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Effect of historical residual hexachlorocyclohexanes and dichlorodiphenyltrichloroethane on bacterial communities in sediment core collected from an estuary in northeastern China by next-generation sequencing ☆

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ABSTRACT

In this study, we evaluate the influence of hexachlorocyclohexanes (HCHs) and dichlorodiphenyltrichloroethane (DDT) on bacterial communities of sediment core from an estuary formed during the period of 1960–2011. Canonical correspondence analysis showed that o,p′-DDT, o,p′-DDD (mitotane), and depth had important influences on bacterial community distributions ($p < 0.05$). Furthermore, our results found variance explained by all variables was 82.9%, while that by o,p′-DDD was 24.4%, and that of o,p′-DDT was 9.8%, indicating that o,p′-DDD had a greater influence on sediment-dwelling bacteria than o,p′-DDT. Also, bacterial diversity was affected and the Shannon index was significantly negatively correlated with total HCHs ($r = -0.579$, $p < 0.05$) and total DDTs ($r = -0.607$, $p < 0.01$), respectively. Furthermore, our results showed that *Flavobacteria* and *Clostridia* content can be considered an indicator of pollution of HCHs and DDTs in sediment core samples.

1. Introduction

Estuaries are important high-energy systems at transitions between freshwater and marine environments, which include surrounding terrestrial environments, that also act as catchments of various pollutants (Ridgway and Shimmield, 2002). Due to sedimentation in estuaries, historical pollutants are stored in vertically in sediment. Therefore, sediment cores are excellent natural laboratories that offer opportunities to monitor historical pollutants. Sediment cores hold historical depositions of pollutants, such as mercury (Muir et al., 2009), lead (Pb) (Eades et al., 2002), brominated flame retardants, hexabromocyclododecanes, polybrominated diethyl ethers (Minh et al., 2007), polycyclic aromatic hydrocarbons (PAHs) (Liu et al., 2005; Wu et al., 2001) and organochlorine compounds (Barra et al., 2001; Sarkar et al., 2008; VanMetre et al., 1997) in lakes, seas, reservoirs and salt marshes (Eades et al., 2002; Fox et al., 2001; Kamman and Engstrom, 2002; Muir et al., 2009; VanMetre et al., 1997).

Agricultural land is a common feature along rivers that flow into estuaries and carry significant amounts of pesticides, such as hexachlorocyclohexanes (HCHs) and dichlorodiphenyltrichloroethane (DDTs), which are typical persistent organic pollutants (POPs), that have been widely used due to low costs and high insecticidal efficacy since the Second World War (Yang et al., 2013).

The Daling River is an important water resource in the Liaohe River delta in northeastern China, which originates in the west and enters the Bohai Sea to the east. Inhabitants along this river are predominately farmers who use pesticides for farming activities. These pesticides collect in the Daling River Basin and may cause long-term adverse effects on coastal resources, thus areas surrounding this basin have undergone rapid ecological changes resulting from agricultural practices. Our previous works have shown that HCHs and DDTs are the most frequently detected contaminants in the surface sediment of the Daling River Basin and the concentrations of HCHs and DDTs in the sediment core reflect the application history and emission of these compounds (Wang et al., 2013a). Microorganisms play important roles in the environment. Although several studies have focused on bacterial communities

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in sediment cores collected from marine environments (Carr et al., 2013), rivers (Xia et al., 2013) and estuaries (Jiang et al., 2009), there is a lack of research on the effect of historical usage of HCHs and DDTs on bacterial communities.

In the present study, next-generation sequencing (NGS) of 16S rRNA genes was used to investigate the effect of historical pollution of HCHs and DDTs on microbial communities in sediment cores. The objectives of this study were (i) to determine the effects of historical pollution on bacterial taxa and diversity and (ii) to screen for microbial indicators of historical pollution. The results of this study provide information on the long-term adaption of bacteria to HCHs and DDTs.

2. Materials and methods

2.1. Sampling

One sediment core was collected using a gravity corer from the Daling River estuary (GPS: 121.563 E, 40.878 N) in May 2011, which was cylindrical in sample with a diameter of 12 cm and a length of 58 cm. The sediment core was immediately sliced into 1 cm-thick sections and each section was then divided into three parts: one for measurement of HCHs and DDTs, one to determine sediment age using a direct gamma assay with ^{210}Pb or ^{137}Cs, and one for analysis of microbial communities. All samples were transported to the hotel within 8 h in a dark sealed container on the ice, and then were stored at −20 °C in the refrigerator for preservation. Next day, they were transported to our laboratory within 8 h, then stored at −80 °C until assayed.

2.2. Measurement of HCHs and DDTs

Sediment core contents of HCHs and DDTs were measured in the laboratory of the International Joint Research Center for Persistent Toxic Pollutants (IJRC-PTS), Dalian Maritime University, Dalian, China. Details of the measurement process are reported elsewhere (Wang et al., 2013a).

