Ecotoxicology and Environmental Safety 165 (2018) 573–581

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

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

Variation of bacterioplankton community along an urban river impacted by touristic city: With a focus on pathogen

Sheng Liu, Chao Wang, Peifang Wang*, Juan Chen, Xun Wang, Qiusheng Yuan

Key Laboratory of Integrated Regulation and Resource Development on Shallow Lakes, Ministry of Education, College of Environment, Hohai University, Nanjing 210098, China

ARTICLE INFO

Keywords:
Bacterioplankton community
Next-generation sequencing
Pathogen
Health risk
Touristic river

ABSTRACT

Bacterioplankton communities play a critical role in ecological processes in river systems, and shifts of their composition may impact microbial levels and raise public health concerns. The aim of this study was to comprehensively analyze the essential factors influencing bacterioplankton community, along with pathogen, and to estimate the health risk caused by the pathogens downstream of the Liushahe River, which is located in the famous touristic city Xishuangbanna. Results showed that wastewater treatment plants (WWTPs) and a subtropical recreational park impacted the bacterioplankton community and pathogen population, and potential pathogen identification demonstrated that 76 of 145 reference genera were present in the river. Moreover, the bacterioplankton community and pathogen were differently impacted by environmental gradients, and SRP, NO_2 and pH were main factors influencing bacterioplankton community while pathogen population was highly correlated with temperature and turbidity. In addition, it is noted that the pathogen population was dominated by bacterioplankton community and this might because the capacity of resistance invasion pathogen was determined by of bacterioplankton community diversity. Therefore, bacterioplankton community diversity can be used to control and predict the amount of pathogens. Quantitative microbial risk assessment (QMRA) also revealed that the infection risks of *Escherichia coli* (*E. coli*), *Mycobacterium avium* (*M. avium*), and *Pseudomonas aeruginosa* (*P. aeruginosa*) during five recreational activities, especially water-based activities in the touristic city, were greater than that in natural areas and mostly exceeded the U.S. EPA risk limit for recreational activities. Our study offered the first insight into the potential relationship between the bacterioplankton community and bacterial pathogens within a touristic river.

1. Introduction

Rivers flowing across cities always play an important role in urban ecological system, especially in touristic cities. As main source of recreational water and drinking water for citizens and tourists, the urban river gathers all kinds of effluents, including urban sewage, storm runoff and recreational discharges. Since fast-growing populations and inefficient sewage treatment, the deterioration of river water quality is inevitably (Suthar et al., 2010).

Bacterioplankton, which promote biogeochemical processes and take part in the aquatic food web, play a vital role in river ecosystems (Cotner and Biddanda, 2002; Pernthaler, 2005). Since bacterioplankton can impact water quality by participating in nutrient cycling and contributing to energy flow, bacterioplankton composition in rivers should be considered an indicator of pollution (Battin et al., 2009; Findlay et al., 2010). As a hazardous part of bacterioplankton communities, pathogens in aquatic systems worldwide could give rise to disease (Sherchand, 2012). It was suggested that the disease incidence is higher in touristic rivers than in other urban waters (Sales-Ortells et al., 2015) because river water is considered a probable reservoir for pathogens and a route of transmission (Aw and Rose, 2012). The sources of bacterial pathogens are very likely sewage effluent and animal feces (Arnone, 2007). The pathogens in rivers may cause disease when citizens and tourists are exposed to water during recreational activities. Thus, the composition of bacterioplankton and pathogen along the touristic river should be investigated.

