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Long-term Effects of Elevated CO_2 on the Proliferation of Cyanophage PP

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Abstract: Much of the research effort focused on the impacts of elevated CO_2 on marine algae but very little work was done on freshwater algae, or on freshwater algal viruses. In this paper, we studied the impacts of elevated CO_2 on the infection of a freshwater cyanobacterium (wild *Leptolyngbya sp.*) by cyanophage PP that have a wide distribution in China. In a 12-month experiment, logarithmic-phase host cells were infected with cyanophage PP at 370 or 740 µatm pCO_2 concentrations; the burst size, lysing cycle and proportion of adsorption were measured. The results showed that the proportion of adsorption, and burst sizes of cyanophage PP increased significantly with elevated CO_2 concentrations, and the proportion of adsorption increased gradually within the 12 months with the gradual increment of cell width. The result indicated that elevated CO_2 concentration may have significantinfluences on the proliferation dynamics of cyanophage–host systems, and some of the influences may increase gradually in a long-term.

Keywords: Adsorption, burst size, global change, phage–host system.

1. INTRODUCTION

Atmospheric CO_2 has increased by 25% in the past 200 years, and projections indicate that it may reach 750 µatm pCO_2 by 2100 [1]. Aquatic ecosystems are an important component of the biosphere, where the amount of CO_2 fixed by planktonic algal photosynthesis accounts for approximately 50% of the total fixed by the entire biosphere. This proportion is expected to increase further with rising temperatures [2].

Algal viruses control the community structure, contribute toward the abundance of planktonic algae, regulate the nutrient cycling of certain elements [3], and mediate the evolution of their hosts *via* horizontal gene transfer [4].

The ecological functions of algal viruses depend on their abundance, andtheir abundance is closely related to the levels of virus proliferation [5]. These processes occur within host cells and are affected by the host physiological status. Much research has focused on the impacts of elevated CO_2 on marine and freshwater algae [6-8], but little is known about cyanophage–host systems, especially in freshwater. Thus, the present study aimed to improve our understanding of the effects of high atmospheric CO_2 on the proliferation of freshwater cyanophage PP.

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2. MATERIAL AND METHODS

Phage-host system and culture conditions: Cyanophage PP is a podovirus with linear, double-stranded DNA, which has been frequently detected at high levels in many eutrophic lakes in China [9]. The following experiments used a cyanophage PP lysate with $>10^8$ PFU· mL-1(PFU, plaque-forming units), which was stored at 4 °C. The host cyanobacterial strain used was isolated from Donghu Lake (the East Lake) (30°31′N, 114°22′E) in Wuhan, China and identified by 16S rDNA analysis as *Leptolyngbya sp*. (the identity score is up to 99% in Genebank). The host was cultured in AA medium at 28 °C, with 3000 lx, and 14:10 h day/night conditions in an ambient CO_2 chamber (to supply a CO_2 concentration of approximately 370 µatm pCO_2, which was measured using a Telaire 7001 CO_2 sensor (USA), or in a 740µatm pCO_2 chamber. Within 1 year, the culture was re-inoculated by adding 35 mL of the cyanobacterium to 115 mL AA medium every 14 days, and the following one-step growth curve, adsorption, and photoreactivation experiments were repeated at 1, 2, 3, 6, and 12 months with in 1 year. Doubling the CO_2 concentration significantly accelerated host growth ($P < 0.01$, paired samples t-test), thus the cell densities of the cultures maintained in two different CO_2 concentrations were normalized to 5.6×10^6 cells· mL^{-1} at pH 7.6 by adding AA medium before the following experiments.

One-step growth experiment: One-step growth experiments were used to measure the lytic cycle and the average burst size, where 50 mL of prepared host cell suspensions were mixed with the cyanophage lysate at MOI of 10^{-5}(MOI,

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multiplicity of infection). The MOI is low enough to avoid numerous phages attach to a single trichome even after the first generation offspring cyanophages were released. After 30min incubation without shaking to allow adsorption, the mixtures were centrifuged at 10000 ×g for 10 min at 28 °C. The pellets were then collected and washed twice in AA medium, before the washed precipitates were resuspended in 50 mL AA medium and incubated in the chambers, as described above. For the plaque assays, 1 mL samples from the culture were plated at 0, 30, 60, 90, 120, 180, and 240 min. After constructing the one-step growth curves, the average burst size was determined as the ratio of the titer after the burst relative to the initial titer (at 0 min), and the corresponding time duration was recorded as the lytic cycle.

