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REVIEW ARTICLE

Antagonistic Interactions Mediated by Marine Bacteria: The Role of Small Molecules

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Abstract Marine bacteria are known to produce a wide variety of structurally diverse and biologically active secondary metabolites. Considerably less is known about the ecological functions of these compounds, in part due to methodological challenges associated with this field of research. Here, we review the antagonistic activities mediated by marine bacteria with a focus on activities linked to structurally defined secondary metabolites. Bacterial antagonism has been documented against other marine bacteria as well as eukaryotes, and includes antibiosis, the inhibition of quorum sensing, larval settlement deterrence, and defense against predation. These compounds likely play important ecological roles that ultimately affect ecosystem structure and function, however, much remains to be learned before these roles can be fully appreciated. Recent technological advances coupled with a better understanding of the diverse processes mediated by secondary metabolites provide new opportunities to expand our understanding of the chemical ecology of bacterial antagonism in the marine environment.

Keywords Antagonism · Secondary metabolites · Marine bacteria · Chemical ecology

Introduction

Early research in marine chemical ecology focused on antagonistic interactions among macroorganisms, particularly predator–prey and seaweed–herbivore interactions (Hay 1996). These studies helped define current thinking about the roles of secondary metabolites in shaping benthic community structure, especially in highly diverse coral reef systems (Hay 2009). In contrast, the ecological roles of small molecules produced by marine microorganisms have been less well studied. None-the-less, it is now well established that secondary metabolites from marine bacteria function in a variety of processes including nutrient acquisition (Hider and Kong 2010) and chemical communication (Straight and Kolter 2009). There is also ample evidence that bacterial small molecules exert antagonistic effects against other marine organisms. The mechanisms responsible for these effects can vary widely, ranging from direct cell killing, as in the case of an antibiotic, to the removal of an essential nutrient, as in the case of an iron chelating siderophore. Antagonism also can result from the production of small organic acids or other compounds that render the environment unsuitable for the growth of competing bacteria (Schnurer and Magnusson 2005). While these compounds may not always fall under the banner of secondary metabolites, their ecological relevance none-the-less warrants consideration.

Bacteria from a wide range of marine environments, including sediments, seawater, biofilms, and those created by living in association with invertebrates and algae, have been shown to possess antagonistic activities. These bacteria may be free-living, epi- or endobiotic, living as commensals, mutualists, or obligate symbionts. In most cases, they are members of complex communities in which competition for limited space and resources can be intense (Hibbing et al. 2010). Antagonistic interactions may play important roles in structuring these communities, where the evolutionary advantages afforded by an effective chemical defense may be crucial for survival. To make the connection between an antagonistic activity observed in the laboratory and an ecologically meaningful effect, however, many factors must be considered including the structures of the active compounds, the concentrations at which they are tested relative to those that occur in nature, and the ecological relevance of the bioassay employed. The methodological challenges associated with defining these variables remain considerable yet

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must be addressed if we are to decipher the ecological roles of microbial natural products in the marine environment. Fueled by advances in molecular biology and analytical chemistry, we are now beginning to appreciate the extent to which bacterial small molecules mediate ecologically relevant antagonistic interactions in the marine environment.

Here, we review the antagonistic effects of marine bacteria on other marine organisms, including bacteria–bacteria interactions such as induced allelopathy and the inhibition of quorum sensing. We also explore the antagonistic effects of bacterial secondary metabolites on eukaryotes, addressing the inhibition of larval settlement and grazing deterrence. In most cases, we have focused on studies that include the chemical basis for the reported activities. The examples provided offer ample evidence that bacterial secondary metabolites display potent and diverse biological activities against various marine organisms. The translation of these activities into ecological function remains a challenge, yet presents exciting opportunities to advance the field of microbial chemical ecology.

Antagonism Among Bacteria

The antibiotic properties of marine bacteria have been recognized since the pioneering research of Claude Zobell (Zobell and Rosenfeld 1947). One of the first comprehensive surveys of antagonism among marine bacteria demonstrated high levels of antibacterial activity among strains belonging to the order *Alteromonadales* (Nair and Simidu 1987). This order includes genera that were consistently identified as producing antibiotic substances in subsequent studies. Employing a forward-thinking experimental design, Lemos and co-workers demonstrated the competitive dominance of antibiotic-producing strains when co-culturing marine bacteria in natural seawater (Lemos et al. 1991). More recently, direct challenge assays were used to address important ecological concepts including allelopathy among free-living pelagic bacteria and those associated with particles such as marine snow (Long and Azam 2001; Long et al. 2005). These authors consistently reported a greater incidence of antagonism in particle- or surface-associated bacteria compared to free-living strains, suggesting that antagonism may function to limit competition in these nutrient-rich microenvironments. Further study of one strain (*Alteromonas* sp. SWAT5) led to the identification of 2-n-pentyl-4-quinolinol (**1**) (Fig. 1) as the major antibiotic compound (Long et al. 2003). When SWAT5 was grown in polysaccharide matrices, which acted as a model particle system, this compound was shown to diffuse within the matrices but not into the surrounding seawater indicating a potential influence on the ecology of particle-associated microbiota. In support of this, compound **1** altered the composition of the bacterial community that colonized the model particle system (Long et al. 2003).

