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Analysis of sterols in selected bloom-forming algae in China

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ABSTRACT

Sterols, a group of stable lipid compounds, are often used as biomarkers in marine biogeochemical studies to indicate sources of organic matter. In this study, sterols in 13 species of major bloom-forming algae in China, which belong to Dinophyceae, Bacillariophyceae, Ulvophyceae, and Pelagophyceae, were analyzed with gas chromatography-mass spectrometry (GC–MS) to test their feasibility in representing different types of harmful algal blooms (HABs). It was found that (24*Z*)-stigmasta-5,24-dien-3β-ol (28-isofucosterol) was a major sterol component in green-tide forming macroalga *Ulva prolifera*. In bloom-forming dinoflagellates *Alexandrium* spp., *Prorocentrum micans* and *Scrippsiella trochoidea*, (22*E*)-4α,23-dimethyl-5α-ergost-22-en-3β-ol (dinosterol) was detected in addition to cholest-5-en-3β-ol (cholesterol), (22*E*)-ergosta-5,22-dien-3β-ol, (22*E*)-stigmasta-5,22-dien-3β-ol and other minor sterol components. In brown-tide forming pelagophyte *Aureococcus anophagefferens*, (24*E*)-24-propylcholesta-5,24-dien-3β-ol ((24*E*)-24-propylidenecholesterol) and (24*Z*)-24-propylcholesta-5,24-dien-3β-ol ((24*Z*)-24-propylidenecholesterol) were detected together with cholesterol, (22*E*)-stigmasta-5,22-dien-3β-ol, stigmast-5-en-3β-ol and campest-5-en-3β-ol. Among the selected bloom-forming diatoms, *Chaetoceros* sp. and *Pseudo-nitzschia* spp. only produced cholesterol, while *Cylindrotheca closterium* produced solely (22*E*)-ergosta-5,22-dien-3β-ol. Sterol content in four bloom-forming algal species correlates well with their biomass or abundance. It's proposed that 28-isofucosterol could serve as a promising biomarker for green algae in green-tide studies. Dinosterol and (24*Z*)-24-propylidenecholesterol can be used as potential biomarkers to represent bloom-forming dinoflagellates and pelagophytes, while (22*E*)-ergosta-5,22-dien-3β-ol is not a good indicator for diatoms.

1. Introduction

Harmful algal blooms (HABs), with an apparent global increase in both frequency and intensity, have become important marine environmental issues in the last several decades (Hallegraeff, 1993). Intensive HABs led to multiple negative impacts on marine ecosystems and human health, and caused severe damages on aquaculture, fisheries, and tourism industries (Anderson, 1997).

The occurrence of harmful algal blooms reflects significant changes of phytoplankton communities. To have a better understanding on the long-term changes of phytoplankton community and harmful algal blooms, investigations based on traditional methods like microscope observation could be used. But this is often hampered by the limited data sets and intensive field sampling efforts. To trace the changes of phytoplankton communities, biomarkers preserved in marine sediments, such as sterols (Volkman et al., 1998; Menzel et al., 2003), phytoplankton pigments (Harris et al., 1996), biogenic silica (Nelson et al., 1995), fatty acids (Canuel et al., 1997) or total organic carbon and nitrogen (Balakrishna and Probst, 2005), have been adopted. Through the analysis of these biomarkers, the structure of phytoplankton community in a specific time, or the long-term changes of specific algal groups, can be deduced.

Among those biomarkers, sterols are often used in biogeochemical studies (Tian et al., 1992; Fattore et al., 1996). Up to date, nearly 160 natural sterols have been detected. Sterols are commonly present in eukaryotic organisms, and are essential components of membranes to maintain the stability of cellular lipid bilayers. They are also involved in biological reproduction and development for signal transduction, and serve as precursors of hormones in many organisms (Hartmann, 1998). Sterols and their

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derivatives present in marine sediment are often used as biomarkers to trace the source of sedimentary organic matter, or assess the pollution status (Volkman et al., 1998). For instances, (22*E*)-stigmasta-5,22-dien-3β-ol (stigmasterol) and stigmast-5-en-3β-ol (sitosterol) have been suggested as indicators of terrestrial higher plants, while 5β-cholestan-3β-ol (coprostanol) have been used to indicate the pollution status of sewage discharge and the impacts on environmental quality in coastal waters (Readman et al., 2005).

Sterols have great potential in harmful algal bloom studies. Some sterols are wide spread among the different groups of algae, while others appear to be restricted in a few classes (Volkman, 1986, 2006). Several sterols have been suggested to represent specific groups of algae, for example, (22*E*)-ergosta-5,22-dien-3β-ol for diatoms (Werne et al., 2000), (22*E*)-4α,23-dimethyl-5α-ergost-22-en-3β-ol (dinosterol) for dinoflagellates (Volkman, 2003), and (24*Z*)-24-propylcholesta-5,24-dien-3β-ol ((24*Z*)-24-propylidenecholesterol) for pelagophyte *Aureococcus anophagefferens* (Giner et al., 2001; Giner et al., 2009). Yu et al. (2013) selected three sterols, epi-brassicasterol, dinosterol and alkenones representing diatoms, dinoflagellates and coccolithophores, to evaluate their contributions to phytoplankton communities, and the long-term changes of phytoplankton communities in the Prydz Bay were demonstrated based on sterols. Besides, sterols have been used to reconstruct productivity of phytoplankton communities (Menzel et al., 2003). The application of sterols in studies of marine ecology, however, are often hampered by the lack of their commercial standards.