Adsorption experiment: In the adsorption experiments, an adsorption mixture was prepared by adding the cyanophage lysate to the prepared host cell suspensions at MOI of 10^{-4}(the MOI is low enough to avoid numerous phages attach to a single trichome). The initial titers of the mixtures were measured using a plaque assay and recorded as P0. The mixtures were then incubated without shaking in the chambers described above. To measure the extent of adsorption, 3 mL aliquots of the adsorption mixture were sampled at 15, 30, and 60 min. The samples were centrifuged at 12000 ×g for 10 min at 4 °C. Then, the supernatant containing free cyanophages was removed, and the titers of the pellets containing adsorbed cyanophages were determined and recorded as *Pt*. The proportion adsorbed at time t (*At*) (%) was calculated using the following formula: $At = P_t/P_0$.

Statistical analysis: 3 biological replicates were carried out in the above experiment,and SPSS 20.0 (IBM, USA) software was used for following tests: Paired sample t-test was performed to measure the differences between tested group and control group, ANOVA (with LSD post-hoc test) was performed to measure the differences among months, and Spearman's correlation test was performed to establish a relationship between proportion of adsorption and cell width.

3. RESULTS

Burst size and lytic cycle: According to the one-step growth curves (Fig. **1**), the cyanophage titer was detected 120 min after adsorption and it reached its maximum at approximately 180 min, regardless of the CO_2 concentration. In contrast to the lytic cycle, the burst size (Table **1**) was significantly higher when the CO_2 concentration was doubled than that in the controls ($P < 0.05$). From Table **1**, doubled CO_2 seemed to cause a gradual increase in the burst size and cell width from the 1st month to the 12th month. However, ANOVA indicated that the difference in the burst size was not significant among months ($P > 0.05$), and the correlation between the burst size and cell width at the doubled CO_2 conditions was not significant too ($P > 0.05$).

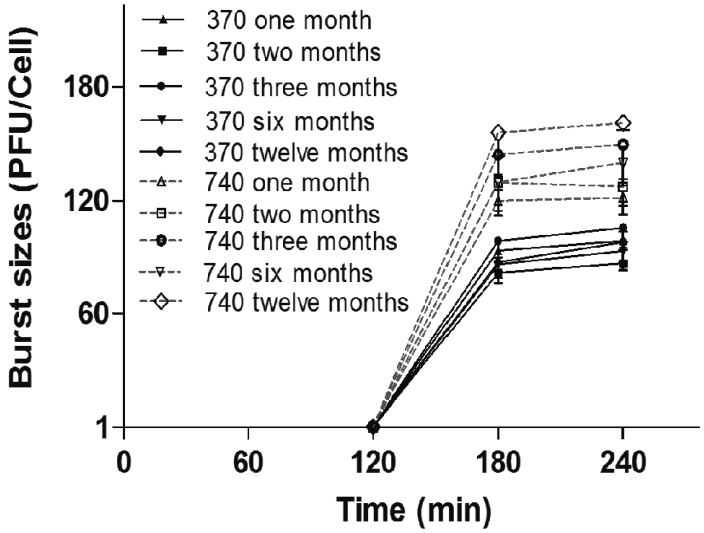

Fig. (1). One-step growth curves of cyanophage PP at different CO_2 concentrations(mean ± SD).

Table 1. Effects of doubled CO_2 onthe burst size of cyanophage PP and the cell width of the host cell (mean ± SD).

Culture Time	370 µatm		740 µatm	
	Burst Size (PFU $cell^{-1}$)	Cell Width (µm)	Burst Size (PFU $cell^{-1}$)	Cell Width (µm)
1 month	93 ± 4	2.51±0.02	120 ± 8	2.52±0.05
2 months	84 ± 5	2.48±0.02	129 ± 26	2.49±0.01
3 months	99 ± 11	2.44±0.08	144 ± 11	2.71±0.07
6 months	93 ± 18	2.50±0.02	140 ± 15	3.02±0.07
12 months	87 ± 8	2.49±0.03	156 ± 15	3.31±0.11

