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Chlorophytes prolong mixotrophic *Ochromonas* eliminating *Microcystis*: Temperature-dependent effect

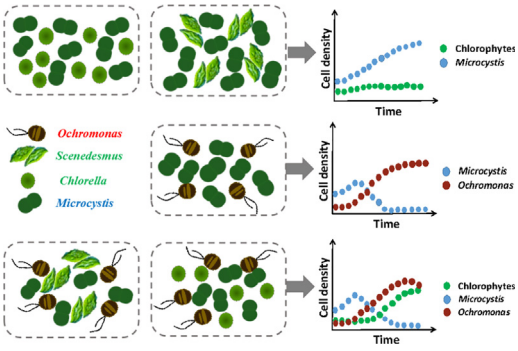
Lu Zhang [a,b], Lei Gu [a], Xinying Hou [a], Qingdan Kong [a], Ke Chen [a], Xuexia Zhu [a], Yuan Huang [a], Yafen Chen [b], Zhou Yang [a,b,*]

[a] Jiangsu Province Key Laboratory for Biodiversity and Biotechnology, School of Biological Sciences, Nanjing Normal University, 1 Wenyuan Road, Nanjing 210023, China
[b] State Key Laboratory of Lake and Environment, Nanjing Institute of Geography and Limnology, Chinese Academy of Sciences, 73 East Beijing Road, Nanjing 210008, China

HIGHLIGHTS

- Chlorophytes prolonged the time for *Ochromonas* to drive *Microcystis* extinct.
- The lag effect of chlorophytes on clearing *Microcystis* was temperature-dependent.
- *Ochromonas* preferred *Microcystis* over chlorophytes.
- *Ochromonas* eliminating *Microcystis* helps the recovery of chlorophytes.

GRAPHICAL ABSTRACT



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ABSTRACT

Cyanobacterial blooms, caused by eutrophication and climate warming, exert severely negative effects on aquatic ecosystem. Some species of protozoans can graze on toxic cyanobacteria and degrade microcystins highly efficiently, which shows a promising way to control the harmful algae. However, in the field, many different species of algae coexist with *Microcystis* and may affect protozoans eliminating *Microcystis*. Therefore, in this study, we assessed the impacts of chlorophytes, a type of beneficial algae for zooplankton and common competitors of cyanobacteria, on flagellate *Ochromonas* eliminating toxin-producing *Microcystis* at different temperatures. Our results showed that *Ochromonas* still eliminated *Microcystis* population and degraded the total microcystins with the addition of chlorophytes, although the time of eliminating *Microcystis* was prolonged and temperature-dependent. Additionally, in the grazing treatments, chlorophytes populations gradually increased with the depletion of *Microcystis*, whereas *Microcystis* dominated in the mixed algal cultures without *Ochromonas*. The findings indicated that although chlorophytes prolong mixotrophic *Ochromonas* eliminating *Microcystis*, the flagellate grazing *Microcystis* helps chlorophytes dominating in the primary producers, which is significant in improving water quality and reducing aquatic ecosystem risks.

1. Introduction

Along with increased human activity, climate warming and water eutrophication induce the frequent occurrence of cyanobacterial

* Corresponding author at: Jiangsu Key Laboratory for Biodiversity and Biotechnology, School of Biological Sciences, Nanjing Normal University, 1 Wenyuan Road, Nanjing 210023, China.
E-mail address: yangzhou@njnu.edu.cn (Z. Yang).

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blooms worldwide (O'Neil et al., 2012; Merel et al., 2013; Heisler et al., 2008). As common food, cyanobacteria have adverse effects on large-sized zooplanktons including rotifers, cladocerans, and copepods (Soares et al., 2010; Lyu et al., 2017; Ger and Panosso, 2014), and increase cyanotoxins production as an induced defense against zooplankton grazing (Jang et al., 2007). The cyanotoxins are not easily degradable and may accumulate in aquatic animals and then transfer in the food chains (Jia et al., 2014; Ferrão Filho et al., 2002), gradually increasing the risk of aquatic production and water safety (Rezaitabar et al., 2017).

