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Composition and influencing factors of bacterial communities in ballast tank sediments: Implications for ballast water and sediment management

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ABSTRACT

This study aims to reveal the composition and influencing factors of bacterial communities in ballast tank sediments. Nine samples were collected and their 16S rRNA gene sequences were analyzed by high-throughput sequencing. The analysis results showed the Shannon index in ballast tank sediments was in the range of 5.27–6.35, which was significantly higher than that in ballast water. Acidobacteria, Actinobacteria, Bacteroidetes, Chloroflexi and Proteobacteria were the dominant phyla and accounted for approximately 80% of all 16S rRNA gene sequences of the samples. Besides, the high contents of sulfate reducing bacteria (SRB) and sulfur oxidizing bacteria were detected in sediments, indicating that the corrosion of metal caused by SRB might occur in ballast tank. In addition, the trace of human fecal bacteria and candidate pathogens were also detected in ballast tank sediments, and these undesirable microbes reduced the effect of ballast water exchange. Furthermore, C and N had significant effects on the bacterial community composition in ballast tank sediments. In conclusion, our findings suggest that the proper management and disposal of the ballast tank sediments should be considered in order to reduce the negative impact and ecological risks related to ballast water and sediments.

1. Introduction

Ballast water is used to maintain the balance of ship and guarantee a safe navigation (Endresen et al., 2004). It is known that organisms can be loaded into ballast tank. These organisms will migrate to other ports with the ship and some of them might survive at the end of the voyage and be discharged into the destination port finally (Altug et al., 2012; Drake et al., 2007). Therefore, ballast water may lead to potential ecological problems at major ports in the world, such as the invasion of species and the disruption of native ecosystems (Drake et al., 2007). Besides, a lot of inorganic or organic suspended matters and even aquatic organisms exist in ballast water. These substances may precipitate at the bottom of ballast tank in the end (Drake et al., 2007). Great loads of sediments were often found at the bottom of ballast tank after long-term navigation (Maglić et al., 2016). It was reported that merchant vessels might contain as much as 200 tons of sediments (Prange and Pereira, 2013). As the ship was maintained, cleaned or disassembled, the sediments were discharged and might seriously threaten the ocean ecosystems surrounding the shipyard if these sediments, especially the sediment containing harmful organisms or pollutants, are not reasonably disposed or treated (Maglić et al., 2016). However, the above risks related to the ballast tank sediments are not well concerned despite the International Convention for the Control and Management of Ships' Ballast Water and Sediments has been promulgated by International Maritime Organization (IMO, 2004).

As well known, there are lots of microorganisms in ballast water, and an estimation of 10^{19} bacteria are transported daily with ballast water around the world (Endresen et al., 2004; Ruiz et al., 2000), including harmful bacteria (Altug et al., 2012; Brinkmeyer, 2016). Compared with ballast water, the sediments contain abundant nutrients. The suspended matters in ballast water, and even the decay of the dead organisms add the nutrients into the sediments. These organic and inorganic nutrients are available to living creatures, especially to the microorganisms (Drake et al., 2007; Wang et al., 2013). As a consequence, various types of microbes may live in the sediment of ballast tank as it can provide a matrix of complex nutrients and solid surfaces (Wang et al., 2012). Moreover, unique microbial communities will be formed in the special ecological environment with darkness, low dissolved oxygen and temperature at ballast tank will breed in the sediments. The microbes should adapt to the special environment of ballast tank, and play an important role in the biogeochemical cycles in sediments (Ikenaga et al., 2010; Ye et al., 2009). On the other hand, these

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microbes might have the risks of biological invasion and environmental pollution. However, the negative influences of ballast tank sediments, especially the occurrence of various microorganisms, have not gained much attention. Therefore, a thorough understanding of microbial communities in ballast tank sediments could provide new insight into the composition and function of organisms in ballast tank sediments for the purpose of mitigating potential adverse effects on marine ecology and human health.

This study aims to determine the diversity and composition of bacteria in ballast tank sediments, identify the function and characteristics of bacterial communities in these sediments, and reveal the relationship between bacterial composition and the sediment properties. Furthermore, the implications of bacteria in ballast water and sediment management were also discussed in the paper. Ballast tank sediments were collected and evaluated based on the high-throughput sequencing results. Our study provides the first insight into the composition of bacterial communities in ballast tank sediments.

2. Materials and methods

2.1. Sample collection

The sediment samples were collected from ship ballast tanks at the shipyard in Jiangyin City, Jiangsu Province, China. Ballast tank sediments from nine ships (BTS-1 to BTS-9) were collected when the ships were repaired or disassembled in the shipyard. When sampling, three different sites in one ballast tank were selected and the surface sediment was acquired by a plastic spatula. Sediment samples were transferred into sterile polypropylene bottle, which was put in ice pack and immediately transported to the laboratory. Each sample was divided into two parts. One part was immediately stored at −20 °C for deoxyribonucleic acid (DNA) extraction. The other part was freeze-dried, ground, and stored in brown bottles for physico-chemical analysis.

