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Targeted deep sequencing reveals high diversity and variable dominance of bloom-forming cyanobacteria in eutrophic lakes

Yongguang Jiang [a,1], Peng Xiao [a,1], Yang Liu [b], Jiangxin Wang [a], Renhui Li [c,*]

[a] Shenzhen Key Laboratory of Marine Bioresource and Eco-environmental Science, College of Life Sciences and Oceanography, Shenzhen University, Shenzhen 518060, PR China
[b] College of Life Sciences, Henan Normal University, Xinxiang 453007, PR China
[c] Key Laboratory of Algal Biology, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, PR China

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ABSTRACT

Cyanobacterial blooms in eutrophic lakes are severe environmental problems worldwide. To characterize the spatiotemporal heterogeneity of cyanobacterial blooms, a high-throughput method is necessary for the specific detection of cyanobacteria. In this study, the cyanobacterial composition of three eutrophic waters in China (Taihu Lake, Dongqian Lake, and Dongzhen Reservoir) was determined by pyrosequencing the *cpcBA* intergenic spacer (*cpcBA*-IGS) of cyanobacteria. A total of 2585 OTUs were obtained from the normalized *cpcBA*-IGS sequence dataset at a distance of 0.05. The 238 most abundant OTUs contained 92% of the total sequences and were classified into six cyanobacterial groups. The water samples of Taihu Lake were dominated by *Microcystis*, mixed Nostocales species, *Synechococcus*, and unclassified cyanobacteria. Besides, all the samples from Taihu Lake were clustered together in the dendrogram based on shared abundant OTUs. The cyanobacterial diversity in Dongqian Lake was dramatically decreased after sediment dredging and *Synechococcus* became exclusively dominant in this lake. The genus *Synechococcus* was also dominant in the surface water of Dongzhen Reservoir, while phylogenetically diverse cyanobacteria coexisted at a depth of 10 m in this reservoir. In summary, targeted deep sequencing based on *cpcBA*-IGS revealed a large diversity of bloom-forming cyanobacteria in eutrophic lakes and spatiotemporal changes in the composition of cyanobacterial communities. The genus *Microcystis* was the most abundant bloom-forming cyanobacteria in eutrophic lakes, while *Synechococcus* could be exclusively dominant under appropriate environmental conditions.

1. Introduction

Cyanobacteria are photosynthetic microorganisms adaptable to various terrestrial and aquatic environments (de Marsac and Houmard, 1993; Whitton, 2012). Water blooms caused by the massive proliferation of cyanobacteria in freshwater ecosystems have drawn considerable attention around the world (Harke et al., 2016; Paerl and Otten, 2013). Eutrophication of water bodies is the main factor promoting the development of cyanobacterial bloom in the past decades (Bonilla et al., 2012; Gobler et al., 2016; O'Neil et al., 2012). Recently, climate warming and rising atmospheric carbon dioxide have significantly increased the occurrence, intensity and duration of cyanobacterial blooms (Deng et al., 2014; Paerl and Huisman, 2009; Sinha et al., 2012; Visser et al., 2016; Wiedner et al., 2007). Certain species of bloom-forming cyanobacteria are capable of producing toxic metabolites, such as hepatotoxic microcystins and cylindrospermopsins and neurotoxic saxitoxins and anatoxins (Dittmann et al., 2013; Neilan et al., 2013; Pearson et al., 2010). Therefore, harmful cyanobacterial blooms are of serious concern for public health due to the toxic effects of cyanotoxins (Havens, 2008; Paerl and Otten, 2013). Thus, daily monitoring of cyanobacterial composition in freshwater bodies is important for predicting and controlling harmful cyanobacterial blooms to ensure drinking water safety.

Aquatic cyanobacterial community is usually composed of highly diverse cyanobacterial species with some of them dominating in the community (Kim et al., 2006; Liu et al., 2016b; Miller et al., 2013). The most prevalent bloom-forming species are *Synechococcus*, *Microcystis*, *Dolichospermum*, *Aphanizomenon*, *Cylindrospermopsis*, and *Planktothrix*. Although morphological characteristics are

* Corresponding author at: Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, Hubei Province, PR China.
E-mail address: reli@ihb.ac.cn (R. Li).
[1] These authors contributed equally to this work.

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widely used in traditional identification of cyanobacteria in water samples, extensive training is required and identification procedure is also labor-consuming considering the high diversity of cyanobacterial morphologies (Whitton, 2012). Besides, microscopical identification is much difficult for small-sized picocyanobacteria (e.g., *Synechococcus*), which play an important role in freshwater ecosystems (Callieri, 2008). To solve this problem, molecular tools have been developed to efficiently investigate cyanobacterial diversity. Several housekeeping loci on the cyanobacterial genome are selected as molecular markers, such as 16S rRNA gene (Nübel et al., 1997), RNA polymerase gene (*rpoC1*) (Palenik and Swift, 1996), intergenic spacer between phycocyanin subunit genes *cpcB* and *cpcA* (*cpcBA*-IGS) (Neilan et al., 1995), and internal transcribed spacer between the 16S rRNA and 23S rRNA genes (ITS) (Huang et al., 2014). In particular, the 16S rRNA gene and *cpcBA*-IGS were proven useful for phylogenetic analysis of cyanobacteria at different taxonomic levels (Dadheech et al., 2010; Dojani et al., 2014; Dyble et al., 2002; Ishida et al., 2001; Robertson et al., 2001; Teneva et al., 2005; Wang et al., 2013). PCR amplification of partial 16S rRNA gene followed by denaturing gradient gel electrophoresis (DGGE) can provide a general overview of the cyanobacterial diversity in environmental samples (Boutte et al., 2006; Ye et al., 2011). The clone libraries of *cpcBA*-IGS or 16S rRNA gene can also be constructed and sequenced to roughly reveal cyanobacterial composition (Dojani et al., 2014; Kim et al., 2006). However, only limited information regarding cyanobacterial community could be obtained by these methods.