The consistent detection of antimicrobial activities among marine bacteria suggests that antagonism plays a role in structuring microbial communities. This has been demonstrated for coral-associated bacteria (Rypien et al. 2010), where inhibitory activities towards known coral pathogens have led to the suggestion that bacteria associated with healthy corals play a protective role for the coral holobiont (Nissimov et al. 2009; Ritchie 2006; Shnit-Orland et al. 2012). An ecological role of antagonism also has been implied for bacteria associated with brittle stars (Strahl et al. 2002), sponges (Mangano et al. 2009), and marine aggregates (Grossart et al. 2004). However, there are relatively few examples where the specific compounds responsible for these activities have been identified. In one such case, the antibiotic activity of a *Roseobacter* strain against an indigenous *Bacillus* sp. was linked to the production of tropodithietic acid (**2**) (Brinkhoff et al. 2004), the tautomeric form of the antibacterial compound thiotropocin, originally isolated from a marine *Pseudomonas* strain (Tsubotani et al. 1984). Members of the *Roseobacter* clade are commonly associated with marine phytoplankton, and their ability to produce antibacterial compounds may lead to their common association with biotic surfaces (Bruhn et al. 2007). In another example, the antibiotic korormicin (**3**) produced by a *Pseudoalteromonas* strain was shown to selectively inhibit the Na^+-dependent NADH:quinone reductase in Gram-negative marine bacteria, while not inhibiting Gram-positive or non-halophilic strains (Yoshikawa et al. 1999). These results imply that some marine bacterial secondary metabolites have evolved specifically to act on targets in other marine bacteria.

A final example comes from the ability of marine bacteria to inhibit the pathogen *Vibrio cholerae* (Long et al. 2005). Bioassay-guided fractionation of an extract from a particle-associated *Vibrio* sp. (strain SWAT3) led to the identification of andrimid (**4**) as the active constituent. Interestingly, this compound was reported originally from an intracellular bacterial symbiont (*Enterobacter* sp.) isolated from a brown planthopper (Fredenhagen et al. 1987), suggesting that it may function in multiple ecosystems. Andrimid producing strains completely inhibited the colonization of particles by *V. cholerae*, while particles inoculated with a non-andrimid producing mutant (SWAT3-111) had no inhibitory effect, thus linking this compound to the observed biological activity. These findings suggested that bacterial antagonism regulates the proliferation of *V. cholerae* on marine particles (Long et al. 2005). A follow-up microfluidics study revealed that *V. cholerae* responded to sub-lethal andrimid concentrations by increasing its swimming speed, turning rate, and run lengths in the process of directing its movements away from the metabolite source (Graff et al. 2013). Analyzing this type of behavioral response may be more realistic than the measurement of toxicity (Kelly et al. 2005) and better reflect the ecological functions of antibiotics in