In China, harmful algal blooms have been frequently recorded in the coastal waters, and different types of blooms were reported. For instances, red tides of diatoms formed by *Skeletonema costatum* and *Pseudo-nitzschia* spp. have been recorded for a long time in many bays and estuaries (Zhou et al., 2001), although most of them have no apparent deleterious impacts. Recurrent blooms of dinoflagellates, including *Prorocentrum donghaiense*, *Karenia mikimotoi* and *Alexandrium catenella*, have been reported in the sea area adjacent to the Changjiang River estuary from the beginning of the 21st century (Zhou and Zhu, 2006; Zhou et al., 2008), and lead to significant impacts on both mariculture industry and natural ecosystems. Large-scale green tides formed by green alga *Ulva prolifera* occurred for ten consecutive years in the southern Yellow Sea from the year 2007 (Zhou et al., 2015). Brown tides caused by *A. anophagefferens* have been recorded in the coastal waters of Qinhuangdao in the Bohai Sea since 2009 (Kong et al., 2012).

Despite many studies performed on harmful algal bloom events in China, understandings on their ecological consequences are still limited. For example, large scale green tides have been reported in southern Yellow Sea for ten years (Zhou et al., 2015), and the biomass of floating green algae in the Yellow Sea could reach several million tons in a single green tide event (Liu et al., 2010). During the green tides, the floating green algae would be transported a long distance from the Subei Shoal to the coastal region of Shandong province under the force of wind and current (Bao et al., 2015). Part of green algae accumulated along the coastal line will be removed by local governments, but a large amount of green algae will deposit into the bottom under unfavorable environmental conditions. The formation, development and final dissipation of green tides will affect the biogeochemical processes of nutrients, and inevitably impact phytoplankton communities and marine ecosystems (Wang et al., 2012). But this process is still poorly understood due to the lack of knowledge on the final destination of floating green algae in the sea. With an effective biomarker to trace the area affected by the green algae, it would be expected to have a better understanding on the ecological consequences of green tides in the Yellow Sea.

For the sea areas with recurrent red tides or brown tides, the changes of phytoplankton communities associated with these harmful algal blooms are also not very well understood, due to the lack of long-term monitoring data in those areas. The adoption of sterols specific for different algal groups, together with other biomarkers, would improve the understandings on long-term changes of HABs and their ecological consequences. Therefore, the sterols in several most representative bloom-forming algal species in China were analyzed with gas chromatography coupled with mass spectrometry, and the relationship between sterol content and algal biomass were also examined. It was aimed to test the feasibility of specific sterol biomarkers in representing different HABs to support studies on their changes and ecological consequences.

2. Materials and methods

2.1. Bloom-forming algae used in the experiments

Altogether 13 representative bloom-forming algal species (19 strains) were studied in this experiment, including dinoflagellates *Scrippsiella trochoidea*, *Alexandrium minutum*, *Alexandrium pacificum* (previously identified as either *A. tamarense* or *A. catenella* based on their morphological features) and *Prorocentrum micans*, diatoms *Skeletonema costatum, Cylindrotheca closterium, Chaetoceros* sp., *Pseudo-nitzschia pungens, P. delicatissima* and *P. multistrata*, green algae *Ulva prolifera*, and pelagophyte *A. anophagefferens* and *Aureoumbra lagunensis*. Most of the strains were isolated from the coastal waters of China, while some related species or strains collected in sea areas outside China were also included. The algae were identified or confirmed by their morphological features observed under the light microscope or electron microscope, sequences of ribosomal RNA gene, and pigment analysis in the Institute of Oceanology. Details of the algae species and strains were listed in Table 1. The algae were cultured in Erlenmeyer flasks with different culture media. Diatoms were cultured with f/2 medium (Guillard and Ryther, 1962), dinoflagellates and green algae with f/2-Si medium, and pelagophytes with L1-Si medium (Guillard and Hargraves, 1993). Natural seawater pumped from a clean sea area near the Institute of Oceanology in Qingdao was used to prepare the culture media after filtration with glass filber membranes (GF/C, 48 mm, Whatman), and the salinity of seawater was 30 ± 1 and pH was 8.2–8.3. The media were autoclaved at 120 °C for 20 min before inoculation of algae. All the cultures were maintained in the laboratory at 20 ± 1 °C, and light intensity was 50 μmol m^{-2} s^{-1} with a light:dark cycle of 14:10.

2.2. Sample preparation and analysis

2.2.1. Chemical reagents and standards

All the experimental reagents, including methanol, hexane, and dichloromethane, were purchased from Merck (Germany). The standards, including cholest-5-en-3β-ol (cholesterol), (22*E*)-stigmasta-5,22-dien-3β-ol (stigmasterol), stigmast-5-en-3β-ol (sitosterol), campest-5-en-3β-ol (campesterol), (22*E*)-ergosta-5,22-dien-3β-ol (brassicasterol), (24*E*)-stigmasta-5,24-dien-3β-ol (fucosterol), 5β-cholestan-3β-ol (coprostanol), hexamethylbenzene, 5α-cholestane and the derivatization reagent bis(trimethylsilyl) trifluoroacetamide and trimethylchlorosilane (99% BSTFA + 1% TMCS), were purchased from Sigma-Aldrich (Steinheim, Germany). Other related sterols, including (24*Z*)-stigmasta-5,24-dien-3β-ol (28-isofucosterol), dinosterol, (24*E*)-24-propylidenecholesterol and (24*Z*)-24-propylidenecholesterol are not commercially available. Chemical structures of the representative sterols were illustrated in Fig. 1.