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Chapter 25
Interactions Between Harmful Algae and Algicidal and Growth-Inhibiting Bacteria Associated with Seaweeds and Seagrasses

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Abstract Phytoplankton and bacteria are the main components of marine ecosystems and hence comprise abundant numbers and biomass in the sea. There exist close and diverse interactions between phytoplankton and bacteria in marine ecosystems. Among these relationships, bacteria possessing algicidal and growth-inhibiting activities against phytoplankton have been increasingly gathering attention and are expected to control phytoplankton dynamics especially harmful algal blooms in coastal environments. Algicidal activities are divided into two types, i.e., the direct attack type, needing direct attachment to host algal cells, and the indirect attack type, producing algicidal matter. Some bacteria kill part of algal populations, and they are growth-inhibiting bacteria. In harmful algal blooms (HABs) of such as the raphidophytes *Heterosigma akashiwo* and *Chattonella* spp., bacteria play an important role in the bloom terminations. Most algicidal bacteria are particle-associated forms in coastal seas. A new finding is that huge numbers of algicidal bacteria against HAB species (dinoflagellates and raphidophytes) were attached to the surface of seaweeds such as the green alga *Ulva pertusa,* the red alga *Gelidium* spp. and the brown algae *Sargassum muticum* and *S. thunbergii*. The densities of these algicidal bacteria reached as many as 10^6 g^{-1} wet weight. Further, algicidal bacteria were found in the biofilm of the seagrass *Zostera marina* with high densities of 10^7 g^{-1} wet blade or more. In the case of the toxic dinoflagellate *Alexandrium tamarense*, growth-inhibiting bacteria with strong activities were isolated from the blade of *Z. marina*. In developed countries, seagrass- and seaweed beds have been lost by reclamation for increasing land of commercial uses. We here propose restoration of seagrass- and seaweed beds, not only for increasing the nursery grounds of various marine lives but also for creating preventative strategies for HAB occurrences. This is a kind of activity in conformity with the Sato-Umi concept recently proposed.

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Abbreviation

AB	Algicidal bacteria
FLB	Free-living bacteria
GIB	Growth-inhibiting bacteria
HA	Harmful algae
HAB	Harmful algal bloom
MPN	Most probable number
PAB	Particle-associated bacteria

25.1 Introduction

In marine ecosystems, planktonic microalgae (phytoplankton) and bacteria are the most numerous and abundant components. Bacteria may stimulate or inhibit phytoplankton growth through nutrient regeneration, endosymbiosis, and production of stimulatory or inhibitory substances (Cole 1982; Riquelme and Ishida 1989), and may kill in extreme cases (Ishio et al. 1989; Sakata 1990; Furuki and Kobayashi 1991; Sakata et al. 1991; Imai et al. 1991, 1993, 1995; Fukami et al. 1992). Since harmful algal blooms (HABs) have occurred and caused huge fishery damage to aquaculture industries through mass mortality of caged fish by noxious red tides and toxin contamination of bivalves by toxic blooms, especially in temperate coastal waters, those bacteria possessing killing activities against phytoplankton have gathered attention as possible terminators of HABs (Ishida 1994; Imai et al. 1998a; Doucette et al. 1998; Skerratt et al. 2002; Mayali and Azam 2004; Sohn et al. 2004; Nakashima et al. 2006; Imai and Kimura 2008; Liu et al. 2008; Mayali et al. 2008; Roth et al. 2008; Kim et al. 2009; Park et al. 2010; Bai et al. 2011; Inaba et al. 2014; Onishi et al. 2014).

In freshwater ecosystems, algal-lysing agents (viruses, bacteria, protists, etc.) have traditionally been studied by using a soft-agar overlayer technique, utilizing microalgae to make a lawn on agar plates (Safferman and Morris 1963; Daft et al. 1975; Yamamoto 1978; Manage et al. 2000; Kim et al. 2008a; Yang et al. 2013), and their roles have been discussed. On the other hand, there are few species of marine phytoplankton, especially those of harmful flagellates, capable of growing and making a lawn on agar plates, and algal-lysing or killing microorganisms have not been investigated as well as freshwater microalgae. After the development of a method for detecting microorganisms that kill and/or inhibit the growth of marine

HAB species which cannot grow on agar plates by applying the most probable number method (MPN method), many strains of bacteria showing activities of killing and/or growth inhibition of host microalgae have been isolated and reported. These bacteria active against microalgae had no distinct names (Baker and Herson 1978; Ishio et al. 1989; Furuki and Kobayashi 1991) before addressing those bacteria as algicidal bacteria (Sakata 1990; Sakata et al. 1991; Imai et al. 1991, 1993). The term "algicidal bacteria" is widely used in related papers published nowadays.

Papers on algicidal bacteria and growth-inhibiting bacteria have increasingly been published in recent years, and the definition of algicidal bacteria (and growth-inhibiting bacteria) in those papers has sometimes appeared to be rather obscure. For example, the bacterium *Pseudoalteromonas* strain SP48 barely killed the toxic dinoflagellate *Alexandrium tamarense* with the initial addition of 9×10^6 cells ml^{-1} or more (Su et al. 2007), and the growth of the killer dinoflagellate *Pfiesteria piscicida* was reported to be inhibited with the inoculation of a bacterial density of about 10^8 cells ml^{-1} (Hare et al. 2005). Added bacterial densities are unrealistic in coastal sea environments where the total bacterial concentrations are usually between 10^6 and 10^7 cells ml^{-1} (van Es and Meyer-Reil 1982; Imai 1989), and the algicidal activities shown at these impractically high bacterial densities need reconsideration as artifact phenomena. I would like to define that algicidal bacteria kill phytoplankton with inoculation cell densities realistic in the sea and grow using the organic matter derived from killed phytoplankton cells.

25.2 Characteristics of Algicidal Bacteria

Algal-lysing agents such as viruses and bacteria have been well investigated in freshwater ecosystems (lakes and ponds) since the 1960s or earlier, and it is strongly suggested that these agents play an important role in terminating blooms of cyanobacteria and others (Safferman and Morris 1963; Daft et al. 1975). Therefore, it is easily supposed that similar microbial agents exist in marine coastal ecosystems and that those agents also terminate harmful algal blooms at the final stage. The Fisheries Agency of Japan supported these investigations during the 1990s for about 10 years, and rather many algicidal bacteria (AB) and growth-inhibiting bacteria (GIB) were actually isolated from Japanese coastal waters. Analyses of small subunit ribosomal DNA (SSU rDNA) revealed that common ABs and GIBs are usually Gram-negatives belonging to the alpha- and gamma-proteobacteria (mainly the genera *Alteromonas*, *Pseudoalteromonas, Pseudomonas* and *Vibrio*) or the phylum Bacteroides (mainly the genera *Cytophaga* and *Saprospira*) (Yoshinaga et al. 1998; Salomon and Imai 2006; Imai 2011).

