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Algicidal Bacteria in the Sea and their Impact on Algal Blooms[1]

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ABSTRACT. Over the past two decades, many reports have revealed the existence of bacteria capable of killing phytoplankton. These algicidal bacteria sometimes increase in abundance concurrently with the decline of algal blooms, suggesting that they may affect algal bloom dynamics. Here, we synthesize the existing knowledge on algicidal bacteria interactions with marine eukaryotic microalgae. We discuss the effectiveness of the current methods to characterize the algicidal phenotype in an ecosystem context. We briefly consider the literature on the phylogenetic identification of algicidal bacteria, their interaction with their algal prey, the characterization of algicidal molecules, and the enumeration of algicidal bacteria during algal blooms. We conclude that, due to limitations of current methods, the evidence for algicidal bacteria causing algal bloom decline is circumstantial. New methods and an ecosystem approach are needed to test hypotheses on the impact of algicidal bacteria in algal bloom dynamics. This will require enlarging the scope of inquiry from its current focus on the potential utility of algicidal bacteria in the control of harmful algal blooms. We suggest conceptualizing bacterial algicidy within the general problem of bacterial regulation of algal community structure in the ocean.

Key Words. Algal-killing, Bacillariophyceae, *Cytophaga*, Dinophyceae, pathogen, phytoplankton, *Pseudoalteromonas*, Raphidophyceae.

THE ecological importance of heterotrophic bacteria and archaea in the ocean is well established, from their utilization of dissolved organic matter (DOM) to their contribution of energy to higher trophic levels through the microbial loop (Azam et al. 1983; Fuhrman and Azam 1980; Hagström et al. 1979; King, Hollibaugh, and Azam 1980; Pomeroy 1974). Incorporating these findings into marine ecosystem models requires elucidating the mechanisms that underlie the variability in organic matter fluxes from phytoplankton to bacteria. How is a large fraction of the carbon fixed by phytoplankton converted to DOM, thus becoming accessible mainly to bacteria? Do bacteria depend on DOM being produced by other organisms, e.g. phytoplankton exudation and ''sloppy feeding'' on them by herbivores (Hellebust 1974) or, do bacteria exert their own biochemical activities on the particulate phase, including live algal cells, to generate DOM? An emerging view (e.g. Azam and Smith 1991; Guerrini et al. 1998) contends that the flow of energy and nutrients between algae and bacteria involves dynamic ecological relationships. Algae and bacteria may establish commensalisms that, under nutrient stress, shift to competition and eventually lead to killing and lysis of algae by bacteria. This dynamic scenario is consistent with reports, over the last two decades, of the occurrence of algicidal bacteria that kill marine microalgae. Furthermore, some of these bacteria may specialize in and even require an algicidal lifestyle (rather than being opportunistically algicidal), as the bacteria-killing *Bdellovibrio* genus and other predatory bacteria (Martin 2002).

Here, we synthesize the knowledge on algicidal marine bacteria and the algae susceptible to them. We also direct the reader to general reviews of bacterial-algal interactions (Cole 1982; Doucette 1995; Doucette et al. 1998). Our emphasis is to assess the significance of algicidal bacteria for marine ecosystems in general and for algal blooms in particular. Further, we stress the need to develop a conceptual framework and quantitative methods for considering algicidal bacteria in an ecosystem context.

Definition. The literature lacks a clear definition of algicidal bacteria. One might argue that many, if not all, heterotrophic bacteria in the sea have the biochemical potential to kill algae. These bacteria would turn algicidal unless, or as long as, the algae can defend themselves against bacterial attack. However, there is evidence that certain bacteria specialize in an algicidal life style. One might define such true algicidal bacteria (and possibly archaea as well) as algal pathogens that satisfy Koch's postulates in an environmental context. The requirement of an environmental context poses conceptual and technical challenges (Fredricks and Relman 1996). For example, most marine bacteria are as yet uncultured, making it challenging to demonstrate their algicidal activity in mixed natural assemblages. A direct test would be to observe individual algal cells ''under attack'' in seawater and determine the identity of the bacterium by culture-independent, molecular techniques. If the alga is in axenic culture, one might inoculate it into seawater, retrieve it (e.g. by micromanipulation or flow-cytometry) to test for algicidal attack, and identify the associated bacteria. As in animal hosts where the virulence of a bacterial pathogen depends on host susceptibility (e.g. Salyers and Whitt 2001), the alga's physiological state might be an important variable in algicidal interaction. Thus, only some individuals of an algal species in a seawater sample might be susceptible, complicating interpretation. Physiological state of the algicidal bacteria may also be a variable in ''virulence''. All these variables must be considered in determining whether a marine bacterium, culturable or not, satisfies the criteria of a pathogen of algae in an ecosystem context.

