Removal effect of algicidal modified clay on *Phaeocystis globosa* blooms in culturing enclosure experiments: A short communication

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Abstract. With the increased scale of marine aquaculture in the Beibu Gulf, as well as accelerating urbanization and industrialization, frequent harmful algal blooms (HABs) have occurred in this area, especially those formed by *Phaeocystis globosa* in the past several years. As the *P. globosa* bloom has been a serious marine ecological disaster in the Beibu Gulf, research on quick and effective methods to eliminate *P. globosa* blooms is a hot research topic. In this study, the bacteria *Streptomyces yatensis* B4503 combined with modified diatomite was used to prepare algicidal modified clay, which was then used to study the removal effect on *P. globosa* blooms in field culture enclosures. The results showed that after 6 h of treatment with algicidal modified clay, compared with the blank control group, the cell density and chlorophyll a content of *P. globosa* decreased by 26.86% and 64.03%, respectively, and they decreased by 75.23% and 84.81%, respectively, after 24 h. The study indicated that algicidal modified clay can be applied to eliminate HABs caused by *P. globosa* in coastal water.

1. Introduction

Guangxi is one of the most important mariculture provinces in China, and the high-quality water in the Beibu Gulf provides advantageous conditions for the rapid development of aquaculture [1]. The total aquaculture area in the Beibu Gulf reached 550 km² during the 12th five-year plan period [2, 3]. However, with the enlargement of marine aquaculture in the Beibu Gulf, as well as accelerating urbanization and industrialization, eutrophication has taken a rapid and negative turn, which has led to frequent harmful algal bloom (HAB) events with increasing scale, duration and harmful species, causing great damage to Guangxi marine aquaculture and having serious impacts on the healthy sustainable development of mariculture [4–7]. HABs, especially those caused by toxic algae, often lead to the death of farmed animals and even cause great harm to humans [4, 8]. Therefore, the prevention and control of HABs has become a hot topic. *Phaeocystis globosa*, which has frequently caused algal blooms in the Beibu Gulf of Guangxi since November 2011, has become a typical harmful species in this sea area [9]. The blooms caused by *P. globosa* may last for two months, which seriously affects the marine aquaculture industry, marine ecological environment and coastal natural landscape. Therefore, it is particularly important to find a rapid and effective method to eliminate *P. globosa*.

At present there are physical, chemical and biological methods to control HABs. In addition to eliminating bloom organisms, the key problem is to avoid secondary pollution. *Phaeocystis globosa* has a heteromorphic life cycle, forming tiny flagellated solitary cells as well as giant colonies. This complex life cycle makes it difficult to kill the blooms by physical isolation method which is the most...
environmentally friendly way. Chemical and biological methods show advantages of removal effects but also have disadvantages such as overreliance on manual labor and secondary pollution. Thus it's critical to find solutions which are environmentally friendly and practical. Spreading of clay or modified clay is recognized internationally as an effective method [10, 11]. However, there are still some problems, such as large consumption of materials, secondary HABs caused by the germination of algal cysts, and the influence of sediment and its toxins on benthic organisms [12–14]. Studies on the relationship between algae and bacteria have resulted in the isolation of actinomycetes capable of inhibiting or killing HAB species. The reported algicidal actinomycetes are mostly Streptomyces species [15]. Actinomycetes can produce various bioactive substances and are considered as potential and effective biological agents to eliminate HAB species [16]. Thus, the combination of algicidal bacteria and the modified clay method is expected to improve elimination efficiency of HABs without causing secondary blooms or secondary pollution. In this study, a P. globosa bloom was simulated in field culture enclosures, and then algicidal modified clay made of modified clay combined with an algicidal bacterium was used to eliminate the bloom to provide a new idea for the control of P. globosa blooms in the sea. Material and Methods