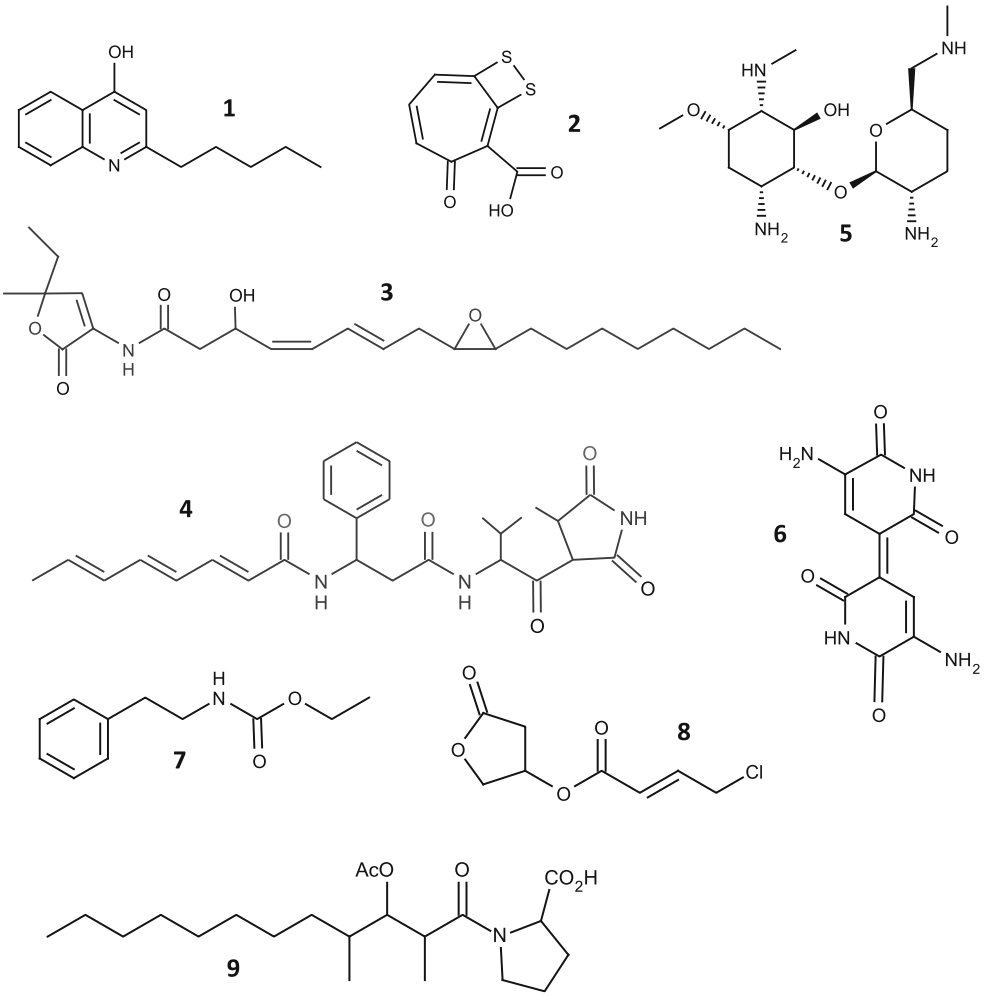

Fig. 1 Secondary metabolites produced by marine bacteria that mediate antagonistic interactions with other marine bacteria. Examples include the antibiotics 2-n-pentyl-4-quinolinol (**1**), tropodithietic acid (**2**), korormicin (**3**), andrimid (**4**), and istamycin (**5**), the biofilm inhibitors indigoidine (**6**), and ethyl N-(2-phenethyl) carbamate (**7**), and the quorum sensing inhibitors honaucin A (**8**), and tumonoic acid F (**9**)

nature, which may seldom achieve toxic concentrations (Yim et al. 2006, 2007).

Induced Allelopathy In contrast to more general surveys of antagonism among marine bacteria, there is increasing evidence that antagonism can be induced in response to microbial challenge. One of the first such studies demonstrated enhanced antimicrobial activity among epibiotic marine bacteria following exposure to terrestrial pathogens (Mearns-Spragg et al. 1998). A subsequent study demonstrated that 12 of 53 marine bacteria isolated from diverse substrates induced the production of the aminoglycoside antibiotic istamycin (**5**) by a marine *Streptomyces* sp., suggesting that this compound targets specific competitors and functions as an inducible defense that prevents invasion (Slattery et al. 2001). Protecting resources by preventing invasion is likely a more relevant mean of antagonism than creating opportunities to invade, in particular for slow-growing, non-motile bacteria such as many marine actinomycetes. Antagonism was also induced in a marine *Bacillus* strain when co-cultured with another *Bacillus* isolated from the same sample of the alga *Ulva californica* (Trischman et al. 2004). In one case where the specific type of advantage was addressed, it appears to be linked more to preventing invasion (i.e., protecting resources) than creating opportunities to invade (Slattery et al. 2001), a suggestion that may be particularly relevant for slow-growing, non-motile bacteria such as many marine actinomycetes. This activity was traced to the increased production of indole and diketopiperazine metabolites, which increased three and four fold, respectively, in response to co-culture conditions. The basis for co-culture induction was examined in more detail in the case of pyocyanin production by *Pseudomonas aeruginosa* (Angell et al. 2006). This compound was originally observed in a mixed culture with *Enterobacter* sp., and subsequently was produced even when a permeable membrane separated the two cultures. In this case, induction may be associated with a diffusible molecule, which contrasts observations that direct contact with bacteria can be required for the induction of fungal secondary metabolites (Schroeckh et al. 2009). Bacterial secondary metabolite production also can be induced in response to algal and invertebrate-generated cues. For instance, antibiotic production by a marine actinomycete was

dependent on the addition of seaweed extract to the culture medium (Okazaki et al. 1975). More recently, a strain of *V. coralliilyticus* was shown to double production of the antibiotic andrimid (**4**) when cultured with chitin as the sole nutrient source (Wietz et al. 2011).

Iron Depletion as a Mechanism of Antagonism Competition for nutrients is one of the major factors determining microbial community composition, particularly in environments where space is limited (Hibbing et al. 2010). In marine systems, iron is often a limiting nutrient (Falkowski et al. 1998), suggesting that the production of efficient iron-chelating molecules will provide a competitive advantage over bacteria unable to access insoluble iron (Butler 1998). Siderophore production has been linked to antagonistic activities among marine *Vibrio* (Pybus et al. 1994) and *Pseudomonas* (Simoes et al. 2008) spp. by the comparison of growth inhibition in iron-limited versus iron-replete conditions, and confirmed using the chrome azurol sulphate assay (Schwyn and Neilands 1987), which provides a rapid method to assess siderophore-mediated iron depletion. While the chemistry and biology of siderophores has been studied in detail (Hider and Kong 2010; Miethke and Marahiel 2007), the extent to which specific siderophores mediate antagonistic activities and how these activities affect the structure of marine bacterial communities remains largely unknown.

Antagonistic Interactions in Marine Biofilms Biofilms are complex microbial assemblages that are generally embedded in an extracellular matrix (Hall-Stoodley et al. 2004). They form on essentially all submerged surfaces and provide important cues for invertebrate larval settlement (Hadfield 2011), which subsequently create biofouling problems for maritime and aquaculture industries (Qian et al. 2007). In addition, they can act as a refuge within which bacteria avoid predation (Matz et al. 2008). Biofilm development has been studied in detail and results in the formation of integrated microbial communities within which cell–cell communication plays a central role (Stoodley et al. 2002). Given the high density of bacteria in biofilms, small molecule mediated antagonism could have a major effect on the associated microbial communities. While considerable effort has gone into the identification of natural products that inhibit biofilm formation, many of which originate from marine organisms (Worthington et al. 2012), these studies generally have focused on the industrial or biomedical applications of antifouling metabolites and thus provide little insight into biofilm chemical ecology.