The current literature, based on both algal and bacterial culture-dependent methods, does provide important insights and poses new questions. Nevertheless, these culture systems are not so simple either and create a different set of difficulties than the highly complex ocean microbial ecosystem. Furthermore, various authors have used different protocols, making results difficult to interpret and to compare. Some studies have emphasized the need to wash the bacterial culture before addition to the algae (Doucette, McGovern, and Babinchak 1999; Furusawa et al. 2003), presumably to remove waste products of bacterial metabolism in organically rich media. Others have considered bacterial culture filtrate as the source of algicidal activity (Lovejoy, Bowman, and Hallegraeff 1998; Skerratt et al. 2002), although it remains unknown if adding washed bacterial cells would also result in algal death. Still others did not specify whether bacterial additions to the algae contained the spent medium, and it is unknown whether the bacterial medium played a role in algicidal activity in those studies. Interestingly, the time to algal culture death is drastically different whether a washed bacterial culture or a culture filtrate is added: days (Doucette, McGovern, and Babinchak 1999) vs. minutes (Skerratt et al. 2002). The reports using algicidal bacteria culture medium and algicidal bacteria cells are not necessarily comparable: the former examines the production of fast-acting anti-

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algal compounds in a bacterial monoculture, whereas the latter assays the ability of bacterial cells to kill algae in co-culture over longer time scales. Further, an algicidal bacterium might kill algae in one assay but not the other. Although both types of studies provide valuable insights, the ecological and evolutionary significance of these phenomena should be recognized as distinct.

Taxonomy. Before the advent of ribosomal DNA sequencing for microbial identification (Woese et al. 1985), biochemical and morphological methods defined bacterial taxa and also provided physiological information about the organism, e.g. carbon source utilization and antibiotic resistance profiles. Although such methods may not be optimal for phylogenetic taxonomy, they provide information with potential ecological relevance. Today, sequencing of the 16S rDNA gene is the standard for phylogenetic analysis of bacteria, but it provides little insight into the organism's physiological ecology. Nonetheless, several studies have used 16S rDNA data to design molecular probes able to detect algicidal bacteria in the environment (Iwamoto et al. 2001; Kondo and Imai 2001; Kondo et al. 1999; Maeda et al. 1998), although field studies utilizing such probes are still lacking. Also, since marine algicidal bacteria belong to different taxa, these authors could design probes specific to a limited number of groups of algicidal bacteria, often only one strain.

To date, there has not been a comprehensive phylogenetic analysis of all known marine algicidal bacteria. Thus, many fascinating questions on the evolution of algal hosts and their bacterial pathogens remain unanswered. For example, are there metabolic properties common to broad bacterial taxa that allowed specific subgroups of bacteria to become algicidal? Are specific phytoplankton species more susceptible to certain types of algicidal bacteria? Studies specifically addressing such evolutionary hypotheses will be needed in order to begin to understand how algicidal bacteria have become adapted to their environment.

