19 Relationships Between Bacteria and Harmful Algae

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19.1 Introduction

The interactions of harmful algal (HA) species with their physico-chemical and biological environments ultimately determine their abundance and distribution. While an algal taxon's physiological tolerance limits (e.g., temperature, salinity, light) and intrinsic phenotypic traits (e.g., growth rate, nutrient uptake, vertical migration) largely define its ecological niche, relationships with the biological components of an ecosystem (e.g., grazers, microbes, pathogens, competing algal species) play a critical role in its ability to achieve concentrations that lead to the many negative impacts characterizing harmful species. A biological factor of potentially great significance in regulating the population and even toxin dynamics of HA that has received increasing yet comparatively little attention to date is their relationship with the ubiquitous and diverse bacterial community. These microbes, which exist as free-living forms as well as securely attached to algal cells, occasionally with a high degree of taxonomic specificity, have now been demonstrated to modulate (either positively or negatively) algal growth rates and transitions between life history stages, influence toxin production, and even induce the rapid lysis of algal cells.

Previous reviews of this topic (e.g., Doucette 1995; Doucette et al. 1998) described an exciting, emerging field in which molecular-based approaches were just beginning to supplement traditional bacteriological techniques, yielding new insights into the nature and ecological implications of bacterial-algal interactions. During the intervening time, continued application of molecular techniques, novel experimental methods, and targeted studies of natural bacterial communities have contributed valuable details of the relationships between bacteria and HA that will be the focus of this chapter.

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19.2 Diversity of Algal-Associated Bacteria

Through the last decade, molecular biological methods have increasingly been used to investigate algal-bacterial diversity due to their finer level of discrimination as compared with commonly used bacteriological identification protocols (e.g., biochemical tests). The culture-independent nature of most molecular techniques allows the importance of the numerous non-cultivable bacteria present in marine ecosystems to be evaluated. Moreover, the coupling of molecular-based detection strategies to automation represents a real potential for the development of in situ real-time monitoring as is starting to be realized for HA species (Babin et al. 2005). Nonetheless, the process of isolating and culturing bacteria continues to be a prerequisite for examining bacterial physiology, and although genomics and metagenomics will to a certain extent minimize the reliance on pure cultures, close partnering of genomics and pure culture studies will likely be the most valuable route to elucidating the functional significance of bacteria associated with harmful algae.

19.2.1 Bacteria Associated with Harmful Algal Species

There is now a growing list of studies of bacteria associated with HA taxa, especially within the dinoflagellate group. Most represent 'snapshots' of the bacteria present in the culture at a given point in time and the emerging picture suggests that these bacterial communities are more notable for their similarities than their differences (e.g., Fig. 2 of Hold et al. 2001). The bacteria associated with these dinoflagellates resemble an ordered and structured community rather than a random assemblage of species recruited from the marine bacterial metacommunity (Curtis and Sloan 2004). Figure 19.1 illustrates one such phylogenetic 'micro-cluster' of bacteria associated with a range of dinoflagellate taxa originating from distinct regions around the globe. One interpretation of this seemingly conserved community structure is that it may reflect a physiological requirement of the dinoflagellates (Alavi et al. 2001; Green et al. 2004). Nonetheless, most of these studies involve laboratory cultures that may not accurately reflect field populations and many of these micro-clusters are in common to both toxic and non-toxic algal species.

The bacterial group most often associated with dinoflagellates and diatoms, irrespective of their toxicity, is the alpha Proteobacteria, and most frequently, the *Roseobacter* clade and its relatives (e.g., Hold et al. 2001; Allgaier et al. 2003; Green et al. 2004). The *Roseobacter* clade alone can dominate the bacterial diversity of microalgal cultures (Alavi et al. 2001), but can also be an abundant feature of field blooms (Fandino et al. 2001, cited in

