Biochemical Systematics and Ecology 50 (2013) 286–292

Contents lists available at SciVerse ScienceDirect

Biochemical Systematics and Ecology

journal homepage: www.elsevier.com/locate/biochemsyseco

Grazer density-dependent response of induced colony formation of *Scenedesmus obliquus* to grazing-associated infochemicals

Xinyan Wu, Jing Zhang, Baoli Qin, Guilian Cui, Zhou Yang*

Jiangsu Key Laboratory for Biodiversity and Biotechnology, College of Life Sciences, Nanjing Normal University, 1 Wenyuan Road, Nanjing 210023, China

A R T I C L E I N F O

Article history:
Received 15 February 2013
Accepted 4 May 2013
Available online 1 June 2013

Keywords:
Colony formation
Daphnia magna
Inducible defenses
Rectangular hyperbolic function
Scenedesmus obliquus

A B S T R A C T

To investigate the effects of infochemicals from *Daphnia magna* reared at different densities on the growth and morphological development of *Scenedesmus obliquus*, we cultured *S. obliquus* with filtrates from *D. magna* with a density of 0, 6, 30, 150, 300, and 600 ind L^{-1}, respectively. Results showed no significant differences in *S. obliquus* growth rates among the treatments with different *Daphnia* densities. On days 3 and 5, the proportion of eight-celled colonies in the treatments constituted 30% of the *S. obliquus* populations. Importantly, the number of cells per particle increased significantly with increasing density of *Daphnia*, indicating a grazer density-dependent response. Specifically, the density-response relationship followed a rectangular hyperbolic model in the first five days of treatment, and then switched to a linear model, which implies that the relationship between the inducible colony size of *S. obliquus* and *Daphnia* density may also be dependent upon incubation time.

1. Introduction

Many algal species have developed multiple defenses, including constitutive defenses and inducible defenses, to avoid predation by species at higher trophic levels (van Donk et al., 2011). Inducible defenses are a ubiquitous form of phenotypic plasticity that can be an important adaptive strategy in aquatic environments, especially when herbivory is variable (Tollrian and Harvell, 1999; Verschoor et al., 2004). There are extensive studies about inducible defenses in algae (Hessen and van Donk, 1993; Lampert et al., 1994; Lürling, 2003; Yang et al., 2006; Toth and Pavia, 2000), and van Donk et al. (2011) have excellently reviewed the induced defenses in marine and freshwater phytoplankton. Among these, the family of *Scenedesmaceae* is by far the best studied phytoplankton taxon with respect to grazer-induced defenses (Hessen and van Donk, 1993; Lampert et al., 1994; Lürling, 2003; Verschoor et al., 2004; Yang et al., 2007). Most of these studies have discovered that the presence of herbivorous zooplankton in the filtered culture media increases the formation of colonies in species of the genus *Scenedesmus* (Hessen and van Donk, 1993; Lampert et al., 1994; Lürling, 2003; Lürling and van Donk, 2000; van Holthoon et al., 2003). After exposure to chemicals released from grazers such as *Daphnia*, unicellular *Scenedesmus* rapidly produced populations with eight-celled colonies (Hessen and van Donk, 1993; Lampert et al., 1994; Lürling, 1998). Grazer induced colony formation was interpreted as a defense mechanism against grazing because colonies can experience a lower grazing losses than single cells (Hessen and van Donk, 1993; Lürling and van Donk, 1997; Lürling, 1999).

---

* Corresponding author. Tel.: +86 25 85891671; fax: +86 25 85891526.
E-mail addresses: yangzhou@njnu.edu.cn, yangzhouff@vip.sina.com (Z. Yang).

0305-1978/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bse.2013.05.001

In aquatic systems, algae experience rapid variations in abundance of grazers (Sommer et al., 1986), which means the grazing risk that the algae are facing is not constant and the grazing-associated infochemical is also variable, thus the degree of inducible colony formation in *Scenedesmus obliquus* maybe depend on the grazer's density. Some studies have indicated a positive relationship between the number of zooplankton in culture and the degree of induced colony formation in *S. obliquus* (e.g. Lampert et al., 1994; Lürling and van Donk, 1997; Lürling, 2003; Verschoor et al., 2004), however, a few studies did not find any correlation between induced colony formation and *Daphnia* biomass in the laboratory (e.g. von Elert and Franck, 1999). To eliminate such uncertainty, we conducted an experiment to test the effects of exudates released from *Daphnia* reared at different densities on growth and morphology in *Scenedesmus*. Specifically, we focused on testing the grazer density-dependent response of inducible colony formation in *S. obliquus* and distinguished the responses at different stages of incubation. As models can provide estimates of biological meaningful parameters that allow us to make quantitative predictions about the change tendency (Xiang et al., 2012), in this study, thus, grazer density- response functions of induced colony formation in *S. obliquus* were fitted where appropriate.

