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Variation pattern of terrestrial antibiotic resistances and bacterial communities in seawater/freshwater mixed microcosms

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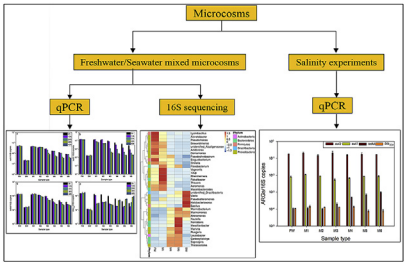
HIGHLIGHTS

- Terrestrial ARGs influence the corresponding ARGs content in coastal areas.
- High salinity could elevate *tet*M content for almost one order.
- Freshwater could pose strong impact to seawater on bacteria composition.
- The potential risk caused by terrestrial ARGs could not be neglected.

GRAPHICAL ABSTRACT



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ABSTRACT

The ocean is the final place where pollutants generated by human activities are deposited. As a result, the long-range transport of the ocean can facilitate the diffusion of terrestrial contaminants, including ARGs. However, to our knowledge, little research has been devoted to discussing the content change of terrestrial ARGs and the reason for the change in coastal area. This study established various microcosms, in which seawater and freshwater were mixed at different ratio to simulate the environmental conditions of different regions in coastal areas. Four ARGs were quantified, and 16S pyrosequencing was conducted. The results showed that the terrestrial ARGs influenced the concentration of the corresponding ARGs in coastal areas, and the content change pattern of each ARG was distinct. The influence of salinity on the ARG content was limited in most cases. Moreover, most dominant bacteria from freshwater had significant positive correlation ($p < 0.05$) with selected ARGs, except for bla_{TEM}. The dominant bacteria in freshwater diminished dramatically in microcosms with a high proportion of seawater. Freshwater may have a strong impact on the bacteria composition of seawater, and the materials from freshwater may prompt the growth of some bacteria (include potential hosts of ARGs) in coastal area.

1. Introduction

During the past decades, abuse and overuse of antibiotics have led to a constant increase of ARGs, which could be transferred to pathogens by horizontal gene transfer (HGT) and therefore pose a threat to human health. Thus, ARGs are regarded as emerging

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contaminants and have drawn increasing attention (Pruden et al., 2006). ARGs are ubiquitous in the environment and have been frequently detected in various environmental medium, such as municipal wastewater, reclaimed water, reservoir water and drinking water, and sulfonamide ARGs (e.g., *sul*1 *and sul*2), tetracycline ARGs (e.g., *tet*M) and β-lactam ARGs (e.g., bla_{TEM}) are the common ARGs in the environment (Huerta et al., 2013; Karkman et al., 2016; Makowska et al., 2016; Pärnänen et al., 2016; Xu et al., 2016; Yuan et al., 2016). Additionally, the transfer mechanism of ARGs between different bacteria consist of three mechanisms by which genes are transferred between bacteria horizontally, including conjugation within bacteria, natural transformation, and transduction. The major mechanism is conjugation, through which, ARGs in bacteria can be acquired by mobile genetic elements (MGEs, e.g., plasmids and integrons) and gene acquisition elements, and then ARGs are disseminated with the MGEs (Gatica et al., 2016; Guo et al., 2015; Qiu et al., 2012).

The ocean is the final place where pollutants generated by human activities are deposited. However, the long-range transport of the ocean can facilitate the diffusion of terrestrial contaminants, including ARGs, and ultimately contribute to the contamination in different regions (Souza et al., 2006; Zou et al., 2011). Therefore, the pollution status of some classes of ARGs in coastal areas has been discussed in recent years. The highest values of ARGs have been detected in estuary area and aquaculture area (Niu et al., 2016). The concentration of antibiotic and heavy metal could influence the abundance of ARGs (Lu et al., 2015; Na et al., 2014). Moreover, the role of class 1 integrons in the prevalence of ARGs had been discussed (Muziasari et al., 2014). However, the study of ARGs in coastal areas mainly focused on monitoring the levels.

Compared with a freshwater environment, the ocean has unique physicochemical properties, including pH, salinity, micronutrient levels, etc. Among which, salinity is one of the main differences between seawater and fresh water. The high salinity may influence the physiological state of terrestrial bacterial, which may include ARB, and thus influence the abundance of terrestrial ARGs (Lu et al., 2015). Furthermore, there are numerous indigenous flora in the coastal environment. The freshwater bacteria may be disadvantageous in competition with indigenous ones, which may lead to the death of terrestrial ARB. However, these factors were rarely discussed in studies on ARGs in coastal area, and the change rule and reasons of terrigenous ARGs content in coastal areas are still unclear.

