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Bacterial community dynamics during a bloom caused by *Akashiwo sanguinea* in the Xiamen sea area, China

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ABSTRACT

Phytoplankton blooms are a worldwide ecological problem and one of the major algae that cause phytoplankton blooms is *Akashiwo sanguinea*. Though much research has addressed the abiotic causes (e.g. growth condition) of *A. sanguinea* blooms, few studies have examined the dynamics of microbial communities associated with these blooms. In this study, polymerase chain reaction (PCR)-based denaturing gradient gel electrophoresis (DGGE) analysis of 16S rDNA genes was used to document changes in the phylogenetic diversity of microbial communities associated with an *A. sanguinea* bloom that occurred in the Xiamen sea in May 2010. Surface sea water was sampled once a day within five consecutive days at four sites, and the microbial community composition was determined using DGGE. Sea water concentrations of chlorophyll *a*, nitrate and phosphate were also measured. The results indicated that the *A. sanguinea* bloom was probably stimulated by low salinity (26–30‰) and ended probably because inorganic nutrients were consumed and resulted in a N/P ratio unfavorable for this alga. Gammaproteobacteria populations increased significantly during bloom declines and then decreased post-bloom. Divergences in the microbial community composition during different bloom periods were the result of changes in *Candidatus*, *Pelagibacter*, *Alteromonas*, *Rhodobacteraceae*, *Vibrio* and *Pseudoalteromonas* populations. *Sediminimonas qiaohouensis* was the first bacterium shown to be significantly negatively correlated with *A. sanguinea* concentration. This study indicated that bacteria may play an important role in *A. sanguinea*–bloom regulation and provides a deeper insight into bacterial community succession during and after an *A. sanguinea*–bloom.

1. Introduction

Phytoplankton blooms have become a widespread problem in the marine environment due to increased anthropogenic pollution and eutrophication (Anderson et al., 2002; Cai et al., 2011; Heisler et al., 2008; Su et al., 2007, 2011; Wang et al., 2010a,b, 2012). The dinoflagellate *Akashiwo sanguinea* is an alga that causes blooms world-wide and frequently causes blooms in the Xiamen sea in summers since 2008. *A. sanguinea* is eurythermal and euryhaline (Matsubara et al., 2007) and commonly causes spring or summer red tides. The bloom forming time differs by location. For example, blooms occur in August–September in Yantai (China) but in February–May in Xiamen (China). More importantly, this bloom often occurs when the seawater salinity and temperature are relatively low, thus *A. sanguinea* could be more likely to bloom during these periods than other bloom-causing phytoplankton which need a higher temperature for rapid growth.

Information on the toxicity of *A. sanguinea* to humans is limited. However, the *A. sanguinea* toxin has been shown to negatively affect birds and fish. *A. sanguinea* produces powerful surfactants, mycosporine-like amino acids (MAAs), which can cause the feathers of marine birds to become saturated with water, making the birds severely hypothermic (Jessup et al., 2009). More importantly, *A. sanguinea* can increase the mortality of abalone larvae although the mechanism is unclear. Abalone is one of the most important commercial fish, and China has the highest production of and demand for abalone in the world (Cook and Gordon, 2010). Because of the warmer seawater, millions of abalone seedlings are transported to the Xiamen sea fisheries from northern China to survive through the winter. As such, the Xiamen sea is very important for abalone farming. *A. sanguinea*–blooms have occurred frequently in this area in the last three years and causing a great threat to abalone larva cultures. So understanding *A. sanguinea*–blooms and potential bloom regulators are needed in order to minimize the risks associated with such algae blooms to the local aquaculture industry.

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Limited research has been reported on *A. sanguinea*. Related studies showed that parasitic dinoflagellates of the genus *Amoebophrya* sp. could infect *A. sanguinea* community (Gunderson et al., 2001), while ciliates grazing on *Amoebophrya* sp. could decrease this infection (Johansson and Coats, 2002). The effect of environmental factors on the growth of *A. sanguinea* was examined and found that the maximum growth rate of *A. sanguinea* occurs at 25 °C and 20‰ salinity (Matsubara et al., 2007). To the best of our knowledge, information on blooms caused by *A. sanguinea* is very limited. The bacterial communities associated with phytoplankton blooms may be important microbial regulators of the blooms. However, the roles that bacteria play in the regulation of blooms are still largely unknown.

Therefore the following questions which need to be addressed were addressed in this study: what bacterial species are predominant in the bloom; how does the microbial community change during and after the bloom event; what correlations exist between the *A. sanguinea* population and biological and environmental parameters? The results of this study provide an insight into the ecological effects brought about by *A. sanguinea* blooms and indicate important bacterial communities which may play a potential role in the regulation of the *A. sanguinea* community and reduce its ecological harm.

2. Materials and methods

2.1. Study sites and sample collection

Bloom sites-M1 (N 24°34′7.36″, E 118°7′27.43″), M2 (N 24°34′32.32″, E 118°8′32.07″), M3 (N 24°34′19.63″, E 118°9′9.51″), and control site-S8 (N 24°33′35.03″, E 118°9′58.96″) were selected in the area where an *A. sanguinea* bloom occurred along the coast of the South China Sea near Xiamen. A total of 20 liters near-surface (0.5 m) sea water samples per site were collected on May 23–27, 2010 during the bloom and post-bloom. Pre-autoclaved polypropylene sampling vials were used for DNA extraction.

