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Resistance of the fish-killing dinoflagellate *Cochlodinium polykrikoides* against algicidal bacteria isolated from the coastal sea of Japan

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Abstract

Noxious red tides of the dinoflagellate *Cochlodinium polykrikoides* tend to be long lasting and cause mass mortalities of cultured and natural fish and invertebrates along the western coast of Japan and the southern coast of Korea. In order to assess the tolerance of *C. polykrikoides* to attack by algicidal bacteria, the effects of algicidal bacteria strains on the growth of three *C. polykrikoides* strains were examined in laboratory culture experiments. Algicidal bacteria used were two strains of *Cytophaga* (J18/M01 and AA8-2, direct attack type and wide prey range), three strains of *Alteromonas* (S, K, D) and one strain of *Pseudoalteromonas* (R, indirect attack type), which were all isolated by using *Chattonella antiqua* as a prey organism. Neither *Cytophaga* strain showed any algicidal activity. In the cases of *Alteromonas* and *Pseudoalteromonas*, some cultures of *C. polykrikoides* were killed, but at least 10 days or more were required for the death of this dinoflagellate. *C. polykrikoides* survived in the presence of algicidal bacteria in concentrations up to 10^6–10^7 cells ml^{-1}, which is enough for other red tide microalgae to be killed. On the contrary, the algicidal effects of bacteria on *C. antiqua* were detected clearly within a few days. These results imply that *C. polykrikoides* is resistant to the six algicidal bacteria examined, which may reflect the capacity for mixotrophy. This resistance of *C. polykrikoides* to algicidal bacteria could provide a selective advantage for survival compared to other microalgae susceptible to attack by algicidal bacteria and hence prolong red tides caused by this harmful dinoflagellate.

Keywords: *Cochlodinium polykrikoides*; Algicidal bacteria; Red tide; *Cytophaga*; *Alteromonas*; Mitigation

1. Introduction

Cochlodinium polykrikoides Margalef (Dinophyceae) is one of the most noxious red tide microalgae that cause mass mortalities of both cultured and natural fish and invertebrates, especially along the western coast of Japan and the southern coast of Korea (Yoon, 2001; Kim et al., 2002; Matsuoka and Iwataki, 2004; Suh et al., 2004; Miyahara et al., 2005; Imai et al., 2006b). The largest reported damage to Japanese fisheries by *C. polykrikoides* occurred in the Yatsushiro Sea during 2000 and totaled about 4 billion yen (~ USD 35 million) (Imai et al., 2006b), while Korean fisheries experienced losses of over 76 billion won (~USD 80 million) in 1995 (Yoon, 2001; Kim et al., 2002).

Shirota (1989) suggested a strategy to control red tides involving treatment with flocculants (e.g., clay) that scavenge particles, including microalgal cells, from the water column and deliver them to bottom sediments. The feasibility of clay treatments has been investigated in Japan, China, South Korea, USA, Sweden and Australia (Shirota, 1989; Sengco and Anderson, 2004). Broadcasting of clays has already been fully implemented at aquaculture sites in both Japan and South Korea during *C. polykrikoides* red tides (Kim et al., 2002; Wada et al., 2002). However, further studies are needed to understand the fates and effects of sedimented cells and associated toxins on benthic animals and the collateral mortality of co-occurring planktonic organisms. Decomposition rates of sedimented biomass and the resulting oxygen depletion also require examination (SCOR-IOC, 1998).

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Chemical and physical controls of red tides are considered to have indiscriminant effects on multiple organisms in seawater, whereas biological controls may represent a more environmentally benign mitigation strategy. The biological control of red tides using grazers such as copepods, bivalves and ciliates has been investigated, but the effects were minimal because of the large spatial scale of red tides (Shirota, 1989). By comparison, microorganisms such as viruses and bacteria seem to be promising red tide control agents, given their abundance in marine ecosystems, rapid growth rate, and often enhanced target specificity (SCOR-IOC, 1998; Salomon and Imai, 2006).

Algicidal bacteria may be effective tools for reducing the negative impacts of noxious red tides (Imai et al., 1995, 1998). During the last two decades, a number of algicidal bacteria have been isolated and identified from marine coastal ecosystems and thus received increasing attention for their possible role in terminating noxious red tides (Imai et al., 1993, 2001; Doucette et al., 1998; Sakata, 2000; Yoshinaga, 2002; Mayali and Azam, 2004; Salomon and Imai, 2006). We have assembled a collection of algicidal bacteria strains isolated from the coastal sea of Japan (Imai et al., 1993, 1995, 2006a,b) and herein tested their effects on the growth of three *C. polykrikoides* strains in laboratory cultures. Our aim was to better understand the relationships between these organisms and evaluate the possibility of using algicidal bacteria to control *C. polykrikoides* red tides. Our findings demonstrate that this noxious dinoflagellate has an extremely high tolerance toward most of the algicidal bacteria examined, which has potential ecophysiological implications for the mechanisms of *C. polykrikoides* red tide formation and maintenance.