The marine bacterium *Pseudoalteromonas tunicata* is a biofilm-forming species that is often found in association with eukaryotic organisms (Rao et al. 2005). In mixed culture experiments, *P. tunicata* effectively outcompeted other biofilm-forming bacteria via the production of the antibacterial protein AlpD, thus providing a competitive advantage during biofilm growth. Other studies have identified marine actinomycete strains that attenuate biofilm formation in *Vibrio* species that cause disease, and thus they may have applications for the aquaculture industry (You et al. 2007). The inhibition of biofilm formation in *Vibrio fischeri* by *Phaeobacter* sp. strain Y4I (*Roseobacter* clade) was linked to the production of indigoidine (**6**), the cyclization product of two glutamine molecules (Cude et al. 2012). The inhibitory effect was dramatically reduced when the indigoidine biosynthetic pathway was inactivated, thus providing a rare example in which genetics has been used to establish a relationship between a marine bacterial secondary metabolite and its biological activity against other marine organisms.

Other examples of biofilm-inhibiting secondary metabolites include phenylbutanoic acid and ethyl N-(2-phenethyl) carbamate (**7**) from a marine *Bacillus pumilus* strain (Nithya et al. 2011) and a *Cytophaga* sp., respectively (Yamada et al. 1997). There also is evidence that antagonistic activities are induced in biofilms based on the observation of significantly greater antimicrobial activity in the supernatants of biofilm cultures compared to the same strains grown in suspended liquid culture (Wilson et al. 2011). Furthermore, the antimicrobial activities of these same biofilm cultures were increased by the addition of "conditioned" growth media from the sensitive strains. These results support the concept that chemically mediated antagonism can provide a competitive advantage in complex biofilms.

Inhibition of Quorum Sensing Quorum sensing (QS) is a process by which bacteria use signaling molecules known as autoinducers to recognize cell densities and thereby regulate processes such as swarming motility, biofilm formation, bioluminescence, and antibiotic production (Miller and Bassler 2001). Gram-negative bacteria predominantly use acyl homoserine lactones (AHLs) to mediate QS, the chemical specificity of which is arbitrated through acyl side chain modifications (Miller and Bassler 2001). Alternatively, most Gram-positive bacteria use ribosomally produced peptides as autoinducers or, in the case of the genus *Streptomyces*, γ-butyrolactones (Waters and Bassler 2005). Given the fundamental importance of QS in the regulation of bacterial physiology, it is not surprising that mechanisms known as "quorum quenching" have evolved to interfere with this process (Waters and Bassler 2005). Quorum quenching can occur by the secretion of enzymes that cleave the lactone ring of the AHL (Dong et al. 2001) or by the production of small molecule antagonists that competitively bind to the AHL receptor site. This has been demonstrated in the case of the furanones produced by the red alga *Delisea pulchra* (Manefield et al. 1999).

The production of QS inhibitors by marine bacteria may provide an effective method of competition that does not require the producing strain to be resistant to a toxic metabolite as is generally the case with antibiotic production.

Examples include honaucins A-C (**8**) isolated from the marine cyanobacterium *Leptolyngbya crossbyana* (Choi et al. 2012). The honaucins bear structural resemblance to the AHLs produced by the marine bacterium *Vibrio harveyi* and are potent inhibitors of bioluminescence, a QS phenotype in this species. In addition, a series of tumonoic acid analogues from *Blennothrix cantharidosmum* represent another group of cyanobacterial secondary metabolites that structurally resemble AHLs and inhibit bioluminescence in *Vibrio harveyi*. Tumonoic acid F (**9**) was found to be the most active, inhibiting QS at non-toxic concentrations without affecting bacterial growth (Clark et al. 2008). In another example, two QS inhibitors isolated from a marine *Halobacillus salinus* strain were identified as phenethylamide metabolites (Teasdale et al. 2009). These compounds inhibited bioluminescence in *Vibrio harveyi* and QS-regulated violacein biosynthesis in *Chromobacterium violaceum* at nontoxic concentrations by competing with AHLs for receptor binding (Teasdale et al. 2009). In a separate study, 24 of 166 strains of marine bacteria exhibited quorum quenching activity and, among those evaluated further, all were confirmed to enzymatically inactivate the AHLs (Romero et al. 2011).

Antagonism in Bacteria–Eukaryote Interactions

The interactions among marine bacteria and eukaryotes are complex and include predator–prey relationships and associations ranging from commensal surface colonization to obligate symbioses. While many of these interactions are likely mediated by secondary metabolites, this area of research is not well developed. The ecological effects of bacterial secondary metabolites can range from reduced fouling on the surface of a host to defense against predators that may target the host or, in the case of free-living bacteria, the bacteria themselves. Bacterial secondary metabolites also can be sequestered by the organisms that feed on them (Pennings and Paul 1993), providing the consumer with acquired mechanisms of defense against potential pathogens and predators. A more unusual type of bacterially mediated antagonism is associated with microbe–animal competition, as evidenced by the bacterial production of non-esterified fatty acids and other minor metabolites that render animal carcasses chemically repugnant to large scavengers (Burkepile et al. 2006). These types of interactions suggest the presence of complex mechanisms by which bacterial metabolites mediate antagonistic interactions with eukaryotes.

Inhibition of Invertebrate Larval Settlement Microbial biofilms are fundamental to the colonization of surfaces by higher marine organisms (Hadfield 2011; Qian et al. 2007). The effects of biofilm-associated bacteria on larval settlement have been studied in detail in the case of *Hydroides elegans* and likely involve chemical cues (Chung et al. 2010; Huang et al. 2012). While increased settlement has been observed in response to biofilm associated bacterial products (Harder et al. 2002), inhibitory effects also have been observed (Dobretsov and Qian 2002; Maki et al. 1988; Wieczorek and Todd 1997). Bacteria associated with biotic surfaces have been shown to inhibit larval settlement (Nasrolahi et al. 2012) and, in the case of certain marine algae, have been suggested to contribute to the defense of the host against fouling (Rao et al. 2007).

