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Successional trajectories of bacterioplankton community over the complete cycle of a sudden phytoplankton bloom in the Xiangshan Bay, East China Sea

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ABSTRACT

Phytoplankton bloom has imposed ecological concerns worldwide; however, few studies have been focused on the successional trajectories of bacterioplankton community over a complete phytoplankton bloom cycle. Using 16S pyrosequencing, we investigated how the coastal bacterioplankton community compositions (BCCs) respond to a phytoplankton bloom in the Xiangshan Bay, East China Sea. The results showed that BCCs were significantly different among the pre-bloom, bloom, and after-bloom stages, with the lowest bacterial diversity at the bloom phase. The BCCs at the short-term after-bloom phase showed a rapid but incomplete recovery to the pre-bloom phase, evidenced by 69.8% similarity between pre-bloom and after-bloom communities. This recovery was parallel with the dynamics of the operational taxonomic units (OTUs) affiliated with *Actinobacteria*, *Bacteroidetes*, *Cyanobacteria*, *Alphaproteobacteria* and *Gammaproteobacteria*, whose abundance enriched when bloom occur, and decreased after-bloom, and *vice versa*. Collectively, the results showed that the BCCs were sensitive to algal-induced disturbances, but could recover to a certain extent after bloom. In addition, OTUs which enriched or decreased during this process are closely associated with this temporal pattern, thus holding the potential to evaluate and indicate the succession stage of phytoplankton bloom.

1. Introduction

In recent decades, the frequency of phytoplankton blooms has increased on a global scale due to the intensification of coastal pollution (Breitburg, 2002; Zhou et al., 2008). Coastal phytoplankton blooms can dramatically change ambient biogeochemical factors, such as exhaustion of oxygen, release toxins and other secondary metabolites into surrounding waters (Bricelj and Lonsdale, 1997; Glibert et al., 2005). As a result, phytoplankton blooms have caused serious ecological consequences by affecting the trophic structure of ecosystems and altering the dynamics of bacterioplankton communities (Herlemann et al., 2011; Vieira et al., 2008) and the toxins can also cause indiscriminate mass mortalities of sea birds, marine mammals, and fish, loss of wild and cultured seafood resources and threatening human health by bioconcentration (Anderson et al., 2012).

For these reasons, efforts have been made to investigate the dynamics of BCCs in response to phytoplankton blooms (Sintes et al., 2013; Thomas et al., 2014), and its abiotic effects on the surrounding environments (Engström-Öst et al., 2012; Mulholland et al., 2009). Recently, increasing evidences have shown a close association between the emergence of phytoplankton blooms and the variations of ambient BCCs in microcosm/mesocosm experiments (Pinhassi et al., 2004; Riemann et al., 2000; Tada et al., 2012) and *in situ* survey (Lamy et al., 2010; Tada et al., 2011). For example, the relative abundance of a few dominant phylotypes were

Abbreviations: BCCs, bacterioplankton community compositions; ECS, East China Sea; PCoA, principal coordinate analysis; ANOSIM, Analysis of similarity; PB, pre-bloom; B, bloom; AB, after-bloom; MANOVA, permutational multivariate analysis of variance.

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significantly altered when a phytoplankton bloom occurred both at microcosmic and mesocosmic scales (Pinhassi et al., 2004; Tada et al., 2012). However, most of those studies ignored the dynamics of relatively rare species that are undetectable by traditional techniques, such as denatured gradient gel electrophoresis (DGGE) and subsequent sequencing (Riemann et al., 2000; Teeling et al., 2012). This information is critical because rare species may hold significant functional roles, as keystone species in microbial consortia or maintaining biogeochemical functions (Alonso-Sáez et al., 2015; Galand et al., 2009), and they could quickly respond to altered environmental conditions and become abundant (Shade et al., 2012). In addition, field surveys on phytoplankton blooms focused on the seasonal or long-term succession of BCCs (Behrenfeld et al., 2013; Teeling et al., 2012), in which showed that phytoplankton blooms, as a recurring seasonal phenomena, could lead to predictable patterns of bacterial successions (Teeling et al., 2012). This pattern could be largely due to the seasonal nutrient availability and temperature those are well known to affect BCCs (Behrenfeld et al., 2013; Sintes et al., 2013). As a result, it is difficult to disentangle the direct phytoplankton bloom effects from the seasonal abiotic factors on the dynamics of BCCs. However, a relative short-term survey, such as an entire cycle of a phytoplankton bloom, could minimize the variation of temperature and other rhythmic biogeochemical variables, thus enabling us to describe how phytoplankton bloom directly affects BBCs.