With morphological, biochemical, and molecular methods of taxonomic analyses, studies have recognized four major groups of algicidal bacteria and many strains, as well as some less common groups (Table 1). The most common groups include members of the genus *Cytophaga* (renamed *Cellulophaga* by Skerratt et al. 2002) and *Saprospira* (phylum Bacteroidetes), and the genera *Pseudoalteromonas* and *Alteromonas* (phylum γ-Proteobacteria). Algicidal bacteria kill their prey by two main mechanisms: direct contact or algicide release (discussed below). *Saprospira* are generalist predators of bacteria (Sangkhobol and Skerman 1981) as well as algae (Sakata 1990) and require attachment to their prey (Lewin 1997). Most algicidal *Cytophaga* also require attachment, although there are some exceptions (Table 1). This is consistent with the finding that marine Cytophagales are often particle-associated (reviewed by Kirchman 2002). Further, their ability to degrade high molecular weight organic matter (also present on phytoplankton cell surfaces) supports this hypothesis. Attachment to algae and the ability to degrade cell surface macromolecules make *Cytophaga* well suited for an algicidal lifestyle. In contrast, all *Alteromonas* and *Pseudoalteromonas* algicidal bacteria tested, killed by releasing dissolved substances (Table 1). This is consistent with the finding that many *Pseudoalteromonas* produce extracellular bioactive molecules (Holmström and Kjelleberg 1999).

Mode of action. Release of a freely diffusible algicide is unlikely to be an energetically efficient strategy for killing algal cells suspended in seawater, based on the calculated volume/volume ratio of bacterial cells/seawater of 10^{-7} (Azam, Smith, and Carlucci 1992). However, this approach may be efficient in low-diffusion microhabitats, such as marine snow. A different strategy would be to express cell surface-bound algicides acting through physical contact with the prey alga. Three experimental techniques have distinguished between these two general strategies. The first tests for the presence of algicidal activity in cell-free spent media from algicidal bacteria by its effect on the target algae (Baker and Herson 1978, and others). However, the algicide may be a by-product of the metabolism of a media component, and not produced under environmental conditions. Further, the production of an algicide might occur only in response to the presence of the algal prey (Yoshinaga, Kawai, and Ishida 1995). A second technique tests for dissolved algicide production in cell-free filtrate of an algal culture killed by a bacterium by adding the filtrate to a healthy algal culture (Imai, Ishida, and Hata 1993, and others). If the filtrate kills the algae, it may be that a dissolved compound from algicidal bacteria is responsible. However, the dissolved compound may in fact have been derived post-mortem from the algal cells killed by bacterial attachment. Algae contain vacuoles replete with acids and hydrolytic enzymes that might kill a fresh culture, but this hypothesis remains untested. Further, a toxic metabolite from bacterial degradation of algal organic matter might be produced following cell lysis caused by attached bacteria. In this case, the metabolite would not be originally responsible for the algal lysis. A less ambiguous third method to test for dissolved algicides is to co-incubate the target algae and algicidal bacteria physically separated by a dialysis membrane or fine pore-size filter allowing dissolved compounds to diffuse across (Yoshinaga, Kawai, and Ishida 1995, and others). Use of commercially-available tissue culture inserts (Nalge Nunc International, Rochester, NY, USA) as in Kim et al. (1999b), allows such a protocol to be carried out in 24-well plates. Microscopic examination of the killing event also provides information on whether attachment is necessary for algal lysis to occur.

Characterization of algicidal compounds. The compounds used by algicidal bacteria that require prey contact to kill (e.g. *Cytophaga* and *Saprospira*) remain uncharacterized, although they may be similar to those from bacteria that kill through dissolved algicides (e.g. *Alteromonas* and *Pseudoalteromonas*). Ectoenzymes, particularly ectoproteases, are among the likely candidates. Lee et al. (2000) were the first to document a dissolved algicidal protease using a combination of genetics and biochemistry. They found that the culture filtrate from algicidal *Pseudoalteromonas* strain A28 exhibited high protease activity, whereas that of non-algicidal mutants did not. They subsequently isolated a 50-kDa serine protease from the wild-type strain filtrate that displayed algicidal activity. Further, Mitsutani et al. (2001) found that a stationary culture cell extract of *Pseudoalteromonas* strain A25 showed both algicidal and high protease activities while exponential phase (as well as both growth phases of a non-algicidal mutant) did not have either of the activities. These results support that at least some algicidal bacteria kill their algal prey using proteases. If proteases are indeed involved in algicidal activity, we hypothesize that phytoplankton cell-surface polysaccharides play a role in defense against algicidal bacteria by protecting the cell against proteolytic attack (see also: Azam and Smith 1991; Guerrini et al. 1998). Some putative algicides are resistant to autoclaving and thus unlikely to be enzymes (Skerratt et al. 2002), but their chemical structures remain uncharacterized.

