CHEMICAL ECOLOGY OF MARINE BACTERIA AND ALGAE

Slattery M.
Department of Pharmacognosy, University of Mississippi, USA

Gochfeld D.J.
National Center for Natural Products Research, University of Mississippi, USA

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Summary

The discipline of chemical ecology has developed within the marine environment over the last two decades and, to a large extent, this research has focused on algae and cyanobacteria. Their compounds have been observed to mediate predator–prey interactions, to act as pheromones, and to serve as allelopathic agents against competitors and/or pathogens. Marine algae are comprised of a diverse group of primary producers, including cyanobacteria (blue-green algae), chlorophytes (green algae), phaeophytes (brown algae), rhodophytes (red algae), and even a few angiosperms (i.e., seagrasses). However, from an ecological standpoint, all of these organisms occupy a similar niche and are subject to herbivory and competition. Herbivory is intense in the marine environment, with losses due to grazing accounting for 60–100% of total algal production. Recent evidence indicates the importance of quantitative levels of chemical defenses in mediating the selective grazing patterns that have been observed. Marine algae and bacteria compete for space and nutrients with other algae, bacteria, and/or invertebrates. Recent studies have provided evidence for allelopathic compounds which ward off competitors and/or pathogens. In addition, undefended marine invertebrates may gain a refuge from predation by living in

association with chemically defended algae and cyanobacteria. Algal compounds are used for other purposes besides defense; brown algae produce pheromones that attract gametes from conspecifics and compounds from red coralline algae have been shown to induce settlement of marine invertebrates into adult habitats. Defensive metabolites can vary within an individual, among individuals within a population, and among populations. This variability may occur over temporal and/or spatial scales. Moreover, this variation can be induced by herbivores and/or by a variety of abiotic factors. The brown algae, *Dictyota* spp., provide an important model system to examine the complexity of ecological and evolutionary patterns and processes mediated by natural products.

1. Introduction

The ecological roles of biochemical constituents from plants and animals have been a subject of biological investigation for approximately 50 years. These compounds have been observed to mediate predator–prey interactions, to act as pheromones, and to serve as allelopathic agents against competitors, and/or pathogens. The discipline of chemical ecology has really developed within the marine environment over the last two decades, and to a large extent this research has focused on algae and cyanobacteria. More recently, the chemical ecology of marine microorganisms, such as phytoplankton and true marine bacteria, has also been examined. The purpose of this article is to provide an overview of our current understanding of the chemical ecology of marine bacteria and algae.

Primary producers in the marine environment include phytoplankton, cyanobacteria, chlorophytes, phaeophytes, rhodophytes, and even a few angiosperms; collectively these diverse groups are referred to as "algae." Traditionally, algae include an assortment of primitive plants living in water, representing distinct shapes, sizes, colors, and complexity. Algae lack the true roots, stems, leaves and flowers characteristic of higher plants. Four major divisions of multicellular algae are typically recognized: 1) Cyanophyta (blue-green algae or cyanobacteria), 2) Chlorophyta (green seaweeds), 3) Phaeophyta (brown seaweeds), and 4) Rhodophyta (red seaweeds). As the names imply these algae vary in coloration, by incorporating different photopigments into their cells. All algae contain chlorophyll, but brown algae also contain xanthophyll, while red algae also include phycoerythrin, and/or phycocyanin. Blue-green algae are classified independently of the other three groups based upon cell structure disparity. From an ecological standpoint, all of these organisms occupy a common niche in the marine environment. They typically grow on the benthos where they are subject to predation (herbivory) and competition.

The chemical ecology of phytoplankton (waterborne unicellular and/or colonial algae) and the true marine bacteria has only recently been investigated. This current interest probably represents our increased understanding of the importance of these two groups in issues related to human health and economics (e.g., harmful algal blooms and shellfish poisonings, respectively).

Much of the basis for our research into the chemical ecology of marine algae and bacteria is grounded in hypotheses and models developed to explain biochemical evolution in plant–herbivore interactions in terrestrial communities. Whereas these models clearly represent a starting point, differences between the terrestrial and marine environments may significantly affect the ecological interactions that are mediated by biochemical compounds. For example, the exudation and chemoreception of a particular metabolite depends on the diffusion properties of that compound in air versus water. Moreover, these environments appear to constrain the biochemical diversity of their respective organisms. Nitrogenous compounds, such as alkaloids, are common in terrestrial plants but are rare in marine algae. This is probably due to the fact that the marine environment is typically nitrogen-limited, and only a few groups of marine organisms are able to fix elemental nitrogen. In contrast, halogenated compounds are common in red and in some green algae, whereas the proportion of halogenated compounds in terrestrial plants is much lower. Brominated and chlorinated compounds are most common in algae, and the mixed biosynthetic pathways that produce these compounds have resulted in many structural classes that are unique to the marine environment. For example, the red alga *Laurencia* has yielded hundreds of halogenated terpenoids and acetogenins representing 26 distinct structural classes, of which 16 are unique to marine systems.

In addition to environmental differences between terrestrial and marine systems, there are also differences in the plant–herbivore relationships. In terrestrial systems, much of the plant biomass is of low nutritional value (e.g., woody structural material) or unavailable (e.g., roots) to grazers. In contrast, marine algae consist of relatively undifferentiated foliose and nutritious tissue that is highly exposed to herbivory. On coral reefs, losses to grazing can account for 60–100% of total algal production, and may exceed that of tropical forests by 10 to 100 times. In terrestrial systems, most of the herbivory is the result of insect specialists that may only be present for limited periods of time during the year. Marine systems are dominated by large motile generalist macrograzers that are typically present year-round; these include up to 25% of the reef fish species (e.g., parrotfishes, surgeonfishes, damselfishes, chubs and rabbitfishes) and a variety of invertebrates (sea urchins, gastropods, polychaetes and crustaceans). Thus, the selection pressures in terrestrial versus marine systems should favor defenses against few specialist or many generalist herbivores, respectively.

