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Distribution of ARGs and MGEs among glacial soil, permafrost, and sediment using metagenomic analysis☆

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ABSTRACT

Antibiotic resistance genes (ARGs) and mobile genetic elements (MGEs) can be identified with metagenomic analyses comparing relatively pristine and human-impacted environments. We collected samples from 3 different environments: glacial soil little affected by anthropogenic activity, deep permafrost dated to 5821 BP (before human antibiotics), and sediment from the Pearl River. Sulfonamides, tetracyclines, and fluoroquinolones were common in the sediment samples. Sulfonamides and tetracycline were not found in permafrost; tetracycline was also not found in glacial soil. ARGs from the sediment were more abundant and diverse than those from glacial soil and permafrost. More types of resistance mechanisms were also present in the sediment. The diversity of MGEs was significantly correlated with the abundance and diversity of ARGs. The result will help future workers to better understand the distribution of ARGs among environments more or less impacted by anthropogenic activities.

1. Introduction

The increase in antibiotic resistance has caused great concern due to the reliance of antibiotics for medical, veterinary, and agricultural purposes (Neu, 1992; English and Gaur, 2010). However, genes coding for antibiotic resistance are not a modern phenomenon. For example, diverse and abundant antibiotic resistance genes (ARGs) have also been identified in 30, 000-year-old Beringian permafrost sediments (D'Costa et al., 2011). Indeed, some soil bacteria even subsist on antibiotics as their sole source of carbon (Dantas et al., 2008). ARGs have also been found in areas with no anthropogenic impact, including isolated caves (Bhullar et al., 2012), the ocean depths (Toth et al., 2010), and deep in the terrestrial subsurface (Brown and Balkwill, 2009). These findings suggest that antibiotic resistance is both natural and ancient (D'Costa et al., 2011). Microbes may produce antibiotics to preserve resources by killing or inhibiting neighboring microbes (Hibbing et al., 2010). It is therefore clear that, in any given environment, the presence of ARGs does not conclusively indicate anthropogenic impact (Durso et al., 2012).

In order to better understand ARGs in different environments, previous research has examined both human-impacted and relatively pristine environments, using quantitative polymerase chain reaction (QPCR) to analyze ARGs (Storteboom et al., 2010a, 2010b). Storteboom et al. (2010a) identified patterns of ARG distribution that discriminated between human-impacted and unaffected river environments: levels of *tet*C, *sul*1, and *sul*2 at impacted river sites were markedly higher than those at the pristine river sites. A phylogenetic analysis of *tet*W sequences indicated that ARGs from polluted river areas and point sources were more similar to each other than to ARGs from relatively pristine river environments (Storteboom et al., 2010b).

However, previous QPCR analyses have been hampered by the limited availability of primers targeting ARGs. Ushida et al. (2010) used QPCR to target 83 ARG subtypes attributed to 6 ARG types.

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Metagenomic analyses of ARGs might lead to the identification of more ARG types and subtypes; such information is important for informative comparisons among relatively pristine and seriously polluted environments. For instance, 260 ARG subtypes belonging to 18 ARG types were detected using a metagenomic analysis of high-throughput sequencing products (Li et al., 2015). Diaz et al. (2017) used metagenomic analysis as well as model-based search algorithms, both of which are more sensitive than traditional identity-based approaches, to identifying antibiotic resistance (AR) sequences. This study found no significant different in AR gene product abundance among three habitats (palsa, bog, and fen) along a permafrost thaw gradient in Abisko, Sweden (Diaz et al., 2017).

It is well known that ARGs are transferred among microorganisms via mobile genetic elements (MGEs) such as plasmids, integrons, and insertion sequences (i.e., horizontal gene transfer; Hall and Collis, 1995; Ochman et al., 2000). MGEs act as ARG carriers in aquatic environments and effectively disseminate ARGs (Ouyang et al., 2015). Metagenomic comparisons between samples from the relatively pristine ocean bed of the South China Sea (SCS) and the human-impacted Pearl River Estuary (PRE) showed that ARG genotypes and resistance mechanisms were much more abundant and diverse in PRE sediments than in SCS sediments; there was also a significant correlation between the abundance of ARGs and the abundance of MGEs (Chen et al., 2013).

Although previous workers have used high-throughput metagenomic sequencing to identify ARGs in permafrost (Rascovan et al., 2016; Nesme et al., 2014), we are unaware of any previous studies focusing on MGEs in this environment. Our research may help to clarify the baseline or primitive level of ARGs in the environment, as well as the MGEs and virulence factors (VFs) that influence their distribution in pre-antibiotic, relatively pristine, and anthropogenically-impacted substrates.

