Allelopathic Effect of Scenedesmus on Microcystis Flos-aquae

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Abstract. Allelopathic interactions among phytoplankton species are deemed to be an important factor contributing to phytoplankton species interspecific competition and community succession. However, the specific role of allelopathy of green algae on cyanobacteria species in eutrophic fresh waters is still unknown. This paper examined the allelopathic effect of two Scenedesmus (Scenedesmus quadricauda, Scenedesmus obliquus) on a common specie of freshwater cyanobacteria (Microcystis flos-aquae) by adding culture filtrate from exponential growth period and in co-culture tests. The presence of S.quadricauda and S.obliquus both extremely inhibited the growth of M.flos-aquae in co-cultures within 10 days. The culture filtrate of S.quadricauda and S.obliquus significantly inhibited the growth of M.flos-aquae, and with the increase of filtrate concentration, the inhibitory effect was more obvious. Our results indicate that under the tested environmental conditions, allelopathic effects of S.quadricauda and S.obliquus on M.flos-aquae can significantly contribute to the inhibition of cyanobacteria's growth.

Key words: Allelopathy, Scenedesmus, Microcystis flos-aquae

1. Introduction

Allelopathy refers to the physiological process where the secondary metabolites released by plants including microorganisms could affect the growth of other organisms, and the interactions arising from this secondary metabolites (allelochemicals) could be harmful or beneficial[1]. Allelopathy also plays an important role in interspecific competition. A number of studies have focused on the impact of allelopathy for interspecific competition and community succession [2]. With the universal eutrophication of waterbodies, the allelopathy between algae in marine and freshwater ecosystems is getting more and more attention. For example, the allelochemicals released by some species of algae that inhibit the growth of other aquatic plants has been observed [3], [4]. Allelopathy could be a critical elements that promotes the superiority of marine and freshwater harmful algal bloom-forming species over other algae species [5].A comprehensive understanding of the competition between cyanobacteria and other species of algae can be crucial to control strategies for bloom for outbreaks in aquatic ecosystems [6].

However, most algal allelochemicals do not cause death, and only inhibit some function in the target species' ecophysiology temporarily. For example, grazing inhibition, photosynthesis inhibition, or decrease in growth rate have all been confirmed to a certain extent. So, more researches of allelopathy between different algae species should be done. And how the allelochemicals function in the target species is also a crucial question to understand. Previous researches more focused on the allelopathic effect of cyanobacteria on other phytoplankton species, while paid little attention to the allelopathy of green algae on cyanobacteria.

Therefore, our study aimed to (1) evaluate the potential allelopathic interaction between green microalgae Scenedesmus quadricauda and Scenedesmus obliquus with the cyanobacteria Microcystis

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flos-aquae under controlled conditions and (2) the effect of culture filtrate of green algae on the growth of the Microcystis flos-aquae, and try to understand how the allelochemicals function in the proliferation of Microcystis flos-aquae.

2. Materials and Methods

2.1. Strain Selection and Pretreatment

Scenedesmus quadricauda (FACHB-1468), Scenedesmus obliquus (FHACB-417) and Microcystis flos-aquae (FHACB-1323) are all purchased from the Freshwater Algae Culture Collection of the Institute of Hydrobiology in China (FACHB-collection). Three algae were separately cultured on an extended scale for a month in modified Blue-green (BG11) medium for a month. The modified BG11 medium contained the following components (mg L⁻¹): NaNO₃ (60.71), KH₂PO₄•3H₂O (4.86), MgSO₄•7H₂O (25), CaCl₂•2H₂O (36), C₆H₈O₇ (1.5), EDTA-Na₂ (0.5), Na₂CO₃ (20), FeC₆H₅O₇•5H₂O•2H₃BO₃•4H₂O (3) H₂BO₃ (2.86), MnCl₂•H₂O (1.86), ZnSO₄•7H₂O (0.222), CuSO₄•5H₂O (0.080), Na₂MoO₄•2H₂O (0.39), Co(NO₃)₂•6H₂O (0.049). The pH of the medium was adjusted to 8.0 by adding NaOH and HCl before autoclaving at 121 ºC for 30 minutes.