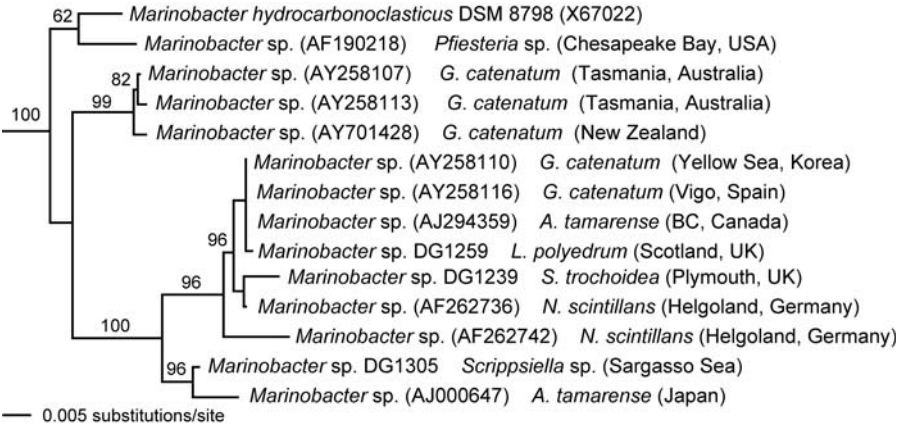

Fig 19.1. Specificity of association: the genus *Marinobacter*. Neighbor-joining tree (with bootstrap support for the branching order) depicting known dinoflagellate-associated *Marinobacter* spp. The dinoflagellate host and its isolation origin are noted in parentheses to the right of each bacterial strain

Long and Azam 2001). One suggested explanation of their predominant association with dinoflagellates is a growth promoting capability (Jasti et al. 2003).

Gamma Proteobacteria, beta Proteobacteria, and Cytophaga-Flavobacteria-Bacteroides (CFB) are the other bacterial groups reported to be associated with HA species. Within the gamma Proteobacteria, *Marinobacter* spp. (Fig. 19.1) and *Alteromonas* spp. appear to have an association with dinoflagellates (Hold et al. 2001; Ferrier et al. 2002). *Marinobacter* have no known function other than currently unpublished data (D. Green and C. Bolch) indicating that they stimulate dinoflagellate growth. Alteromonads have seemingly ambiguous roles, including stimulating dinoflagellate growth (Ferrier et al. 2002) as well as inhibiting *Alexandrium* cyst formation (Adachi et al. 2002). The presence of *Pseudoalteromonas* (gamma Proteobacteria) in algal cultures is more sporadic, which may reflect their propensity toward algicidal activity (Mayali and Azam 2004). Yet, *Pseudoalteromonas* spp. are frequently encountered in the field (Skerratt et al. 2002, cited in Mayali and Azam 2004), where they are a potentially significant factor in bloom decline. Representatives of the beta Proteobacteria are generally considered to be rare in marine systems, but were observed by Alverca et al. (2002) to dominate the intracellular bacterial flora of the dinoflagellate, *Gymnodinium instriatum*. Another significant bacterial phylum associated with many microalgal cultures and field populations is the CFB group. This group is associated with algal surfaces and in the free-living fraction of algal blooms (e.g., Rooney-Varga et al. 2005), where they are important in the degradation of high molecular weight dissolved organic matter (Kirchman 2002). More importantly, they are frequently asso-

ciated with the exhibition of algicidal activity (e.g., Doucette et al. 1999), and like *Pseudoalteromonas*, may be important in bloom termination.

Apart from the ecological implications of bacteria-HAB associations, it is important to note that algal blooms can serve as reservoirs for human pathogens such as *Vibrio cholerae*. In this case, a viable, but unculturable form of *V. cholerae* associated with plankton can become infectious under conditions favoring bloom development. Moreover, this pathogen has been reported to survive for extended periods in the presence of cyanobacteria, diatoms, and dinoflagellates (Rose et al. 2001). Investigations of this relationship as it relates to the growth potential of *V. cholerae* are ongoing (e.g., Mouriño-Pérez et al. 2003).