Figure 25.1 shows an example of algicidal activities against three phytoplankton species by the bacterium *Alteromonsa* sp. strain S (Imai et al. 1995). Co-culture was made for 3 days after the addition of bacterial cells with densities of about 10^3 cells ml^{-1}, and all the phytoplankton cells were entirely killed. Cells of naked species of

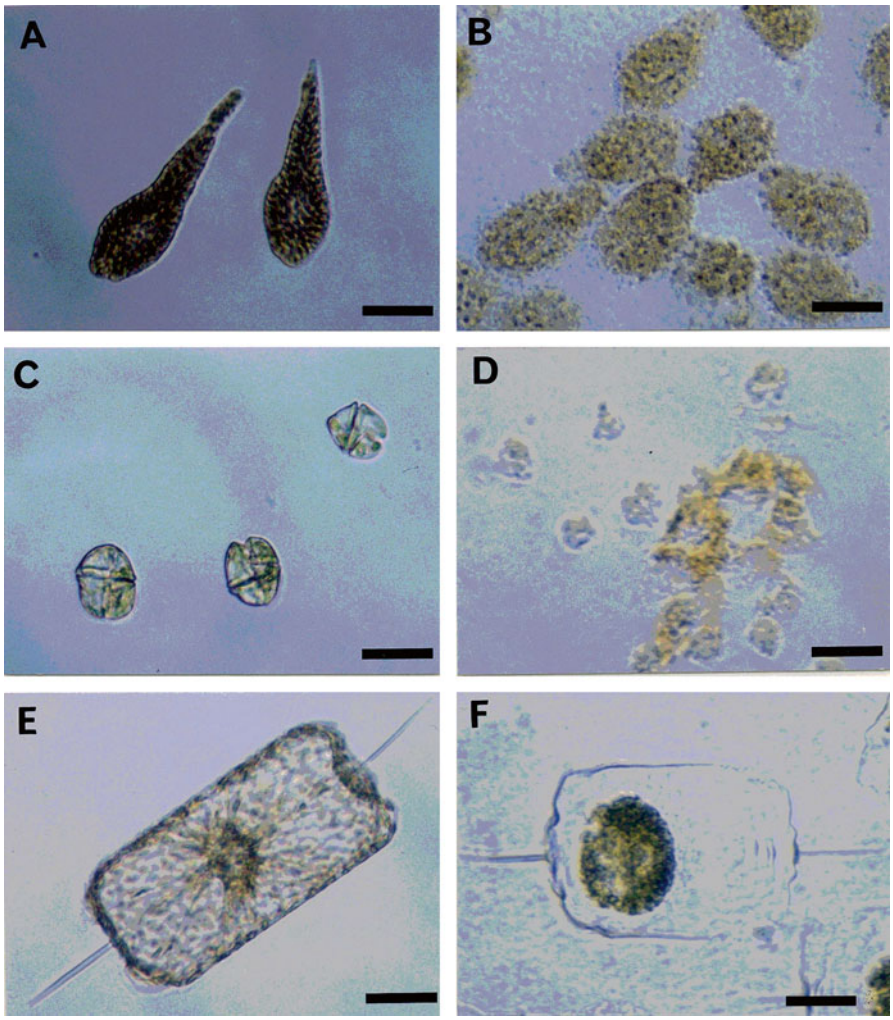

Fig. 25.1 Microscopic observations on the algicidal activities of the bacterium *Alteromonas* strain S against three marine phytoplankton species (Imai et al. 1995). Co-culture was made for three days. *Bars*: 30 μm. (**a**) Live cells of *Chattonella antiqua* (Raphidophyceae). (**b**) Burst cells of *C. antiqua* killed by the bacterium. (**c**) Live cells of *Karenia mikimotoi* (Dinophyceae). (**d**) Dead and burst cells of *K. mikimotoi* killed by the bacterium. (**e**) Live cell of *Ditylum brightwellii* (Bacillariophyceae). (**f**) Dead cell of *D. brightwellii* killed by the bacterium

the raphidophyte *Chattonella antiqua* and the dinoflagellate *Karenia mikimotoi* were destroyed and burst. The bacterium also killed the diatom *Ditylum brightwellii,* but it could not lyse frustules. In the case of the activities of GIBs against *C. antiqua*, two types of growth-inhibiting activities were observed (Inaba et al. 2014). The first is a change of the cells into a roundish form from the normal spindle-shaped

vegetative cell morphology of *C. antiqua*. This altered morphology resulted in a loss of motility and sinking of those cells to the bottom of culture vessels within a week. This eventually caused the death of *C. antiqua*. The second type of inhibition was an elongation of the vegetative cells that was caused by the delay of cell division and also reduced its motility. Elongated *C. antiqua* cells lost their ability to swim straight and rotated around at the bottom of culture plate wells.

The gliding bacteria such as the genera *Cytophaga* and *Saprospira* are generally direct attack type, and those of alpha- and gamma-proteobacteria and firmicutes are the types for extracellular production of algicidal matter (Sakata et al. 1991; Imai et al. 1993, 1995; Kondo and Imai 2001; Skerratt et al. 2002). Concerning the prey specificity, some bacteria kill only one algal species, others kill multiple algal species, and still others kill different algal species from several groups (Mayali and Azam 2004; Park et al. 2010; Inaba et al. 2014). Direct attack-type bacteria tend to prey upon algal species of wide range.

An interesting role of dissolved organic matter was demonstrated in *Alteromonas* E401, the killer of the harmful dinoflagellate *Karenia mikimotoi* (Yoshinaga et al. 1995). The bacterium strain E401 produced a high molecular weight (>10 kD) heat-labile compound showing algicidal activity. This killing substance was specifically produced in response to excreted organic matter (EOM) from *K. mikimotoi*. The algicidal activity was restricted to *K. mikimotoi* and *Gymnodinium catenatum*, but gave no effects on other dinoflagellates, diatoms or raphidophytes examined. This is also a case for experiments using raphidophyte *Heterosigma akashiwo*. If this is frequent in the sea, species-specific algicidal activity against blooming algal species is a common phenomenon induced by the EOM from each microalgal species.

Ectoenzymes, particularly ectoproteases, are the likely candidates for algicidal organic matter excreted by algicidal bacteria. Mitsutani et al. (2001) found that a stationary culture cell extract of *Pseudoalteromonas* A25 showed both algicidal and high protease activities. At least some algicidal bacteria might kill prey algae by using some kinds of proteases. As other algicidal substances, Isatin and red pigments of prodigiosins were identified from marine algicidal bacteria (Nakashima et al. 2006; Kim et al. 2008b; Sakata et al. 2011).

Skerratt et al. (2002) showed that Gram-negative algicidal bacteria appear to use the AI-2 mechanism (quorum sensing) at the mid- to late stage of the log growth phase. The AI-2 mechanism has been thought to be for bacteria communicating between species rather than acetylated homoserinelactones (AHLs). There is a metabolic benefit for these bioactive mechanisms to be activated simultaneously rather than individually.

Imai et al. (1995) observed the swarming of the bacterium *Alteromonas* S to the valve face of the diatom *Ditylum brightwellii* in the early stage of algicidal attack (Fig. 25.2). The swarming capacity to prey algal cells is thought to be an advantageous strategy for algicidal bacteria. Skerratt et al. (2002) also reported the swarming of algicidal bacteria. Swarming is presumably a result of chemotaxis. It is of interest to reveal whether algicidal activities relate to swarming and quorum sensing.