Antifouling activity could provide a benefit for the host by limiting the detrimental effects of surface colonization (Wahl 1989). Possibly due to the commercial implications of fouling, considerable effort has been devoted to the characterization of metabolites from marine bacteria that inhibit invertebrate larval settlement. For example, a branched chain fatty acid (12-methyltetradecanoid acid) isolated from a deep-sea sediment *Streptomyces* sp. strongly inhibited the settlement of *H. elegans* larvae (Xu et al. 2009). Ubiquinones isolated from a marine sponge-associated *Alteromonas* sp. displayed considerable anti-settlement activity against barnacle larvae, with activities varying depending on the length of the polyprenyl side chain (Kon-ya et al. 1995). Two polyethers from a sponge-associated strain of *Winogradskyella poriferorum* were inhibitory towards barnacle and *H. elegans* larval settlement. These compounds were localized in the cells, accounted for up to 17 % of the cellular dry weight, and were nontoxic in a zebrafish assay (Dash et al. 2011). The tropical mat-forming cyanobacterium *Moorea producens*, frequently described in the literature as *Lyngbya majuscula* (Engene et al. 2012), is a rich source of secondary metabolites (Nunnery et al. 2010). Among these, dolastatin 16 (**10**) (Fig. 2) has been identified as a potent inhibitor of barnacle larval settlement (Tan et al. 2010). Field tests confirmed the *in situ* antifouling activity of this compound at concentrations as low as 0.01 μg ml^{-1}. A deep-sea sediment derived *Pseudomonas rhizosphaerae* strain was found to produce a series of aromatic compounds and diketopiperazines that interfered with larval settlement, including two metabolites that also inhibited the growth of bacteria known to encourage *H. elegans* settlement (Qi et al. 2009). In a final example that touches on the putative bacterial origin of metabolites isolated from marine invertebrates, 6-bromindole-3-carbaldehyde (**11**) was isolated both from a marine ascidian (*Stomozoa murrayi*) and a bacterium (*Acinetobacter* sp.) associated with the ascidian's surface (Olguin-Uribe et al. 1997). This compound inhibited barnacle larval settlement and was suggested to account for the lack of fouling observed on the ascidian in the field.

The marine bacterial genus *Pseudoalteromonas* often occurs in association with macroorganisms and is a prolific source of biologically active secondary metabolites (Bowman 2007). *Pseudoalteromonas* spp. have been shown to inhibit