19.2.2 Spatio-Temporal Relationships Between Bacteria and Algae

Aside from a need to identify bacteria and understand their physiological function, a pressing aim in algal-bacteria-HAB research is to understand the role bacteria play in field population dynamics – the 'how' and 'what' interactions are important in HAB ecology (Doucette et al. 1998). Are there 'key' or 'signature' bacteria that define the progress of a HAB event? Several recent studies have begun to provide insights into how bacterial communities can change during the growth of algal populations. Among the more interesting concepts to emerge from such work is that changes in attached (versus free-living) bacterial communities are linked closely with both successional changes in phytoplankton assemblages (Rooney-Varga et al. 2005) and with algal physiological status (Grossart et al. 2005). Moreover, members of the Bacteroidetes phylum tend to predominate the bacteria attached to algal cells, although the alpha Proteobacteria are also represented frequently in both attached and free-living communities over time. These findings strongly suggest that there are very specific, two-way interactions between phytoplankton and their attached microbial flora that can influence such important processes as bacterial-algal succession and the cycling of organic matter in the ocean. Notably, there are relatively few descriptions dealing specifically with the dynamics of bacterial communities associated with HABs (e.g., see Doucette et al. 1998; Töbe et al. 2001, cited in Green et al. 2004; Wichels et al. 2004). Preliminary data from a study of bacterial clone libraries associated with blooms of the dinoflagellate, *Karenia brevis*, on the west Florida shelf show a clear dominance of the alpha Proteobacteria and Bacteroidetes groups, as noted above for non-HA species (K. Jones, C. Mikulski, and G. Doucette unpubl. data). Clearly, additional efforts to describe both the spatio-temporal variability of bacterial communities associated with HABs and the functional attributes of dominant taxa are needed to better define how bacteria may influence algal bloom dynamics.

19.3 Bacterial Influences on Algal Growth, Metabolism, and Toxins

19.3.1 Bacterial Effects on Algal Growth

To what extent bacteria might be involved in regulating transitions between stages of HAB events (i.e., initiation, development, maintenance, decline) is an often neglected issue, despite multiple scenarios suggesting the potentially important influence of bacteria on algal growth. Note that the role of bacteria as prey for phagotrophic algal taxa is considered elsewhere (see Stoecker et al. Chap. 14). As discussed by Doucette et al. (1998), bacteria may contribute to the supply of trace elements such as iron, and recent work has further highlighted the potential significance of bacterially produced siderophores in defining iron chemistry in the coastal zone (Soria-Dengg et al. 2001, cited in Green et al. 2004). Interestingly, the unique chemistry of marine bacterial siderophores (Martinez et al. 2000, cited in Barbeau et al. 2001) can potentially increase the availability of Fe to phytoplankton (Barbeau et al. 2001). Whether bacteria play a fundamental role in mediating the biological availability of Fe and other essential trace elements to phytoplankton, remains poorly understood; however, this becomes an important question within the framework of increasing eutrophication and anthropogenic modification of the coastal zone (e.g., dumping of metal-rich sludge, increased run-off/erosion, etc.), which may lead to increased availability of trace elements, corresponding to an increased frequency of HABs.

Bacteria are generally thought to have an indirect role in phytoplankton growth through re-mineralization of excreted dissolved organic matter (Azam 1998). However, some evidence would suggest that bacteria have more specific and direct effects on algal growth, such as the supply of vitamins (Haines and Guillard 1974). More recently, specific bacterial taxa have been linked with growth stimulation of dinoflagellates such as *Gambierdiscus toxicus* (Sakami et al. 1999), *Pfiesteria* spp. (Alavi et al. 2001), and *A. fundyense* (Ferrier et al. 2002). It is interesting to speculate whether bacterial release of metabolites that may lead to enhanced algal growth is, in fact, a mutualistic strategy capable of maintaining the health of associated algal cells that in turn excrete organic matter required for bacterial growth or, more simply, one of altruism? Perhaps the most compelling evidence of a direct relationship between bacteria and algal growth comes from recent work demonstrating that the dinoflagellate *G. catenatum* has an obligatory requirement for bacteria following excystment from the sexual resting stage (D. Green and C. Bolch unpubl. data). Moreover, it was determined that individual bacterial strains could meet the dinoflagellate's bacterial requirement. Notably, *Marinobacter* was one of these 'growth-promoting' taxa, highlighting the remarkable phylogenetic relatedness observed amongst the dinoflagellate-associated *Mari-*