Prey specificity and preference. Various studies have found different levels of prey specificity with no clear pattern: some bacteria lyse only one algal species, others lyse multiple species within a given taxon, and still others can lyse cells of different species from several groups (Table 1). Algal taxa known to be affected by algicidal bacteria include members of the Chlorophyceae, Rhodophyceae, Bacillariophyceae, Dinophyceae, Hap-

Table **1**. Summary data for marine algicidal bacteria active against eukaryotic microalgae.

Strain name	Genus	Phylum[a]	Method of ID	# spp. tested	# spp. suscep.	Algal target(s)[b]	Type[c]	Reference
ACEM 21	*Cellulophaga*	Bacter.	16S	11	3	Raph., Din.	dissolved	(Skerratt et al. 2002)
A5Y	*Cytophaga*	Bacter.	morphology	6	4	Bacil, Raph.	attachment	(Mitsutani et al. 1992)
41-DBG2	*Cytophaga*	Bacter.	16S	6	3	Din.	dissolved	(Doucette, McGovern and Babinchak 1999)
Unknown	*Cytophaga*	Bacter.	biochemical	1	1	Raph.	attachment	(Furuki 1993)
MC8	*Cytophaga*	Bacter.	16S	1	1	Raph.	attachment	(Yoshinaga et al. 1998)
J18/M01	*Cytophaga*	Bacter.	16S	11	10	Raph., Din., Bacil.	attachment	(Imai et al. 1991)
LR2	*Cytophaga*	Bacter.	biochemical	6	3	Rhod., Chlor.	n/d	(Toncheva-Panova and Ivanova 1997)
AA8-2	*Cytophaga*	Bacter.	16S	1	1	Din.	attachment	(Nagasaki, Yamaguchi and Imai 2000)
5N-3	*Flavobacterium*	Bacter.	16S	4	1	Din.	dissolved	(Fukami et al. 1929)
SS98-5	*Saprospira*	Bacter.	16S	1	1	Bacil.	attachment	(Furusawa et al. 2003)
SS-K1	*Saprospira*	Bacter.	morphology	3	3	Bacil, Hapt.	attachment	(Sakata 1990)
Unknown	unknown	Bacter.	16S	10	1	Bacil.	n/d	(Chan, Kacsmarska and Suttle 1997)
C49	*Flavobacterium*	Bacter.	morphology	5	1	Raph.	n/d	(Yoshinaga, Kawai and Ishida 1997)
ACEM 20	*Zobellia*	Bacter.	16S	11	4	Raph., Din.	dissolved	(Skerratt et al. 2002)
K12	*Alteromonas*	γ-Proteo.	biochemical	11	10	Bacil.	dissolved	(Nagai and Imai 1998)
SR-14	*Alteromonas*	γ-Proteo.	biochemical	10	3	Bacil.	n/d	(Kim et al. 1999a)
ANSW2-2	*Alteromonas*	γ-Proteo.	16S	3	1	Din.	dissolved	(Doucette, McGovern and Babinchak 1999)
E401	*Alteromonas*	γ-Proteo.	16S	10	6	Din., Raph.	dissolved	(Yoshinaga, Kawai and Ishida 1995)
MC27	*Alteromonas*	γ-Proteo.	16S	1	1	Raph.	dissolved	(Yoshinaga et al. 1998)
GY21	*Alteromonas*	γ-Proteo.	16S	1	1	Raph.	dissolved	(Yoshinaga et al. 1998)
GY9501	*Alteromonas*	γ-Proteo.	16S	3	3	Raph., Din.	dissolved	(Kuroda, Yoshinaga and Uchida 2000)
S	*Alteromonas*	γ-Proteo.	16S	6	6	Raph., Din., Bacil.	dissolved	(Imai et al. 1995)
K	*Alteromonas*	γ-Proteo.	16S	6	4	Raph., Din., Bacil.	dissolved	(Imai et al. 1995)
D	*Alteromonas*	γ-Proteo.	16S	6	4	Raph., Din., Bacil.	dissolved	(Imai et al. 1995)
A25	*Pseudoalteromonas*	γ-Proteo.	16S	1	1	Bacil.	dissolved	(Mitsutani et al. 2001)
A28	*Pseudoalteromonas*	γ-Proteo.	16S	4	4	Bacil., Raph.	dissolved	(Lee et al. 2000)
Y	*Pseudoalteromonas*	γ-Proteo.	16S	9	4	Raph., Din.	dissolved	(Lovejoy, Bowman and Hallegraeff 1998)
ACEM 4	*Pseudoalteromonas*	γ-Proteo.	16S	11	3	Raph., Din.	dissolved	(Skerratt et al. 2002)
R	*Pseudoalteromonas*	γ-Proteo.	16S	6	6	Raph., Din., Bacil.	dissolved	(Imai et al. 1995)
T827/2B	*Pseudomonas*	γ-Proteo.	morphology	2	2	Bacil.	dissolved	(Baker and Herson 1978)
LG-2	*Pseudomonas*	γ-Proteo.	biochemical	5	2	Din.	dissolved	(Lee and Park 1998)
EHK-1	*Pseudomonas*	γ-Proteo.	16S	1	1	Din.	dissolved	(Kitaguchi et al. 2001)
T27	*Vibrio*	γ-Proteo.	morphology	18	18	Raph., Din.	n/d	(Ishio et al. 1989)
A47	*Vibrio*	γ-Proteo.	morphology	5	1	Raph.	n/d	(Yoshinaga, Kawai and Ishida 1997)
B42	*Vibrio*	γ-Proteo.	morphology	5	1	Bacil.	n/d	(Yoshinaga, Kawai and Ishida 1997)
C4	*Vibrio*	γ-Proteo.	morphology	5	2	Raph., Bacil.	n/d	(Yoshinaga, Kawai and Ishida 1997)
G42	*Pseudomonas*	γ-Proteo.	morphology	5	1	Raph.	n/d	(Yoshinaga, Kawai and Ishida 1997)
G62	*Vibrio*	γ-Proteo.	morphology	5	2	Raph., Din.	n/d	(Yoshinaga, Kawai and Ishida 1997)
ACEM 32	*Bacillus*	Gram +	16S	11	5	Raph., Din.	dissolved	(Skerratt et al. 2002)
ACEM 22	*Planomicrobium*	Gram +	16S	10	4	Raph., Din.	dissolved	(Skerratt et al. 2002)
LG-1	*Micrococcus*	Gram +	biochemical	5	1	Din.	dissolved	(Park et al. 1998)
KY1	n/d	n/d	n/d	1	1	Din.	dissolved	(Park, Kim and Kim 1999)