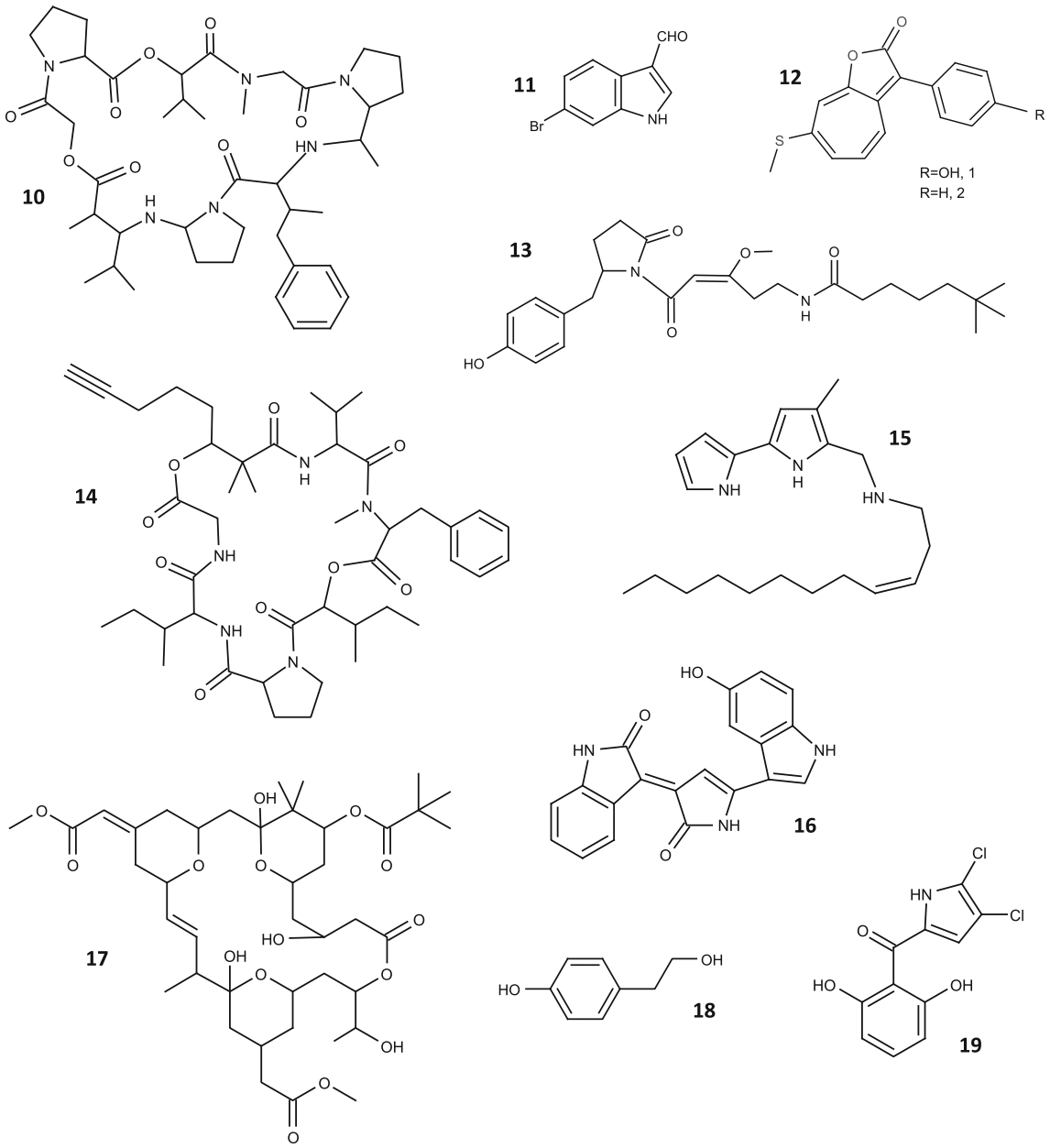

Fig. 2 Secondary metabolites produced by marine bacteria that mediate antagonistic interactions with marine eukaryotes. Examples include the larval settlement inhibitors dolastatin 16 (**10**) and 6-bromindole-3-carbaldehyde (**11**), the algaecides roseobacticides A and B (**12**), the grazing deterrents ypoamide (**13**), pitipeptolide A (**14**), tambjamine (**15**), and violacein (**16**), and the host-defense compounds bryostatin-10 (**17**), isatin (**18**), and pyoluteorin (**19**)

surface colonization by micro- and macroorganisms (Holmström et al. 2002), which has been linked to the lack of macrofouling on the surfaces of algae (Dobretsov and Qian 2002; Egan et al. 2000) and tunicates (Holmström and Kjelleberg 1999). *Pseudoalteromonas tunicata* cell densities as low as 10^2 cm^{-2} can prevent the settlement of algal spores and fungi, while approximately 10^5 cells cm^{-2} can deter invertebrate larvae (Rao et al. 2005). Considering the natural surface abundances of pseudoalteromonads (Skovhus et al. 2007), these bacteria may be present in sufficient quantities to prevent fouling *in situ* (Rao et al. 2007). The antifouling activities of *P. tunicata* have been correlated with pigmented strains and a polar, low molecular weight, heat-stable compound (Holmström et al. 2002; Huang et al. 2011). Interestingly, other *Pseudoalteromonas* strains induce larval settlement (Huang et al. 2011, 2012). The contrasting effects of different *Pseudoalteromonas* strains suggest the presence of specific, chemically mediated relationships with their eukaryotic hosts.

Interactions with Phytoplankton and Algae Marine bacteria also interfere with phytoplankton growth (Amin et al. 2012) and

algal spore germination (Egan et al. 2000). *Pseudoalteromonas* biofilms reduced *Ulva* zoospore settlement by 90 %, while *Vibrio* biofilms completely inhibited spore germination (Bernbom et al. 2011). Other *Pseudoalteromonas* strains were shown to paralyze and lyse *Enteromorpha* zoospores, with spores possessing a strain-specific response that was related to biofilm age (Patel et al. 2003). However, none of these observations have been linked to a specific bacterial compound. Marine bacteria exert antagonistic effects on phytoplankton (Hare et al. 2005; Mayali and Azam 2004; Suikkanen et al. 2004) by reducing swimming motility (Mayali et al. 2008) and producing compounds such as thiotropocin (Kawano et al. 1997) and prodigiosin-like pigments (Jeong et al. 2005; Nakashima et al. 2006). These observations suggest that marine bacteria can have a negative effect on algal bloom dynamics (Mayali and Azam 2004). However, bacteria also can have stimulatory effects on phytoplankton (Bell and Mitchell 1972), algal zoospore attachment (Joint et al. 2002), and seagrass growth (Celdrán et al. 2012).

Phytoplankton cues may induce the antagonistic activities of marine bacteria. For example, the antibiotic effects of bacteria in the *Roseobacter* clade against the fish pathogen *Vibrio anguillarum* were greater when co-cultured with the phytoplankter *Nannochloropsis oculata* (Sharifah and Eguchi 2011). A second example of induction addresses the complex chemical relationship between the bacterium *Phaeobacter gallaeciensis* and the phytoplankter *Emiliania huxleyi*, whose growth benefits from bacterially produced antibiotics and growth-stimulating auxins (Seyedsayamdost et al. 2011). However, *P. gallaeciensis* switches to the production of potent and selective algaecides, the roseobacticides (**12**), in response to *p*-coumaric acid, a cell wall breakdown product of ageing algae. This converts *P. gallaeciensis* into an opportunistic pathogen of *E. huxleyi* once blooms start to decay (Seyedsayamdost et al. 2011).

