Allelopathic effects of Karlodinium veneficum on Rhodomonas salina

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Abstract

In recent years, Karlodinum veneficum algal blooms events had happened from time to time, killed a lot of fish and other aquatic organisms. At present, the formation mechanism of the K. veneficum is not clear, and the effects on other phytoplankton are rarely reported. This study was investigated the allelopathy of K. veneficum on Rhodomonas salina, and the mechanism of allelopathy was preliminary discussed. (1) The results showed that K. veneficum was able to inhibit the growth of Rhodomonas salina. And the inhibiting effects were positively correlated with the cell density of K. veneficum. (2) Four components of K. veneficum culture all inhibited the growth of R. salina. Specifically, the strongest effect was observed in the sonicated culture, followed by the whole-cell culture, the filtrate of sonicated culture, and the cell-free filtrate of whole-cell culture. (3) The allelopathy of K. veneficum on co-occuring phytoplankton observed to be regulated by nutritional conditions. The allelopathy of K. veneficum was enhanced significantly via increasing nutrient concentrations. Meanwhile, N-deficient and P-deficient conditions could enhance the toxicity of K. veneficum. The results of the study showed that K. veneficum may be obtained the important nutrients sources to promote the population growth from phytoplankton cells. Heterotrophy could be an important competing strategy for K. veneficum and thus plays an important role in the formation and maintenance of K. veneficum blooms.

Keywords

Harmful algae blooms, Karlodinum veneficum, Mixotrophy, Allelopathy.

1. Introduction

Harmful algae blooms (HABs) have increased frequently in recent years and caused a series of problems such as death of wild and cultured fishes and other aquatic organisms, which impacts seriously on aquaculture and loss of economy [1]. Meanwhile the HABs could cause degradation and problems of human health[2-4].

Mixotrophic dinoflagellate bloom was a significant trend when HABs happened[5]. Mixotrophic alga can adapt different environmental condition. They can synthesize organics by photosynthesis when the illumination and nutrition are sufficient. Otherwise, when such environmental factors are limited, the mixotrophic alga could ingest the organics from other organism for survival[6]. The complex nutritional strategy makes the mixotrophic alga could dominate in community. Recently, many studies indicated the HABs were mainly caused by mixotrophic algae reproduction due to the nutritional strategy reinforce the usage of nutrients, which promoted HABs finally [7].

Karlodinum veneficum belong to Karlodinium (genus), Kareniaceae (family), Gymnodiniales (order), Dinophyceae (class), Pyrrophyta (phylum) [8]. The morphology of *Karlodinum veneficum* could observed under optical microscope, which is small, oval without cell wall and swims fast. The transverse furrow divides the cell into two same part almost, four irregular cholorplasts distribute in top and bottom parts averagely. The length and width are 10 - 15 μm and 7 – 12 μm respectively [9]. *Karlodinum veneficum* is a mixotrophic algae and exists widely.

The mechanism of phytoplankton allelopathy has been concerned widely in academic world. Many researches showed Karlodinium genus had allelopathic effect on other organisms. Lu et al found that the co-culture of Karlodinium micrum and Prorocentrum donghaiense could inhibit Prorocentrum

donghaiense initially till the Population annihilation over control significantly. While the growth rate of Karlodinium micrum didn't show difference between control group [10]. The diatom Pseudo-nitzschia multiseries and P. pungens had allelopathic effect on phytoplankton in co-culture experiment [11]. Garcia-Portela, Maria et al newest research found Ostreopsis could inhibit the growth of Prorocentrum hoffmannianum and Gambierdiscus excentricus [12].

Karlodinium veneficum blooms frequently with poisoning incidents but few researches reported the allelopathic effect of *Karlodinium veneficum* on co-existed phytoplankton. Therefore, to reveal the ecological function in marine ecosystem, the algal bloom kinetics, evolutionary history of *Karlodinium veneficum*, it is necessary to understand the alimentation mode and allelopathic effect on co-existed phytoplankton. Present study also provide evidence to prevention and control of *Karlodinium veneficum* blooms.

2. Materials and methods

2.1 Experimental materials

The experimental strain *Karlodinum veneficum* JX24 were collected and separated from seawater in Yeli Island, Zhuhai city. The target strain *Rhodomonas salina* CCMP1319 were gifted by Christopher J. Globler (Stony Brook University) .