[a] Bacter. = Bacteroidetes, γ-Proteo = γ-Proteobacteria.
[b] Raph. = Raphidophyceae, Din. = Dinophyceae, Bacil. = Bacillariophyceae, Chlor. = Chlorophyceae, Rhod. = Rhodophyceae, Hapt. = Haptophyceae.
[c] Type of algicidal bacteria effect: by attachment or through the release of dissolved algicides.
n/d = not determined.

tophyceae, and Raphidophyceae. Algae forming harmful algal blooms (HABS) have been studied because of the interest in using algicidal bacteria for biological control of the blooms. A few studies have also examined prey specificity of algicidal bacteria on benign phytoplankton species used in aquaculture (Baker and Herson 1978; Sakata, Fujita, and Yasumoto 1991).

While most algae studied thus far are large flagellates and diatoms that form harmful blooms, it is not known whether

algicidal bacteria also kill the more abundant < 5 μm algae that are major players in global ocean biogeochemical cycles. Indeed, one might speculate that the mortality of smaller algae creates conditions for harmful algal blooms to occur. In any event, it is important to test whether bacteria killing non-bloom-forming algae, irrespective of the alga's size, are more widespread and significant than currently recognized (but see Toncheva-Panova and Ivanova 1997). If so, then bacteria may simply be another common cause of algal mortality (vs. being a rare event), along with virus infection (Bratbak, Egge, and Heldal 1993), eukaryotic pathogenesis (Coats et al. 1996), grazing (Nakamura, Suzuki, and Hiromi 1996), and nutrient stress (Brussaard, Noordeloos, and Riegman 1997).