The Xiangshan Bay, where connects to the East China Sea (ECS), is an eutrophic semi-enclosed basin and has been a typical aquaculture zone for decades (Wang and Wu, 2009). Fisheries have input large amounts of nutrients into the surrounding water, resulting in seasonal occurrences of harmful phytoplankton blooms (Wang and Wu, 2009). Few studies have focused on the succession of BCCs in response to phytoplankton blooms in the coastal ECS (Fu et al., 2011; Liu et al., 2013). Given that bacterioplankton communities play key roles in the mineralization of organic matter and mediation of the energy flow in marine ecosystem (Gibbons et al., 2013), it is likely that changes in bacterial species link to the degradation of phytoplankton-derived organic matter during phytoplankton blooms (Fandino et al., 2001; Riemann et al., 2000). For example, *Bacteroidetes* species are especially relevant in the cycling of organic matter during phytoplankton blooms (Fandino et al., 2001; Pinhassi et al., 2004; Riemann et al., 2000), and *Alteromonas* increase at the early stage of bloom (Tada et al., 2012), while *Roseobacter*/*Rhodobacter*increase increase at the late stage of a phytoplankton bloom (Teeling et al., 2012). Thus, it appears that specific taxa are closely associated with a given stage of phytoplankton bloom and could indicate the successional stages of phytoplankton bloom (von Scheibner et al., 2014; Weinbauer et al., 2011). In this study, we collected samples over a complete cycle of a sudden phytoplankton bloom, using 16S-tag pyrosequencing to survey the dynamics of BBCs. The aims of this study were: (i) to investigate the successional pattern of BCCs over a sudden natural bloom-induced perturbation, (ii) to identify the environmental factors that shape this succession, and (iii) to screen sensitive bacterial taxa that indicate the stage of phytoplankton bloom.

2. Materials and methods

2.1. Sample collection

The sampling site (29°32′N, 121°45′E) is located at a long-term monitoring station, where we monitored the bacterioplankton community at a 2-m depth biweekly. On August 8, 2012, Typhoon Haikui made landfall over Xiangshan Bay, subsequently resulted in *Skeletonema costatum* and *Chaetoceros curvisetus* dominated bloom after five days. We intensified the sampling on the 5th, 13th, 14th, 15th, 17th, and 18th August, with 3–5 replicates at each sampling day. Water samples were collected using a 5-L custom made sampler, then transported into laboratory within 2 h in an icebox. Blooms of *S. costatum* could reach the concentration of 5×10^6 cells L^{-1} (Anderson et al., 2001). In this study, cell density of *S. costatum* was 10^7 cells L^{-1} in these three days (13th, 14th and 15th Aug.), which was 100-fold higher than those on 5th and 17th August. Moreover, *C. curvisetus* density was also up to 10^7 cells L^{-1} during the same period, but it lasted for a shorter duration, and thus we categorized samples from these three days into bloom stage (B, named B13, B14, and B15) according to cell density of *S. costatum*. Cell density of *S. costatum* in other samples were low so that they were categorized into pre-bloom (PB, on 5th Aug., named PB05), and after-bloom (AB, on 17th and 18th Aug., named AB17 and AB18) phases (Fig. 1), respectively.

2.2. Chemical analyses of water samples

Phytoplankton cells were counted under an inverted microscope, using a 50 cm^3 settling chambers (Utermöhl, 1958). Temperature and salinity were measured using appropriate sensors (WTW-LF-197, Brives B.V., Germany). For the measurement of chlorophyll *a* (Chl *a*), a water sample of 100 mL was filtered through a 0.45-μm polycarbonate membrane (Millipore, Boston, MA, USA), then extracted in 80% *N*, *N*-dimethyl formamide for 24 h at 4 °C. The concentration of Chl *a* in the extracts was measured using a spectrophotometer (UV-1601, Shimadzu, Japan) at wavelengths of 647 and 665 nm according to the equation $\text{Chl } a = 12.70A_{665} - 2.79A_{647}$ (Inskeep and Bloom, 1985). The levels of nitrate (NO_3^-), phosphate (PO_4^{3-}), chemical oxygen demand (COD), and dissolved oxygen (DO) were measured according to standard methods (Greenberg et al., 1992).

2.3. DNA extraction and purification

To collect microbial organism, 2 L seawater was pre-filtered through a nylon mesh (100-μm pore size) and subsequently filtered through a 0.22-μm polycarbonate membrane (Type GTTP; Millipore, USA) using a vacuum pump. The filters were cut into pieces and DNA was extracted using a Power Soil® DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA, USA) according to the manufacturer's protocols. The DNA extracts were quantified with a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, USA) and then stored at −80 °C until amplification.

2.4. 16S rRNA gene PCR amplification and 454 pyrosequencing

An aliquot (50 ng) of DNA from each sample was used as template for amplification. The V4–V5 regions of the bacterial 16S rRNA gene were amplified using the primer set: 515F: 5′-GTGCCAGCMGCCGCGG-3′ with the Roche 454 'A' pyrosequencing adapter and a unique 10-bp barcode sequence, and primer 907R: (5′-CCGTCAATTCMTTTRAGTTT-3′) with the Roche 454 'B' sequencing adapter at the 5′-end of each primer. To minimize PCR biases, each sample was amplified in triplicate under the following conditions: denaturation at 94 °C for 1 min, 30 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 30 s; with a final extension at 72 °C for 10 min (Riemann et al., 1999). PCR products for each sample were combined and purified using a PCR fragment purification kit (TaKaRa Biotech, Japan). The purified PCR products were quantified using a Quant-It™ Pico Green kit (Invitrogen, USA). An equal amount of PCR products for each sample were pooled in a single tube and analyzed on a Roche FLX 454 platform (Roche Diagnostics Corporation, Branford, CT, USA), producing reads from the forward direction

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