In recent years, high-throughput sequencing has been widely used for detailed and accurate understanding of microbial community structure in ecosystems by producing a large sequence dataset of molecular markers (Cole et al., 2005). For example, Kleinteich et al. (2014) and Zhang et al. (2016) investigated the cyanobacterial composition in the meltwater ponds and Gurbantunggut Desert, respectively, by pyrosequencing the 16S rRNA gene. Unfortunately, their sequence datasets were contaminated by abundant bacterial sequences although the primers used to amplify the 16S rRNA gene were considered to be cyanobacteria-specific (Kleinteich et al., 2014; Zhang et al., 2016). In another study, plastid 23S rRNA gene was applied for pyrosequencing analysis of phytoplankton in eutrophic lakes, but cyanobacterial populations can only be delineated at order-level unsuitable for accurate identification of bloom-forming species (Steven et al., 2012). Phycocyanin is an exclusive compound of cyanobacteria, and thus genes related to phycocyanin, for instance, *cpcBA*-IGS could be a more specific and reliable molecular marker in deep sequencing compared with 16S rRNA gene. The purpose of this study is to provide a comprehensive understanding of the cyanobacterial community in freshwater ecosystems suffering annual cyanobacterial blooms. Therefore, pyrosequencing method based on *cpcBA*-IGS was developed to reveal cyanobacterial diversity and composition in three eutrophic lakes at molecular level.

2. Materials and methods

2.1. Collection of cyanobacterial samples

Taihu Lake is a large shallow freshwater lake in eastern China and located in a temperate region (30°56′–31°33 N, 119°53′–120°36′ E; water area = 2338 km^2; mean depth = 1.9 m). Dongqian Lake is also a shallow freshwater lake in eastern China (29°44′–29°47′ N, 121°37′–121°41′ E; water area = 20 km^2; mean depth = 2.2 m). A sediment dredging project was conducted in Dongqian Lake from 2009. Dongzhen Reservoir is a deep reservoir in southeastern China and located in a subtropical region (25°28′–25°30′ N, 118°54′–118°59′ E; water area = 17.8 km^2; maximum depth = 36 m). All these three lakes have been suffering from eutrophic problems and annual cyanobacterial blooms (Guo, 2007; Jing et al., 2013; Lv et al., 2013). Surface water (0–0.5 m) was sampled at 9 locations, 5 in Taihu Lake, 2 in Dongqian Lake and 2 in Dongzhen Reservoir (Fig. 1). Water samples at 10 m depth in

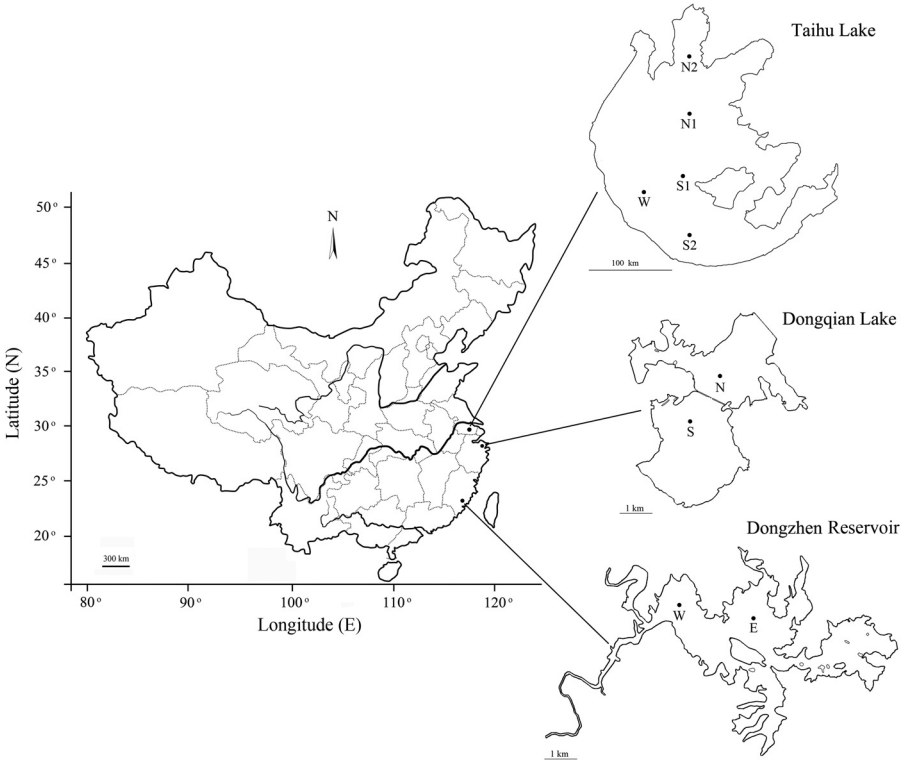

Fig. 1. Map of three lakes showing sampling locations in this study.