibiotic resistant phenotype, still remains a critical means to discover novel ARGs and identify their function that could be missed when using sequence based analysis only. Using functional screening of metagenomic libraries, novel functional ARGs have been identified from human microbiome (Sommer et al., 2009), organic pig gut (Kazimierczak et al., 2009), activated sludge (Mori et al., 2008) and soils (Allen et al., 2009; Lang et al., 2010; McGarvey et al., 2012). Most of these ARGs have not been identified previously and are evolutionarily distant from known resistance genes.

* Corresponding author at: Key Lab of Urban Environment and Health, Institute of Urban Environment, Chinese Academy of Sciences, Xiamen 361021, China. Tel.: +86 592 6190997; fax: +86 592 6190514.
E-mail address: ygzhu@iue.ac.cn (Y.G. Zhu).

0160-4120/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.envint.2013.12.010

Numerous reports describe that agricultural practices, such as direct application of antibiotics or animal manure, provide positive selective pressure for antibiotic resistant bacteria, resulting in increase number and resistant level of these bacteria and ARGs in agricultural soils, and thereby expand the level of native resistance in soil (Knapp et al., 2010; Popowska et al., 2012. In addition, a considerable amount of resistant bacteria added into soil through manure amendment would lead to the spread of resistance to soil bacteria (Ghosh and LaPara, 2007; Heuer et al., 2011). However, there are only a few studies focusing on identification and characterization of ARGs in agricultural soil using functional metagenomics. Using this method, a total of 8 new ARGs were identified from a Spanish agricultural field, conferring resistance to ampicillin, gentamicin, chloramphenicol and trimethoprim (Torres-Cortes et al., 2011). From an apple orchard soil, 13 ARGs were identified including two putative novel bi-functional protein encoding genes (Donato et al., 2010).

Recent studies have been carried out to quantify the ARGs from soil, water and sediment samples in China. In a study conducted in Haihe River, Northern China, Luo et al. revealed that sulfonamide resistance genes, *sul1* and *sul2*, were prevalent in this river, and that the gene abundance in sediments is 120–2000 times higher than in water, suggesting that sediments are an important reservoir of ARG in Haihe River (Luo et al., 2010). In a recent survey of tetracycline resistance genes in soils adjacent to representative swine feedlots in China, 15 tetracycline resistance (*tet*) genes were commonly detected in soil samples and the absolute number of *tet* gene copies was strongly correlated with the concentrations of tetracycline residues in these soil samples (Wu et al., 2010).

Despite the widely recognized potential health risks of over- or misuse of antibiotics in human and animal industries in China, information on abundance and diversity of ARGs is still scarce. Therefore the aim of this study is to investigate ARGs conferring resistance to one of thirteen antibiotics and discover novel ARGs in agricultural soils using functional metagenomic screening. By using three different type of soil, we also aim to assess the impact of agricultural practices, such as manure amendment and application of antibiotics, on the prevalence and diversity of ARGs in agricultural soils.

2. Material and methods

2.1. Soil samples

Four soil samples were collected from different agricultural soils, including 1) one from a field soil (DC soil) grown with *Allium fistulosum* L with a history of manure amendment located at Tongzhou, Beijing, Northern China. 2) Two from paddy fields in Tianjin (QG and WG soils), northern China, without application of manure and organic compost. 3) One from shrimp pond sediment (YZC soil, previously paddy soil) at Zhangzhou, Southern China. For each sample, three 500 g subsamples of soil were taken from 0–10 cm depth and were mixed in situ to form a combined sample. Soil samples were stored in icebox for transport to laboratory and approximately 100 g subsamples were stored at −80 °C for DNA extraction.

