Ecotoxicology and Environmental Safety 157 (2018) 169–175

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

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

Abundances and profiles of antibiotic resistance genes as well as co-occurrences with human bacterial pathogens in ship ballast tank sediments from a shipyard in Jiangsu Province, China

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ARTICLE INFO

Keywords:
Ballast tank sediments
Antibiotic resistance gene
Human bacterial pathogen
High-throughput sequencing
Ballast water exchange
Network analysis

ABSTRACT

Ship ballasting operations may transfer harmful aquatic organisms across global ocean. This study aims to reveal the occurrences and abundances of antibiotic resistance genes (ARGs) and human bacterial pathogens (HBPs) in ballast tank sediments. Nine samples were collected and respectively analyzed by real-time quantitative PCR and high-throughput sequencing technologies. Ten ARGs (*aadA1*, *blaCTX-M*, *blaTEM*, *ermB*, *mefA*, *strB*, *sul1*, *sul2*, *tetM*, and *tetQ*) and the Class-I integron gene (*intI1*) were highly prevalent (10^5–10^9 gene copies/g) in ballast tank sediments. The *sul1* was the most abundant ARG with the concentration of 10^8–10^9 copies/g and *intI1* was much more abundant than the ARGs in ballast tank sediments. The strong positive correlations between *intI1* and ARGs (*blaCTX-M*, *sul1*, *sul2* and *tetM*) indicated the potential spread of ARGs via horizontal gene transfer. In ballast tank sediments, 44 bacterial species were identified as HBPs and accounted for 0.13–21.46% of the total bacterial population although the three indicator pathogenic microbes (*Vibrio cholerae*, *Escherichia coli*, and *Enterococci*) proposed by the International Maritime Organization were not detected. *Pseudomonas pseudoalcaligenes*, *Enterococcus hirae*, *Shigella sonnei* and *Bacillus anthracis* were the dominant pathogens in ballast tank sediments. Zn and P in sediments had positive effects on the ARGs. Network analysis results indicated that *sul1* and *sul2* genes existed in several bacterial pathogens. Ballast tank sediments could be regarded as a carrier for the migration of ARGs. It is important to manage ballast tank sediments reasonably in order to prevent the dissemination of ARGs and bacterial pathogens.

1. Introduction

Ballast water is often used to guarantee the safe navigation of ships (Endresen et al., 2004). In recent years, the spread of harmful aquatic organisms and noxious pathogens via ballast water has been widely concerned (Altug et al., 2012; Drake et al., 2007; Lymperopoulou and Dobbs, 2017). Moreover, suspended particulate matters in ballast water sank to the tank bottom as sediments. After long-term navigation, a large amount of sediments would accumulate at the bottom of ballast tank (Maglić et al., 2016; Prange and Pereira, 2013). The amount of sediments found in the ballast tanks of one merchant vessel was up to 200 t (Prange and Pereira, 2013). The living or dead aquatic organisms could fall to the bottom of ship tank and even colonize the sediment (Brinkmeyer, 2016; Drake et al., 2007).

The spread of antibiotic resistance genes (ARGs) has become a serious global problem in recent years (WHO, 2014). The ARGs have been detected in wastewater, river, offshore fishery area, marine sediment and costal water (Guo et al., 2016; Li et al., 2015a; Lin et al., 2015; Niu et al., 2016; Souissi et al., 2018; Zhu et al., 2017). Moreover, due to the environmental pollution, a large number of human bacterial pathogens (HBPs), such as *Salmonella* and *Shigella*, were detected in estuaries, ports, and coastal areas (Altug et al., 2012; Myers et al., 2003; Prasad et al., 2015). The seawater containing ARGs and HBPs could be pumped into ballast tanks of ships and discharged into surrounding waters of destination ports (Drake et al., 2007; Emami et al., 2012). Therefore, those emerging contaminants might travel worldwide through ballast water. The spread of ARGs could enhance the resistance against antibiotics and the HBPs might make people sick. Various viral communities were found in ballast water from the ships sailing in the Great Lakes and the composition of viruses was different from the

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https://doi.org/10.1016/j.ecoenv.2018.03.053
Received 24 December 2017; Received in revised form 18 March 2018; Accepted 23 March 2018

native species in the Great Lakes (Kim et al., 2015). Besides, 27 pathogens were detected in ballast water of 21 ships sailing in Turkey Marmara Sea, including some antibiotic-resistant bacteria (Altug et al., 2012). These findings indicated that ballast water might transport and introduce pathogens and antibiotic resistant bacteria to a new water area. Undoubtedly, the ARGs–containing microbes and HBPs in ballast water were precipitated at the bottom of the ballast tank and accumulated in the sediment. Therefore, various HBPs could survive in the ballast tank sediment which provided a solid matrix containing rich nutrients (Drake et al., 2007; Wang et al., 2012). More importantly, the ARGs can be transferred among microorganisms and even to the HBPs (Tang et al., 2016). Serious ecological damages may occur in the area surrounding a shipyard if these sediments are not reasonably treated or disposed during ship cleaning or disassembling, especially when ARGs and harmful pathogens existed (Endresen et al., 2004). However, little attention has been paid to the occurrences and abundances of ARGs and HBPs in ballast tank sediments so far.

A systematic understanding of the ARGs and HBPs in ballast tank sediments is helpful to mitigate the potential adverse effects of ballast tank sediments on marine ecology and human health. Therefore, the study aims to reveal the occurrences and abundances of ARGs (*aadA1*, *blaCTX-M*, *blaTEM*, *ermB*, *mefA*, *strB*, *sul1*, *sul2*, *tetM*, and *tetQ*) and HBPs in ballast tank sediments and the relationship between ARGs and HBPs. Our study provides the first insight into the occurrences of ARGs and HBPs in ballast tank sediments.