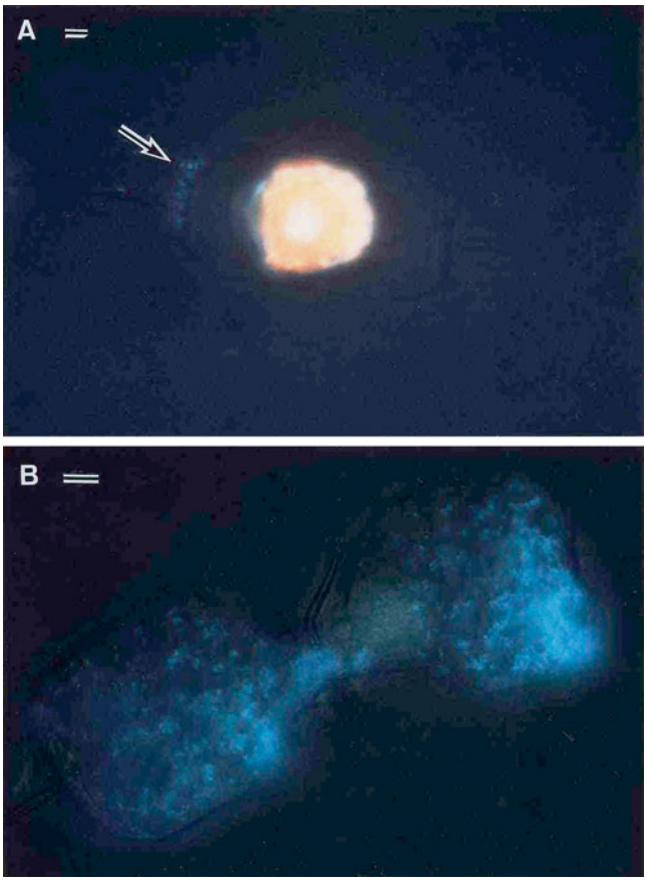

Fig. 25.2 Epifluorescence micrographs of the bacterium *Alteromonas* strain S attacking the diatom *Ditylum brightwellii* (Imai et al. 1995). Bacterial cells are seen as fluorescing spots. *Bars*: 10 µm. (**a**) Cell of *D. brightwellii* and the bacterial cells (*indicated by an arrow*) gathering to a valve face of the diatom. Co-culture was made for 1 day. (**b**) Killed diatom cells attacked by the bacterium. Numerous bacterial cells are seen inside the diatom cell. Co-culture was made for 2 days

25.3 Ecological Relationship Between Algicidal Bacteria and Harmful Algae in Coastal Seas

In northern Hiroshima Bay, the western Seto Inland Sea, the dynamics of the *Heterosigma akashiwo* (Raphidophyceae) killer bacteria revealed a close relationship with that of *H. akashiwo* populations (Imai et al. 1998a; Kim et al. 1998). *H. akashiwo* killers followed the increase of *H. akashiwo* cells, reached a maximal level after the beginning of decline of *H. akashiwo*, maintained a high level for at least 1 week after the crash of the bloom, and then decreased (Fig. 25.3). Similar results were reported between the dynamics of the raphidophytes *Fibrocapsa japonica* and *Chattonella*

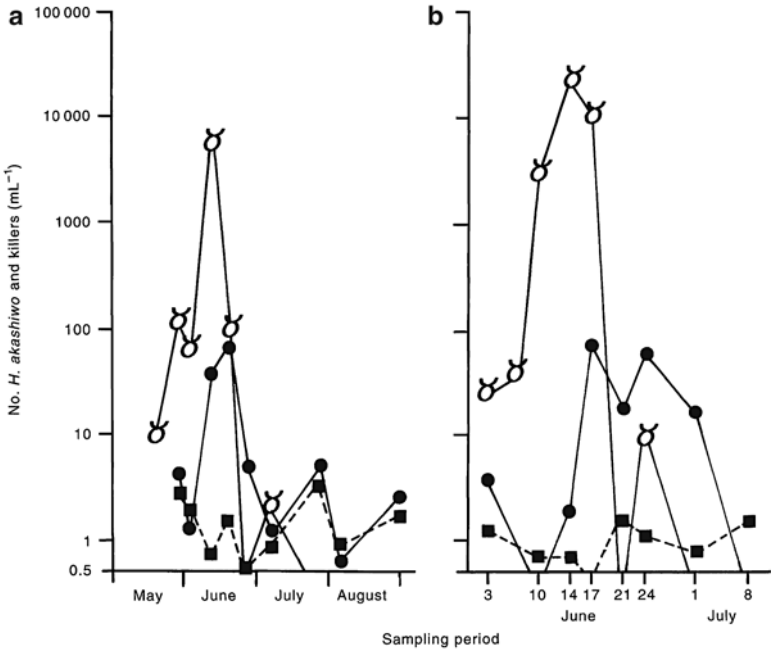

Fig. 25.3 Fluctuations in densities of *Heterosigma akashiwo*, H-killers (*closed circles*: algicidal micro-organisms against *H. akashiwo*) and C-killers (*closed squares*: algicidal micro-organisms against *Chattonella antiqua*) in the surface waters collected at a station in northern Hiroshima Bay (**a**) from May 28 to August 31, 1992; and (**b**) from June 3 to July 8, 1993 (Imai et al. 1998a)

subsalsa and that of the killers of *F. japonica* and *C. subsalsa* in shallow brackish detention ponds in South Carolina, USA (Liu et al. 2008).

The population structure of algicidal bacteria was also determined by restriction fragment length polymorphism (RFLP) analysis of the bacterial 16S rRNA genes during *H. akashiwo* red tides there (Yoshinaga et al. 1998). Bacteria belonging to gamma-proteobacteria were the dominant algicidal agents during the termination period of red tides followed by the strains of the phylum Bacteroides in 1994 and 1995. This result clearly indicates the specific group of algicidal bacteria (gamma-proteobacteria) associated with the termination of *H. akashiwo* red tides in the coastal areas such as Hiroshima Bay.

In Harima-Nada, the eastern Seto Inland Sea, the cell density of algicidal *Cytophaga* sp. J18/M01 (originally isolated from Harima-Nada) increased just after the peak of a small bloom of *Chattonella* spp. in the summers of 1997 (Fig. 25.4). This bacterium has a wide prey range (Imai et al. 1993) and also showed a close relationship with the change of the total microalgal biomass including diatoms and others (Imai et al. 2001). *Cytophaga* sp. J18/M01 (originally isolated from Harima-Nada in the summer of 1990) is the same species as *Cytophaga* sp. AA8-2 and AA8-3, which were originally isolated from Ago Bay in the summer of 1995 (Imai et al. 1999; Kondo et al. 1999). The algicidal bacterium *Kordia algicida* OT-1 with strong activity was also isolated from Korean costal water (Sohn et al. 2004), and this bacterial