In order to reduce the risks of toxic cyanobacteria, different physical, chemical, and biological measures (i.e. nanofiltration, hydrogen peroxide, zooplankton, algicidal bacteria) have been extensively tried to control toxic *Microcystis* or degrading microcystins (Teixeira and Rosa, 2006; Barrington et al., 2013; Li et al., 2017). Given the expensive costs and potential damages of chemical and physical measures, biological measures are considered to be an environment-friendly way. Zooplankton grazing can efficiently reduce cyanobacterial biomass and cyanotoxin production (Ekvall et al., 2014). In natural waters, protozoans play an important role in aquatic ecosystem and are major grazers of cyanobacteria (Novarino et al., 1997; Dryden and Wright, 1987). Quite a lot studies showed that protozoans could eliminate *Microcystis* population and resist microcystins. For instance, amoeba can ingest toxic colonial cyanobacteria competently (Wichelen et al., 2010; Urrutia-Cordero et al., 2013); the flagellates *Ochromonas* sp., *Poterioochromonas* sp., and *Diphylleia rotans* not only rapidly graze on toxic *Microcystis*, but also degrade microcystins (Zhang et al., 2010; Mohamed and Al-Shehri, 2013; Zhang et al., 2017). Wilken et al. (2010) demonstrated that cyanotoxins did not suppress the growth and digestion of flagellates, furthermore, temperature rising can enhance flagellates eliminating *Microcystis* and degrading microcystins (Yang et al., 2016; Zhang et al., 2017). Hence, protozoans play an important role in transferring the primary producers which are difficult to use to higher trophic levels.

However, previous studies were insufficient for understanding more natural factors acting on the protozoans grazing on cyanobacteria. Temperature acts as a pivotal factor driving the changes of phytoplankton community component (Vázquez et al., 2005). Cyanobacteria usually coexist with other microalgae including chlorophytes and diatoms (Chen et al., 2003). Particularly, the chlorophytes, as nutritious food, are generally beneficial for zooplankton to live and reproduce (Lyu et al., 2017). Lürling et al. (2013) suggested that the overall growth rates of chlorophytes in pure culture were similar to those of cyanobacteria at all temperatures, but cyanobacteria became more prevalent than chlorophytes in mixed culture with temperature rising (Yang et al., 2017; Wang et al., 2015), on account of stronger competitiveness of utilizing nutrients and inhibition effects of toxic metabolites (Wang et al., 2015; Żak and Kosakowska, 2014). Also, zooplankton selective predation impacts phytoplankton community component by reducing the ingestion of unselected preys (Wang et al., 2010). In mixed microalgal diets, prey size is the primary factor in food selection of protozoans (Monger and Landry, 1991). Algal defensive colonies can usually avoid small-sized predators grazing (Mayeli et al., 2004). *Scenedesmus* is a typical species forming defensive colonies in chlorophytes (Lürling, 2000; Zhu et al., 2015). Their colonies exceed the prey range ingested by flagellate *Ochromonas* (Kapsetaki et al., 2016). Moreover, stable colonies of *Chlorella* are also immune to predation by flagellates although flagellates can graze on unicellular *Chlorella* (Boraas et al., 1998). Based on above knowledge, it is essential and reasonable to consider temperature and chlorophytes in the study of protozoans grazing on cyanobacteria.