*nobacter*s (see Fig. 19.1), and suggesting that other dinoflagellates may also have an obligatory growth requirement for certain bacteria. The mechanistic basis for this requirement remains uncertain, but examples of bacteria employing sophisticated secretion systems to establish mutualistic relationships with eukaryotes have been reported (e.g., Dale et al. 2002).

Initiation of sexuality in dinoflagellates has largely been thought of as a function of physical or chemical stresses, such as nitrogen or phosphorus deficiency commonly used to induce formation of sexual life history stages (e.g., Blackburn et al. 1989, cited in Green et al. 2004). An involvement of bacteria in dinoflagellate sexuality would not seem immediately obvious. Yet, Adachi et al. (1999) reported that a certain fraction of the naturally occurring bacteria within *Alexandrium* blooms promoted the formation of cysts. This group also observed the co-occurrence of bacteria in natural blooms that inhibited the formation of *Alexandrium* cysts (Adachi et al. 2002). It remains unclear if these bacteria were producing specific compounds that would promote or inhibit cyst formation or whether an indirect mechanism affecting the algal cells' physiology was involved.

19.3.2 The Role of Bacteria in Toxin Production

Historically, the bacteria associated with HA species have been studied through a causal link between algal toxin production and bacterial presence (e.g., Silva 1982, cited in Doucette 1995). This link was reinforced by findings that bacteria were capable of producing paralytic shellfish poisoning-like toxins (PST) (e.g., Kodama et al. 1988; Gallacher et al. 1997). Although a direct bacterial involvement in PST production and PSP events has been questioned (Sato and Shimizu 1998; Baker et al. 2003, cited in Martins et al. 2003; Martins et al. 2003; Green et al. 2004; Wichels et al. 2004), there is evidence that domoic acid (DA) production by *Pseudo-nitzschia* spp. may be stimulated indirectly by bacterial presence.

Bates et al. (1995) showed that non-axenic *P. multiseries* produced high DA levels at late-exponential phase coinciding with growth limiting conditions. The opposite trend in DA production was observed in axenic *P. multiseries* cultures; however, this pattern was reversed upon addition of bacteria isolated from the non-axenic cultures, providing compelling evidence that environmental bacteria can enhance DA production by *P. multiseries*. Recently, polymerase chain reaction (PCR) primers were used to amplify bacterial 16S rDNA associated with cells of axenic *P. multiseries* cultures. Among the PCR products obtained, several were of identical sequence to bacteria isolated from non-axenic *P. multiseries* (Kobayashi et al. 2003).

Work by Bates et al. (2004) has indicated that free-living bacteria associated with *P. multiseries* cultures are not capable of autonomous DA production, even in the presence of algal exudates, suggesting that any bacterial

direct involvement in toxin production likely involves bacteria attached to the diatom cells. Kobayashi and co-workers (unpubl. data) have also examined this bacterial-algal relationship by measuring DA levels in axenic and non-axenic *P. multiseries* strains co-cultured with bacteria derived from the latter separated by cellophane membrane. Their results showed that while concentrations of dissolved DA were similar between cultures, maximum intracellular toxin levels were at least three-fold higher (1.1 pg cell^{-1}) in the non-axenic cultures, again indicating that direct contact with bacteria was essential for *P. multiseries* to produce large amounts of domoic acid. More recently, Kaczmarska et al. (2005) has proposed that a specific bacterial community composition or its density may be important to explaining the variable DA levels associated with some *P. multiseries* cultures. They hypothesized that some bacteria were antagonistic to the alga, which provoked an increase in DA production as a specific response to the bacteria's presence.