2. Materials and methods

2.1. Sample collection

The Chongce Glacier is located in the eastern part of the West Kunlun Mountains, China, and is one of the most densely concentrated glacial regions on the Tibetan Plateau. With a length of 27 km and a maximum width of 3 km, the Chongce Glacier is one of the most famous glaciers in the region (Yan et al., 2016). The area around Chongce Ice Cap is uninhabited and is mostly free from anthropogenic impact, with the exception of scientific activities. Glacial soil samples from 3 random points (points were 10–20 m apart) were collected in the glacial terminus zone (35°13′06.82″N, 81°07′13.03 E; altitude 5, 800 m) during September and October 2013. To reduce the influence of birds, mammals, or human tourists on our results, surface samples less than 0.01 m deep were discarded; the underlying samples were collected and placed into separate sterile Whirl-Pak bags (Nasco, Salida, CA, USA). We were extremely careful not to contaminate the samples: we wore non-particulating sterile suits, sterile gloves, and masks at all times during the entire sampling process.

Field work was conducted on Eboling Mountain in the upper reaches of the Heihe River basin, part of the inland drainage area of the Eastern Qilian Mountains on the Qinghai-Tibet Plateau. The mean annual air temperature of this basin is −10 °C to −6 °C (Peng et al., 2013). One deep permafrost core (#B site: 38°00′11.76″N, 100°54′24.66″E; altitude 3, 615 m), about 11.7 m deep, was machine drilled on the north slope of Eboling Mountain in March 2012. The core was broken into smaller samples. We selected the bottom of the core, and used ^{14}C analysis (Mu et al., 2014) to date it to 5, 821 BP.

Sediment samples were collected at 3 random points (100 m apart) from a depth of 0–20 cm in the Pearl River (23°5′41.79″N, 113°14′59.98″E, 18 m) on 30 April and transported to our lab on 1 May 2016. The Pearl River samples represent a typical level of anthropogenic antibiotic pollution (Zhang et al., 2015a). Sediment samples were taken using a grab sampler, and stored in polyethylene plastic bags.

The collected samples were transferred to a refrigerator at −20 °C immediately after collection. Each type of sample was ground separately and sieved in a class 100, 000 clean room before antibiotic analysis and DNA extraction.

2.2. Extraction and identification of antibiotics

We analyzed 8 commonly used antibiotics, including 4 fluoroquinolones (ciprofloxacin, enrofloxacin, norfloxacin, and ofloxacin), 3 sulfonamides (sulfadiazine, sulfamethazine, and sulfamethoxazole), and tetracycline. Antibiotic standards were purchased from Sigma-Aldrich (St. Louis, MO, USA). Methanol (MeOH) and acetonitrile (ACN) were obtained from Merck (Darmstadt, Germany). Milli-Q water was prepared using a Milli-Q water purification system (Millipore, USA). Stock solutions of the sulfonamides and tetracycline (100 mg L^{-1}) were prepared in methanol. Stock solutions of the fluoroquinolones (100 mg L^{-1}) were prepared in methanol containing 0.5% NaOH (1 M). All stock solutions were stored in the dark at −20 °C. Working solutions were prepared fresh daily.

Antibiotics were extracted from samples following the method described by Fang et al. (2014). The final extract was analyzed using High-Performance Liquid Chromatography–Tandem Mass Spectrometry (HPLC-MS, Varian 356-LC, Varian, USA). The chromatographic separation of antibiotics was conducted using a Varian Diamonsil C18 column. Following separation, the elution was introduced into a mass spectrometer for antibiotic identification.

2.3. DNA extraction and quality control

We extracted DNA from 3 replicates of 0.5 g soil each using the FastDNA Spin Kit for Soil (MP Biomedicals, USA), following the manufacturer's instructions.

We checked the quality of the extracted DNA in two ways: (1) the degree of DNA degradation was determined by running 1% agarose gels. (2) DNA concentration and potential RNA or protein contamination were determined by microspectrophotometry (NanoDrop ND-1000, NanoDrop Technologies, Willmington, DE). OD values for all samples were between 1.8 and 2.0.

2.4. DNA sequencing

A total of >1 μg of DNA from each sample was used to construct shotgun library with a NEBNext Ultra DNA Library Prep Kit for Illumina (NEB, USA). The library preparations were then sequenced on an Illumina HiSeq 2000 platform using PE101 + 8+150 cycle sequencing (paired-end sequencing, 150-bp reads, and 8-bp index sequence). Approximately 12 Gb of metagenomic data were generated from each DNA sample, a total of 116 Gb. Metagenomics data sets have been deposited in the NCBI Short Read Archive (SRA), accession no. SRP114734.

2.5. Data quality control

The metagenomic data sets were filtered with Fastqc (download in the following website: http://www.bioinformatics.babraham.ac.uk/projects/fastqc/), removing any reads where >10 bases were ambiguous (N), where the average quality score was <30, and that overlapped with the adapter sequences.