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The antibiotic resistome of free-living and particle-attached bacteria under a reservoir cyanobacterial bloom

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ABSTRACT

In freshwater systems, both antibiotic resistance genes (ARGs) and cyanobacterial blooms attract global public health concern. Cyanobacterial blooms can greatly impact bacterial taxonomic communities, but very little is known about the influence of the blooms on antibiotic resistance functional community. In this study, the ARGs in both free-living (FL) and particle-attached (PA) bacteria under bloom and non-bloom conditions were simultaneously investigated in a subtropical reservoir using high-throughput approaches. In total, 145 ARGs and 9 mobile genetic elements (MGEs) were detected. The most diverse and dominant of which (68.93%) were multidrug resistance genes and efflux pump mechanism. The richness of ARGs in both FL and PA bacteria was significantly lower during the bloom period compared with non-bloom period. The abundance of ARGs in FL bacteria was significantly lower under bloom condition than in the non-bloom period, but the abundance of ARGs in PA bacteria stayed constant. More importantly, the resistant functional community in PA bacteria was more strongly influenced by the cyanobacterial bloom than in the FL bacteria, although > 96% ARGs were shared in both FL and PA bacteria or both bloom and non-bloom periods. We also compared the community compositions between taxonomy and function, and found antibiotic resistant communities were highly variable and exhibited lower similarity between bloom and non-bloom periods than seen in the taxonomic composition, with an exception of FL bacteria. Altogether, cyanobacterial blooms appear to have stronger inhibitory effect on ARG abundance in FL bacteria, and stronger influence on antibiotic resistant community composition in PA bacteria. Our results further suggested that both neutral and niche processes interactively affected the ARG composition dynamics of the FL and PA bacteria. However, the antibiotic resistant community of FL bacteria exhibited a higher level of temporal stochasticity following the bloom event than PA bacteria. Therefore, we emphasized the bacterial lifestyles as an important mechanism, giving rise to different responses of antibiotic resistant community to the cyanobacterial bloom.

1. Introduction

Nowadays, the increasing emergence and spread rate of, and rate of spread of antibiotic resistance genes (ARGs) has become one of the biggest global public health concerns (Chee-Sanford et al., 2001; He et al., 2016; Levy and Marshall, 2004; Zhu et al., 2017b). People are more likely infected by antibiotic resistant bacteria (ARB) from natural water, particularly freshwater environments which are considered to be a key reservoir of ARGs (Czekalski et al., 2015). This can happen *via* consumption of aquatic products, direct contact (*e.g.* swimming) and drinking (Feng et al., 2011; Witte, 2000; Xi et al., 2009). The occurrence and distribution of ARGs have been investigated in various aquatic environments, including rivers (Garcia-Armisen et al., 2011; Luo et al., 2010; Ouyang et al., 2015), lakes (Czekalski et al., 2015) and the influent and effluent of sewage treatment plants (Bondarczuk et al., 2016; Chen and Zhang, 2013; Munir et al., 2011). Exploring the dynamics of ARGs in inland water in a changing environment is fundamental to understanding of ecological processes and mechanisms underlying ARGs spread in aquatic ecosystems. To date, however, research on the diversity and distribution of ARGs during cyanobacterial blooms has been lacking.

The antibiotic resistome of bacterioplankton in aquatic environments are closely correlated with the dynamics and functions of bacterial taxonomic communities (Chen et al., 2016; Garcia-Armisen et al., 2011), which are normally shaped by many abiotic and biological factors (Eiler and Bertilsson, 2004; Liu et al., 2013), including different lifestyles (*i.e.* free-living and particle-attached) (Savio et al., 2015), water stratification (Yu et al., 2014) and algal blooms (Von Scheibner

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et al., 2014; Woodhouse et al., 2016). However, if and how these factors affect the dynamics of ARGs has not been well investigated. For instance, although taxonomic surveys have compared free-living (FL) and particle-attached (PA) bacterial taxonomic communities in a variety of ecosystems (Jackson et al., 2014; Milici et al., 2016; Millar et al., 2015; Orsi et al., 2015; Savio et al., 2015), we are not aware of any studies comparing ARGs between these communities. Although there are a wide range of pore-sizes (ranging from 0.8 μm to 30 μm) used to separate PA from FL bacteria, as particulate matter in waters is normally in a continuum of sizes (Fuchsman et al., 2011; Ganesh et al., 2014, 2015; Jackson et al., 2014; Mestre et al., 2017; Milici et al., 2016; Salazar et al., 2016). However, the 3.0 μm pore-size is the most prevalent and widely used for the definition of PA bacterial assemblage. In the light of numerous investigations (D'Ambrosio et al., 2014; Jackson et al., 2014; Milici et al., 2016; Millar et al., 2015; Orsi et al., 2015; Savio et al., 2015), we used traditional size-fractionated filtering to explore ARGs distribution and dynamics in both FL (0.2–3 μm) and PA (> 3 μm) bacteria under different environmental conditions in order to elucidate the processes and mechanisms behind it.

High-throughput approaches are currently revolutionizing our understanding of microbial ecology on an unprecedented scale (Caporaso et al., 2011). These powerful, fast and reliable techniques (*e.g.* high-throughput qPCR and high-throughput sequencing) have been successfully applied for the qualitative and quantitative assessment of ARGs and operational taxonomic units (OTUs) within bacterial taxonomic communities in the environment (Chen et al., 2016; Liu et al., 2015b; Looft et al., 2012; Su et al., 2015; Zhu et al., 2013). To date, most studies exploring changes in ARG diversity in response to environmental change have focused on deterministic (selective) processes including mobile genetic elements (MGEs), but the contribution of neutral process in structuring ARG community composition is poorly understood (Chen et al., 2016; Zhu et al., 2017b). As there is evidence that both deterministic (selective) and stochastic (neutral) processes shape microbial taxonomic communities simultaneously (Liao et al., 2016; Liu et al., 2017; Roguet et al., 2015), both processes need to be considered when investigating the community assembly of ARGs.