2. Materials and Methods

2.1. Isolation and identification of Streptomyces yatensis B4503
The bacterium Streptomyces yatensis B4503 was isolated from rhizosphere soil and sampled from Guangxi Shangkou Mangrove Nature Reserve (108°36′14″E, 21°44′53″N). Fresh plant samples were air-dried and washed thoroughly, then cut into pieces (4–5 cm). The dried plant samples were surface sterilized by using 5% (w/v) sodium hypochlorite and 75% (v/v) ethanol as described by Li et al. [17]. After drying, the surface-sterilized samples were smashed and spread on different media to isolate the bacterial strains. The genomic DNA of strain B4503 was extracted using 10% (w/v) chlexe-100 solution (biorad Laboratories, Inc.) after boiling for 10 min. The 16S rRNA gene was amplified as described by Bai et al. [18]. The EzBio-Cloud Identify Service (https://www.ezbiocloud.net/identify) was used to identify similarities of 16S rRNA gene sequence [19, 20]. Our previous experiments showed that B4503 had a good algicidal effect on P. globosa in the laboratory (data not shown).

2.2. Preparation of algicidal modified clay
Diatomite (1000 g) was dispersed in 5000 mL of Al(NO₃)₃ solution (12.7 mg/mL), and after magnetic stirring to achieve homogenization, 3000 mL of NaOH (0.4 mol/L) was added slowly. Then, the mixed suspension was filtered, and diatomite was laid on a tray and placed in a 60°C air-blowing drying oven for complete drying. The B4503 fermentation broth was incubated at 28°C and 180 rpm for 8 d. The modified diatomite was added to the fermentation broth at a ratio of 1:5 (m:V) and magnetically stirred at room temperature for 7 h, and then the suspension was filtered. The filtered, modified diatomite containing bacteria was laid on a tray, kept at -80°C for 4 h, and then freeze-dried. The final product was algicidal modified clay.

2.3. Time and site
The field enclosure culture was conducted in December 2019. The place was selected in the eastern sea area of Dongwan, Fangchenggang (Figure 1, 108°22′48.77″E, 21°37′16.92″N).

2.4. Experimental design
The culturing enclosures were fixed before the experiment, and approximately 1000 L of field water, 75 g of NaNO₃ and 5 g of NaH₂PO₄·H₂O (according to f/2 medium) were pumped into each enclosure. After mixing, 15 L of of P. globosa at the exponential growth stage was added to the enclosures. The 6 enclosures were divided into three groups: the experimental group, unmodified clay control group and blank control group, with two parallel subgroups in each group. Water samples in the enclosure were taken at the same time every day to observe the changes in the phytoplankton population structure and
cell density, the colony density and diameter of *P. globosa* and environmental physical and chemical factors (temperature, pH, chlorophyll *a* and DO) were analyzed to detect indicators. At the same time, water outside the enclosures was sampled as a control. After *P. globosa* was observed to reach a bloom density >10^7 cells/L, 500 g of algicidal modified clay was added to the experimental treatment group, and 500 g of unmodified clay was added to the clay control group, while the blank control group did not undergo any processing. Samples were taken at 0 h, 6 h and 24 h after treatment.

![Figure 1](image.png)

**Figure 1.** The figure showing the site of culturing enclosures for this research (the red star)

2.5. Measurement of indicators

Seawater samples of 1 L were mixed with 1.5% acid Lugol’s solution and concentrated gradually to 20 mL. A 100 μL concentrated sample was transferred to a 0.1 mL counting chamber for phytoplankton qualitative and quantitative analysis. Cells of *P. globosa* and other phytoplankton species were identified and quantified under an inverted microscope (Nikon Ti-S, Japan). Density and diameter of *P. globosa* colonies were measured under the same microscope mentioned above.

Temperature, DO and pH were read directly from multi-parameter controller (JFE AAQ171, Japan) in field. The concentration of chlorophyll *a* was determined using a fluorescence spectrophotometer (PE LS-55, America) following extraction using 90% acetone in the dark for 24 h according to Parsons et al. [21].

