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Roles of mixotrophy in blooms of different dinoflagellates: Implications from the growth experiment

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ABSTRACT

Studies over the last two decades suggested that mixotrophy could be an important adaptive strategy for some bloom-forming dinoflagellates. In the coastal waters adjacent to the Changjiang River estuary in the East China Sea, recurrent blooms of dinoflagellates *Prorocentrum donghaiense*, *Karenia mikimotoi* and *Alexandrium catenella* started to appear from the beginning of the 21 century, but roles of mixotrophy in the formation of dinoflagellate blooms were not well understood. In the current study, mixotrophy-based growth of four selected bloom-causative dinoflagellate species, i.e. *K. mikimotoi*, *A. catenella*, *P. donghaiense* and *Prorocentrum micans*, were studied. Dinoflagellates were co-cultured with different prey organisms, including bacterium *Marinobacter* sp., microalgae *Isochrysis galbana* and *Hemiselmis virescens*, under a variant of nutrient conditions. It was found that growth of dinoflagellate *K. mikimotoi* was significantly promoted with the presence of prey organisms. Growth of *P. donghaiense* and *P. micans* was only slightly improved. For *A. catenella*, the addition of prey organisms has no effects on the growth, while both of the two prey microalgae *I. galbana* and *H. virescens* were killed, probably by allelochemicals released from *A. catenella*. There was no apparent relationship between nutrient conditions and the mixotrophy-based growth of the tested dinoflagellates. Based on the results of the growth experiment, it is implicated that mixotrophy may play different roles in the growth and bloom of the four dinoflagellate species. It can be an important competitive strategy for *K. mikimotoi*. For the two *Prorocentrum* species and *A. catenella*, however, the role of mixotrophy is much limited. They may depend more on other competitive strategies, such as phototrophy-based growth and allelopathic effect, to prevail in the phytoplankton community and form blooms.

1. Introduction

Harmful algal blooms (HAB) have been increasing drastically in both frequency and scale in China over the last two decades. Most of the HAB events recorded recently were caused by dinoflagellates, such as *Prorocentrum* spp., *Karenia* spp. and *Alexandrium* spp., which significantly threat marine ecosystems and human health (Zhou and Zhu, 2006). The coastal waters adjacent to the Changjiang River estuary in the East China Sea has turned to be an area with high frequency HAB events since the beginning of the 21st century. Large-scale blooms of *Prorocentrum donghaiense* have been found in this region every year from 2000 to now. Recurrent blooms of *Alexandrium* spp., which can produce paralytic shellfish toxins (PST), were also observed in this region from 2002 to 2011 (data of the two National Basic Besearch Priority Programs of China, CEOHAB I, II). In 2005, a large-scale bloom of *Karenia mikimotoi* occurred in this region and lasted for more than one and half months, leading to mass mortality of cultured animals (Zhou and Zhu, 2006).

Data have been accumulating to explain the occurrence of intensive dinoflagellate blooms in this region. It is the comprehensive effects of physical, chemical and biological factors that give rise to these uncommon ecological phenomena. However, cultural eutrophication is by any means the most important reason for the intensive dinoflagellate blooms. Significant changes in nutrient composition and concentration in the coastal waters adjacent to the Changjiang River estuary occurred since the 1950s, due to the increasing nitrate input and decreasing silicate input from the Chagnjiang river (Jiang et al., 2012; Zhou et al., 2008). It was found that large-scale dinoflagellate blooms in the East China Sea were closely related to the change of nutrient concentration and composition in seawater. Besides, enhanced stratification of seawater driven by global warming may also contribute to the occurrence of intensive dinoflalgellate blooms. Compared to

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diatoms which have no autonomic moving ability, dinoflagellates capable of vertical migration tend to prevail in stratified waters (Escaravage and Prins, 2002). Moreover, dinoflagellages have many adaptive stratigies, such as the ability in using dissolved organic material (DOM), luxurious consumption of nutrients, and allelopathy, in competing against diatoms (Huang et al., 2007; Kubanek et al., 2005; Ou et al., 2006; Prince et al., 2008)

Studies hypothesized that blooms of some dinoflagellates were closely related to their mixotrophy capability (Burkholder et al., 2008; Glibert et al., 2009; Jeong et al., 2005a). HAB causative dinoflagellates, which dominate marine phytoplankton community during the blooms, used to be considered as exclusively autotrophic species. But recent studies revealed that more than 50 species previously defined as phototrophic dinoflagellates had phagotrophic ability, and were confirmed as mixotrophic species (Burkholder et al., 2008; Stoecker et al., 2006; Stoecker, 1999). They ingest a variety of prey organisms, including bacteria, cyanobacteria, diatoms, ciliates and even other dinoflagellates. Phagotrophic characteristics of some dinoflagellates have been studied (Berge et al., 2008a; Bockstahler and Coats, 1993; Glibert et al., 2009; Hansen, 2011; Hansen and Nielsen, 1997; Jeong et al., 2004, 2005a, 2005b, 2005c, 2011; Jezbera et al., 2005; Kim et al., 2008; Lewitus et al., 1999; Li et al., 1999, 2000a; Lindehoff et al., 2010; Nygaard, 1993; Park and Kim, 2010; Skovgaard, 1996, 2000; Stibor and Sommer, 2003; Stoecker et al., 1997; Yoo et al., 2009, 2010; Zhang et al., 2011). It was suggested that mixotrophic dinoflagellates could acquire essential substances for growth by ingesting prey organisms. Therefore, the mixotrophic ability can be an important strategy affecting the dynamic of dinoflagellate blooms (Jeong et al., 2005a).