2.2. Experimental Design and Culture Condition

Three algae cells in logarithmic phase after extended culture were used as algae species in the experiments. The modified BG11 medium (pH=8.0) was used in this study. The cultures were kept at 25 ºC with photosynthetically active radiation intensity of 30 μmol quantam 2m⁻² s⁻¹ under a light: dark regime of 12:12 h. All experimental utensils were autoclaved (LDZX-50KBS, Shenan, Shanghai) at 121 ºC for 30 min. During the experiment period, all the flasks were shaken twice very day to avoid agglomeration and then placed statically. All tests were cultured for 10 days.

2.2.1. Experiment 1: Mixed culture tests

M.flos-aquae was co-cultured with S.quadricauda and S.obliquus, respectively, with an initial ratio of M. M.flos-aquae to other phytoplankton species of 10:1(based on the size of algal cell volume). Another group of M.flos-aquae was pure cultured as control group. The initial density of the M.flos-aquae culture was 1.3× 10⁵ cells /mL. Each treatment containing the medium of 250mL was conducted in triplicate in flasks.

2.2.2. Experiment 2: Addition of filtrate of two Scenedesmus

Filtrate of S.quadricauda and S.obliquus were harvested in their exponential growth period by filtrating through fibre filter (0.45 mm) under axenic conditions. S.quadricauda and S.obliquus filtrate were then diluted into five concentrations (0% (control), 25%, 50%, 75%, and 100%) using modified BG11 medium. Concentrations of nitrogen and phosphorus were adjusted to the same level as in the modified BG11 medium. The pH of all medium was modified to 8.0 using HCl and NaOH. The initial density of the M.flos-aquae culture was 1.3×10⁵ cells /mL. Total volume is 100 mL for each sample.

2.3. Analytical Method

2.3.1. Calculating cell numbers

As M.flos-aquae is a filamentous cyanobacterium, its cell number is difficult to count efficiently. Therefore, we studied the relationship between cell number and optical density of the wavelength at 686 nm absorbance (OD686 nm). A 1-mL dense M. flos-aquae culture in the exponential phase was diluted to different multiples. Cell numbers in all suspensions were counted under a microscope (Ott Optical Instrument, BK5000, Chongqing), and then measured the corresponding OD686 nm via photospectrometry (Puxi General Instrument, T6, Beijing).The mixed culture groups were counted cell numbers under a microscope every two days.

2.3.2. Molecular weight distribution and fluorescent substance of algae filtrate

A high performance size exclusion chromatography (Waters e2695,USA)—UV/visible detector (Waters 2489, USA)—total organic carbon analyzer (Sievers 900 Turbo, USA) system combined with a TSK—GEL G3000PWXL column (7.8mmx 30 mm) was used to measure the Molecular weight distribution of samples with the HPSEC-UV-TOC method. A fluorescence spectrometer (Cary Eclipse, Varian, USA) was used to note down the fluorescence EEM of samples following the method described by Chen et al [7].
3. Results and Discussion

