Co-culture of fish with macroalgae and associated bacteria: A possible mitigation strategy for noxious red tides in enclosed coastal sea

ICHIRO IMAI, DAISUKE FUJIMARU, AND TOMOKAZU NISHIGAKI

Laboratory of Marine Environmental Microbiology, Division of Applied Biosciences, Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan (imai1ro@kais.kyoto-u.ac.jp)

SUMMARY: Algicidal bacteria play an important role in terminating red tides in coastal sea. On the surface of macroalgae such as *Ulva* sp. and *Gelidium* sp., algicidal microorganisms (mainly bacteria that kill red tide flagellates) were found to be attached numerously. Maximum number of algicidal bacteria were about $10^5 - 10^6$ / g (wet weight macroalgae). Algicidal bacteria were also much abundant in seawater collected at a seaweed bed in a coastal area of Osaka Bay. It is hence proposed that co-culturing of fish with macroalgae is a new and gentle prevention strategy for the occurrences of noxious red tides in enclosed and small-scale embayments by virtue of continuous release of many algicidal bacteria from the surface of macroalgae.

KEY WORDS: red tide, mitigation, algicidal bacteria, macroalgae, co-culture of fish and macroalgae

INTRODUCTION

The incidents of red tides had drastically increased in frequency and scale in Japanese coastal waters, especially in the Seto Inland Sea, during 1960s and 1970s, and huge fishery damages had recurred especially on cultured fish.^{1,2)} The average economic loss caused by the red tides is about one billion yen per year. Therefore, there is an urgent and compelling need for bloom mitigation strategies in aquaculture areas.

Rather many methods for direct control of red tides had been attempted before 1985, but no physical and chemical control was successful on the whole.³⁾ Thereafter, chemical and physical control options have received little attention.

Microorganisms such as bacteria, viruses, and parasites appear to be promising control agents against harmful red tides, as they are abundant in marine ecosystems, proliferate rapidly, and sometimes are host-specific.^{4,5)}

Recently algicidal microorganisms (ACM), mainly bacteria, are found to be abundantly floating in seawater in macroalgal bed (Imai and Fujimaru, submitted). We have continued to investigate the algicidal bacteria (ACB) in macroalgal bed at

intertidal shore, and found that numerous ACM (mainly bacteria that kill red tide flagellates) attached on the surface of macroalgae. Here we discuss a possibility of utilization of macroalgae as a source of ACB which may prevent occurrences of red tides in coastal areas.

MATERIALS AND METHODS

Samples of seawater and macroalgae in inshore were collected using 500-ml sterilized bottles once a month as a rule at a station (34° 20.3' N, 135° 10.9' E) in the coast of Misaki-cho, Osaka Prefecture, facing to Osaka Bay, during the period from 26 April to 25 November in 1999. A green alga *Ulva* sp., a red alga *Gelidium* sp., and the brown algae *Sargassum thunbergii* and *S. muticum* were chosen for the study. Samples were chilled and transported to the laboratory within 5 hrs.

Enumeration of ACM in seawater samples were carried out by the microplate MPN method.⁶⁾ Seven species (8 clones) of red tide microalgae belonging to Raphidophyceae, *Chattonella antiqua* (NIES-1), *C. marina* (MS-3-P), *C. ovata* (HR-93-P), *Fibrocapsa japonica* (Fib-1), and *Heterosigma akashiwo* (893 and IWA), and Dinophyceae, *Heterocapsa circulari-*

squama (HU9433) and *Gymnodinium* (=Karenia) *mikimotoi* (G303) were used as susceptible prey organisms. These microalgal strains were all axenic and kept in modified SWM-3 medium⁷⁾ at 22°C and under light intensity of about 100 - 150 μ mol photon/m²/sec with a 14h:10h L:D photo-cycle. Precultures grown to the early stationary phase were diluted appropriately with the same medium, and then 0.5ml aliquots of the diluted culture of each prey clones were inoculated into the wells of a disposable sterilized tissue culture microplate (48 wells). The seawater samples were filtered through 0.8 μ m pore size Nuclepore filter to eliminate organisms larger than bacteria. Serial 10-fold dilutions were made for the filtered seawater samples with filtered and autoclaved seawater, and 0.5ml aliquots at each dilution level were added into the 5 wells of the microplate containing prey algal cells in each well. The inoculated microplates were incubated for about 2 weeks under the conditions described above. The wells of the microplates were observed every 2 days using an inverted microscope. Wells in which more than 99% of the algal cells were killed were scored as positive, and the most probable number (MPN) of ACM against each algal clone in seawater sample was calculated with a microcomputer.⁸⁾

The collected macroalgae were put in bottles containing 300-ml autoclaved seawater and shaken 100 times by hand to detach microorganisms from the surface of the macroalgae. After taking out macroalgae, these samples were filtered through 0.8 μ m pore size Nuclepore filter, and ACM were enumerated by microplate MPN method as described above. The wet weight of macroalgal samples were measured after wiping with paper.