Both strains were cultured in illumination incubator (RXZ-smart, purchased from Jiangnan instrument factory, Ningbo). They were cultured in F/2 medium (28 ± 1 PSU) under a photoperiod of 12 h (light): 12 h (dark) at 20 ± 1 ℃ and an irradiance of 100 μmol·m^{-2}·s^{-1} approximately.

2.2 Experimental methods

2.2.1 Allelopathic effect on Rhodomonas salina by Karlodinum veneficum

Cell density of all experimental alga were calculated by hemocytometer under light microscope after collected 0.1 mL well–shaken microalgae fixed by 1 % Lugol's Solution.Then diluted target alga with F/2 to similar ESD of Karlodinum veneficum according to individual ESD of target alga before experiment. The initial cell densities were 3000 cell mL^{-1} and 2000 cells mL^{-1} for *Karlodinum veneficum* and *Rhodomonas salina* resepectively.

The experiments were performed in 6 - well plate. Briefly, the total volume of each well was 10 mL, with 9 mL *Karlodinum veneficum* and 1 mL *Rhodomonas salina* in each well. The positive control group comprised 9 mL *Karlodinum veneficum* and 1 mL f/2 medium. Each negative one comprised 1 mL target alga and 9 mL f/2 medium. All groups were performed triplicate. Samples of all groups were collected in 24 h and 48 h, collected and fixed 1 mL for calculation of cell density.

2.2.2 Different effect on growth of Rhodomonas salina by different compositions of Karlodinum veneficum

Karlodinum veneficum and *Rhodomonas salina* were cultured till the middle of logarithmic growth before performing experiment. Obtained 1 mL well-shaken culture then fixed by 1 % Lugol's Solution to calculate cell density, then diluted *Karlodinum veneficum* and *Rhodomonas salina* to 3333 cells mL^{-1} for use.

50 mL Karlodinum *veneficum* were filtrated by 0.22 μm filter membrane to obtain extracellular filtrate. The intracellular liquid was obtained from 100 mL *Karlodinum veneficum* culture after ultrasonication (60% 10min) until none of intact cell appeared under microscopy.

Intracellular filtrate was filtrated by 0.22 μm filter membrane. The experiment was performed in 6-well plate. 9 mL extracellular filtrate and intracellular filtrate of *Karlodinum veneficum* were add into well respectively, then put in 1 mL *Rhodomonas salina* culture in each well. The total volume in each well was 10 mL.

The positive control was obtained by mixture of 9 mL *Karlodinum veneficum* and 1 mL *Rhodomonas salina* culture. Negative control included 9 mL f/2 medium and 1 mL *Rhodomonas salina* culture in

one plate. Each group was triplicated. Cell density of *Rhodomonas salina* was calculated after 24 h, 48 h and 72 h. The method was mentioned above.

2.2.3 Allelopathy of Karlodinum veneficum impacted by nutrient limited factor

The nutrient levels of *Karlodinum veneficum* culture medium were set at three: 180 μg L^{-1} N,20 μg L^{-1} P, 448 μg L^{-1} N,50 μg L^{-1} P, 12 mg L^{-1} N,1.3 mg L^{-1} P. Themicroelement and vitamin were added as f/2 formula.

The initial cell densities of *Karlodinum veneficum* were adjusted to 2000 cells mL^{-1} and 8000 cells mL^{-1}, 2000 cells mL^{-1} for *Rhodomonas salina*. Intracellular filtrate of *Karlodinum veneficum* under different nutrient levels were added into *Rhodomonas salina* culture for 48 h.

Three N/P ratio levels were set in the *Karlodinum veneficum* culture medium, including N: P = 10 : 1（226 μg L^{-1} N,50 μg L^{-1} P）， N: P = 20 : 1（448 μg L^{-1} N,50 μg L^{-1} P）， N: P = 30 : 1（672 μg L^{-1} N , 50 μg L^{-1} P）. The microelement and vitamin were same as f/2 formula.

Experiments were performed in 6-well plates. *Karlodinum* veneficum intracellular filtrate and *Rhodomonas salina* culture (9:1 v/v) were mixed together (10 mL) in single well. Blank f/2 medium and *Rhodomonas salina* culture (9:1 v/v) for control group. Each group was triplicated.

