

Research Progress in Dinoflagellate Prevention and Control Through Algae-lysing Bacteria

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Abstract. As environmental pollution intensifies, the frequency of dinoflagellates incidents gradually increases at home and abroad in recent years. It causes serious harm to aquatic ecological environment, aquatic animals, tourism industry and human health. This paper introduces the features and harm of dinoflagellates, and related methods to prevent and control dinoflagellates. Among them, the prevention and control of dinoflagellates by algae-lysing bacteria, including species and dissolve mechanisms of algae-lysing bacteria, and the prospect of algae control through algae-lysing bacteria, are mainly introduced.

Introduction

Microalgae are one of the most important components in the ecosystem of shrimp ponds. They play an important role in material circulation and energy flow in balance aquaculture ecosystem. [1] Species composition and abundance variation of planktonic microalgae not only determine the bait base and dissolved oxygen level of ponds, but also directly or indirectly affect related biological and chemical processes. [2] Most microalgae are autotrophic amphitrophy, a few of them are heterotrophic [3]. By using nutrients in the water, microalgae can cause water bloom and red tide. In lakes, reservoirs and ponds, dinoflagellate can become the dominant genus through sudden proliferation and aggregation, and then change the color of water body. The phenomenon is called as dinoflagellate bloom. At present, water blooms have become a worldwide public nuisance [4]. The most common and most influential kind of water bloom is dinoflagellate bloom. As one of the most effective methods to control algae, algae-lysing bacteria should be used to control these phenomena. At present, there is little study on prevention and control of *Peridinium perardiforme* and *Peridiniopsis penardii* through algae-lysing bacteria. It is necessary to find algae-lysing bacteria which can be used to dissolve common seen dinoflagellate, and to investigate solutions on dinoflagellate prevention and control.

Harm of Dinoflagellate

With the increasing contamination in recent years, dinoflagellate blooms have produced great negative influence on water environment, tourism industry, human health and aquaculture. [7] For instance, *Oodinium acidophilum* can cause Oodinirosis of fish. Summer fingerlings which have just moved into the pool, and fish which has just moved into the wintering ponds are most vulnerable to this disease. Grass carp is particularly sensitive [8]. In Florida and California coast of The United States, water become brown because of the rapid reproduction of gyrodinium and vine ditch algae, which killed millions of fish and other marine animals [9]. In Taiwan, 1989, massive *Pennaeus monodon* are killed in red tide caused by *Alexandrium tamarense* [10]. In Xiamen seas, 1994, all shrimps in culture ponds were dead within a few days due to *Alexandrium tamarense* [12]. From a 96 hours acute experiment, Xue-hong Wang found that, the semilethal density of *Alexandrium tamarense* on larval whiteleg shrimp is 7500/mL [12]. Zhong-xiu Liang and his colleagues found that, *Alexandrium tamarense* can cause oxidative damage of *Fenneropenaeus chinensis* [13]. Research

team lead by Zhuo-jia Li found that the dominant dinoflagellate, *Peridinium pusillum*, effects the survival rate of larval whiteleg shrimp [14-16].

It has been found that, different species of dinoflagellates have different mechanisms, and can produce different kinds of damage. The first environmental toxin caused by dinoflagellate is the phenomenon of hypoxia caused by explosive proliferation of dinoflagellate; then, some minor environmental toxins are created, such as certain kinds of dinoflagellates and *Heterosigma carterae*, which can not only coat fish gills, but also damage the epithelial cells of fish gills through hemolysin glycolipids, and lead to suffocation of fish.

The most important environmental toxin caused by dinoflagellate is microcystin [17, 18]. According to differences in symptoms and mechanism, microcystin can be classified as paralytic shellfish poisoning (PSP), diarrhetic shellfish poisoning (DSP), neurotoxic shellfish poisoning (NSP) and ciguatera (*Ciguatera*). [9] The effects of *Alexandrium tamarense* on the survival rate of larval whiteleg shrimp are caused by paralytic shellfish toxins [12].

Progresses in Algae-lysing Bacteria Research

Now the eutrophication of water body is becoming increasingly serious, harmful algal blooms frequently happen. The exploration of effectively ways to control and remove algae is extremely urgent. As a new biological method, algae-lysing bacteria shows wide application prospect. Algae-lysing bacteria can control dinoflagellate through direct or indirect attacks, such as competition for oxygen or direct parasite. Algae-lysing bacteria have good control effects, which are especially suitable to be employed in initial stage of water bloom. They can control algal biomass and prevent algal blooms in short term.

Features of algae-lysing bacteria. Algae-lysing bacteria are a kind of bacteria which can inhibit the growth of microalgae or kill microalgae through direct or indirect dissolving of algae cells [19]. Since some water blooms cannot be effectively controlled through physical, chemical or other biological methods, algae-lysing bacteria method, as a kind of biological solution on water blooms and red tide, has attracted more and more attention. In recent years, new kinds of algae-lysing bacteria are constantly reported abroad.