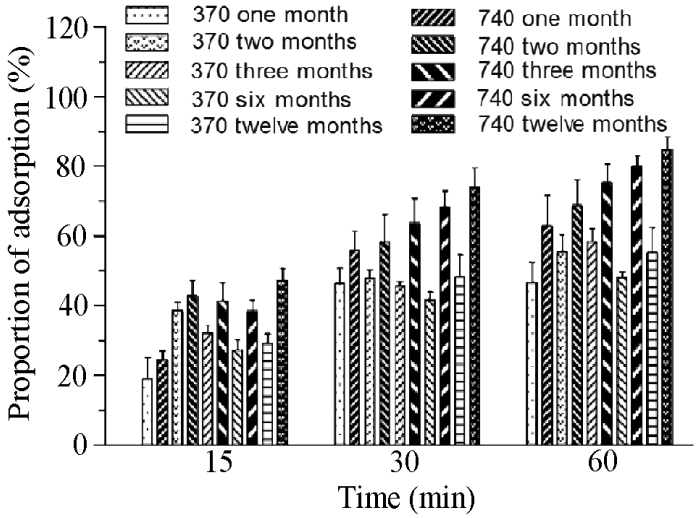

Fig. (2). Proportion of adsorption at different CO_2 concentrations(mean ± SD).

Proportion of adsorption: The results of the adsorption experiments are shown in Fig. (**2**). The proportion of adsorption that occurred when the CO_2 level was doubled was significantly higher than that in the controls ($P < 0.05$). Moreover, based on the proportion of adsorption at 60 min, it is clear that doubled CO_2 caused a gradual increase in the proportion of adsorption from the 1st month until the 12th month. ANOVA also indicated that the difference in the proportion of adsorption at 60 min was significant among months ($P < 0.05$). In addition to the continuous increase in the adsorption capacity throughout the year, a continuous increase in the cell width was observed during the year (Table **1**, $P < 0.05$, ANOVA), and there exists a significant positive correlation between the proportion of adsorption at 60 min and cell width at the doubled CO_2 conditions ($P < 0.05$).

4. DISCUSSION

Adsorption and the burst size, which are important indicators of cyanophage infectivity, can be affected by the host's physiological status [10] in numerous ways. Firstly, viruses had higher adsorption rates and burst sizes when the host was supplied with sufficient nutrients or was in the rapid growth phase [11], and we observed a doubling the CO_2 concentration significantly accelerated host growth in the present study.Secondly, enlarged host cells may provide a greater surface area for adsorption [12], and we observed a significant positive correlation between the proportion of adsorption and cell width at the doubled CO_2 conditions in the present study.

Many studies have reported the effects of elevated CO_2 concentrations on marine and freshwater algae, but only three previous studies have considered the effects of different CO_2 concentrations on algal viruses. In a 30-day mesocosm experiment, Larsen *et al*. [13] showed that the abundance of small viruses (mostly phages) did not respond to changes in the CO_2 levels, whereas the abundance of the *Emilianiahuxleyi* virus and an unidentified large dsDNA virus decreased with increasing CO_2 levels. In another short-term (only a few weeks) laboratory batch monoculture experiment [14], no impact was found on viral lysis of *Phaeocystispouchetii* while increased burst size and slightly delayed lysis was observed for *E. huxleyi* with increased CO_2. In a 39-day laboratory batch culture experiment [15], CO_2 and NaOH were used to adjust the pH concentration, where the eclipse period of cyanophage S-PM2 increased as the pH decreased, whereas the latent period and burst size decreased. The present study was conducted over the course of a year, and a continuous increase in the adsorption capacity was observed throughout the year, which suggests that long-term experiments are more representative and useful than short-term experiments for understanding the effects of long-term global change [14].

Changes in the species composition of freshwater phytoplankton communities have already been observed in areas with elevated CO_2 levels [16]. However, the roles of phytoplanktonic viruses in these changes are poorly understood. The contributions of phytoplanktonic viruses to changes in aquatic ecosystems during global change needs to be studied in terms of significant shifts in viral infectivity (as observed in the present study) and the species-specific viral response to increasing CO_2 concentrations [14]. It is also necessary to establish more accurate climate and ecosystem models [15], which have previously excluded the abundant phytoplanktonic viruses and their major ecological functions [5].

CONCLUSION

Elevated CO_2 concentration influenced the infectivity of cyanophage PP significantly, and some of the influences may increase gradually in a long-term.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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