2.3 Data statistics and analysis

All data were visualized by Origin 8.5 and analyzed by SPSS 13.0. Inhibition rate were calculated by following formula:

Inhibition Rate=（1- $N_{treatment}$ / $N_{control}$）×100%

$N_{treatment}$ and $N_{control}$ stand for cell number of *Rhodomonas salina* in treatment group and control group.

3. Results

3.1 Allelopathic effect of Karlodinum veneficum on Rhodomonas salina

Karlodinum veneficum has an inhibitory effect on the growth of *Rhodomonas salina*, with inhibition rates of 26% and 36% at 24 h and 48 h, respectively (Fig. 1). The growth of *Rhodomonas salina* in the co-culture group was lower than that in the single culture group at 24 h and 48 h, and there was a significant difference in the density of *Rhodomonas salina* between the two groups at 24 h and 48 h ($P < 0.05$). *Karlodinum veneficum* inhibits the growth of *Rhodomonas salina* itself, and as the co-culture time prolongs, the inhibitory effect is increasing.

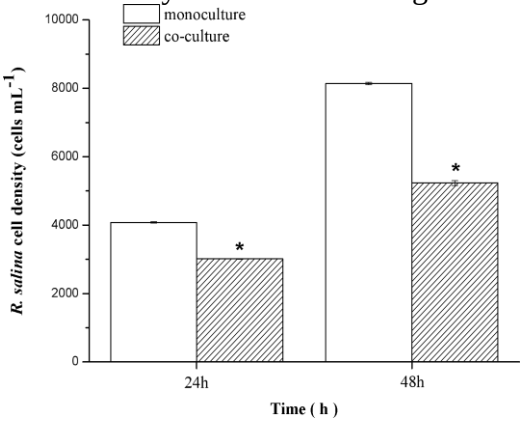

Fig. 1 *Rhodomonas salina* cell density and specific growth rate changes
(*represents a significant difference from the control, $P < 0.05$)

3.2 Effects of different components of Karlodinum veneficum culture solution on the growth of Rhodomonas salina

The four algal components of *Karlodinum veneficum* significantly inhibited *Rhodomonas salina* ($P < 0.05$). The inhibition rate from strong to weak was ultrasonic sonication, whole cell algae, sonication filtrate, whole cell algae filtrate. (Fig. 2).

The inhibition rate of *Karlodinum veneficum* cell sonication on *Rhodomonas salina* was 26 %, 37 %, 32 % at 24 h, 48 h, and 72 h, respectively. The inhibition rate of the sonication group at 24 h was consistent with the whole cell algae group. Significant difference ($P < 0.05$). The intact *Karlodinum veneficum* cell culture was co-cultured with *Rhodomonas salina* for 72 h. The inhibition of *Rhodomonas salina* growth increased with time, and the inhibition rate of *Rhodomonas salina* was 21%, 28% and 32% at 24 h, 48 h and 72 h, respectively. The inhibitory effect of *Karlodinum veneficum* cell filtrate and *Karlodinum veneficum* cell disrupted filtrate on *Rhodomonas salina* decreased with time. The algal cell filtrate was 16 %, 12 % and 8% at 24 h, 48 h and 72 h, respectively. The algal cell disrupted filtrate was 24%, 20%, and 18% at 24 h, 48 h, and 72 h, respectively, and was significantly lower than the other experimental groups ($P < 0.05$).

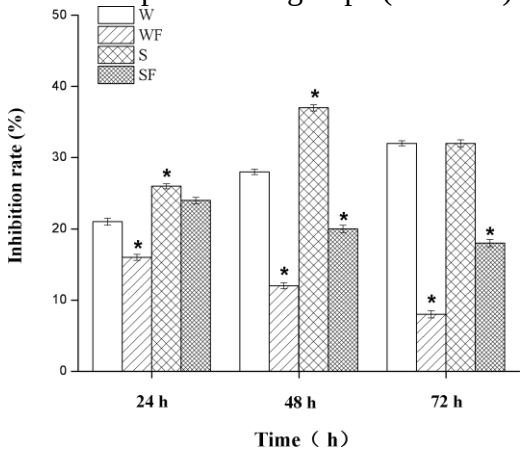

Fig. 2 Effect of different components of Karlodinum veneficum algae solution on Rhododonas salina

(W is whole cell algae liquid, WF is whole cell algae liquid filtrate, S is ultrasonic broken algae liquid, SF is ultrasonic broken algal liquid filtrate; * represents significant difference with control, $P < 0.05$)

Comparing the effects of these four components on the growth of *Rhodomonas salina*, the inhibitory effect of *Karlodinum veneficum* on *Rhodomonas salina* does not depend on the presence of living cells. Allelochemicals are present in cells, and they are unstable outside the cell, without intact cells in time. Supplementation of allelochemicals, the allelopathic effect will decrease with time.