2. Materials and methods

2.1. Ballast tank sediment sampling

The ballast tank sediment samples (BTS-1 to BTS-9) were obtained from nine ocean-going vessels in a shipyard in Jiangyin City, Jiangsu Province, China. The routine voyage of those vessels was between Asia and South America. A plastic spatula was used to grab the sediment at three different sites in one ballast tank. Then the grabbed three subsamples were mixed thoroughly to obtain a composite sample (approximately 2.0 kg). Ballast tank sediment samples were put into sterile bottles in an ice box and transported to the laboratory immediately. Partial samples were freeze-dried for the analysis of physical/chemical properties and the remaining samples were stored at − 20 °C for DNA extraction.

2.2. Analytical methods of physico-chemical properties of sediment samples

The aqueous suspension of sediment (sediment: water = 1:5 w/v) was prepared and then pH was measured by a pH meter. The elemental analyzer Vario EL III (German) was used to measure the contents of C, N, and H in ballast tank sediments. The total phosphorus in samples was determined according to the alkali fusion-Mo-Sb anti-spectrophotometric method. Fe and Zn in sediments were digested by the HNO_3-HF-$HClO_4$ and then determined with an inductive coupled plasma emission spectrometer (Agilent720ES, America). The specific physico-chemical properties of ballast tank sediments had been determined in our previous study (Lv et al., 2017).

2.3. DNA extraction and ARGs measurement

Genomic DNA was extracted from ballast tank sediment samples (each 0.5 g) with the FastDNA® SPIN kit for soil (MP Biomedicals, Illkirch, France) according to the standard protocols. In this study, ten frequently detected ARGs encoding the resistances to macrolides (*ermB* and *mefA*), sulfonamides (*sul1* and *sul2*), tetracyclines (*tetM* and *tetQ*), aminoglycosides (*aadA1* and *strB*), and β-lactams (*blaCTX-M* and *blaTEM*) were quantified by Bio-Rad CFX96 Real-Time PCR System according to the method by Su et al. (2017). The Class-I integron gene (*intI1*) and 16S rRNA gene were also determined by qPCR. The primers used for qPCR assays were given in Table S1 (in Supplementary material). The 25-μL real-time PCR reaction mixture contained 12.5 μL of SYBR® Premix Ex TaqTM (Zoman, Beijing), 0.5 μL of forward primer (10 μM), 0.5 μL of reverse primer (10 μM), 1 μL of DNA extract, and 10.5 μL of ddH_2O. Each PCR reaction was conducted in triplicate. The PCR was programmed as follows: 95 °C for 2 min, followed by 40 cycles at 94 °C for 20 s and 40 s at the annealing temperatures (Table S1). Six- or seven-point standard curves were obtained according to the same routine with 10-fold serially diluted plasmids carrying target genes. The quantification limits of qPCR were 130–780 copies for each gene. The amplification efficiencies of standard curves for qPCR were in the range of 91.7–107.7% and the R^2 values of the standard curves ranged from 0.992 to 1.000. The absolute abundance of ARGs was presented as the gene copies per gram dry sediment. The relative abundance of ARGs was calculated as the proportion of ARG to 16S rRNA gene in order to minimize the interference from various background bacterial abundances and this index could reflect the proportion of antibiotic-resistant bacteria in the bacteria population (Duan et al., 2017; Qian et al., 2016; Thomas and Nielsen, 2005).

2.4. High-throughput sequencing and human potential pathogen analysis

The V3–V5 regions of 16S rRNA were sequenced with MiSeq platform. The sequencing data were deposited into the NCBI short reads archive database under the project number PRJNA412156. QIIME was used to optimize the raw sequences, and the clustering of the high-quality reads into operational taxonomic units (OTUs) was conducted using Usearch with 97% similarity (Edgar, 2010). Compared to our previous study (Lv et al., 2017), the pathogenic bacteria were identified at the species level in order to give a more accurate assessment of potential pathogens. A 16S rRNA gene database containing more than 500 species of known pathogens were constructed with the corresponding sequences downloaded from Greengenes Website (http://greengenes.lbl.gov) according to the reports of Li et al. (2015b) and Woolhouse et al. (2006). The local BLAST program was conducted to detect the potential pathogen sequences by aligning the sequences of representative OTU to the above pathogen databases. The threshold for pathogen alignment by BLAST was set as follows: aligned length ≥ 420, e-value cutoff $< 1 \times 10^{-20}$, and similarity ≥ 98% (Cai and Zhang, 2013).

2.5. Statistical analysis

Independent-sample T test was performed to analyze the difference among various ARGs in each sample. Pearson correlation was conducted to identify the relationships among the ARGs. The influences of environmental factors on the occurrence and relative abundance of ARGs in ballast tank sediments were determined by redundancy analysis (RDA) with the Vegan package of R software. The correlation-based network analysis between ARGs and the HBPs was firstly performed by R software and then the networks were plotted by Gephi 0.9.2.

3. Results and discussion

3.1. Occurrences and abundances of ARGs in ballast tank sediments

All the 10 ARGs were detected in ballast tank sediments although their abundances were different in those samples. As shown in Fig. 1a, sulfonamide resistance genes (*sul1* and *sul2*) were the most abundant ARGs and their absolute abundances were in the ranges of 8.36×10^7–1.61×10^9 copies/g and 2.55×10^7–4.31×10^8 copies/g, respectively. The absolute abundances of *tetM*, *tetQ*, *ermB*, *strB*, and *aadA1* were in the order of magnitude of 10^6–10^7 copies/g. The *mefA* was the lowest ARG as its copy number ranged from 1.46×10^5 to 2.47×10^6 copies/g in ballast tank sediments. This result indicated that