Abbreviations: ANOSIM, analysis of similarities; ANOVA, analysis of variance; CFU, colony-forming unit; DOC, dissolved organic carbon; DSi, dissolved silicate; EC, electric conductivity; MRT, multivariate regression tree; NGS, next-generation sequencing; ORP, oxidation-reduction potential; OTUs, operational taxonomic units; PCA, principal component analysis; QMRA, quantitative microbial risk assessment; qPCR, quantitative polymerase chain reaction; RDA, redundancy analysis; SRP, soluble reaction phosphorous; TN, total nitrogen; TP, total phosphorous; WWTPs, wastewater treatment plants

* Corresponding author.
E-mail address: pfwang2005@hhu.edu.cn (P. Wang).

https://doi.org/10.1016/j.ecoenv.2018.09.006
Received 8 June 2018; Received in revised form 29 August 2018; Accepted 1 September 2018

Furthermore, bacterioplankton, including pathogen, are diverse in composition, and its population may change in response to temporal and spatial variation of environmental conditions,such as nutrient concentrations (Fisher et al., 1998; Liu et al., 2012), temperature (Staley et al., 2015), and flow rate (Wang et al., 2016). And these studies mainly focused on correlating the physical and chemical factors with the variation of bacterioplankton communities and revealed how bacterioplankton respond to environmental changes in rivers whereas the pathogens was always been ignored. However, it is doubtful whether bacterioplankton communities and pathogen populations have identical alteration in rhythm with environmental gradients. Bacterioplankton communities play important roles in regulating pathogen survival by means of biotic interactions including predation (Sørensen et al., 1999), antagonism from indigenous microorganisms (Garbeva et al., 2004) and competition for resources (Yoshiko et al., 2006). The relationship between bacterioplankton communities and pathogens should be studied in order to monitor pathogens in rivers and providing strategies for water quality management. A previous study had investigated the impact of microbial community and physico-chemical properties on enteropathogens in soil from different land-uses and indicated that microbial community composition predominantly regulated enteropathogens survival (Moynihan et al., 2015). It is also reported that the invasion of bacterial pathogen was determined by microbial diversity in soil (Elsas and Salles, 2012). However, these researches only focused on a single pathogen solely, the relationship between bacterioplankton communities and total bacterial pathogens had not yet been investigated, especially in river.

Although next-generation sequencing (NGS) was commonly conducted in recent studies investigating bacterioplankton communities and pathogens (Drury et al., 2013; Fang et al., 2017; Ibekwe et al., 2016) since it could reveal the comprehensive structure of bacterioplankton community due to its high-throughput feature, it was inefficient to measure specific target pathogens. Due to the sensitivity, quantitative polymerase chain reaction (qPCR) is a reliable method to quantify pathogens in urban water (Byappanahalli et al., 2016; De et al., 2014). Therefore, NGS and qPCR could be combined to screen bacterioplankton community and pathogens distribution.

Xishuangbanna is located in Yunnan Province and features unique tourism. In 2016, Xishuangbanna received 25.19 million domestic and foreign tourists, with a total tourism revenue of 42.03 billion yuan (Xu et al., 2014). The Liushahe River is an important water resource of Xishaungbanna. Moreover, because the Liushahe River is the last urban tributary flows into the Lancang River before it gets across the Chinese border, the deterioration of water quality and increase of pathogens in the Liushahe River poses international risks in both public health and economic aspects (Wang et al., 2016), and bacterioplankton community and pathogen population in the Liushahe River has not yet been investigated. It is hypothesized that the bacterioplankton community and pathogens would shift variously in response to environmental variation along touristic river and pathogen population could be dominated by bacterioplankton community. In this study, we investigated (1) how the bacterioplankton community and pathogen population were influenced by the touristic city and the crucial environmental factors and (2) whether their existed a relationship between bacterioplankton communities and pathogens, as well as the identification of public public health risk caused by pathogens.

2. Materials and methods

2.1. Study area, sampling procedure, and physicochemical analysis

The Liushahe River is located in Xishuangbanna, Yunnan Province, Southwest China, with a length of 92 km and a watershed area of 2064 km^2. The Liushahe River flows downstream through Jinghong before joining the Lancang River (Fig. 1).