Prey preference in algal lysis by algicidal bacteria is not conclusively known. Studies have not examined the consequence of incubating an algicidal bacterium in the presence of two or more algal prey species together. A bacterium might lyse two prey species presented individually, but kill only one when the two prey are offered together. Conversely, an alga in monoculture may be resistant to an algicidal bacterium, but be susceptible in the presence of another susceptible prey alga. For example, an algal species may release a substance that induces bacterial virulence against one or more other algal species. Such prey preference hypotheses need to be tested if we are to eventually predict the effect of algicidal bacteria on phytoplankton community structure in the ocean.

Ecosystem considerations. As mentioned, the studies of distribution and abundance of algicidal bacteria have been motivated by, and related to, algal-bloom cycles in the coastal ocean. Quantitative studies of algicidal activity in an ecosystem context have not been done for lack of a suitable method. Algicidal bacteria are generally enumerated by their ability to kill a specific algal prey in the MPN (most-probable number) format (Imai et al. 1998a). This method showed the abundance of algicidal bacteria increased during the decline of several algal blooms (Fukami et al. 1991; Imai et al. 1998b; Kim et al. 1998; Kim et al. 1999b; Yoshinaga et al. 1995), consistent with their involvement in bloom decline. In a culture-independent, fluorescent antibody-based approach, Imai et al. (2001) quantified the abundance of an algicidal *Cytophaga* sp. over the course of a *Chattonella* sp. bloom. They also found an increase in the abundance of the specific bacterium following the bloom peak. Despite such documentation of population increases of algicidal bacteria at bloom's end, there remains no conclusive causal evidence for the killing of algae by algicidal bacteria in the ocean! Perhaps the situation is no different than for virus-induced mortality of algae, where laboratory demonstration of the phenomenon still needs to be followed up with conclusive and quantitative field measurements.

Whether algae constitute the sole prey of algicidal bacteria in the ocean is largely unstudied. Some algicidal bacteria may conceivably be generalist microbial predators, and kill bacteria and heterotrophic protists as well. The only published accounts of algicidal bacteria interactions with organisms other than their prey algal species involve other bacteria preventing the algicidal bacteria from killing their prey. Nagasaki et al. (2000) found that a bacterium isolated from an algal culture decreased the algicidal activity of a *Cytophaga* (strain AA8–2) against its dinoflagellate prey *Heterocapsa circularisquama,* although it did not completely prevent it. Mayali and Doucette (2002) found that bacterial communities associated with cultures of the dinoflagellate *Karenia brevis* fully prevented algicidal activity by *Cytophaga* (strain 41-DBG2), while communities from other *K. brevis* cultures did not. Although the exchange of these communities among different dinoflagellate cultures reversed susceptibility to the algicidal bacterium, no cultured bacteria were identified as the cause of inhibition. Interestingly, in the co-cultures where *Cytophaga* strain 41-DBG2 did not cause algal lysis, this bacterium grew following inoculation, but never to abundances greater than 10^6 cells ml^{-1}. In all other incubations, algal lysis was noticeable only after the bacterium reached concentrations $> 10^6$ cells ml^{-1}. Other studies have observed a similar threshold abundance of algicidal bacteria before algal lysis is manifested (Fukami et al. 1992; Imai, Ishida, and Hata 1993; Mitsutani et al. 1992; Yoshinaga, Kawai, and Ishida 1995).

The reason for this apparent threshold remains unknown, but it raises the question of whether a single species of algicidal bacteria can attain such high abundance in the ocean. The typical abundance of the entire diverse assemblage of bacteria in seawater is 10^6 ml^{-1}. However, bacterial abundances can reach over 10^7 ml^{-1} (Heidelberg, Heidelberg, and Colwell 2002; Kamiyama, Itakura, and Nagasaki 2000). Further, even a small number of algicidal bacteria cells, if aggregated around a single phytoplankton cell or concentrated in marine snow, can create an algicidal hot spot. Such microscale patchiness in bacterial communities has been documented on marine snow (Rath et al. 1998) and in seawater (Long and Azam 2001). These hot spots may be important in the ecology of algicidal bacteria and their significance for carbon cycling and sequestration in the ocean.