2.3. DNA extraction, polymerase chain reaction (PCR) analysis and NGS sequencing

Genomic DNA was extracted using the PowerSoil® DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, CA, USA) according to the manufacturer's recommendations. The V5–V6 regions of the 16S rRNA gene was amplified in 25-μl PCR reactions containing 0.5 μmol l^{-1} of each primer (Invitrogen Corporation, Carlsbad, CA, USA), 0.2 mmol l^{-1} dNTPs (Thermo Scientific, Waltham, MA, USA), 40 μg of bovine serum albumin (Thermo Scientific), 4 mmol l^{-1} $MgCl_2$ (Thermo Scientific), 1 U of *Taq*-DNA Polymerase with the recommended PCR buffer (Thermo Scientific) and 1 μl of DNA extract (5–10 ng DNA). Amplification of the 16S rRNA gene was performed using the primers Bac_410-F (AGGATTAGATACCCTGGTA) and Bac_685-R (CRRCACGAGCTGACGAC) with seven barcode bases fused to the 5′ end of the Bac_410-F primer during synthesis. The PCR primers were designed using an in-house program for broad-range 16S rRNA primer design. Bac_410-F matches perfectly to 1,157,646 of 1,408,126 bacterial sequences of 'Good' quality score spanning *Escherichia coli* positions 400–500 and Bac_685-R matches perfectly to 1,370,071 of 1,408,126 bacterial sequences of 'Good' quality score spanning positions 650–750 in the Ribosomal Database Project (RDP) release 11.3 using the RDP Probe Match tool (http://rdp.cme.msu.edu/probematch/search.jsp).

Samples were amplified using an initial denaturing step of 2 min at 94 °C, followed by 23 cycles consisting of denaturation for 30 s at 94 °C, annealing for 30 s at 55 °C, elongation for 1 min at 72 °C and a final elongation step for 10 min at 72 °C. Each PCR reaction included a negative control. PCR products were run on a 1% agarose gel and purified using the Gel Extraction Kit (Axygen Scientific, Inc., Salem, MA, USA). The purified PCR products were quantified using the Bio TekFlx800 with Quant-iT Pico Green dsDNA Assay Kit (Invitrogen Corporation) and pooled equimolar for sequencing using the MiSeq Sequencing System (Illumina, Inc., San Diego, CA, USA).

2.4. Sequence processing

Sequences with an average Phred quality score of <25, containing ambiguous bases, homopolymer runs exceeding six bases, mismatched primers, or sequence length <100 bp were removed. Then, only sequences that overlap by more than 10 bp and with no mismatches were assembled according to overlap sequences. Reads that could not be assembled were discarded. Barcode and sequencing primers were trimmed. Unique sequences were aligned and clustered into operational taxonomic units (OTUs) using the pyrosequencing pipeline at RDP (Cole et al., 2009) and OTUs were defined using a sequence similarity cut-off value of 97%. Representative sequences of OTUs were compared to the RDP, Greengene and small subunit (SSU) databases using the best hit classification option to classify the abundance count of each taxon (Zhao et al., 2013). Each sample was rarefied to 15,000 sequences. The Shannon index (H′) was used to estimate bacterial diversity. The H′ was determined with the following equation: $H' = -\Sigma$ pi ln Pi. The term Pi was calculated as follows: Pi = ni/*N*, where ni is the number of reads for individual OTUs and *N* was equal to 15,000.

2.5. Statistical analysis

Environmental distance was measured as Euclidean distance using all environmental variables standardised to have a mean of zero and a standard deviation of one. We performed non-metric multidimensional scaling based on environmental distance to depict pesticide pollution in two dimensions in the R environment with Vegan (v2.0-10) for descriptive community ecology.

The relationships between environmental variables and bacterial community composition were studied. We performed canonical correspondence analysis (CCA) to compare correlations between species and environment using CANOCO 4.5 software (http://canoco.software.informer.com/4.5/). In this study, dominant classes (comprising >0.1% of 447,123 sequences) were used to analyze the correlations with environmental variables by CCA. The Monte Carlo permutation test (9999 permutations) with unrestricted permutation was performed to identify statistically significant (at the 5% level) correlations between taxa data and environmental variables. Other statistical analysis were performed in package "Stats" in the R environment.

2.6. Nucleotide sequence accession numbers

All bacterial 16S rRNA gene sequences generated for the present study were deposited to the National Center for Biotechnology Information Sequence Read Archive (http://www.ncbi.nlm.nih.gov/sra) under the accession number SRP028532.

3. Results

3.1. Environmental pollutants

The average sedimentation rate of the core sections was 1.1 cm $year^{-1}$ and the sediment age was 1960–2011 (Wang et al., 2013a). As shown in Table S1, the environmental pollutants in the