Marine Pollution Bulletin xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Marine Pollution Bulletin

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

Bacterial community structure in the intertidal biofilm along the Yangtze Estuary, China

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ARTICLE INFO

Keywords:
Intertidal biofilm
Bacterial community
Yangtze Estuary
MiSeq sequencing

ABSTRACT

In this study, the 16S rRNA-based Illumina MiSeq sequencing was used to investigate the bacterial community structure and composition of intertidal biofilm taken along the Yangtze Estuary. The results showed that 680,721 valid sequences of seven samples were assigned to 147,239 operational taxonomic units, which belonged to 49 phyla, 246 family and 314 genera. Compared to other studies on water and sediments in the study area, biofilms showed highest index of bacterial diversity and abundances. At different taxonomic levels, both dominant taxa and their abundances varied among the seven samples, with *Proteobacteria* as the dominant phylum in general. Principal component analysis and cluster analysis revealed that bacterial communities at WSK differed from those at other sampling sites. Salinity, dissolved oxygen, pH and nutrients were the vital environmental factors to influence the bacterial community structure of biofilms. These results may provide a new insight into the microbial ecology in estuarine environments.

1. Introduction

Biofilms are complex communities comprised of bacteria, protozoa, microalgae, and their extracellular polysaccharide matrix (Rao et al., 1997; Hans-Curt and Jost, 2010). They are ubiquitous on submerged natural and artificial surfaces in the aquatic environments (Palmer and White, 1997; Thiyagarajan et al., 2006; Wahl et al., 2012). As primary producers and consumers, microbial communities are important components of the microbial food loop (Sun et al., 2014), and most of their functions may be associated with special microbial-community composition and structure (Lyautey et al., 2005; Despland et al., 2012). In addition, bacteria affiliated to different groups can show different degrees of activity in a given ecosystem (Cottrell and Kirchman, 2003). Therefore, understanding the composition and dominant groups of bacterial community in biofilm may be particularly important in ecological studies.

In marine environments, several studies have proven that the composition and structure of biofilms play crucial roles in various biological and ecological processes, including organic matter decomposition (Quan et al., 2012), carbon cycling (Jiao et al., 2014), nitrogen fixation (Barlett and Leff, 2010), sulfate reduction (Santegoeds et al., 1998), and larval recruitment of invertebrates (Wang et al., 2012; Li et al., 2014). For fresh water environments, the composition and structure of biofilms are also studied in terms of denitrification of a bioreactor (Chu and Wang, 2013; Wu et al., 2013), in a drinking and waster water treatment system (Martiny et al., 2003; Sagberg et al., 2011; Liao et al., 2013; Heijnen, 2014) and in some natural river networks (Marti et al., 2013). Nonetheless, only a few research groups have reported the composition and structure of biofilms formed in estuarine systems (Nocker et al., 2007), especially for the intertidal biofilm (Piccini and Garcíaalonso, 2015).

Bacteria are the first recruits and most abundant microbes in biofilms (Qian et al., 2007); therefore, the composition and structure of their community are influenced by a variety of biological or non-biological factors (Burmolle et al., 2006; Marti et al., 2013; Zhang et al., 2014; Li et al., 2014). As for the non-biological factors, Marti et al. (2013) and Nocker et al. (2007) found that the richness and diversity of the bacterial community in a biofilm are influenced by the effluents with lower levels of dissolved oxygen (DO) and higher concentrations of nutrients from a waste water treatment plant (WWTP), indicating that water quality is essential to the growth and diversity of bacteria in biofilms. Salinity is a major environmental determinant of bacterial-

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http://dx.doi.org/10.1016/j.marpolbul.2017.07.051
Received 1 June 2017; Received in revised form 20 July 2017; Accepted 20 July 2017
0025-326X/ © 2017 Published by Elsevier Ltd.

community composition (Lozupone and Knight, 2007; Zhang et al., 2014; Ye et al., 2016), and could directly affect the richness, growth, and activity of bacterial communities (Bendov et al., 2008; B. Zheng et al., 2014b; Y. Zheng et al., 2014a; Gao et al., 2016). Complex hydrodynamics have also been demonstrated to significantly influence bacterial diversity in biofilms and to alter the state of bacterial aggregation at solid-liquid interfaces (Rickard et al., 2004).

The Yangtze Estuary is the region where the Yangtze River flows into the East China Sea, which has great ecological and economic significance for east China. Nonetheless, the Yangtze Estuary has been strongly influenced by human activities, such as ranges of pollutants (Liu et al., 2001; Kuotung et al., 2009; Shi et al., 2014; Lin et al., 2015) and nutrients (Liu et al., 2003; Chai et al., 2006; Zhang and Jiao, 2007), which have been released into this estuary along with urban river runoff and sewage outfalls, thereby posing an environmental risk to the ecosystem. Studies on microbial ecology are increasing in number, and most of them are focused on water or sediment in the Yangtze Estuary and its adjacent area (Zhang and Jiao, 2007; Feng et al., 2009; Sun et al., 2014; Liu et al., 2015; Ye et al., 2016). Nevertheless, microbial communities in intertidal biofilms along the Yangtze Estuary have not been explored until now.