In this study, we simultaneously investigated ARG functional diversity and distribution in both FL and PA bacterioplankton along a water depth gradient during both cyanobacterial bloom and non-bloom periods in a subtropical reservoir. The objectives of this study were to 1) determine the responses of ARGs in FL and PA bacteria to the cyanobacterial bloom and thermal stratification (surface, middle, bottom and mixing water); 2) reveal the community assembly mechanisms of the antibiotic resistant community following the cyanobacterial bloom; and 3) quantify the relationships between ARGs and MGEs, ARGs and microbial taxa (or OTUs). The research questions of this study were: 1) How does the abundance, richness, and community composition of ARGs change during a cyanobacterial bloom? 2) Are there different responses between ARGs in FL and PA bacteria to the cyanobacterial bloom? And 3) What roles do selective and neutral processes have in driving the antibiotic resistant community assembly of FL and PA bacteria?

2. Materials and methods

2.1. Sample collection and environmental variables

Water samples were collected twice a month during October, November and December 2014 from Xidong Reservoir (24°49′N, 118°10′E; Fig. S1), which is located in north of Xiamen City, Fujian province, southeast China. The general limnological characteristics of the Xidong Reservoir have been described in our previous study (Xue et al., 2017). Samples (about 20 L water for each sample) were collected both during a cyanobacterial bloom period, with high-density *Microcystis aeruginosa* (days 297 and 304 of the year), and during the following post-bloom period (days 325, 332, 346 and 363 of the year). The water samples were simultaneously collected at the surface (0.5 m), middle (12–20 m), bottom (25 m) layers of the water column. Our sampling covered both periods of water stratification (days 297, 304, 325, 332 and 346 of the year) and unstratified mixing periods (day 363 of the year) (Fig. S2A). In total, 17 environmental variables of the water were taken directly from our previous study (Xue et al., 2017).

There is no general agreement on a standard definition of the pore-size to fractionate the free-living (FL) and particle-attached (PA) bacterioplankton. We selected the 3 μm pore-size to separate both types of bacterioplankton to facilitate comparisons between studies, because most previous studies have used this pore-size to distinguish between FL and PA fractions (Jackson et al., 2014; Milici et al., 2016). For the particle-attached bacteria ($n = 18$) depending on the biomass concentration, 300–400 mL water was filtered through 3.0 μm pore-sized polycarbonate filters (47 mm diameter, Millipore, Billerica, MA, USA) by vacuum filtration. The filtrate, which represented the bacterioplankton fraction smaller than 3.0 μm (hereafter referred to as 'free-living' bacteria, $n = 18$), was collected in a sterile glass bottle and subsequently filtered through 0.22 μm pore-sized polycarbonate filters (47 mm diameter, Millipore, Billerica, MA, USA). The filters with bacterial cells were stored at −80 °C until deoxyribonucleic acid (DNA) extraction (Liu et al., 2015b; Savio et al., 2015). Based on the bacterial lifestyle and bloom status, we artificially defined four categories of bacterioplankton samples: ARGs in free-living bacteria during bloom period (FLB), free-living bacteria during non-bloom period (FLN), particle-attached bacteria during bloom period (PAB) and particle-attached bacteria during non-bloom period (PAN).

2.2. DNA extraction

Total DNA was extracted from filters using a FastDNA SPIN Kit and the FastPrep Instrument (MP Biomedicals, Santa Ana, CA, USA) according to the manufacturer's instructions. The concentration and quality of extracted DNA were determined through spectrophotometric analysis using NanoDrop ND-1000 device (Thermo Fisher Scientific, Waltham, MA, USA). Then the DNA was diluted to equal concentrations: 30 ng/μL for high-throughput quantitative PCR reactions, and 3 ng/μL for real-time quantitative PCR reactions, respectively.

2.3. Real-time quantitative PCR (qPCR)

The absolute copy number of 16S rRNA gene of samples was quantified by a Lightcycler 480 instrument (Roche, Basel, Switzerland) using a SYBR® Green approach. Each sample was quantified in triplicate using a standard curve and a negative control (Schmittgen and Livak, 2008). The thermocycling steps for qPCR amplification and calibration standard curves for positive controls were generated as described in a previous study (Ouyang et al., 2015). The qPCR reactions were performed in 20 μL system, including 10 μL reaction mixtures (iQ™ SYBR Green Supermix, Bio-Rad, Indianapolis, IN, USA), 7.5 μL H_2O, 2 μL template DNA and 0.25 μL each primer.

2.4. High-throughput quantitative PCR (HT-qPCR)

The primer design followed methods described by Zhu et al. (2013). HT-qPCR of antibiotic resistance genes (ARGs) was performed using the SmartChip Real-time PCR (Warfergen Biosystems, Fremont, CA, USA) as described previously (Su et al., 2015; Wang et al., 2014). A total of 296 primer pairs were used to target the genes including one 16S rRNA gene, 285 ARGs, 10 mobile genetic elements (MGEs) which contain 8 transposase genes, one universal class I integron-integrase gene (*intI*), and one clinical class 1 integron-integrase gene (*cintI*; Table S1). The 285 ARGs confer resistance to major classes of antibiotics (Su et al., 2015). All qPCRs were performed in triplicate, and a non-template negative control was included. The results of the HT-qPCR were analyzed with the standard settings: any wells with multiple melting peak

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