Types of algae-lysing bacteria. As early as 1924, Geitler reported that a kind *Polyangium* parasitium which can kill *Cladophora fracta* through parasitism. Different types of algae-lysing bacteria have been reported at home and abroad recently, mainly include: *Myxobacter* sp., *Cytophaga* sp., *Cellvibrio* sp., *Arthrobacter* sp., *Flexibacter* sp., *Bdellovibrio bacteriosus* sp., *Flavobacterium* sp., *Vibrio*, sp., *Saprospira* sp., *Pseudomonas* sp., *Sphingomonas* sp., *Alteromonas* sp., *Pseudoalteromonas* sp., etc. [20]

Algae-lysing mechanism of algae-lysing bacteria. Methods of dissolving bacteria is divided into direct and indirect ways. Direct ways mean that, algae-lysing bacteria directly attack the host, and contact with algae cells. They can even penetrate through the interior of algae cells. Indirect ways mean that, bacteria compete limited nutrition with algae, or dissolve bacteria through extracellular material they secreted. [28]. *Muricauda* in *Flavobacteriaceae* can inactive, expand and deform the algal cells of *Prorocentrum micans*. Materials in the cells then move to one end, and the algal cell eventually rupture. However, there are no algae-lysing activities in filtered fluid of bacterium suspension or bacterium suspension after high temperature heating treatment. The results show that *Muricauda* sp. use direct ways to dissolve algae [21]. Single cell of *Aquimonas* does not have algicidal effect, while its filtered fluid does. *CA* dissolve algae through releasing algicidal substances, which belongs to indirect ways [22].

Screening of algae-lysing bacteria. The screening of algae-lysing bacteria includes primary screening and secondary screening. In primary screening, algae liquids are spread to plates after gradient dilution, then cultured for a certain period of time under suitable conditions. Afterwards, bacterial colonies with different colors are separated and purified to obtain single bacterial colony. Then bacteria are crossed and saved with nutrient agar slant. Afterwards, the isolated bacteria are cultivated in sterile nutrient medium for an appropriate period of time. Then the bacteria absorb solutions of bacteria and algae. The bacteria which can make the algae liquid yellow are algae-lysing

bacteria. The secondary screening is similar to primary screening, but in secondary screening, samples should be taken out and fixed at regular intervals, and the morphological and number changes of algae cells should be observed [1]. The algae-lysing effects could be determined after secondary screening.

Detection and effectiveness evaluation of algae-lysing bacteria. Generally, the methods of bacteria detecting include plate count, direct microscopic count and fluorescence microscopy count. The plate counting method can only count the number of culturable bacteria. Direct microscopic count and fluorescence microscopy count can count the total number of bacteria in the system, but the specificity and sensitivity of these methods are low. In recent years, a number of new methods have been developed, include real-time quantitative PCR, PCR- denaturing gradient gel electrophoresis, fluorescence in situ hybridization, immunofluorescent test and microplate -MPN method [23].

The evaluation indexes of algae-lysing effects include, the forms and sizes of spots in solid algicidal experiment; algal cell number changes in liquid algicidal experiment; morphology changes of algal cells; changes of chlorophyll content; changes of chlorophyll fluorescence efficiency and changes in MDA content [1].

Conclusion and Foresight

For the dinoflagellates which are commonly seen in ponds, the following treatment measures should be adopted. Bacterial strains which can dissolve harmful dinoflagellate should be screened; characteristics of these strains should be analyzed to find their impacts on the growth of dominant dinoflagellate; algae-lysing mechanisms of proven strains should be identified; the effective concentrations of algicidal strains on different dominant dinoflagellate should be determined; their adaption ability to breeding environment should be identified to make preliminary judgment on the growth conditions of these strains. Thus, theoretical basis for the application of algae-lysing bacteria in regulating and controlling of dominant dinoflagellate in aquaculture environment can be provided, in order to regulate the community structure of planktonic microalgae in ponds, and maintain the balance of aquatic ecosystem.

The prospect of algae-lysing bacteria is very broad. Compared with other chemical, physical and biological methods, algae-lysing bacteria have advantages of safety, reliability, high efficiency and low environmental pollution. The research of algae-lysing bacteria will provide theoretical foundation for the prevention and control of harmful algae, and the protection of aquatic ecosystem balance. At the same time, the study on algae-lysing substances secreted by bacteria can provide new ideas for biological algicide exploration, which has important theoretical significance and potential application value. At present, there are many researches on algae-lysing bacteria in China, but these researches have not been applied to the practice of environmental management. Meanwhile, researches on the prevention and control of dinoflagellate are still in initial stage, technique and method breakthroughs are still needed. In view of advantages of algae-lysing bacteria and current research progresses, it can be concluded that, the isolation of safe algae-lysing bacterial strains with high efficiency and wide application range has great significance on prevention and control of dinoflagellates.

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