The aim of the study is to evaluate the effect of chlorophytes on flagellates grazing on toxin-producing cyanobacteria. *Microcystis aeruginosa* is commonly reported as a major bloom-forming species. Previously, we isolated a species of mixotrophic protozoans *Ochromonas* from Lake Taihu and found it was able to rapidly reduce *Microcystis* and microcystins (Zhang et al., 2017). Therefore, two different species of chlorophytes, namely, *Scenedesmus* and *Chlorella*, were added into culture media containing *Ochromonas* and *Microcystis* at three temperatures. We then formulated the following hypotheses: (1) the addition of chlorophytes prolongs the time to *Microcystis* extinction; (2) the food selection of flagellates shifts the outcome of competition between *Microcystis* and chlorophytes; (3) *Microcystis* releases more microcystins under competition and grazing pressures. To evaluate these hypotheses, the abundances of microorganisms, the growth and grazing rates of *Ochromonas*, and the changes in microcystins were examined in the study.

2. Materials and methods

2.1. Microorganism culture

The mixotrophic grazer *Ochromonas gloeopara* (strain YZ1) isolated from Lake Taihu was maintained in a monoclonal culture containing a BG-11 medium (Zhang et al., 2017). The three species of microalgae, namely, the toxin-producing cyanobacteria *M. aeruginosa* (PCC7806), the chlorophyte *Scenedesmus obliquus* (FACHB-416), and *Chlorella pyrenoidosa* (FACHB-11), used in the experiment were purchased from the Freshwater Algae Culture Collection at the Institute of Hydrobiology, China. Microorganism cultures were all grown in sterilized BG-11 media at 25 °C under fluorescent light of 40 μmol m^{-2} s^{-1} photons and photoperiod of 12 h:12 h (light: dark). All the treatments in the experiment were carried out under this light condition. Before the experiment, all the algae were maintained in the logarithmic growth phase, and appeared as a single cell or paired cells.

2.2. Experimental design

There were two experiments in this study. ***Experiment 1***: in order to determine whether *O. gloeopara* can graze on chlorophytes, prior to the experiment of impacts of chlorophytes on *Ochromonas* grazing *Microcystis*, *O. gloeopara* were respectively cultured with *S. obliquus* and *C. pyrenoidosa* at 25 °C for 10 days, and only chlorophytes were present in the controls. In this experiment, four treatments were set up, including *Ochromoans* cultured with *Scenedesmus*, *Ochromoans* cultured with *Chlorella*, only *Scenedesmus*, and only *Chlorella* (three replicates per treatment, see Fig. 1). ***Experiment 2***: *S. obliquus* and *C. pyrenoidosa* were separately added to the mixed cultures of mixotrophic *O. gloeopara* and *M. aeruginosa*, i.e. *Ochromoans* exposed to *Microcystis* and *Scenedesmus* (OMS), *Ochromoans* exposed to *Microcystis* and *Chlorella* (OMC), at 20, 25, and 30 °C, respectively. Correspondingly, each treatment without *S. obliquus* and *C. pyrenoidosa* served as controls, i.e. only *Ochromoans* exposed to *Microcystis* (OM). Moreover, each treatment without mixotrophic *O. gloeopara*, i.e. *Microcystis* cultured with *Scenedesmus* (MS), *Microcystis* cultured with *Chlorella* (MC), and only *Microcystis* (M) was considered as controls of grazing treatment to analyze the ingestion rate of *O. gloeopara*. Overall, eighteen treatments were performed (three replicates per treatment, see Fig. 1). This experiment was performed in 250 mL flask filled with 150 mL of sterilized BG-11 medium for 10 days in OM treatment and for 14 days in OMS and OMC treatments. Considering that cyanobacteria account for a large proportion in phytoplankton communities and are much more than protozoan predators in cyanobacteria-occurring eutrophic waters (Chen et al., 2003; Vázquez et al., 2005; Chen et al., 2008), we selected the following concentrations of microorganisms. In all treatments, *M. aeruginosa* PCC7806 was inoculated at an initial concentration of ~3×10^5 cells mL^{-1}. The concentrations of *S. obliquus* and *C. pyrenoidosa* were both ~6×10^4 cells mL^{-1}. In grazing treatments, *O. gloeopara* was added at a final concentration of ~1×10^3 cells mL^{-1}, which was also based on our previous studies showing strong ability