19.3.3 Bacterially-Mediated Release and Metabolism of Algal Toxins

In addition to their role as modulators of algal toxin production, as well as putatively autonomous sources of some toxins as discussed above, certain bacteria can mediate the release of phycotoxins into marine ecosystems through the lysis of algal cells. In the case of two such algicidal bacteria targeting the brevetoxin (PbTx)-producing dinoflagellate, *Karenia brevis*, disruption of the algal cell wall exposed previously intracellular toxin to the surrounding seawater (Roth 2005). By tracking the toxin present in several size fractions (>5.0, 0.22–5.0, <0.22 μm) following treatment of *K. brevis* cultures with these algicidal bacteria, a 50 ng mL^{-1} pulse of dissolved (<0.22 μm) brevetoxin was observed co-incident with the onset of cell lysis and a rapid decline in the >5.0-μm fraction, which initially accounted for 50 to 90 % of the total toxin present (~125 ng PbTx-3 equiv mL^{-1}). The peak in dissolved toxin (initially ca. 60 % of total toxin) dissipated in about 5 days, likely due to natural and/or bacterial degradation. Nonetheless, it was clear that attack of *K. brevis* cells by algicidal bacteria resulted in a marked redistribution of brevetoxin to different size fractions and would be expected to alter the routes and efficacy of toxin trophic transfer.

It is well established that many bacteria are capable of metabolizing algal toxins to yield either known congeners or novel, chemically modified derivatives within a given toxin class. The sources of such bacterial isolates range from laboratory cultures of both toxic and non-toxic algae (Sakamoto et al. 2000; Smith et al. 2002) to those originating from natural collections of shellfish (Smith et al. 2001) or other invertebrates (Kotaki et al. 1985) known to accumulate phycotoxins. Interestingly, Stewart et al. (1998) reported that the capacity for domoic acid metabolism was higher in bacteria isolated from shellfish species that rapidly depurate toxins as compared to those known to retain toxins for extended periods, suggesting a linkage between such bacte-

ria and toxin clearance rates in shellfish. In addition, the products of bacterial toxin metabolism may be either more or less potent than the starting compound(s) (e.g., PSP toxins; Kotaki 1989; Smith et al. 2001), with the former leading potentially to increased public health risks for commercially harvested seafood above that expected based solely on the algal toxin source (Bricelj and Shumway 1998).

In terms of actual metabolic transformations of PSP toxins, Sakamoto et al. (2000) reported evidence for the involvement of glutathione (GSH) in certain reactions, leading to production of stable toxin-GSH conjugates by covalent linkage of the GSH cystein moiety (involving the sulfur atom) at the toxin C-11 position (Sato et al. 2000). Such conjugates are formed between PSP toxins with 11-O-sulfate such as gonyautoxins (GTXs) and various thiol compounds, including those of biological origin. Notably, when these conjugates are treated with an excess of thiols, bound toxins are released without the 11-O-sulfate group, yielding more potent congeners such as saxitoxin. PSP toxins could bind with the cystein moiety of various proteins or amino acids and be co-metabolized in toxic organisms as well as in bacteria. The latter suggests a possible explanation for the difficulty in demonstrating autonomous bacterial production of these toxins and a potential justification for re-visiting this issue.

19.4 Potential Implications of Interactions Among Bacteria

The majority of this chapter has focused on characterizing relationships between bacteria and species of HA. However, interactions between members the microbial community (e.g., production of antibiotics; Holmström and Kjelleberg 1999) may play an important role in determining composition and thus functional attributes of the bacterial assemblage occurring in association with algal cells. This, in turn, can have a direct effect on the nature of algal-bacterial interactions. For example, Long and Azam (2001) reported that greater than 50 % of 86 pelagic marine bacterial isolates tested exhibited antagonistic properties against other bacteria via the production of growth inhibiting compounds. Perhaps, not surprisingly, particle-associated isolates, which must compete for available surfaces to colonize, were far more likely than free-living forms to produce such substances and the gamma Proteobacteria contained the most representatives either producing inhibitory materials or resistant to their effects. These findings have important biogeochemical implications (e.g., carbon cycling), since changes in species composition resulting from antagonistic interactions between bacteria will affect both the quality and quantity of particulate organic material degraded by microbial communities.