2.2. Metagenomic library construction

High molecular weight community DNA was extracted by the freeze-grinding, SDS-based methods (Zhou et al., 1996) and was purified using a low melting agarose gel followed by phenol extraction. DNA concentration and quality was determined with NanoDrop ND-1000. 5 μg purified DNA was partially digested with 10 U *Sau*3AI (Takara) in a 100 μL reaction at 37 °C for 2 h. Digested DNA with a size range of 1 kb–3 kb was selected by agarose gel electrophoresis and extracted from agarose gel using QIAquick Gel Extraction Kit (Qiagen 28704). Recovered DNA was ligated with *Bam*HI digested pUC19 vector using the following protocol: 1 μL 10× Ligation buffer, 80 ng partially digested DNA, 20 ng *Bam*HI cut pUC19 vector and 0.5 μL NEB T4 ligase (M0202) in a 10 μL ligation reaction, followed by incubation at 16 °C for 16 h and heat inactivation at 65 °C for 20 min. 2 μL fresh ligation product was transformed by electroporation into 50 μL prepared electro-competent *Escherichia coli* DH5α cells (Sambrook and Russell, 2000). After transformation using 2500 V for a 2 mm electroporation cuvette, cells were recovered with 1 mL SOC medium in a shaker at 37 °C, 200 rpm for 1 h. For each sample, recovered cells were combined as a library after all the ligation products were transformed.

Libraries were titered by plating 1 μL and 0.1 μL recovered cells onto LB agar plates containing 100 μg mL^{-1} ampicillin followed by incubation at 37 °C for 16 h. The LB agar plates were spread with 40 μL 2% X-gal and 7 μL 20% IPTG before titering. After incubation, white colonies were selected for amplifying the insert using M13 primers flanking the *Bam*HI site of the pUC19 vector. For each library, average insert size was calculated by PCR amplifying 15 random chosen inserts using M13 primers and agarose gel electrophoresis. Total library size was determined by multiplying average insert size with the number of colony forming unit (cfu). The rest of recovered cells were grown in 30 mL LB broth containing 100 μg mL^{-1} ampicillin at 37 °C 200 rpm for 6 h, cells were collected by centrifugation at 5000 rpm for 15 min and re-suspended in 10 mL LB containing 100 μg mL^{-1} ampicillin, which were frozen in liquid nitrogen with 15% glycerol and stored in −80 °C.

2.3. Screening of antibiotic resistant clones

For each library, 2 mL frozen stock was grown in 20 mL LB with 100 μg mL^{-1} ampicillin at 37 °C for 6 h. 100 μL amplified library was spread on LB agar plates containing 100 μg mL^{-1} ampicillin and one of the antibiotics listed in Table S1 and incubated at 37 °C (16 h), 30 °C (2 d) and 25 °C (4 d), with 3 replicates for each temperature. Resistant clones were transferred to fresh LB agar plates containing 100 μg mL^{-1} ampicillin and relevant antibiotics of which resistance had been selected. Insert of each clone was amplified with M13 primers. Insert size was determined by agarose gel electrophoresis. Amplified inserts with identical size were digested with *Msp*I. Inserts with different RFLP pattern and different insert size were picked and sequenced using Sanger sequencing at BGI Shenzhen (BGI, Shenzhen, China). Picked clones were grown in LB medium containing 100 μg mL^{-1} ampicillin and relevant antibiotic to verify resistance before sequencing.

2.4. Identification of antibiotic resistance genes

Open reading frames (ORFs) of full length inserts were identified using ORFfinder (http://www.ncbi.nlm.nih.gov/projects/gorf/). Identified ORFs were compared to a non-redundant protein database using blastp and non-redundant nucleotide database using tblastx. The closest hit for each ORF was collected with blastp and phylogenetic analysis for all genes was performed categorized by the antibiotic class resistant phenotype. The amino acid sequences were downloaded and aligned using ClustalW methods, phylogenetic trees were constructed by the neighbor-joining method and bootstrap analysis (1000 replicates) was performed with MEGA 5.05 software package (Tamura et al., 2011).

3. Results and discussion

3.1. Soil metagenomic library construction and antibiotic resistant clone isolation

Four metagenomic libraries were constructed using DNA extracted from different agricultural soils: one from a shrimp pond sediment (YZC library), one from a field soil grown with *A. fistulosum* L (DC library) and two from paddy fields in Tianjin (QG and WG library). Each library contained about 2×10^5 recombinant clones with average

Download English Version:

https://daneshyari.com/en/article/4422801

Download Persian Version:

https://daneshyari.com/article/4422801

Daneshyari.com