Chemical Defense Against Eukaryotic Grazers Protozoan grazing has a major effect on bacterioplankton biomass (Pernthaler and Amann 2005) and influences the structural diversity of bacterial communities (De Mesel et al. 2004). Marine bacteria have evolved a variety of mechanisms that defend against predation (Jousset 2012), with the benthic, mat-forming cyanobacterial genera *Lyngbya* and *Microcoleus* being among the best studied for the production of deterrent metabolites (Berry et al. 2008; Capper et al. 2006). These include the lipopeptide ypoamide (**13**) (Nagle et al. 1996) as well as the malyngamides and majusculamides, which deter feeding by reef fish and invertebrates at ecologically relevant concentrations (Pennings et al. 1996). Natural concentrations of pitipeptolide A (**14**) proved deterrent to sea urchins, amphipods, and crabs, but not the sea hare *Stylocheilus longicauda* (Cruz-Rivera and Paul 2007). *Stylocheilus longicauda* preferentially feeds on *L. majuscula* (Paul and Pennings 1991) and, like other opisthobranch molluscs, sequesters diet-derived secondary metabolites that serves as anti-predator defenses (Paul and Pennings 1991; Pennings et al. 1996). Anti-feeding metabolite production can be used to distinguish cyanobacterial chemotypes with different habitat specificities, with the deterrent compound constituting the major secondary metabolite in some chemotypes (Cruz-Rivera and Paul 2007; Thacker et al. 1997).

Among heterotrophic marine bacteria, anti-grazing molecules are less known. A functional genomic screen of *Pseudoalteromonas tunicata* D2 identified a recombinant *E. coli* clone that killed the nematode *Caenorhabditis elegans* (Ballestriero et al. 2010). End sequencing of the associated fosmid and mapping onto the *P. tunicata* D2 genome revealed a gene cluster involved in the production of the antifungal compound tambjamine (**15**). While *C. elegans* is a terrestrial species, tambjamine may likewise deter predation by marine nematodes (Ballestriero et al. 2010). Protection against protozoan grazing has been demonstrated also for the purple pigment violacein (**16**) produced by marine *Pseudoalteromonas luteoviolacea* (Matz et al. 2008) and freshwater bacteria (Matz et al. 2004). This compound causes the rapid autolysis of bacterivorous nanoflagellates at nanomolar concentrations or following the uptake of 1–2 violacein-producing cells, and has been proposed to function as a chemical defense in biofilms (Matz et al. 2008). In a strain of *V. cholerae*, a quorum-regulated antiprotozoal factor was shown to inhibit the growth of the flagellate *Rhynchomonas nasuta* and thus limit grazing losses (Erken et al. 2011). This grazing resistance was linked to the QS response regulator HapR and *Vibrio* polysaccharide production (Sun et al. 2013). Additionally, the extracellular protease PrtV from *V. cholerae* provides grazing resistance against the flagellate *Cafeteria roenbergensis* and the ciliate *Tetrahymena pyriformis* and was further identified as lethal to *C. elegans* when *V. cholerae* colonized worm intestines (Vaitkevicius et al. 2006).

Host Protection by Bacterial Metabolites Structural similarities between compounds isolated from marine invertebrates and those produced by bacteria has led to the widely discussed hypothesis that some of these compounds originate from bacterial symbionts. One of the challenges in addressing this issue is that many symbionts are not readily obtained in culture. Despite this obstacle, there is mounting evidence for the microbial origin of some metabolites (Piel 2004), starting with the pioneering cellular localization studies from the lab of the late John Faulkner (Bewley et al. 1996; Unson and Faulkner 1993; Unson et al. 1994). Progress is being made towards identifying the roles of these compounds in antagonistic interactions. For example, a series of detailed studies of the bryozoan

Bugula neritina led to the identification of the bacterial symbiont '*Candidatus* Endobugula sertula' and its production of the bryostatins (Davidson et al. 2001; Lopanik et al. 2004b; Sharp et al. 2007), a series of biomedically important compounds (Sun and Alkon 2006) originally ascribed to the host (Pettit et al. 1982). Several bryostatin analogues, including bryostatin-10 (**17**), can comprise up to 1 % of larval dry weight and have been shown to reduce pinfish feeding on *B. neritina* larvae by up to 75 %. This has been reported as the first example of a marine bacterial symbiont that produces an anti-predator defense for its host (Lopanik et al. 2004a, b).

A second example comes from an isopod species from the coral reefs of Papua New Guinea. This isopod harbors a dense carpet of cyanobacteria on its dorsal surface, which renders it unpalatable to common reef fish predators (Lindquist et al. 2005). While the chemical basis for this defense has not been defined, it has been proposed that the symbionts allow the isopods to occupy exposed, well lit reef areas, and that the host "cultivates" the symbionts, using them as a food source as well as for defensive purposes (Lindquist et al. 2005). The protective roles of symbiotic bacteria are also associated with the prevention of infection in the host. For example, isatin (**18**) produced by a shrimp-associated *Alteromonas* sp. defends the host embryos from pathogenic fungi (Gil-Turnes et al. 1989), while 4-hydroxyphenethyl alcohol similarly defends embryos of the lobster *Homarus americanus* (Gil-Turnes and Fenical 1992). It has likewise been suggested that the egg capsules of the commercially important squid *Dorytheutis opalescens* are chemically defended by symbiotic bacteria (Kaufman et al. 1998).