2. Materials and methods

2.1. Algae and zooplankton

The strain of *S. obliquus* (FACHB-416) used in the experiment was obtained from the Institute of Hydrobiology, Chinese Academy of Sciences. The alga was cultured axenically in liquid BG-11 medium (Rippka et al., 1979) in 1.0 L Erlenmeyer flasks at 25 °C under fluorescent light at 2500 Lux with a light–dark period of 12: 12 h. The zooplankton species, *Daphnia magna*, a laboratory clone maintained in the laboratory for several years, was cultured in beakers and fed with *S. obliquus* as food under the same conditions as culturing *S. obliquus*.

2.2. Experimental design

To obtain test water containing dissolved infochemicals from different densities of zooplankton, 5-day-old *D. magna* was washed to remove surface contamination and allowed to evacuate their guts in sterile distilled water for 12 h and then incubated at treatment densities of 6, 30, 150, 300, and 600 individuals per liter water with sufficient *S. obliquus* (2×10^6 cells mL^{-1}) as food. After 24 h, the animals were removed and the culture medium was filtered through a 0.10 μm membrane filter (Millipore Corporation, USA) and used as test water that contained some infochemicals exuded by the *Daphnia* (Yang et al., 2007). Another suspension culture of *S. obliquus*, not subjected to grazing by any zooplankters, was filtered and used as control water. Nitrate and phosphate concentrations in the treatment and in the control were adjusted to the same levels.

An aliquot of exponentially growing unicellular *S. obliquus* was transferred into 250 mL Erenmeyer flasks containing 150 mL of medium. Each batch culture contained 135 mL suspension of *S. obliquus* and either 15 mL of additional control water or 15 mL of the *Daphnia* infochemical containing test water. The initial algal concentration in these treatments and control was 1.883×10^5 cells mL^{-1}. The control and five different treatments (6, 30, 150, 300, and 600 *D. magna* per liter) were run in triplicate for nine days in a climate-controlled chamber under the culture conditions described above. To reduce effects caused by minor difference in photon irradiance, the flasks were shaken three times each day and rearranged randomly.

2.3. Growth and colony formation

Samples (2 mL) were taken every other day and fixed in Lugol's solution (2%), and abundance was determined using a haemocytometer (Tianlong XB-K-25; Jiangsu, China) under a microscope (Olympus 6V20WHAL; Tokyo, Japan). Growth rate was determined as the slope of *ln* abundance vs. time from samples collected every other day (Yang et al., 2010). At the same time, the numbers of cells per particle were also recorded by counting at least 600 particles, which were comprised of single cells, two-celled forms or colony formations. The mean number of cells per particle and the mean proportion of cells in different particles (unicells, two-celled, four-celled, eight-celled, and the rest) were calculated from these counts, respectively.

2.4. Statistical analysis

All data, presented as mean ±1SE, were analyzed by repeated measures ANOVA ($\alpha = 0.05$). Statistical analyses were conducted with SigmaPlot 11.0. To assess the impact of *Daphnia* density on colony formation, assuming that increasing *Daphnia* density will increase inducible colony formation up to a maximum, above which there will be no further increase, and that there will be some colonies produced even in the absence of *Daphnia* test water, a semi-mechanistic model, analogous to Michaelis–Menten enzyme kinetics was fit to the data: the rectangular hyperbolic function, $C_p = C_0 + a^*D/(b + D)$, was applied to the data, where C_p is cells per particle, D is density of *Daphnia*, C_0 is predicted normal colonies produced in the absence of *Daphnia* test water, a is the maximum increase of colony formation with adding *Daphnia* test water, $C_0 + a$ is the maximum cells per particle produced, if the response becomes asymptotic, and b is a constant. If the rectangular hyperbolic function is not suitable for fitting the data, other functions will be tried to fit the data.