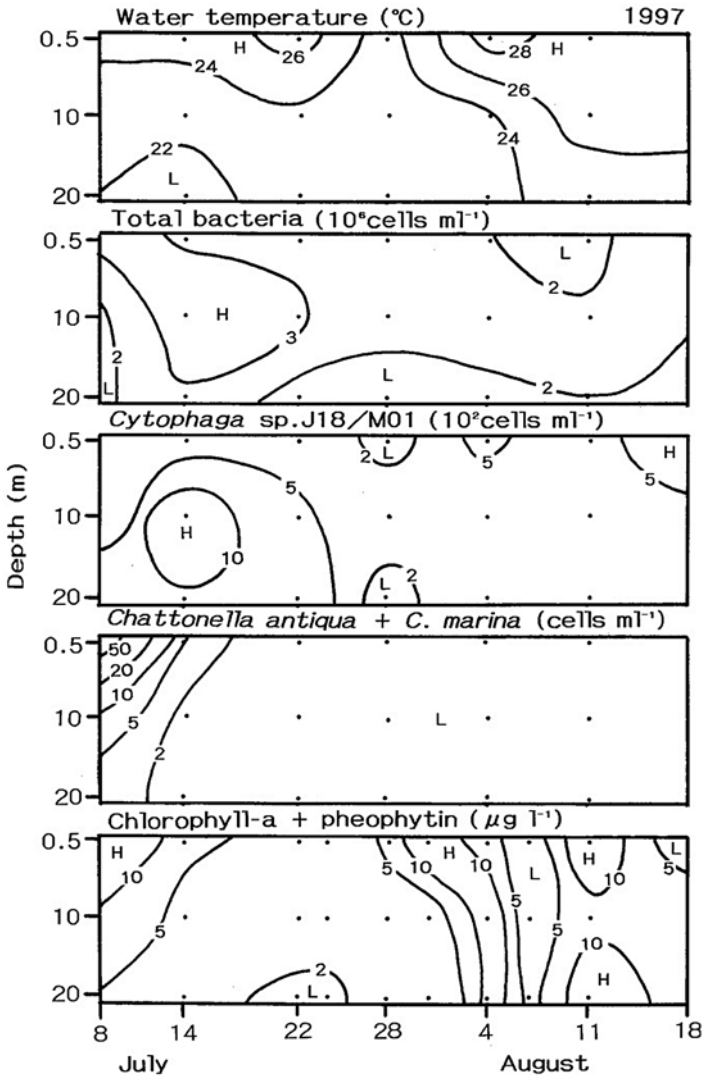

Fig. 25.4 Changes in vertical profiles of water temperature, total bacteria, algicidal bacterium *Cytophaga* sp. J18/M01, *Chattonella* spp. (*C. antiqua* and *C. marina*), and microalgal biomass (chlorophyll a and pheophytin) at the NH3 station in northern Harima-Nada, the eastern Seto Inland Sea, during the summer of 1997 (Imai et al. 2001)

strain has the same base-pair sequence of a 16 s rRNA gene to *Cytophaga* sp. AA8-2 and AA8-3 (Mayali 2007). Hence this algicidal *Cytophaga* species shows wide distribution in coastal areas of Japan and Korea. Yooseph et al. (2010) investigated the sequences of 197 diverse marine genomes collected from around the world along with previously published marine prokaryotic genomes in the context of marine

metagenomic data. It is interesting to notice that the bacterium *K. algicidal* (hence *Cytophaga* sp. AA8-2 and AA8-3) was detected from almost all samples from the global ocean. The algicidal bacterium *Cytophaga* sp. J18/M01 and the same species clones are consequently thought to be globally distributed both in the ocean and in the coastal seas of the world. It is hence considered that algicidal processes by bacteria are not peculiar phenomena in marine environments as previously expected.

Bacterial populations were fractionated into two groups (PABs and FLBs) by the filtration of water samples through a 3 μm pore filter, and algicidal activities and/or growth-inhibiting activities against HAB species were checked for each isolated bacterial strain of both fractions collected in the Yatsushiro Sea and Harima-Nada. The results clearly demonstrated that algicidal bacteria were more abundantly found in the fraction of PAB (particle-associated bacteria) (Imai et al. 2009a; Park et al. 2010; Inaba et al. 2014).

In general, macroaggregates are heavily colonized by bacteria as compared with the surrounding water, and are important for biochemical processes as "hot spots" (Simon et al. 2002; Azam and Malfatti 2007). It is interesting to note that the *Cytophaga* and gamma-proteobacteria groups are among the dominant bacterial flora attached to macroaggregates or marine snow (Doucette et al. 1998; Azam and Malfatti 2007). Swarming was observed in the algicidal process by *Alteromonas* sp. and *Cytophaga* sp. (Imai et al. 1995; Skerratt et al. 2002). When a small number of algicidal bacteria cells aggregated around a single microalgal cell or on a macroaggregate, such microscale patchiness could create an algicidal hot spot in the sea. These hot spots are thought to be important in the ecology of algicidal bacteria (Doucette et al. 1998; Imai et al. 1998a, b; Mayali and Azam 2004).

25.4 Seaweed Beds as Prevention Strategies for Red Tides

A new aspect of the ecology of algicidal bacteria is the discovery that huge numbers of algicidal bacteria attach onto the surface of seaweeds such as *Ulva* sp. (Chlorophyceae), *Gelidium* sp. (Rhodophyceae), *Sargassum muticum* and *Sargassum thunbergii* (both Phaeophyceae) (Imai et al. 2002). The abundance of algicidal bacteria on these macroalgae was determined with the MPN method (Imai et al. 1998b) on the coast of Osaka Bay in the Seto Inland Sea, and the maximum numbers of about 10^5–10^6 g^{-1} (wet weight) were detected for *Karenia mikimotoi, Fibrocapsa japonica* (Raphidophyceae), and *Heterosigma akashiwo*. Algicidal bacteria were also abundant (occasionally > 10^3 MPN ml^{-1}) in seawater at seaweed beds regardless of the occurrences of the relevant HAB species. Strains of algicidal bacteria were isolated from *Ulva* sp., *Gelidium* sp. and seawater at the seaweed bed, and these strains were identified on the basis of the analyses of 16S rDNA genes. These bacteria also belonged to gamma-proteobacteria and the phylum Bacteroides, and the strains belonging to alpha-proteobacteria were newly identified (Imai et al. 2006a). Table 25.1 shows the prey range of algicidal bacteria isolated from the surface of seaweeds and seawater in the seaweed bed of Misaki-kouen of Osaka Bay in 1999 (Imai et al. 2006a). The dinoflagellate *K. mikimotoi* was the most susceptible

Table 25.1 Prey range of algicidal bacteria isolated from the surface of seaweeds (*Ulva* sp. and *Gelidium* sp.) and seawater in seaweed bed (Imai et al. 2006a)

	Prey algae							
Bacterial strain (origin)	*Chattonella antiqua*	*C. marina*	*C . ovata*	*Fibrocapsa japonica*	*Heterosigma akashiwo* (893)	*H. akashiwo* (IWA)	*Karenia mikimotoi*	*Heterocapsa circularisquama*
Pseudoalteromonas sp. 46 (*Ulva* sp.)	–	–	–	++	–	++	++	–
Pseudoalteromonas sp. 47 (*Ulva* sp.)	–	–	–	++	–	++	++	–
Octadecabacter sp. 49 (*Ulva* sp.)	++	–	–	–	–	–	–	–
Pseudoalteromonas sp. 53 (*Ulva* sp.)	–	–	–	++	–	++	++	–
Rhodobacteraceae 63 (seawater in seaweed bed)	–	–	+	+	+	–	++	–
Alteromonas sp. 57 (*Gelidium* sp.)	–	+	++	–	+	+	–	–
Vibrio sp. 55 (*Ulva* sp.)	–	–	++	–	–	+	++	–
Vibrio sp. 58 (*Gelidium* sp.)	–	–	++	–	–	+	++	–

++ Decrease below initial cell density; + lower growth than control (no addition of bacteria); – no effects

species, followed by the raphidophyte *F. japonica*, *H. akashiwo* (IWA) and *C. ovata*. The armored dinoflagellate *Heterocapsa circularisquama* was not killed by any of the algicidal bacteria examined. Each strain tended to show a rather limited prey range, causing mortality in at most three microalgal species of the eight strains of seven red tide species. *Vibrio* sp. 55 (isolated from *Ulva*) and *Vibrio* sp. 58 (isolated from *Gelidium*) were the same species based on the same 16S rDNA sequences, and revealed the same pattern of algicidal efficiency (Imai et al. 2006a). This fact implies that some kinds of algicidal bacteria freely attach to the surface of seaweeds regardless of the seaweed species common to their habitat.