In this study, we collected water samples from five areas downstream of the Liushahe River. To avoid deviation, three water samples were collected within meters along the river in each area. Areas 1 and 2 were natural areas (1 − 1, 1 − 2, 1 − 3, 2 − 1, 2 − 2, 2 − 3); area 3 was a suburban area that was impacted only by urban land use and not by sewage discharge (3 − 1, 3 − 2, 3 − 3); areas 4 and 5 were considered part of the touristic city, with area 4 downstream of the subtropical recreational park (4 − 1, 4 − 2, 4 − 3); and area 5 was impacted by WWTPs (5 − 1, 5 − 2, 5 − 3) and influenced by both urban land use and sewage discharge.

Each water sample of one L volume was collected at the water depth of approximately 30 cm with a Ruttner sampler (Hydro-Bios, Altenholz, Germany) for the subsequent physicochemical analysis in the laboratory. To acquire the bacterioplankton, 1.5-L water samples were collected at each site and filtered through 0.22-μm pore prewashed polycarbonate filters (47 mm diameter, Millipore, Burlington, MA, USA) by vacuum filtration. The filters were stored in sterile 50-mL centrifuge tubes on ice bags during transport and then stored at −80 ℃ prior to extraction of bacterioplankton DNA.

The global positioning (GPSMAP 62 s, Garmin, KS, USA) information of the sampling area is listed in Table S1. The electric conductivity (EC), pH, temperature, dissolved oxygen (DO), and oxidation-reduction potential (ORP) were measured in situ during sample collection using a multiparameter water quality analyzer (HQ40d, Hach, Loveland, CO, USA). The turbidity was also determined during the sampling process using a turbidimeter (2100 P, Hach). The physicochemical analysis was conducted in the laboratory. The analysis of dissolved organic carbon (DOC) was conducted with an Elementar Liquid-TOC analyzer (Frankfurt, Germany). Dissolved silicate (DSi) was estimated with the molybdate blue spectrophotometric method base on previous study (Mortlock and Froelich, 1989). Total nitrogen (TN) and total phosphorus (TP) were measured colorimetrically according to previous research (Wang et al., 2017). Nitrate nitrogen (NO_3), nitrite nitrogen (NO_2), ammonium nitrogen (NH_4), and soluble reactive phosphorus (SRP) were measured colorimetrically by an AA3 Auto-Analyzer (Seal, Norderstedt, Germany).

2.2. DNA extraction, sequencing, data processing and pathogen identification

DNA extraction was conducted using the PowerWater DNA Extraction Kit (Mo Bio, Carlsbad, CA, USA) according to the manufacturer's instructions. Agarose gel (1%) electrophoresis and spectrophotometry were conducted to determine the quality and the quantity of DNA (Nano Drop ND 2000, Termo Scientifc, DE, USA).

The V4 region of the microbial 16 S rRNA gene, which contains accurate taxonomic information (Bates et al., 2011), was amplified by polymerase chain reaction (PCR) as follows: 94 ℃ for 5 min, 31 cycles at 94 ℃ for 30 s, 52 ℃ for 30 s, 72 ℃ for 45 s, and a final extension at 72 ℃ for 10 min with the primers 515 F (5′-GTGCCAGCMGCCGCGGTAA-3′) and 806 R (5′-GGACTACHVGGGTWTCTAAT-3′). PCR was conducted in triplicate for all samples. The amplicons were purifed before sequencing.

The obtained DNA was then sequenced by the Illumina Hiseq. 2000 platform according to the manufacturer's instructions. Data processing was performed referred to previous study (Li et al., 2017). Briefly, the high-quality sequences were sliced into lengths of 245–260 bp for subsequent analysis. Sequences were clustered to operational taxonomic units (OTUs) at 97% identification. And then taxonomic assignment of OTUs was conducted using RDP classifier (Cole et al., 2009).

The potential pathogen identification was also conducted with a reference database of human pathogenic bacteria including 145 genera, which was established based on the German Culture Collection (DSMZ, Braunschweig, Germany) and previous studies (Fang et al., 2017). The potential pathogens were identified at the genus level.