3.3 Effects of nutrients on allelopathic effects of Karlodinum veneficum

3.3.1 Under different nutrient concentrations

Under low, medium and high nutrient conditions, Karlodinum veneficum has a certain degree of inhibition on *Rhodomonas salina* (Fig. 3). *Karlodinum veneficum* has a higher inhibition rate on *Rhodomonas salina* when the nutrient concentration is higher. Increased as the density of *Karlodinum veneficum* cells increased.

The inhibition rates of *Rhodomonas salina* in the low, medium and high nutrient conditions of *Karlodinum veneficum* with initial density of 2000 cells mL^{-1} were 18%, 36% and 53%, respectively. There were significant differences among the three groups ($P < 0.05$). The inhibition rate of *Rhodomonas salina* in *Karlodinum veneficum* with initial density of 8000 cells mL^{-1} was 30%, 48% and 64% under low, medium and high nutrient conditions. There were also significant differences among the three groups ($P < 0.05$).

3.3.2 Under different nutrient ratios

Different ratios of nitrogen to phosphorus affect the inhibitory effect of Karlodinum veneficum on Rhodomonas salina, and the inhibition rate of nitrogen-phosphorus imbalance is higher than that of nitrogen-phosphorus balance (Fig. 4). When the initial cell density of Karlodinum veneficum is 2000 cells mL-1, the inhibition rate of Rhodomonas salina is 40%, and the inhibition rate of nitrogen-phosphorus ratio is 30% under the condition of 30:1. The ratio of nitrogen to phosphorus is 20%, that is, the lowest inhibition rate of nitrogen-phosphorus balance is 36%. When the initial density is 8000 cells mL^{-1}, the inhibition rate of *Rhodomonas salina* is 57%, and the inhibition rate of nitrogen to phosphorus ratio is 55 %, the ratio of nitrogen to phosphorus is 55%. The inhibition rate was the lowest at 20:1, which was 48%. The inhibition rate of nitrogen-phosphorus imbalance was significantly higher than that of nitrogen-phosphorus balance ($P < 0.05$).

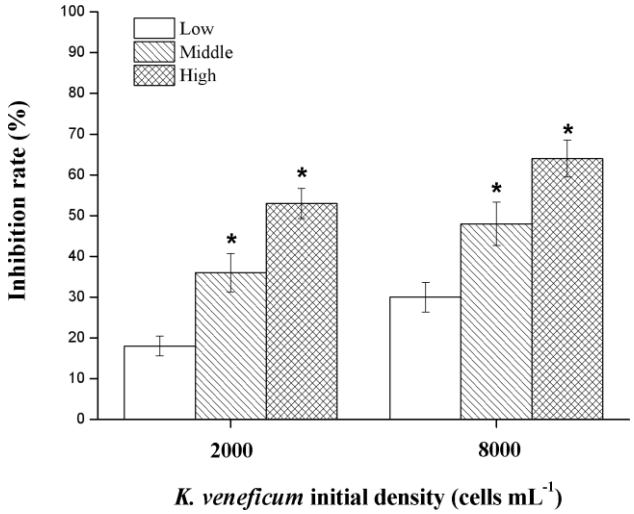

Fig. 3 Inhibitory effect of *Karl*odinum veneficum on Rhododonas salina at different nutrient concentrations(* represents a significant difference from the control, $P < 0.05$)