RESULTS AND DISCUSSION

Figure 1 shows the changes of ACM against 5 species (6 clones) of raphidophytes and 2 dinoflagellates in shore seawater at the station. Higher maximum values were obtained for *G. mikimotoi* (4.3×10^3 /ml), *F. japonica* (4.3×10^2 /ml) and *H. akashiwo* (IWA) (1.6×10^2 /ml). ACM for other species were low level (< 33 /ml). Higher densities of ACM for *G. mikimotoi* and *F. japonica* are similar to the results

obtained in seawater in macroalgal bed of Tomari Port, Obama Bay (Imai and Fujimaru, submitted).

Fig. 1. Seasonal fluctuations of algicidal microorganisms in shore seawater against *Chattonella* (A, $\square$: *C. antiqua*, $\circ$: *C. marina*, $\bullet$: *C. ovata*), other raphidophytes (B, $\blacksquare$: *Fibrocapsa japonica*, $\triangle$: *Heterosigma akashiwo* 893, $\blacktriangle$: *H. akashiwo* IWA), and dinoflagellates (C, $\bigcirc$: *Gymnodinium mikimotoi*, $\bullet$: *Heterocapsa circularisquama*).

Figures 2 and 3 show the fluctuations of ACM associated with the surfaces of *Ulva* sp., *Gelidium* sp., *S. thunbergii*, and *S. muticum*. Samplings of brown algae (*S. thunbergii* and *S. muticum*) were not feasible in the summer and thereafter. ACM inhabited on the surface of macroalgae in huge numbers, especially *Ulva* sp. and *Gelidium* sp. Maximum values were 1.4×10^2 /g for *C. marina* and 2.4×10^2 /g for *C. ovata* on *Ulva* sp. On *Gelidium* sp., maximum values were 2.6×10^3 /g for *C. antiqua*, 1.3×10^6 /g for *F. japonica*, 4.7×10^3 /g for *H. akashiwo* (893), 2.6×10^5 /g for *H. akashiwo* (IWA), and 4.9×10^5 /g for *G. mikimotoi*. *G. mikimotoi* was most susceptible to ACM associated to macroalgae examined. We could isolate ACB from

Fig. 2. Seasonal fluctuations of ACM against *Chattonella* (A, $\square$: *C. antiqua*, $\circ$: *C. marina*, $\bullet$: *C. ovata*), other raphidophytes (B, $\blacksquare$: *F. japonica*, $\triangle$: *H. akashiwo* 893, $\blacktriangle$: *H. akashiwo* IWA), and dinoflagellates (C, $\odot$: *G. mikimotoi*, $\bullet$: *H. circularisquama*), associated with the macroalgae *Ulva* sp. and *Gelidium* sp.

Fig. 3. Seasonal fluctuations of ACM against *Chattonella* (A, $\square$: *C. antiqua*, $\circ$: *C. marina*, $\bullet$: *C. ovata*), other raphidophytes (B, $\blacksquare$: *F. japonica*, $\triangle$: *H. akashiwo* 893, $\blacktriangle$: *H. akashiwo* IWA), and dinoflagellates (C, $\odot$: *G. mikimotoi*, $\bullet$: *H. circularisquama*), associated with the brown algae *Sargassum thunbergii* and *S. muticum*.

the wells of microplate in the higher dilution levels (data not shown), therefore most of ACM were presumably ACB.

Many bacteria colonize on the surface of macroalgae, and some of the bacteria are pathogens of macroalgal disease^{9,10} and some bacteria play an major role in degrading macroalgae.¹¹ Bacteria antagonistic to macroalgae are considered to function as ACB for microalgae.

Based on the present findings, here we can propose a new prevention strategy of red tide occurrences by using of macroalgae in aquaculture area (Fig. 4). Co-culturing of *Gelidium* sp. and/or *Ulva* sp. and fish such as red sea bream or yellowtail is proposed to be effective in cage culture. Many ACB will be continually released from the surface of macroalgae to seawater, and contribute to reduce cell densities of phytoplankton including harmful species by algicidal activity. Consequently, these bacteria probably play an important role in preventing the occurrences of noxious red tides. This strategy may be effective in enclosed and small-scale inlets. The most excellent merit of this strategy is that macroalgae give no negative images to fishermen of aquaculture and consumers. Moreover, *Ulva* sp. is actually utilized as supplementary foods for red sea breams in some cage cultures of Mie and Ehime Prefectures in Japan. Feasibility studies will be needed for the practical use of macroalgae in the future.

Fig. 4. A possible prevention strategy from red tides by using of macroalgae in aquaculture area. Co-culture of macroalgae and fish is proposed in the same cage and/or adjacent water.

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