Therefore, the objective of this study was to characterize the bacterial community structure and composition of intertidal biofilms taken along the Yangtze Estuary by the MiSeq Illumina sequencing method, and then to elucidate environmental factors influencing the abundance, diversity and composition of bacterial communities in biofilms.

2. Materials and methods

2.1. Sample collection

Seven sampling sites along the Yangtze Estuary were selected for collection of biofilm samples (Fig. 1): Chaoyangnongchang (CY, a tidal flat), Sanchakou (SCK, a tidal flat), Wusongkou (WSK, the junction of the Huangpu River and the Yangtze River), Shidongkou (SDK, a sewage outfall of a WWTP into the Yangtze River), Liuhekou (LHK, the junction of Liu River and the Yangtze River), Qiyakou (QYK, a junction of the Urban River and the Yangtze River) and Xupu (XP, a tidal flat). In October 2016, natural biofilm samples were scraped from the surface of artificial cement substrata with sterile shovels. The biofilm samples were collected from embankment facilities at QYK, LHK, SDK, WSK, SCK and CY, and from the cement stones at XP. All the samples were immediately transferred into sterile plastic bags and stored on ice in the field. After transportation to the laboratory, a part of the biofilms was sub-packaged into 1.5 mL sterile tubes and stored at −20 °C for DNA extraction, and the remaining samples were stored at 4 °C before the analysis of physiochemical properties.

2.2. Physicochemical properties analysis

Environmental parameters are listed in Table 1, including DO, temperature, oxidation reduction potential (Eh), salinity, pH, dissolved organic carbon (DOC), total nitrogen (TN) and total phosphorus (TP). DO, Eh, temperature, salinity and pH of surface water were measured using a portable water quality analyzer (HQ 40d, HACH, USA). DOC in the surface water was measured by means of a TOC automatic analyzer (TOC-L, Shimadzu, Japan) directly after filtration via 0.45 μm filters. The concentrations of TN and TP were measured by an auto discrete analyzer (EasyChem Plus, Systea, Italy) by the methods of EPA n. 351.2 and EPA n. 365.3, respectively. Water content of each biofilm was measured according to the weight loss of a known amount of wet biofilm dried at 80 °C to a constant weight (B. Zheng et al., 2014b; Y. Zheng et al., 2014a). All physiochemical parameters of the samples were analyzed in triplicate.

2.3. DNA extraction PCR amplification

Microbial genomic DNA of the biofilm samples were extracted in triplicate using the OMEGA Mag-Bing Soil DNA Kit (Omega Bio-Tek, Norcross, GA, USA). The V4–V5 region of the 16S rRNA gene was amplified with the forward primer 515F (5′-GTGCCAGCMGCCGCGG-3′) and reverse primer 907R (5′-CCGTCAATTCMTTTRAGTTT-3′), where the barcode is an eight-base sequence unique to each sample (Ye et al., 2016). Polymerase chain reaction (PCR) was conducted in

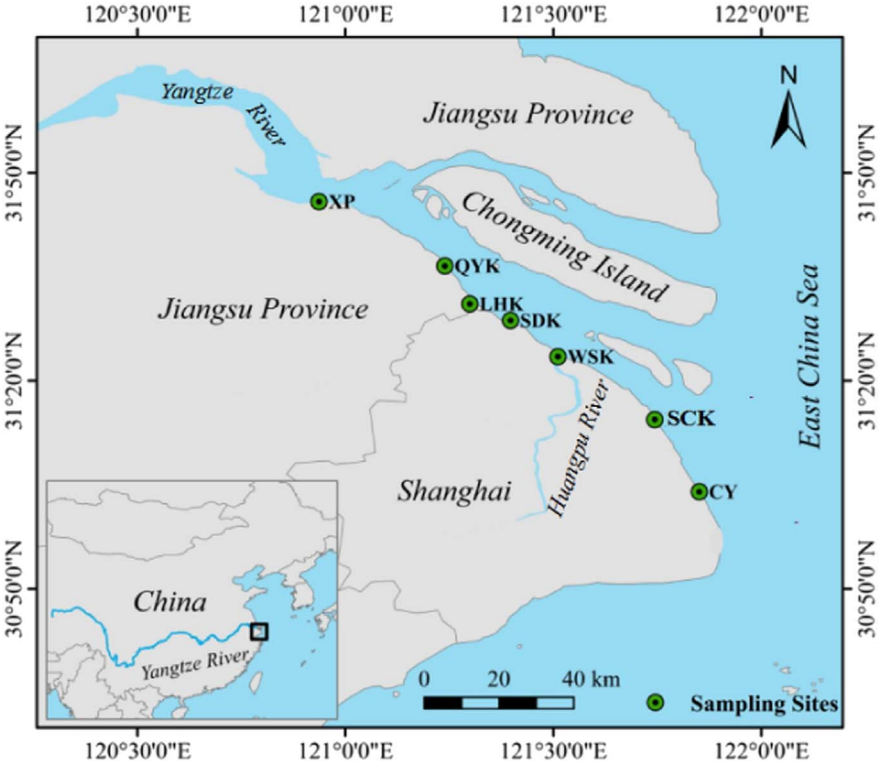

Fig. 1. Sampling sites along the Yangtze Estuary.

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