In this study, seawater/freshwater mixed microcosms were established to simulate various regions of coastal areas, and qPCR was employed to quantify the content of 16S rDNA, *sul*1, *sul*2, *tet*M and bla_{TEM}. In addition, pyrosequencing of the 16S rDNA gene was employed to reveal the shift of the bacterial community in each microcosm. The aims of this study were: 1) to investigate the abundance variation of terrigenous ARGs in different mixed microcosms; 2) to discuss the influence of salinity on terrigenous ARGs; 3) to illuminate the reason why terrigenous ARG content variation is found in mixed microcosms. To our knowledge, this is the first study focusing on the variation of terrigenous ARGs in coastal areas.

2. Method and material

2.1. Setup of seawater/freshwater mixed microcosms

The seawater/freshwater mixed microcosms experiment was based on the OECD 308 test to survey the abundance variation of terrestrial ARGs in coastal areas (Luo et al., 2014; Zou et al., 2016). The seawater/freshwater mixed microcosms were set up with an illumination incubator (light-treated for 12 h per day). The freshwater was collected from the Beitang Drainage River (detailed information of the river is shown in supplemental information, S1), and the seawater was sampled from coastal areas far away from the estuary (38.38N, 117.86E) to avoid freshwater. The two water properties are described in Table 1. Freshwater and seawater were supplemented with 2% (v:v) Luria-Bertani (LB) media and 2% (v:v) 2216E media, respectively. After incubation overnight (12 h) in a constant temperature shaker (BSD-400, Boxun), the freshwater and seawater were mixed in different volume proportion (5:1, 3:1,1:1, 1:3, 1:5, 1:9) in 500 mL flasks to simulate the different regions in coastal areas (each treatment was performed in triplicate). The mixed proportion was determined according to previous studies (Bricker et al., 2003; Hu et al., 2016). All mixed microcosms were maintained for 48 h at 28 °C in the incubator. Cycloheximide (Sigma, America, ≥94% purity) was added to each microcosm (100 mg/L) to prevent fungal growth. Samples were collected periodically from all treatment (0 h, 12 h, 24 h, 36 h, and 48 h). The sample collection was performed on a super clean bench (SW-CJ-1D) to avoid bacterial pollution. For each time point, a 10 mL sample was collected and centrifuged, and then stored in −80 °C until DNA was extracted.

2.2. DNA extraction and quantitative polymerase chain reaction (qPCR)

DNA was extracted with a bacterial Genomic DNA miniprep Kit (AxyGEN, America) according to the manufacturer's instructions. The qPCR assays were performed with a LightCycler 96 instrument (Roche, Switzerland), and the procedure was set as the follows: initial denaturation (95 °C, 2 min), followed by 40 cycles consisting of denaturation (95 °C, 10 s), annealing (annealing temperature described in Table S2, 30 s), extension (72 °C, 45 s), and a final extension (72 °C, 6 min). The abundance of 16S rDNA, *sul*1, *sul*2, bla_{TEM}, and *tet*M were quantified (the established standard curve method is shown in supplemental information, S2), and the primers and standard curve information was listed in Tables S1 and S2, respectively.

2.3. Effect of salinity on the abundance of terrestrial ARGs

The salinity of each seawater/freshwater mixed microcosm in 2.1 was measured by a salinity meter (PR-100SA, Atago, Japan). A new sample was collected from Beitang Drainage River, and a freshwater microcosm was established according to the description of 2.1. A certain amount of seawater crystal was added to the microcosm to meet the salinity in each mixed microcosm, and the main components of the seawater crystal were described in a previous study (Wang et al., 2013). The microcosms were incubated in 28 °C for 2 days, and then 10 mL of sample for each treatment was centrifuged, and DNA extraction was conducted as described in Section 2.2. The seawater crystal dosage and salinity of each microcosm were listed in Table S3.

2.4. Pyrosequencing of 16S rDNA gene and bioinformatics analysis

One microcosm was chosen to conduct 16S rDNA pyrosequencing to study the evolution of the community at the beginning and the end of the experiment. Then five microcosms were chosen on the second day, and triplicate samples were collected from each microcosm to conduct the 16S rDNA pyrosequencing. A set of primers 515f and 806r, which target the hypervariable V4 region of 16S rDNA, was used to amplify the 16S rDNA (He et al., 2016). Amplification and sequencing were subsequently conducted on an Illumina MiSeq platform of Novogene (Beijing, China). A PCR mixture (30 μL) contained 15 μL of Phusion® High-Fidelity PCR