2.2. Environmental parameters

Immediately after collection, water samples for phytoplankton species identification and phytoplankton counts were preserved with 1% Lugol's iodine solution (Lack, 1971) and a separate sample was fixed with neutral formalin (Irigoien et al., 2000) for determination of *A. sanguinea* concentration. Phytoplankton cells were counted under an inverted microscope, using 50 cm^3 settling chambers.

Chlorophyll *a* concentration was determined fluorimetrically according to Yentsch and Menzel (1963). A 100 mL sample was filtered through GF/F filters (Whatman, NJ, USA). The filters were kept at −20 °C for at least 2 h and pigments were extracted in 6 mL 90% acetone under 4 °C in the dark. Fluorescence of the extracts was measured using a fluorometer (Turner Designs, CA, USA).

Concentrations of soluble reactive phosphorus (SRP), nitrate, nitrite, and ammonia were measured according to Grasshoff et al. (1999). Total inorganic nitrogen (TIN) was calculated by summing the molarity of NO_3, NO_2 and NH_4. The N:P ratio was calculated by dividing TIN values by SRP values. COD measurements were made according to the dichromate method (Jardim and Rohwedder, 1989).

Bacteria on black polycarbonate filters were counted under an epifluorescence microscopy after being fixed with 2% formaldehyde and stained with DAPI (0.5 mg mL^{-1}) (Porter and Feig, 1980). Bacterial productivity was determined by measuring the rate of incorporation of [^{3}H]thymidine into bacterial DNA (Riemann et al., 1987).

2.3. Phylogenetic analysis

2.3.1. DNA extraction

The water (500 mL) was filtered through 0.22-μm diameter pore-size filters (Millipore, USA) to collect both attached and free-living bacteria. Filtration was conducted as soon as the samples were taken to the lab and filters were held at −70 °C until analysis. DNA extraction was performed as described in detail by Wichels et al. (2004). Filters were washed using DNA extraction buffer (Tris 50 mmol L^{-1}, EDTA 20 mmol L^{-1}, NaCl 100 mmol L^{-1}, CTAB 2%, pH 8.0), and cell lysis was facilitated by adding lysozyme (2 mg mL^{-1}) and SDS (10%). Proteins were removed by phenol–chloroform–isoamyl alcohol (25:24:1) extraction. After isopropanol precipitation, DNA was resuspended in sterile water (50 μL) and stored at −20 °C until further analysis. Before PCR amplification, the quality of DNA was checked by using a 1% agarose gel electrophoresis in 1 × TAE (20 mM Tris, 10 mM acetate, 0.5 mM Na_2-EDTA, pH 8.2) buffer. After electrophoresis, photographs were taken on a GelDoc XR (USA).

2.3.2. Amplification of 16S rDNA genes and denaturing gradient gel electrophoresis (DGGE)

A 16S rDNA gene fragment (169–194 bp in length) was amplified by PCR using a universal primer complementary positioned at 517–534 (59-ATTACCGCGGCTGCTGG-39) and a bacterial primer positioned at 341–358 with a 40-bp GC clamp (underlined) (5′-CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCACGGGGGGCCTACGGGAGGCAGCAG-3′) (Muyzer et al., 1993) following the protocol of Riemann et al. (1999). In brief, after an initial denaturation for 5 min at 95 °C, samples were amplified by touchdown PCR [94 °C, 5 min; 94 °C, 0.5 min (decreased 1 °C every cycle); 72 °C, 0.5 min, 10 cycles; 94 °C, 0.5 min; 55 °C, 0.5 min; 72 °C, 0.5 min; 20 cycles; 72 °C, 10 min].

PCR products were precipitated with ethanol, resuspended in TE buffer, PCR product was loaded on 8% polyacrylamide gels (acrylamide–*N*, *N*9-methylenebisacrylamide [37:1]) containing denaturant gradients of 35–55% from top to bottom (where 100% is defined as 7 mol L^{-1} urea and 40%, v/v formamide). Electrophoresis was performed with the Gel Doc 2000 digital gel documentation system (Bio-Rad), using 1 × TAE running buffer at 60 °C for 7 h at 110 V. Gels were stained for 30 min in ethidium bromide, and photographed with UV transillumination.

2.3.3. Sequencing and phylogenetic analysis

Dominant DGGE bands were excised using a sterile razor blade, and DNA was eluted by incubating in 50 μL sterile water and left in a 4 °C fridge overnight. The supernatant was used as a template to amplify partial 16S rDNA with primers 341F without GC-clamp and 517R, then sequenced. Sequences were aligned with closely related 16S rDNA sequences from GenBank. Phylogenetic analysis was performed using MEGA version 4 (Tamura et al., 2007) after multiple data alignment with CLUSTAL X (Thompson et al., 1997) by DNAMAN (version 5.1; Lynnon Biosoft).

2.3.4. Data analysis

The DGGE banding profiles were analyzed with Quantity One gel documentation software (Bio-Rad, USA) to determine the contribution of each band to the overall intensity of the bands in one lane and in the same position in the whole DGGE image, respectively. The lanes were analyzed site by site to make the results more comparable. After the bands structure of related lanes from different sites were reported by Quantity One, the data was more deeply analyzed with principal component analysis (PCA) by MATLAB 7.10 (MathWorks, USA) to identify the key bacterial species that contribute to the bacterial community structural differences at different bloom stages. PCA is a statistical tool that