Another unstudied trophic interaction is that between algicidal bacteria and viruses. The abundance of viruses in seawater is usually one order of magnitude greater than bacteria, and phages are thought to keep bacterial communities diverse (Thingstad and Lignell 1997). Algicidal bacteria are likely to be subject to phage lysis and this may regulate their populations and therefore their algicidal activity. This potentially important interaction should be explored in order to place the impact of algicidal bacteria on phytoplankton community structure in an ecosystem context.

Heterotrophic protists are an additional component of the microbial loop that directly affects the activity of algicidal bacteria. They prey on algae and in this way compete with algicidal bacteria. In addition, protists might accidentally ingest algicidal bacteria caught in the process of killing or consuming an algal prey cell. Studying the time it takes for an algicidal bacterium to kill an algal prey and produce progeny may clarify this interaction. Does this process occur fast enough to be successful before an algal cell is grazed? If not, what is the fate of an algicidal bacterium accidentally ingested by a protist grazing on an algal cell? Further, smaller bacterivorous protists may control the populations of algicidal bacteria through direct grazing. On the other hand, bacterial attachment to algal cells may be a refuge against predation by these smaller protists, but they subsequently become subject to grazing by larger protists and herbivorous zooplankton. We need to clarify the interactions between algicidal bacteria, algal prey, and protist grazers in order to understand the ecological role of algicidal bacteria in marine ecosystems.

Conclusions. Studies of marine algicidal bacteria have uncovered a potentially significant ecological phenomenon, but more research is necessary to assess their impact in shaping phytoplankton community structure. Previous studies have thoroughly demonstrated the existence of bacteria that can kill phytoplankton cultures. Subsequent studies have demonstrated that the abundance of such bacteria increases following the peak of some algal blooms. However, it is possible that algicidal bacteria do not affect phytoplankton mortality in nature, even taking into account the available evidence. Phytoplankton bloom declines may be initially due to another cause, such as nutrient limitation, viral lysis, eukaryotic pathogens, or a combination thereof. The detritus resulting from the cell lysis increases bac-

terial community metabolism, and studies have documented increased bacterial abundances following algal blooms (Romalde, Toranzo, and Barja 1990). The bacterial bloom may be associated with an increase in algicidal bacteria, causing a secondary infection of the remaining moribund algal cells as well as the high abundances of algicidal bacteria found by the studies mentioned above.

The next step in algicidal bacteria research will be to document the phenomenon of bacteria killing phytoplankton in nature. A crucial step toward that goal is to gain a mechanistic understanding of how algicidal bacteria kill their phytoplankton prey. Several studies have indicated that proteases may be involved in the killing activity, but genetic and biochemical studies need to test this hypothesis. Once signature DNA sequences or proteins for the process of algicidal activity have been discovered, they can be used to develop molecular probes specific to those signatures to search for the algicidal process in nature.

Future directions. An essential goal for the future is to conclusively demonstrate that the phenomenon of phytoplankton death due to bacteria does indeed occur in the ocean and that it is a significant process in the marine assemblages under natural conditions. This will require a mechanistic understanding of how algicidal bacteria kill their phytoplankton prey as well as in situ process rate measurements. Identification of the biochemical bases of the phenomenon, and the identification of the responsible molecules, should help constrain the phenomenon in an ecosystem context. It is also of interest whether algicidal bacteria are obligate or facultative in their lifestyle of killing algae, and whether the relevant phenotype is expressed only during algal blooms. This is important in understanding whether there are energetic restrictions on the proliferation of algicidal bacteria. Finally, a goal for the future is to determine the significance of trophic interactions for the population dynamics of algicidal bacteria in various ecosystem scenarios. These are challenging problems, but their resolution is important in view of the considerable interest in understanding the potential role of bacteria in the decline of harmful algal blooms. The knowledge gained will also be important in incorporating the consequences of bacteria-algae interactions in our concepts and models of the oceanic carbon cycle.

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