Mixotrophic ability of the bloom causative dinoflagellate speceis in the sea area adjacent to the Changjiang River estuary, i.e. *Prorocentrum donghaiense*, *Karenia mikimotoi* and *Alexandrium catenella*, have been confirmed (Glibert et al., 2009; Jeong et al., 2005a, 2005b; Nygaard, 1993; Stoecker et al., 1997; Yoo et al., 2009; Zhang et al., 2011, 2012). However, the role of mixotrophy in the outbreak of dinoflagellate blooms is poorly understood. Our previous studies revealed that *K. mikimotoi*, *Prorocentrum micans* and *A. catenella* collected from the coastal waters of China could ingest bacterium *Marinobacter* sp. and microalga *Isochrysis galbana* (Zhang et al., 2011, 2012). *K. mikimotoi*, *P. donghaiense* and *A. catenella* could also feed on cryptophyte *Hemiselmis virescens* (data unpublished). Cryptophytes are widely distributed in the ocean. Based on the pigment analytical results, cryptophytes characterized by alloxanthin was important constituent of the phytoplankton community in the waters adjacent to the Changjiang River estuary, which could contribute more than 1 $\mu g\, l^{-1}$ chlorophyll *a* in spring and summer (Kong, 2010). Cryptophyte *Hemiselmis* sp. has been isolated from the Changjiang River estuary and its adjacent waters (Xing et al., 2008). Haptophytes represented by *I. galbana* are also widely distributed in the Changjiang River estuary and its adjacent waters (Hu, 2004). Pigment 19′-hexanoyloxyfucoxanthin (Hex-fuco), which was considered as a characteristic pigment for haptophytes, was widely detected in phytoplankton samples collected from the Changjiang River estuary and its adjacent waters (Kong, 2010). In the current study, therefore, we focused on the mixotrophy-based growth of these dinoflagellates with the presense of prey organisms (*Marinobacter* sp., *I. galbana* and *H. virescens*) under different nutrient conditions. The potential roles of mixotrophy in the formation of dinoflagellate blooms in the sea area adjacent to the Changjiang River estuary were also discussed.

2. Materials and methods

2.1. Strains and culture conditions

Four dinoflagellate species, *Karenia mikimotoi* (strain KM-lv), *Prorocentrum micans* (strain PMNH), *Prorocentrum donghaiense* (strain PDDH), *Alexandrium catenella* (strain ACDH) were selected as the targeted species. Two microalgal species, chrysophyte *Isochrysis galbana* (strain 3011) and cryptophyte *Hemiselmis virescens* (strain CCMP443), and bacterium *Marinobacter* sp. isolated from the dinoflagellate culture medium, were adopted as the prey organisms (Table 1). Culture conditions of algae were described before (Zhang et al., 2012). *K. mikimotoi* was cultured under the light intensity of 30 $\mu E\, m^{-2}\, s^{-1}$, and other algae were cultured under the light intensity of 45 $\mu E\, m^{-2}\, s^{-1}$.

2.2. Experiment 1 – co-culture of dinoflagellates with Marinobacter sp. and Isochrysis galbana

Karenia mikimotoi, *Prorocentrum micans* and *Alexandrium catenella* at the exponential growth stage were inoculated into 5 l conical flasks containing 3 l sterile seawater, respectively, with the initial algal cell density at about 5000 cells ml^{-1}. On the 6th day, every dinoflagellate culture was divided into 36 bottles, each containing 70 ml algal culture medium. These bottles were separated into three groups, the blank group (no prey organism added), the bacteria group (using bacteria as prey organism), and the microalgae group (using microalga *Isochrysis galbana* as prey organism). Four different nutrient conditions were assigned in each group, which were nitrogen limited (N-limited), phosphorus limited (P-limited), nitrogen and phosphorus limited (N, P-limited) and nitrogen and phosphorus replete (N, P-replete), all in triplicate. Besides, a bacteria control group and a microalgae control group were also setup, in which dinoflagellate cultures were substituted by dinoflagellate culture medium filtered through a 0.45 μm membrane before the addition of prey organisms. Details on setup of the experiments were shown in Table 2. Before addition of the prey organisms and nutrients on the 6th day, about 100 ml filtered culture medium of dinoflagellate was collected to determine the concentrations of dissolved inorganic nitrogen (DIN) and phosphate (DIP), using a SKALAR sa3000/5000 chemistry unit (SKALAR Company, The Netherlands).

In the bacteria groups, bacterium *Marinobacter* sp. isolated previously and cultured with ORI solid medium was used as prey organisms (Zhang et al., 2012). In the microalgal group, *Isochrysis*

Table 1
List of algal species used in the experiment.

Algal species	Algal strain	Original place	Algal size	
			Length (μm)	Diameter/width (μm)
Karenia mikimotoi	KM-lv	The South China Sea	29.6 ± 3.4	25.1 ± 3.0
Alexandrium catenell	ACDH	The East China Sea	25.6 ± 2.4	25.8 ± 2.2
Prorocentrum micans	PMNH	The South China Sea	38.6 ± 3.6	32.9 ± 3.2
Prorocentrum donghaiense	PDDH	The East China Sea	16.1 ± 1.8	8.2 ± 0.7
Isochrysis galbana	IG3011	The Yellow Sea	4.9 ± 0.5	4.9 ± 0.5
Hemiselmis virescens	CCMP443	The Gulf of Mexico	6.0 ± 1.1	3.9 ± 0.5