3.1. Algae Biomass

The algae biomass of *M.flos-aquae* during 10 Days under different conditions is presented in Fig. 1. In the general scale. The effects of *S.quadricauda* and *S.obliquus* on *M.flos-aquae* were similar; both of them obviously inhibited the growth of *M.flos-aquae*. A lot of studies have considered the competition between cyanobacteria and other microalgae caused by chemical and physical factors. [8], [9], and shown that the phytoplankton succession in mixed cultures is not always determined by nutrient concentration of the medium [6]. In this study, the concentrations of the major nutrient elements (nitrogen and phosphorus) were measured in the last day of the incubation period and showed no significant difference between the pure culture group and mixed culture groups. It shows that nutrient concentration is not the major factor to cause the inhibition of the growth of *M.flos-aquae*. Some studies have mentioned that allelopathy also plays an important role in phytoplankton competition [10], [11]. So allelopathy may play an major role in this study. Some experiments have been done in this study to test the hypothesis above. *S. quadricauda* and *S. obliquus*’s culture filtrate were added to the BG11 medium which was used to culture the *M.flos-aquae* with different concentrations (25%, 50%, 75%, and 100%). The culture results are presented in Fig. 2. The growth curves showed that the algae biomass of the *M. flos-aquae* decreases with increasing *S. quadricauda* and *S. obliquus*’s culture filtrate concentration. Fig.3 shows the inhibition of the growth of *M. flos-aquae* in different concentration of *Scenedesmus*’s culture filtrate at the tenth day. Every concentrations of the culture filtrate inhibits the growth of *M.flos-aquae*. When the concentration was 25%, the inhibition of *S.quadricauda*’s culture filtrate is Slightly larger than *S.obliquus*’s. While in the other concentrations (50%,
75%, and 100%), the result is opposite. The results above proved that \textit{S.quadricauda} and \textit{S.obliquus} both have Allelopathic effect on \textit{M.flos-aquae}. Moreover, this allelopathy acts as an inhibitory action. And with the increase of allelochemicals concentration, the inhibitory effect was more obvious.

![Fig. 3: The inhibition of the growth of \textit{M.flos-aquae} in different concentration of \textit{Scenedesmus}'s culture filtrate](image)

### 3.2. Molecular Weight Distribution of Algae Filtrate

Molecular weight distribution of algae filtrate can be seen in Fig. 4 (mixed with \textit{S.obliquus} as an example). The TOC molecular weight distributions of samples are showed in Fig.4A. The EPS of the mixed culture group compared with the pure culture group was significantly reduced. Previous studies have shown that EPS is mainly made up of proteins and sugar. Proteins and sugar are important components of cell structure and essential for living cells. The reduced EPS means the synthesis of intracellular proteins and sugar reduce. So the allelochemicals may inhibit algae cells proliferation by inhibiting the synthesis of proteins and sugar. The UV molecular weight distributions of samples are showed in Fig.4B. The humic substances with low molecular weight of the mixed culture group compared with the pure culture group were obviously reduced. It can be explained in the same way mentioned above. The allelochemicals may inhibit the synthesis of humic substances with low molecular weight which may play an important role in cellular activity to inhibit the growth of \textit{M. flos-aquae}.

![Fig. 4: Molecular weight distribution of algae filtrate (A:TOC, B:UV)](image)

### 3.3. Fluorescence EEM Spectra of Algae Filtrate

The fluorescence EEM spectra of algae filtrate in this study (mixed with \textit{S. obliquus} as an example) are presented in Fig.5. Both the pure culture group and the mixed culture group were found to contain four fluorescence peaks. Peak A and C represent humic-like fluorescence, whereas peak B and T represent
protein-like fluorescence. Peak A, peak C, peak T and peak B of pure culture group has a greater intensity than the mixed culture group. This shows that both humic-like fluorescence substances and protein-like fluorescence substances of mixed culture group was less than the pure culture group. This result is in agreement with the analysis of molecular weight distribution. The allelochemicals of *S. obliquus* can inhibit the growth of *M. flos-aquae* by inhibiting the synthesis of proteins and humic substances.

![Fluorescence EEM spectra of pure culture group](image1)

A: Fluorescence EEM spectra of pure culture group

![Fluorescence EEM spectra of mixed culture group](image2)

B: Fluorescence EEM spectra of mixed culture group (mixed with *S. obliquus*)

**Fig. 5: Fluorescence EEM spectra of of algae filtrate**

4. Conclusion

We conclude that both *S. quadricauda* and *S. obliquus* can significantly affect the growth of *M. flos-aquae* and these effects depend on the allelopathy. Our experiments revealed negative effects of *S. quadricauda* and *S. obliquus* on the growth of *M. flos-aquae* by inhibiting the *M. flos-aquae* to synthesise protein, sugar and humic substances. The *M. flos-aquae* bloom can be controlled in a more environmental friendly method if the allelochemicals of *S. quadricauda* and *S. obliquus* on *M. flos-aquae* can be identified in the future tests.

5. References


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