3. Result and Discussion

Before the start of the experiment, the phytoplankton in seawater were mainly diatoms such as *Skeletonema* sp. and *Pseudo-nitzschia delicatissima*. The total cell abundance of phytoplankton was 6.5×10^4/L, and that of *Skeletonema* sp. was 1.8×10^4/L, accounting for 27.7% of the total cell abundance. The HABs caused by *P. globosa* are different from those of most species, and their particularity is reflected in their complex heteromorphic life history; that is, their cells can exist in two forms: free single cells and colony cells. During the outbreak of blooms, the alga is mainly colony cells [22]. Because *P. globosa* has a special glial vesicle structure, it is necessary to observe the changes in the size and number of colonies at the same time the total cell density was monitored. The number and diameter of *P. globosa* colonies increased gradually in each group from 1–3 d (Fig. 2a-b). On d 4, the amount of *P. globosa* began to increase rapidly, and the total cell density increased to 5.56×10^7 cells/L and reached a mean value of 8.53±0.63×10^7 cells/L on d 5 (Fig. 3a). The color of the water in the enclosure became
brown, and different treatments for each group were started. In contrast with $P. \text{globosa}$ cell density, the chlorophyll $a$ content began to increase greatly on $d$ 3 (Fig. 3b), with an average value of 16.61±2.82 μg/L, which was 3 times that on day 0. This observation was due to the massive reproduction of diatoms such as $\text{Skeletonema}$, $\text{Chaetoceros}$ and $\text{Leptocylindrus danicus}$, which benefited the supplementation of nutrients. The chlorophyll content increased to 71.16±2.82 μg/L on $d$ 5.

**Figure 2.** Changes in the density (a) and diameter (b) of $\text{Phaeocystis globosa}$ colonies over time. The black arrow shows the time at which the three groups began to receive different treatments. Control means the group did not undergo any processing; clay control means both enclosures of this group contained 500 g of unmodified clay; experimental means 500 g of algicidal modified clay was added to this group.

**Figure 3.** Changes in $\text{Phaeocystis globosa}$ cell density (a) and Chl $a$ concentration (b) over time. The black arrow shows the time at which the three groups began to receive different treatments. Control means the group did not make any processing; clay control means both enclosures of this group contained 500 g of unmodified clay; experimental means 500 g of algicidal modified clay was added to this group.

After 6 h of treatment with algicidal modified clay, compared with the blank control group, the cell density and chlorophyll content of $P. \text{globosa}$ decreased by 26.86% and 64.03%, respectively, and after 24 h, they decreased by 75.23% and 84.81%, respectively. With the growth of $P. \text{globosa}$, the seawater
in the enclosures turned brown but became clear again when algicidal modified clay was added. This result showed that algicidal modified clay has a good effect on the control of *P. globosa* blooms. At present, there are many methods for the removal of *P. globosa*, including modified clay [23, 24]; the extract of a substance such as rice stalks, wheat stalks or calamus [25, 26]; chemical reagents such as chlorine dioxide, nonylphenol and flavonoid compounds [27–29]; and algicidal bacteria [30, 31]. However, most of the methods mentioned above are focus on algal lysis mechanism and removal efficiency base on laboratory experiments, and modified clay has been applied in field research and has achieved good results [23]. Algicidal bacteria are microorganisms that can directly or indirectly destroy the cell structure or change the physiological state of algae to inhibit or kill algal cells [32, 33]. Both B4505 cells and its culture solution show high algicidal effect on *P. globosa* cells in the laboratory (data not shown). Combining the advantages of modified clay and algicidal bacteria may help to eliminate the problems of using modified clay, such as the large consumption of materials, secondary HAB caused by the germination of algae cysts, and the influence on benthic organisms. Diatomite was modified in this study because modified clay has been affirmed to control HABs, and the porous structure of diatomite may absorb more algicidal bacteria and algino-lytically active substances to improve the efficiency of the algicidal effect.

4. Conclusion
Combining the advantages of modified clay and algicidal bacteria may be a quick and effective method to eliminate *P. globosa* blooms in the field, and the modified diatomite is a natural source of material for environmental protection. This method breaks through the limitation of algicidal bacteria researched only in the laboratory and helps to solve the problems of using modified clay, thus providing a new idea for the study of the removal of HABs.

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