Ulva pertusa was cultivated with red sea bream (*Pagrus major*) in a pen cage in Shimo-Haya Bay, Wakayama Prefecture, and algicidal bacteria were investigated for the dinoflagellates *K. mikimotoi* and *H. circularisquama*, and the raphidophytes *C. antiqua*, *H. akashiwo* and *F. japonica* (Imai et al. 2012a). ABs were enumerated with co-culturing experiments using bacterial strains forming colonies on nutrient agar plates, considering the indication of underestimation of ABs with the MPN method (Imai et al. 1998a, b). Table 25.2 shows densities of ABs on the surface of *U. pertusa*. *K. mikimotoi* killers were most abundant, followed by *F. japonica* killers. The detected densities of ABs were in the range of 10^4–10^6 cells g^{-1} wet weight, and the total algicidal bacteria (number of bacteria that killed at least one HAB species examined with co-culture experiments) were 2.46×10^5–9.12×10^5 cells g^{-1} wet weight occupying the ratio of 33–80 % of the total number of colony-forming bacteria on the examined leaves of *U. pertusa* (Table 25.2). Figure 25.5 represents the numbers of ABs enumerated with a co-culturing experiment in seawater samples collected at the *U. pertusa* cage (station 1), coastal point (station 2) and 1-km offshore point (station 3). Killers for *K. mikimotoi* and *F. japonica* tended to be more abundant than those for other HAB species, and killers tended to be more abundant at cage and coastal point than offshore. There is a possibility that ABs were supplied from seaweed areas to offshore areas.

Based on these studies, we can propose a new prevention strategy for red tides by using seaweeds in aquaculture areas (Fig. 25.6). Co-culturing of *Gelidium* sp. or *Ulva* sp. and finfish such as red sea bream or yellowtail is proposed to be effective

Table 25.2 Fluctuations of algicidal bacteria ($\times 10^5$ cells g^{-1} wet weight) on the surface of the green alga *Ulva pertusa* cultivated in a cage at station 1 in Shimo-Haya Bay during the period of April to August 2003 (Imai et al. 2012a). Enumeration was made with a co-culturing experiment using bacterial strains forming colonies on nutrient agar plate and five species of red tide plankton

Target red tide plankton species	April 24	June 13	August 28
Karenia mikimotoi	3.51	7.98	1.13
Heterocapsa circularisquama	1.87	<0.28	0.57
Chattonella antiqua	0.94	0.86	0.38
Heterosigma akashiwo	0.47	<0.28	0.19
Fibrocapsa japonica	1.64	2.28	1.70
[a]Total algicidal bacteria	4.91	9.12	2.46
Total colony-forming bacteria	7.01	11.4	7.56

[a] Number of bacteria that killed at least one species of red tide phytoplankton. Some bacteria killed two or more plankton species

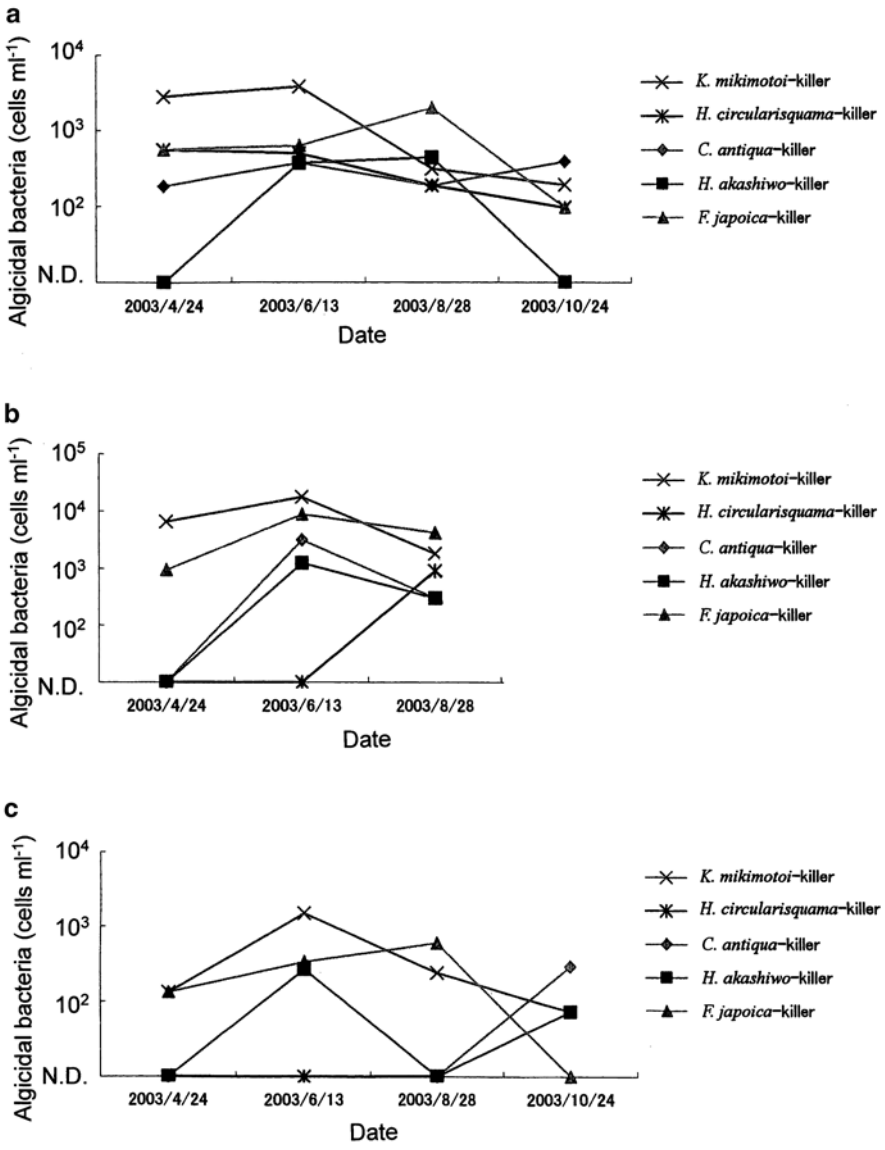

Fig. 25.5 Fluctuations of algicidal bacteria in surface seawater at the stations in Shimo-Haya Bay during the period of April to October 2003 (Imai et al. 2012a). Enumerations were made with co-culturing experiments using bacterial strains forming colonies on agar plates and HAB species. (**a**) Station 1, cage of culture of *Ulva pertusa* with red sea bream. (**b**) Station 2, coastal water. (**c**) Station 3, about 1 km offshore from station B

