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Fast-growing algicidal *Streptomyces* sp. U3 and its potential in harmful algal bloom controls

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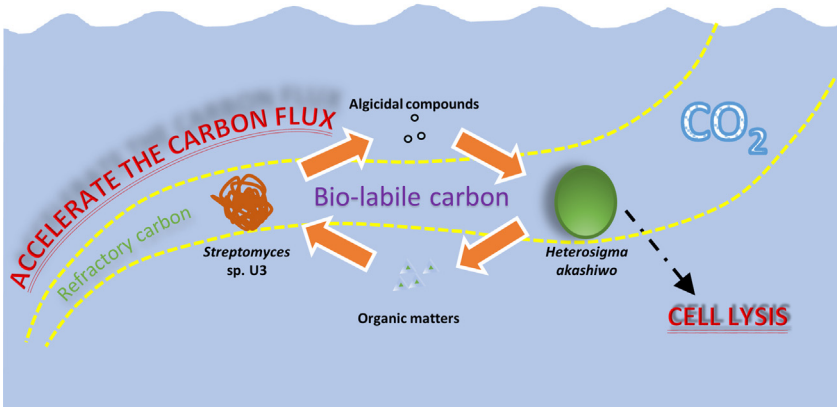
HIGHLIGHTS

- *Streptomyces* sp. U3 could kill *H. akashiwo* by producing active compounds.
- U3 can utilize alga-derived nutrition to support its growth and achieve constant algicidal effect.
- The algicidal compounds possibly caused the autophagy of algal cells.
- The mycelia of U3 could convert bio-labile DOM into refractory DOM during the algicial process.

GRAPHICAL ABSTRACT



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ABSTRACT

To find the potential algicidal microorganisms and apply them to prevent and terminate harmful algal blooms (HABs), we isolated an actinomycete U3 from Mangrove, which had a potent algicidal effect on the harmful alga *Heterosigma akashiwo*. It could completely lyse the algal cells by producing active compounds, which were highly sensitive to high temperature and strong alkaline, but resistant to acid. One μg/mL of crude extract of the fermentation supernatant could kill 70% of *H. akashiwo* cells in 3 d. Unlike most of the other known algicidal *Streptomyces*, U3 showed strong ability of proliferation with the algal inclusion as the nutrient source. The washed mycelial pellets also gradually exhibited significant algicidal effect during the visible growth in the algal culture. It suggests that U3 could efficiently absorb nutrients from algal culture to support its growth and produce algicidal compounds that might cause the autophagy of algal cells. Therefore, applying U3, as a long-term and environmentally friendly bio-agent to control the harmful blooms of *H. akashiwo*, would be effective and promising. And the decrease of

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bioavailable DOM and increase of bio-refractory DOM during the algicidal process of U3 provided new insights into the ecological influence of algicial microorganisms on marine ecosystem.

1. Introduction

With the continuous development ofeconomy and human activities, harmful algal blooms (HABs) caused by eutrophication occur more and more frequently [1]. HABs have been considered as ocean disasters that can destroy the marine ecological environment, affect the survival of marine organisms, damage coastal aquaculture industries and even threaten human health [2]. *Heterosigma akashiwo* is widely distributed in both northern and southern hemispheres, because of its eurythermy and euryhalinity [3]. It is one of the most notorious red tide-causing algae and the blooms have caused heavy damage in Canada, Korea, Japan and other countries almost every year [4,5]. Therefore, it is urgent to search for potent and harmless methods to control the harmful blooms of *H. akashiwo*.

Compared with the physical and chemical methods, biocontrol technologies for the regulation of HABs have become the research hotspots because of their potential effectiveness, species specificity, and eco-friendly characteristics [6]. Decades of studies have revealed that algicidal microorganism is one of the most promising biological agents because of its great variety, large quantity and extensive distribution [7]. There are two ways about how microorganisms act on target algal cells: direct attack, which needs cell-to-cell contact [8,9]; indirect attack, where the interaction between microorganisms and algal cells is mediated by the algicidal compounds, such as antibiotics from actinomycete and fungi [10,11]. Therefore, both algicidal microorganisms and their algicidal compounds have been considered as effective agents that could control HABs and following marine disasters.