Another fascinating but less well-developed example comes from the sponge *Theonella swinhoei*. The Compounds discovered from this sponge include structural classes such as polyketides, which are best known as being of microbial origin (Piel et al. 2005). Based on metagenomic and bioinformatic analyses, the production of polyketides in the theopederin and onamide classes in *T. swinhoei* were linked to unidentified bacterial symbionts in the sponge. While the ecological roles of these compounds in the symbiosis have not been resolved, the closely related compound pederin produced by bacterial symbionts of *Paederus* spp. beetles are known to deter predation on the eggs and larvae of the host (Kellner and Dettner 1996), suggesting similar defensive functions are provided to the sponge. Host protection also has been shown for algal-associated bacteria. For instance, *Pseudomonas* strains regularly isolated from the brown alga *Saccharina latissima* (synonym *Laminaria saccharina*) produced 2,4-diacetylphloroglucinol and pyoluteorin (**19**), both of which displayed antibiotic activity against *Pseudoalteromonas elyakovii* and *Algicola bacteriolytica*, two bacterial pathogens of the related species *S. japonica* (Nagel et al. 2012). Host protection also is implied for bacterial lipopolysaccharides from *Marinobacter* sp. that trigger early events of algal defense reactions by inducing oxidative bursts (Küpper et al. 2006).

An interesting example of the challenges associated with identifying ecologically relevant antagonistic activities mediated by host-associated bacteria comes from tetrodotoxin (TTX), a potent neurotoxin best known as the causative agent of food poisoning associated with eating raw pufferfish (i.e., fugu). TTX has been isolated from a range of marine and non-marine organisms including marine bacteria (Simidu et al. 1987; Wang et al. 2010; Yu et al. 2011). TTX may function as an anti-predator defense, venom, and/or signaling molecule; however, none of these hypotheses have been tested rigorously. The potential for a bacterial origin of TTX in marine animals also remains controversial, as it has been shown to accumulate through dietary sources (Williams 2010) and to be localized in various cell types, tissues, and vesicles (Tanu et al. 2002, 2004). To the best of our knowledge, the association of TTX with bacteria in animal hosts has yet to be supported by co-localization studies.

Future Prospects

Linking biological activities to specific metabolites and their ecological functions remains a major challenge. New technologies such as imaging mass spectrometry (IMS) are providing unprecedented opportunities to study small molecule mediated antagonism in a visual context (Moree et al. 2013 this issue; Watrous and Dorrestein 2011). IMS applications include the visualization of natural products on living surfaces (Kubanek et al. 2003) and the identification of molecules associated with zones of inhibition on agar plates (Yang et al. 2011). Additional advances come from a better understanding of the molecular genetics of natural product biosynthesis (Fischbach and Walsh 2006) and the development of tractable genetic systems for a growing number of marine bacteria (Eustaquio et al. 2009). The latter are providing more efficient approaches to generating secondary metabolite-deficient mutants, which can be used to link the production of specific metabolites to their associated biological activities. With marine bacteria, this has been accomplished only in a limited number of cases (Ballestriero et al. 2010; Cude et al. 2012; Long et al. 2005), yet it represents an important advance for the field.

Unlike studies of marine invertebrates and plants, where "natural" concentrations can be estimated using volumetric extraction methods (Pawlik 1993), these approaches generally are not available for bacteria. Exceptions include mat-

forming cyanobacteria, which can be readily collected in the field and extracted using the techniques applied to macro-organisms. Cyanobacteria have provided excellent examples of the ecological relevance of bacterial secondary metabolites (Capper et al. 2006) and offer useful models for future studies. In the case of most bacteria, however, estimating ecologically relevant test concentrations remains a particular challenge, as we have little information about the *in situ* concentrations of bacterial metabolites. Furthermore, the results of laboratory-based bioassays may not be indicative of ecological function, especially given that sub-inhibitory metabolite concentrations can have dramatic effects on bacterial gene expression (Goh et al. 2002). In many cases, the ecological interpretation of antagonism has been left to speculation.

Summary

Marine bacteria are widely recognized as a resource for natural product discovery (Fenical and Jensen 2006). While considerable effort has gone into the search for commercially relevant secondary metabolites from marine bacteria, considerably less attention has been paid to their ecological functions. Laboratory studies have consistently demonstrated the antagonistic effects of marine bacteria against macro- and microorganisms. In many cases, these activities have been linked to specific secondary metabolites, suggesting they provide a competitive advantage to the producing organism.

The widespread observation of bacterially mediated antagonism suggests that these effects may influence bacterial microscale heterogeneity, predator–prey relationships, symbioses, the development of fouling communities, and other processes that impact ecosystem function. Certain bacterial taxa including members of the *Roseobacter* clade (Wagner-Döbler and Biebl 2006) and *Pseudoalteromonas* spp. (Skovhus et al. 2007) are consistently linked to observations of antagonism. These bacteria are commonly found in marine microbial communities and may be adapted to employ antagonism as a survival strategy. However, the antagonistic activities reported for members of the rare biosphere, such as the marine actinomycete genus *Salinispora* (Mincer et al. 2002), also are of interest, as less abundant organisms can serve as a reservoir of genetic and functional diversity (Yachi and Loreau 1999). The antagonistic activities of marine bacteria also have been capitalized on for their commercial potential, for instance as probiotics in the aquaculture industry (Balcazar et al. 2006) and component of antifouling coatings (Burgess et al. 2003).

Marine bacteria produce natural products that affect how they interact with each other and the community of macro- and microorganisms with which they co-occur. While the ecological interpretation of laboratory based studies has largely been left to speculation, new analytical techniques and experimental designs (see Moree 2013) that take into account environmentally meaningful variables, such as compound concentrations and assay organisms, provide exciting opportunities to make significant gains in this area of research. Although antagonism represents only one potential effect of bacterial secondary metabolites, there is mounting evidence that these effects can be important for the structure and function of marine ecosystems.

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