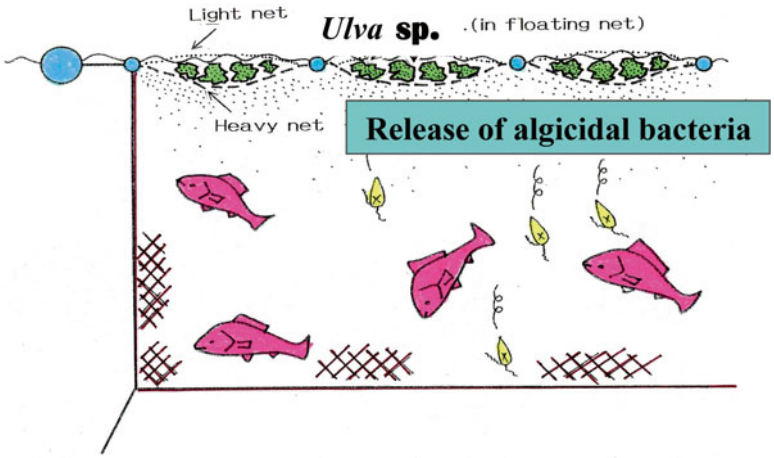

Fig. 25.6 Possible prevention strategy against noxious red tides by use of seaweeds such as *Ulva pertusa* that release algicidal bacteria into the surrounding seawater in aquaculture areas. Co-culture of seaweeds and fishes is proposed in the same cages and/or adjacent water (Imai et al. 2002)

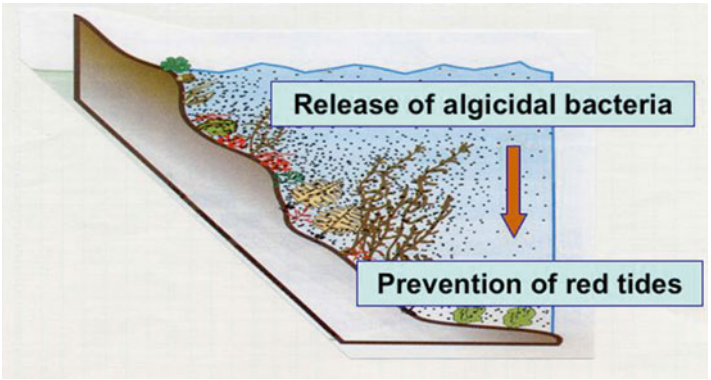

Fig. 25.7 Seaweed beds as sources of algicidal bacteria that help prevent the occurrence of red tides in coastal areas (Imai et al. 2006b)

in cage cultures (Imai et al. 2002). Many algicidal bacteria would be continually released from the surface of seaweeds to the surrounding seawater, and would contribute to reduce cell densities of HA species. Consequently, these bacteria probably play an important role in preventing HAB occurrences. This strategy is thought to be effective in enclosed and small-scale inlets.

When we artificially develop and restore the natural seaweed beds under the large-scale plan, these newly recovered seaweed beds would presumably function as tools to prevent occurrences of HABs by virtue of the continuously released algicidal bacteria (Fig. 25.7). This is regarded as a kind of bioremediation (biostimulation and bioaugmentation, simultaneously). Further, seaweed beds also serve as nursery grounds for important fishery resources such as fish and invertebrates for commercial use.

25.5 Seagrass Beds as Prevention Strategies for Harmful Algal Blooms

Seagrass beds have an important function in coastal ecosystems to maintain biodiversity and to provide feeding, housing, and spawning grounds for marine life (Nakaoka and Aioi 2001; Williams and Heck 2001). As an interesting feature, the seagrass *Zostera marina* and *Z. noltii* possess growth-inhibiting activity against phytoplankton through allelopathy (Harrison and Chan 1980; Wit et al. 2012). For example, an extract from *Z. marina* was lethal at a concentration of 0.25 mg dry leaf/mL to eight species of examined microalgae, i.e., the pennate diatoms *Cylindrotheca fusiformis, Nitzschia angularis, N. frustulum, N. longissima*, the centric diatom *Skeletonema costaum*, the dinoflagellates *Gonyaulax polyedrum*, *Protogonyaulax tamarensis* (*Alexandrium tamarense*), and the green alga *Platymonas* sp. (Harrison and Chan 1980). The growth of phytoplankton was delayed by the addition of *Z. noltii* in mesocosm experiments (Wit et al. 2012). However, since highly diverse microorganisms possessing various activities live in seagrass beds, it is expected that there exist various kinds of algicidal and/or growth-inhibiting bacteria against phytoplankton. Interestingly, algicidal bacteria against red tide-causing flagellates were actually discovered with high densities in the biofilm on the surface of *Z. marina* blades (Imai et al. 2009b). The densities of those algicidal bacteria were 9.19×10^6–64.3×10^6 cell g^{-1} wet weight for the raphidophyte *Chattonella antiqua* and the dinoflagellates *Heterocapsa circularisquama*, *Karenia mikimotoi* and *Cochlodinium polykrikoides* (Table 25.3). Consequently, it is considered that seagrasses are favorite habitats for algicidal bacteria, and they have an ability to autonomously proliferate via algicidal activity and utilization of resultant organic matter. Hence it is expected that it will be possible to autonomously exterminate and/or prevent occurrences of red tides. Future studies are essential to determine whether allelopathy or algicidal bacteria are more effective to kill or inhibit the growth of phytoplankton.

Concerning toxic species such as paralytic shellfish poisoning (PSP)-causative *Alexandrium tamarense*, growth-inhibiting bacteria possessing strong activity were

Table 25.3 Abundance of algicidal bacteria against five species of harmful algal bloom (HABs) detected from the surface of seagrass (Zostera marina) leaf and from the seawater of the seagrass bed (particle-associated [PAB] and free-living [FLB] fractions) collected on July 13, 2006 (Imai et al. 2009b)

	Algicidal bacteria		
	Zostera leaf	Sea water ($\times 10^3$/ml)	
Target HAB species	($\times 10^6$/g wet leaf)	PAB	FLB
Chattonella antiqua	9.19	4.8	0
Heterosigma akashiwo	0	2.4	0
Heterocapsa circularisquama	9.19	0	0
Karenia mikimotoi	64.3	2.4	0
Cochlodinium polykrikoides	27.6	2.4	0

discovered from the surface of blades of *Zostera marina* (Onishi et al. 2014). The isolated bacterial strain (E9) markedly inhibited the growth of *A. tamarense* even with an initial inoculum size as small as 2.9 cells ml^{-1} (Fig. 25.8). The variety of the morphology affected by the bacterium was shown in Fig. 25.9. After addition of the cells of inhibiting bacterial strain E9, thecal plates were often detached from the algal cell after around 3 days, and spherical cells, presumably temporary cysts, were also frequently observed at the same time in experimental vessels. These naked cells were eventually killed by the bacterium (Fig. 25.9). Small subunit ribosomal DNA