Inhibition of a bacterial taxon by other bacteria may also indirectly modulate the population dynamics of HA species. Indeed, Mayali and Doucette

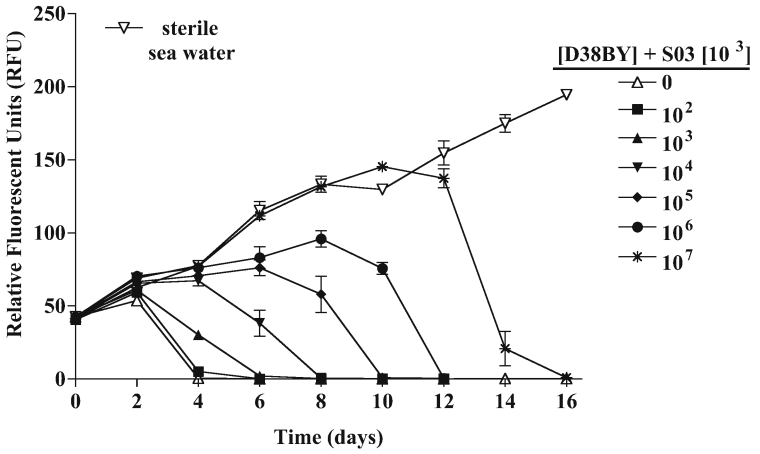

Fig 19.2. A 'protective' bacterium (strain D38BY, Flavobacteriaceae) was added at concentrations ranging from 0–10^7 cells mL^{-1} to bacteria-free *Karenia mikimotoi* co-cultures. D38BY inoculations followed immediately the addition of algicidal strain S03 at 10^3 cells mL^{-1} on Day 0. A positive control consisted of sterile seawater and culture growth and was monitored by in vivo fluorescence (after Roth 2005)

(2002) established that resistance of a *Karenia brevis* culture to attack by an algicidal bacterium was not an intrinsic property of the algal cells but instead was mediated by the associated bacterial community and that this resistance could be conferred to susceptible *K. brevis* cultures simply by switching ambient bacteria with a resistant culture. Recently, a representative of this "protective" bacterial community belonging to the Flavobacteriaceae was isolated and shown to inhibit the killing activity of this algicidal bacterium in a concentration-dependent manner (Fig. 19.2, Roth 2005), although the antagonistic mechanism involved remains to be determined. Nevertheless, given the potentially important role of algicidal bacteria in regulating algal population growth, it would appear that this algal-bacterial interaction may ultimately be regulated by antagonistic relationships between bacterial taxa. These findings, in turn, raise questions of whether algal species are able to selectively promote the growth of bacteria that may inhibit the killing activity of algicidal strains and if such ecological relationships are common in the marine environment.

19.5 Future Directions/Research Needs/Critical Questions

Discussion of algal-bacterial communication was outside the scope of the present summary. Nonetheless, it is evident from numerous examples throughout

this chapter that bacterial-algal, cell-to-cell interactions are likely to be pivotal factors regulating HAB ecology, whether via cell signaling or micronutrient exchange (e.g., Miller et al. 2004). Parallel strategies employing model algal-bacterial communities in conjunction with classical physiological as well as gene expression (i.e., transcriptomics/proteomics/metabolomics) approaches are needed to reveal the micro-scale processes operating between cells that have meso- and meta-scale consequences (e.g., Moran et al. 2004). There does not appear to be any question that bacteria are involved in HAB ecology, yet the challenge we now face is to produce compelling, quantifiable evidence of their role in natural systems. This effort will require identification of specific mechanisms of action coupled with measurements of the chemicals being exchanged, regulated, or modified, in order to establish their significance throughout a bloom event.

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