2. Materials and methods

2.1. Organisms

Three clonal cultures of *C. polykrikoides* (strains WK96621, GT03-01, GT03-02) were used in this study. The strain WK96621 was isolated by M. Yamaguchi in June 1996 from a small bloom event in Tanabe Bay, Wakayama Prefecture, Japan, and strains fGT03-01 and GT03-02 were isolated by I. Imai in September 2003 from a bloom in Imazato Inlet, Nagasaki Prefecture, Japan (Fig. 1). These algal strains have been maintained in modified SWM-3 medium (Chen et al., 1969; Imai et al., 1996) at 25 °C under an illumination of 100–150 μmol photons m^{-2} s^{-1} on a 14 h light:10 h dark photo-cycle. Although these clones were washed repeatedly with micropipettes and treated with antibiotics of AM9 medium (Provasoli et al., 1959), the presence of intracellular bacteria and their release from algal cells prevented the establishment of axenic cultures (Imai et al., unpublished observation).

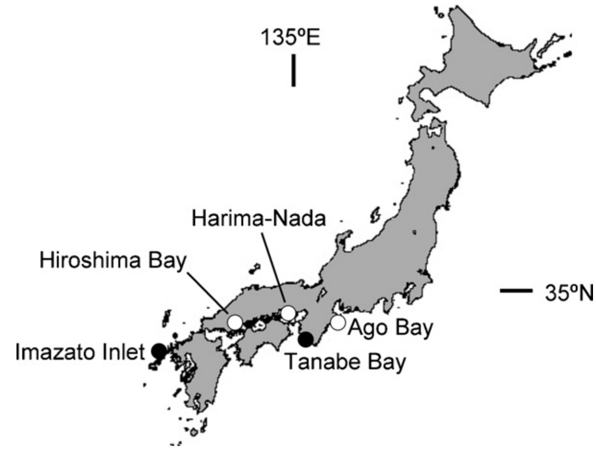

Fig. 1. Geographic locations of Tanabe Bay and Imazato Inlet for isolation of *Cochlodinium polykrikoides* (closed circles), and Harima-Nada, Ago Bay and Hiroshima Bay for isolation of algicidal bacteria (open circles).

The six strains of algicidal bacteria employed in this study are listed in Table 1 and the locations from which they originated are shown in Fig. 1. Two strains belonged to the genus *Cytophaga* (direct attack type – i.e., require physical contact with target algal cells) and were isolated from Harima-Nada in 1990 (strain J18/M01, Imai et al., 1993) and Ago Bay in 1995 (strain AA8-2, Imai et al., 1999) by using *Chattonella antiqua* (Hada) Ono (strain NIES-1) and *Heterocapsa circularisquama* Horiguchi (strain HA92-1), respectively, as prey organisms. The other four algicidal bacteria strains (*Alteromonas* and *Pseudoalteromonas*; indirect attack type – i.e., produce extracellular algicides) were isolated from Hiroshima Bay in 1991 using *C. antiqua* as the prey (Imai et al., 1995). These bacteria kill microalgae such as *C. antiqua*, utilizing organic material from the dead cells as nutrients to support growth. All bacterial strains were cultured in a peptone medium (Ishida et al., 1986) containing 0.5 g Trypticase peptone (BBL) and 50 mg yeast extract (DIFCO) per liter, and stored at −80 °C until used for experiments.

Table 1
Strains of algicidal bacteria used in the present study

Species/strain	Year of isolation	Location of isolation	Prey algae for isolation	Killing manner
Cytophaga J18/M01	1990	Harima-Nada, Hyogo Pref.	*Chattonella antiqua*	Direct attack
Cytophaga AA8-2	1995	Hiroshima Bay	*Heterocapsa circularisquama*	Direct attack
Alteromonas S	1991	Hiroshima Bay	*Chattonella antiqua*	Indirect attack
Alteromonas K	1991	Hiroshima Bay	*Chattonella antiqua*	Indirect attack
Alteromonas D	1991	Hiroshima Bay	*Chattonella antiqua*	Indirect attack
Pseudoalteromonas R	1991	Ago Bay, Mie Pref.	*Chattonella antiqua*	Indirect attack

'Direct attack' refers to bacteria requiring physical contact with target algal cells. 'Indirect attack' refers to bacteria producing extracellular algicides