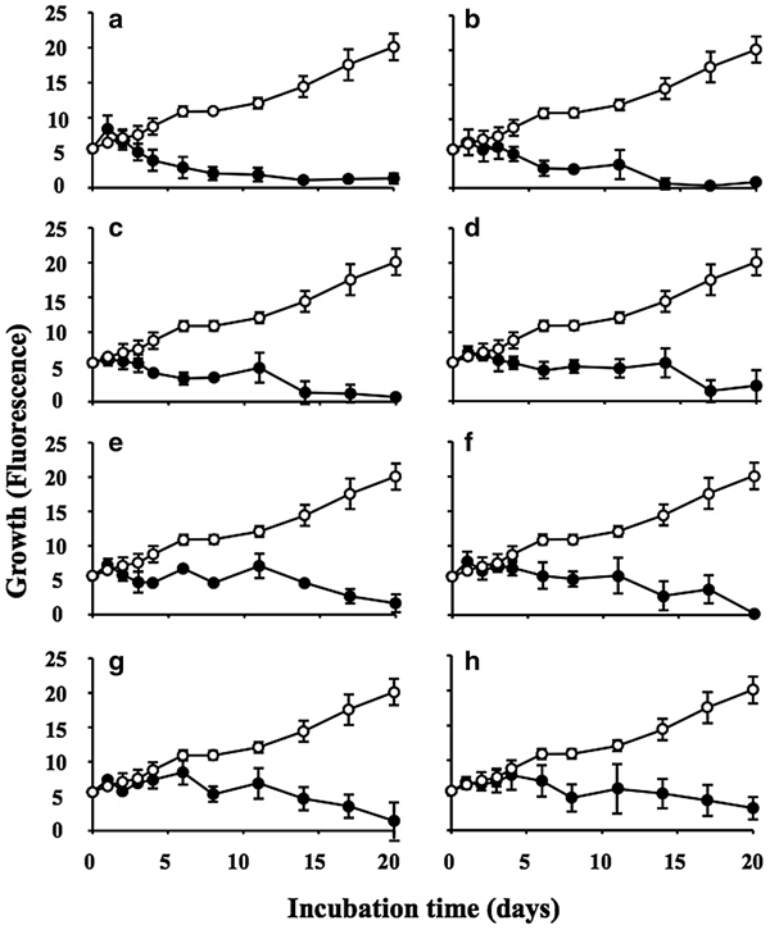

Fig. 25.8 Effects of growth-inhibiting bacterial strain E9 with different inoculum sizes on *Alexandrium tamarense* in the modified SWM-3 medium (Onishi et al. 2014). The initial cell density of *A. tamarense* was 3.6×10^2 cells ml^{-1}. The initial bacterial densities (cell ml^{-1}) were (**a**) 2.9×10^7, (**b**) 2.9×10^6, (**c**) 2.9×10^5, (**d**) 2.9×10^4, (**e**) 2.9×10^3, (**f**) 2.9×10^2, (**g**) 2.9×10^1, and (**h**) 2.9×10^0. Control (*open circle*) indicates the growth of *A. tamarense* with no addition of bacterial cells (axenic culture)

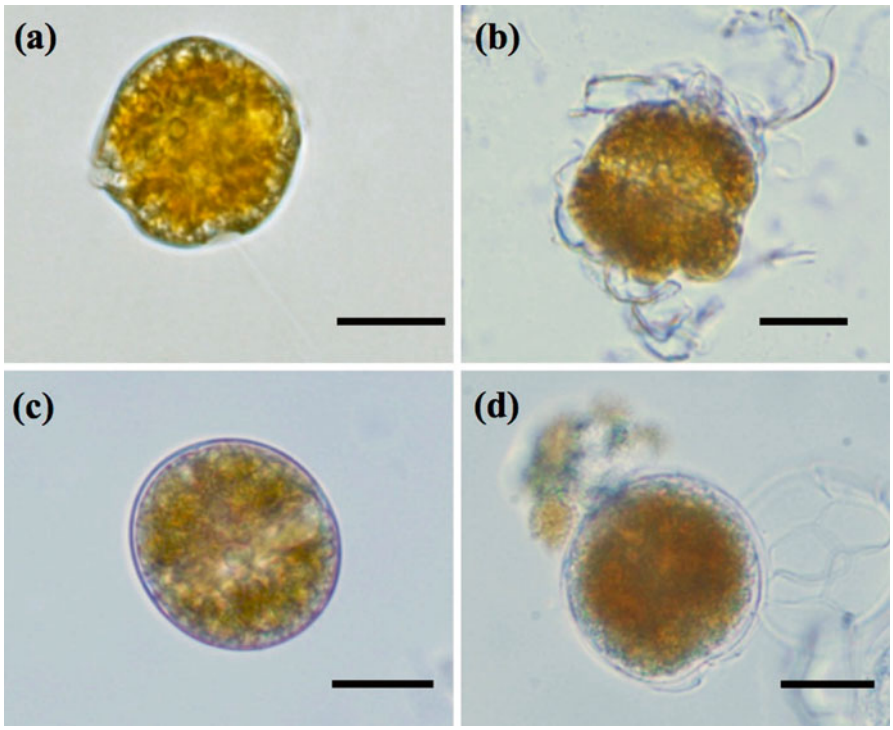

Fig. 25.9 Effects of bacterial strain E9 on morphology of *Alexandrium tamarense*: (**a**) control, no addition of bacterial cells; (**b**) *A. tamarense* cell with detached thecal plates after 3 days' incubation; (**c**) spherical cell of suspected temporary cyst observed after 3 days' incubation; (**d**) disrupted cell releasing cell contents observed on day 7 after addition of bacterial cells (Onishi et al. 2014). Scale bar 20 μm

(SSU rDNA) sequencing analysis demonstrated that the most probable affiliation of this strain (E9) was a member of the genus *Flavobacterium* belonging to the phylum Bacteroides, and proved that another inhibitory bacterial strain (E8) against *A. tamarense* showed the same sequence as the strain E9, indicating the same species (Fig. 25.10). Two other bacterial strains (E4-2 and E10) isolated from the same seagrass sample, showing a different colony color from E9, revealed no growth-inhibiting activity against *A. tamarense*. Interestingly, the strain E4-2 revealed the same sequence of SSU rDNA as E8 and E9 (100 %), and the strain E10 matched E8 and E9 with 99.8 % similarity. In conclusion, it is considered that seagrass beds have the potential to prevent occurrences of not only harmful red tides (Imai et al. 2009b; Imai and Yamaguchi 2012), but also toxic dinoflagellate blooms by virtue of the association of strong growth-inhibiting bacteria (Onishi et al. 2014).

Seagrass beds have been rapidly disappearing at a rate of 110 km^2 $year^{-1}$ in the world since 1980, and 29 % of the initial area has disappeared since 1879, when seagrass areas were first approximately estimated (Waycott et al. 2009). On the