2.2. Physico-chemical analysis

The pH was measured in aqueous suspension (sediment: water = 1:5 w/v) by a pH meter. The contents of C, H, N and S elements in ballast tank sediments were measured with an elemental analyzer Vario EL III (German). Total phosphorus was determined by the alkali fusion-Mo-Sb Anti spectrophotometric method. The concentrations of Fe and Zn in sediments were determined by an inductive coupled plasma emission spectrometer (Agilent720ES, America) after digestion with the mixture of HNO_3-HF-$HClO_4$.

2.3. DNA extraction

Total genome DNA of the samples from ballast tank sediments was extracted with the FastDNA® SPIN kit for soil (MP Biomedicals, Illkirch, France) according to the protocols of manufacturer. The concentration and purity of the DNA extract were checked based on the absorbance at 260 and 280 nm determined with a Nanodrop® ND-1000 spectrophotometer (Labtech International, UK). The DNA samples were stored at −20 °C for next analysis.

2.4. Quantitative PCR

Quantitative polymerase chain reaction (qPCR) was used to determine the total bacteria in ballast tank sediment though the Bio-Rad CFX96 Real-time PCR System. The primer pair 357F/518R was chosen for the determination of 16S rRNA gene. 25 μL real-time PCR reaction mixture contained 12.5 μL SYBR® Premix Ex TaqTM (Zoman, Beijing), 0.5 μL forward primer, 0.5 μL reverse primer, 1 μL DNA extract, and 10.5 μL ddH_2O. Each reaction was conducted in triplicate. The plasmids of *Escherichia coil* which integrated with target gene were used for standard curve calibration. The PCR workflow was 95 °C for 2 min, followed by 40 cycles at 95 °C for 20 s, 60 °C for 40 s. The total number of 16S rRNA gene was normalized to per dry weight of sediment.

2.5. Illumina MiSeq sequencing

The V3–V5 region of 16S rRNA gene was chosen for PCR amplification with the specific primer pair of 338F (5′-ACTCC TACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′). The PCR reaction was conducted with ABI GeneAmp® 9700 according to the following scheme: 95 °C for 2 min, followed by 25 cycles at 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s, and a final extension step at 72 °C for 10 min. Three replicates were conducted with extracted DNA template for PCR reaction and the triplicate PCR products were mixed and checked by gel electrophoresis. Then the amplified DNA was re-extracted from agarose gel by AxyPrepDNA Kit (Axygen, MA, USA). The extracted PCR products were mixed after quantification with a QuantiFluor™ -ST (Promega, WI, USA) in order to acquire an equal DNA concentration for each sample. Finally, these samples were subjected to library preparation and sequenced by a MiSeq platform (Illumina, CA, USA). The sequencing data have been deposited into the NCBI short reads archive database (accession number: PRJNA412156).

2.6. Processing of the sequences

The processing of 16S RNA high-throughput sequencing results was conducted as described previously. In brief, the raw sequences were optimized according to length, quality and chimera by the QIIME pipeline (Caporaso et al., 2010). The high-quality sequences with 97% similarity were assigned into the same operational taxonomic unit (OTU) using Usearch and the most abundant sequence of each OTU was selected as its representative sequence (Edgar, 2010). Rarefaction curve, Shannon diversity index, and species richness estimator of Chao1 were calculated in the software of Mothur at a sequence divergence of 3%. The Ribosomal Database Project (RDP) Classifier (Version 2.2) was used for taxonomic identification at a confidence threshold of 0.7. If there was no strong match for the representative sequence within the RDP database, the OTUs should be considered as an unclassified type. The relative percentage of a given phylogenetic division was set as the quotient of its sequence number to the total number of sequences per sample.

2.7. Statistical analysis

The relationship between bacterial community (the whole of OTU in the sample) of the nine sediments and its physico-chemical parameters was analyzed by redundancy analysis (RDA) with the Vegan package in R software. RDA was conducted to determine the relationship between bacterial species (the top 20 abundant genera) and environmental factors.

3. Results

3.1. Physico-chemical characteristics of ballast tank sediments

The pH value of ballast tank sediments ranged from 7.62 to 9.34, indicating that these sediments belonged to alkaline substances (Table 1). Ballast tank sediments also had a certain amount of organic matters and the content of C ranged from 1.01% to 4.85%. The suspended organic matters, the residues of aquatic organisms in ballast water and the deciduous anticorrosion coatings of ballast tank were the main sources of organics in sediments. Moreover, the N concentration in ballast tank sediments ranged from 0.15% to 0.25%, whereas P concentration ranged from 465 to 700 mg/kg. The contents of N and P in ballast tank sediments were similar to those in conventional marine sediments (Lin et al., 2013). Besides, ballast tank sediments contained high contents of heavy metals and the concentrations of Fe and Zn were