At present, studies on the death process of algae triggered by algicidal compounds or microorganisms are relatively few and there is no uniform conclusion about algicidal mechanism. The death processes of algae triggered by different algicidal microorganisms or algicidal compounds seemed different. However, the rise of oxidative stress, the damage of cellular structures, and the suppression of photosynthesis could be often observed in most of the studies concerning the algicidal mechanism [12]. They were also regarded as the features of programmed cell death (PCD) [13,14]. However, whether these stresses would cause PCD were still debatable. Not to mention the unclear meaning of PCD in unicellular organisms, apoptosis, necrosis and autophagy could only explain part of the phenomenon. More specialized studies are needed to make a comprehensive assessment of the significance of PCD under the circumstance.

Ocean is one of the most important carbon pools in the world, which plays a significant role in the global carbon cycle. Dissolved organic matters (DOMs), which participate in the biogeochemical carbon processes deeply, have an approximately equivalent stock as the atmospheric CO_2 [15] and are one of the largest biologically active carbon reservoirs. Most of DOMs are molecularly-uncharacterized components because of their diversity and complicated structures [16], thus it is estimated that only 1% ~ 10% of DOMs can be characterized as specific compounds [17]. However, different fractions of DOMs have various optical properties. Colored dissolved organic matters (CDOMs) can absorb light at both ultraviolet (UV) and visible wavelengths [18]. A subfraction of CDOMs that emit induced fluorescent light is called fluorescent dissolved organic matter (FDOM) [19]. Along with the development of fluorescence excitation emission matrix (EEM) spectroscopy and multivariate data analysis techniques, fluorescence properties could be characterized qualitatively and quantitatively and then used as a tracer for the dynamics and characteristics of the total DOM pool [20–22]. There are two main groups of FDOMs: protein-like compounds and humic-like compounds [23]. FDOMs can be formed or degraded through various ways. Recent studies have shown that the abundance of phytoplankton can directly contribute to the change of FDOM composition [24]. Meanwhile, bacteria also will consume and produce various FDOMs [25,26]. However, there is no report about how the composition and abundance of FDOMs change when algal blooms were interfered by algicidal microorganisms.

In this study, a potent algicidal *Streptomyces* U3, which could produce algicidal compounds to remove several harmful algae, was isolated from Zhangjiangkou Mangrove National Nature Reserve. U3 could also efficiently absorb nutrients from algal culture to support its growth and production of algicidal compounds. And the changes of FDOMs during the algicidal process of U3 were also studied to demonstrate potential ecological impact.

2. Materials and methods

2.1. Algal cultures

Cultures of *H. akashiwo* and other algal species including *Platymonas subcordiformis*, *Chlorella autotrophica*, *Platymonas helgolandica*, *Dunaliella salina*, *Nannochloropsis*, *Alexandrium minutum*, *Prorocentrum donghaiense*, *Scrippsiella trochoidea* XM01, *Thalassiosira pseudonana*, *Phaeodactylum tricornutum*, *Nitzschia closterium*, *Amphiprora alata*, *Thalassiosira weissflogii*, *Skeletonema costatum*, *Phaeocystis globosa*, *Microcystis aeruginosa* 7806, *Microcystis aeruginosa* 7803, *Microcystis aeruginosa* 7820, *Microcystis aeruginosa* 1752, *Synechocystis* sp. 6803 that used in this study were supplied by the State Key Laboratory of Marine Environmental Science, Xiamen University. All algae for the experiments were cultured in fresh sterilized f/2 medium (without silicate) at 20 ± 1 °C and the light condition was set to a 12 h: 12 h light-dark cycle with the light intensity of 50 μmol photons m^{-2} s^{-1}. They were inoculated weekly to ensure that the algae were tested in exponential phase.

2.2. Isolation and screening of algae-lysing microorganisms

Sediment samples collected from Fujian Zhangjiangkou Mangrove National Nature Reserve, China (23°53′–23°56′ N and 117°24′–117°30′ E) were suspended in sterilized seawater and then serially diluted. Diluted suspension was spread onto the Zobell marine agar 2216 and incubated for 72 h at 28 °C. Morphologically different colonies were picked and further streaked several times in order to obtain pure cultures. The algae-lysing microorganisms were screened on the plates containing algal culture and judged by the inhibition zones. Single colony of each isolate was transferred to Zobell marine broth for 72 h cultivation (150 rpm, 28 °C), and then inoculated in double-layer plates, which consisted of first layer with only f/2 medium (1.5% agar) and over layer with 2 mL algae cells suspension and 3 mL f/2 medium (0.7% agar). After incubation under light for 7 d, plaques suggest the existence of algicidal