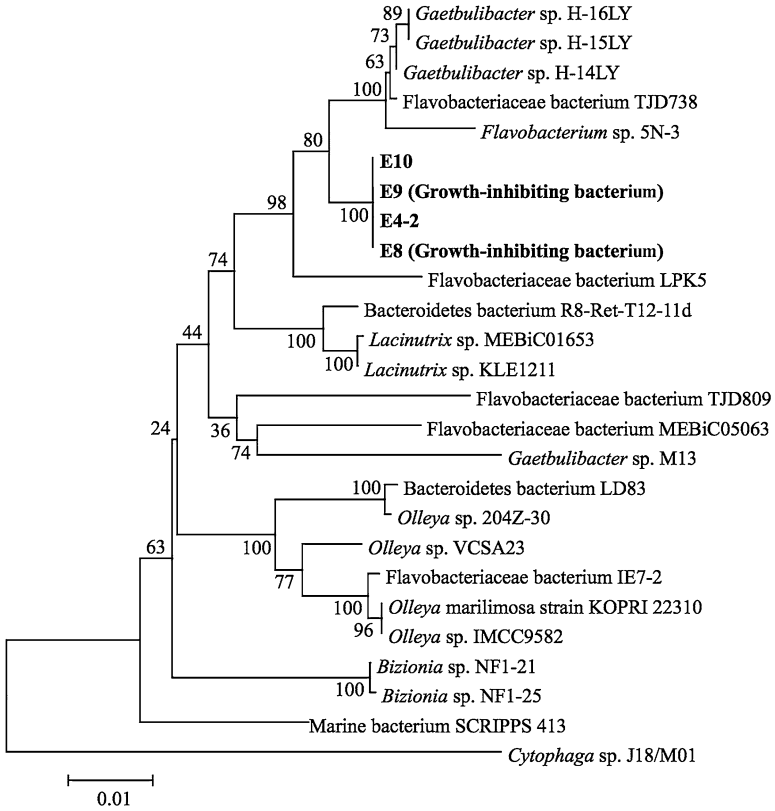

Fig. 25.10 Phylogenetic tree including the growth-inhibiting bacteria E8 and E9 and two closely related bacterial strains (E10 and E4-2) based on 16S rRNA gene sequences (Onishi et al. 2014). The tree was constructed using the neighbor-joining method and maximum likelihood method (NJ/ML)

other hand, the scale and frequency of occurrences of harmful algal blooms have been increasing globally (Hallegraeff 1993). There is an interesting report from the Mediterranean coast that the large-scale decline of seagrass beds was accompanied by increasing frequency of the toxic dinoflagellate blooms of *Alexandrium minutum* (Abdenadher et al. 2012). Also, on the coast of the Seto Inland Sea, Japan, incidents of HABs markedly increased (from <50 to the maximum of 299 in 1976) during the 1960s and 1970s, and seagrass beds had dramatically been reduced to one quarter (from 22,635 ha in 1960 to 6381 ha in 1989) during the same period (Imai and Yamaguchi 2012). This is thought to be a case of catastrophic shifts (Scheffer et al. 2001) with regard to the function of controlling phytoplankton by seagrass beds in coastal ecosystems. Therefore, it is proposed that restoration and/or creation of seagrass beds is potentially and urgently important to prevent HABs.

25.6 Perspectives

Algal blooms produce much abundant organic matter. After the rapid bloom termination by algicidal bacteria, killed algal cells must be decomposed rapidly and enter into the food web mainly through microbial loops (Kamiyama et al. 2000) without delay. If not, bloom terminations directly contribute to the deterioration of coastal environments such as anoxia. Seagrass beds and seaweed beds are expected to be hot spots of microbial processes such as algicidal activity, decomposition of excessively produced organic matter, and hence the function of microbial loops. More extensive studies are needed on the effects of algal blooms and decay on food webs in these hot spot areas in the future.

In freshwater ecosystems, one of the most dramatic state shifts is the sudden loss of transparency and vegetation in shallow lakes subject to human-induced eutrophication (Scheffer et al. 1993, 2001). Experimental study suggested that water plants increased water clarity, thereby enhancing their own growing conditions. The reduction of phytoplankton biomass and turbidity by water plants involves various mechanisms, i.e., reduction of nutrients, protection of phytoplankton grazers (*Daphnia* etc.) against fish predation, and prevention of sediment resuspension. A real example of an increase in vegetation and accompanying transparency was observed in Lake Biwa in the 1990s (Haga and Ohtsuka 2008).

Similarly, in marine coastal ecosystems, seagrass (*Zostera marina*) beds also occasionally disappear due to dense red tides such as the raphidophyte *Heterosigma akashiwo* (Lee et al. 2007). Seagrass (*Z. marina*) bed can recover by itself through new shoot recruitment from the seed bank under sufficient light conditions. Since seagrass harbors huge numbers of algicidal bacteria against HAB species, restoration and/or creation of seagrass beds should be effective for keeping the phytoplankton biomass in an adequately low range and for preventing occurrences of HABs in adjacent waters.

Sato-Umi is a newly proposed concept for sustainable fisheries, identified as "high productivity and biodiversity in the coastal sea with human interaction" (Yanagi 2008). As mentioned above, the creation and/or restoration of seagrass beds and seaweed beds are considered to contribute to preventing HAB occurrences by virtue of the activities of algicidal bacteria released from seaweeds and seagrasses to adjacent coastal waters (Fig. 25.11). These strategies are ultimately environment friendly, and restoration and maintenance of seaweed and seagrass beds are important to maintain the health of the coastal sea, preventing the occurrence of HABs (Imai et al. 2006b; Imai and Yamaguchi 2012). This is a kind of harmony between humankind and nature in conformity with the concept of "Sato-Umi" (Yanagi 2008; Imai and Yamaguchi 2012; Onishi et al. 2014).

The Sato-Umi concept would be also applicable to freshwater ecosystems. An algicidal bacterium (*Agrobacterium vitis*) active against the toxic cyanobacterium *Microcystis aeruginosa* was actually isolated from the surface of the water plant *Egeria densa* (Imai et al. 2012b). Therefore, creating an adequate scale of water-plant zone is expected to keep lake conditions in a healthy state with lower

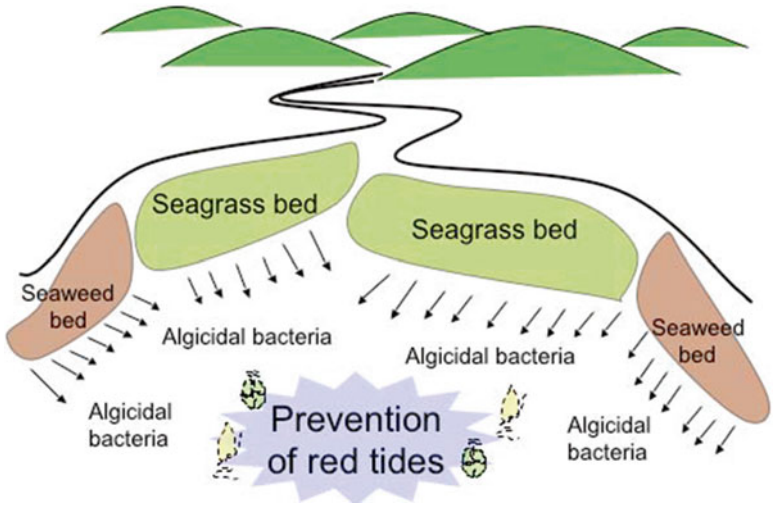

Fig. 25.11 Schematic representation of strategies for preventing red tide (harmful algal bloom) occurrences by restoration and/or development of seaweed- and seagrass beds in coastal areas (Imai et al. 2009b). Algicidal bacteria would be supplied to seawater and prevent red tide occurrences by virtue of controlling phytoplankton populations within some moderate levels

frequencies of toxic cyanobacterial blooms by virtue of algicidal bacteria (Imai et al. 2012b) and with a clear state (Scheffer et al. 2001). Studies on interrelationships among higher plants, seaweeds, phytoplankton, and microorganisms such as bacteria and viruses promise to solve environmental problems in aquatic (both marine and freshwater) ecosystems in the future.

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