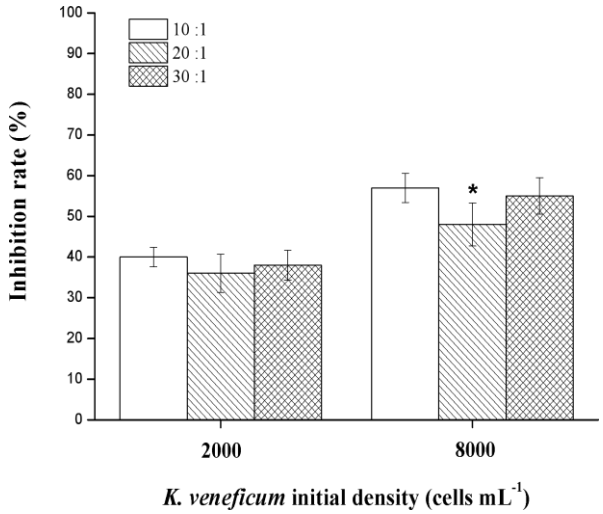

Fig. 4 Inhibitory effect of Karlodinum veneficum on Rhododonas salina at different nutrient ratios (* represents a significant difference from the control, $P < 0.05$)

4. Discussion

4.1 Allelopathic effects of Karlodinum veneficum on Rhododonas salina

Karlodinum veneficum inhibited the growth of *Rhodomonas salina*, and the inhibition rate was positively correlated with the cell density of *Karlodinum veneficum*. As the co-cultivation time prolongs, this difference is also changing.

Previous studies have found that *D. sylvestris* has a certain allelopathic effect on coexisting phytoplankton, which shows strong inhibitory effects on the growth of *Prorocentrum*, *Haberophyta*, *Karlodinum veneficum* and *Rhodomonas salina*. The growth of the algae has no significant effect and can promote the growth of *Prymnesium parvum*. The allelopathic effect intensity of allelochemicals on target algae is related to the cell density of both susceptible algae and target algae. The *Alexandrium* New York strain has the greatest allelopathic effect on *Rhodomonas salina*, while the Canadian strain is relatively weak [13]. In this experiment, due to the restriction of the species of the tested strain *Kallodinum veneficum*, the allelopathic effects of different strains of *Karlodinum veneficum* could not be further studied.

4.2 Effects of different components of Karlodinum veneficum culture solution on the growth of Rhodomonas salina

The four different algal fluid components of *Karlodinum veneficum* JX24 can significantly inhibit the growth of *Rhodomonas salina*. The inhibition rate is from ultrasonic to weak, whole cell algae, sonication filtrate and whole cell algae filtrate. The inhibitory effect of cell sonication was higher than that of other components, and the inhibition rate reached 37%, indicating that the inhibitory effect of *Karlodinum veneficum* on the coexisting phytoplankton does not depend on the presence of living cells. It can produce allelochemicals in the cells and secrete them extracellularly. And thus, acting on other co-cultivated algae. However, the allelopathic substance does not exist stably outside the cell, and no intact cells are promptly supplemented with secreted allelochemicals, and the allelopathic effect will decrease with time.

Granéli and Johansson also found that within 36 h, the small *Prymnesium parvum* cell filtrate significantly inhibited Thalassiosira weissflogii, *Rhodomonas salina* (*Rhodomonas cf. baltic*) and *Prorocentrum minimum*. But after 36 h, the tested algae began to resume growth [14]. The cell-free filtrate of *C. elegans* can also inhibit the growth of *P. tricornutum*, *Prototheca platensis*, and *Alexandrium tamarense*.

4.3 Effects of nutrients on allelopathic effects of Karlodinum veneficum

This study shows that high nutrient salt concentration can increase the inhibitory effect of *Karlodinum veneficum* on *Rhodomonas salina*. *Karlodinum veneficum* has a greater inhibition rate of *Rhodomonas salina* at higher nutrient concentrations and increases with the increase of *Karlodinum veneficum* cell density. At the same time, nitrogen limitation and phosphorus restriction can also increase the inhibitory effect of *Karlodinum veneficum* on *Rhodomonas salina*. Different ratios of nitrogen to phosphorus affect the inhibitory effect of *Karlodinum veneficum* on *Rhodomonas salina*, and the inhibition rate of nitrogen-phosphorus imbalance is higher than that of nitrogen-phosphorus balance.

Granéli and Johansson also found a similar phenomenon when studying the allelopathic effect of *Prymnesium parvum* (*P.* parvum), that is, *P. parvum* under nitrogen-limited culture significantly increased the amount of allelochemicals it produced, but under N sufficient conditions for other The allelopathic effects of phytoplankton are almost non-existent[14]. It can be speculated that certain specific red tide algae under the condition of nitrogen auxotrophy can increase the release of allelochemicals to inhibit other algae in the same water area, and then compete for limited nutrition to achieve self-protection mechanism.

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