Like their terrestrial counterparts, algae have invested in either chemical (defensive compounds) or morphological (calcification and/or toughness) resistance mechanisms. In some cases, the use of both mechanisms simultaneously may act to deter multiple grazers or may increase effectiveness (i.e., additively or synergistically) against a particular grazer. In many plants, the concentration of a defensive metabolite is genetically predetermined; these are called constitutive defenses. However, some plants respond to herbivory by enhancing their synthesis of a defensive metabolite or by synthesizing a novel metabolite. Two patterns have been described: 1) activation, which involves the enzymatic conversion of a nontoxic precursor compound into a related toxic metabolite, and 2) induction, which involves the expression of new metabolite and/or the proteins involved in metabolite production via molecular processes. Activation is typically a rapid (seconds to minutes) response to localized grazing, while induction tends to require more time (hours to days). Toxicity and/or feeding deterrence are not necessarily a function of structure. In fact, very slight chemical modifications can have significant effects on the bioactivity of a particular structural class. Thus, these compounds interact in unique ways with the particular physiology of the consumer. In some cases, the consumption of chemically defended plants has been shown to affect the fitness of the herbivores, in other cases specific herbivores are able to consume diets containing broadly deterrent compounds with impunity.

Herbivores in marine systems exhibit distinct preferences for algae, based on observations of grazing in the laboratory and field, as well as on gut content analyses. This selective grazing has the potential to effect major changes in community species' compositions and abundances. Recent evidence indicates the importance of quantitative levels of chemical defenses in mediating the preferences observed. In some cases, the effects of these chemical defenses operate in a dose-dependent fashion. Defensive metabolites can vary within an individual and among individuals within a population; they can also vary temporally and/or spatially. In addition, as grazing pressure increases across biogeographic ranges, the production of defensive metabolites by some species responds in a directly proportional manner.

Two complementary theories have been developed to explain quantitative chemical variation in plants at the individual and population levels in terrestrial systems. The Optimal Defense Theory and the Carbon–Nutrient Balance Hypothesis were developed to explain intraspecific chemical variation. In the case of the former theory, defenses are hypothesized to be costly, and thus defensive metabolites are allocated to the most valuable tissues and/or the tissues that are most susceptible to herbivores. In the case of the latter hypothesis, the availability of resources is suggested directly to influence the allocation to chemical defenses. Thus, as an environmental variable such as light or nutrient level changes, so too would the levels of chemical defenses within the plant. The Plant Apparency Model and the Resource Availability Model were developed to explain interspecific chemical variation. The plant apparency model is similar to the optimal defense theory in positing that plant defenses evolved in response to the value of the various tissue groups of the plants, to the costs of production of the defensive metabolite(s), and to the chance of being encountered by a herbivore. The resource availability model proposes that access to nutrients from the environment limits plant growth rates; for slower growing plants, which are favored when resources are scarce, the value of plant tissues is proportionately higher and thus the need for chemical defenses is greater.

2. Ecological Roles of Bacterial and Algal Compounds

2.1. Feeding Deterrence

Selection pressures for feeding deterrent compounds in cyanobacteria and algae are extreme in the marine environment; this is particularly true in coral reef ecosystems that are dominated by a diversity of large motile macroherbivores. Numerous studies using ecologically relevant grazers in either laboratory or field settings have demonstrated that many species of cyanobacteria and algae are chemically defended. Cyanobacteria have traditionally been known to produce a variety of toxic or pharmacologically active metabolites, but the ecological relevance of these metabolites is only now becoming fully appreciated. Compounds from the filamentous blue-green algae *Lyngbya majuscula*, *Hormothamnion enteromorphoides*, and *Microcoleus lyngbyaceus* have been the subjects of feeding deterrent assays in recent years on Guam. Generalist grazers, including the parrotfish *Scarus schlegeli*, the rabbitfishes *Siganus argenteus* and *S. spinus*, the sea urchins *Diadema savignyi* and *Echinometra mathaei*, and the crabs *Leptodius* spp., were all deterred by natural concentrations of blue-green algal metabolites embedded in artificial diets. More interesting were the results of feeding assays that used a cyanobacterial specialist, the opisthobranch mollusc *Stylocheilus longicauda*. This species is now known to sequester cyanobacterial defensive metabolites within its tissues, possibly as a defense against predators. However, the ability of this sea hare to consume chemically defended blue-green algae was only discovered after feeding assays were conducted over increasingly narrow concentration gradients, using metabolite concentrations much lower than 2% per gram dry weight. At these lower concentrations many of the feeding deterrent compounds were actually feeding stimulants to *S. longicauda*. It now seems apparent that development of extensive cyanobacterial mats during blooms, often in the presence of intensive grazing pressure, may be facilitated by the production of novel defensive metabolites by these blue-green algae.

Figure 1. Mat-forming cyanobacteria, such as *Lyngbya majuscula*, respond quickly to favorable environmental conditions; their persistence is thought to be a function of the broad-spectrum deterrence provided by their chemical diversity.

Feeding deterrent compounds are also distributed widely throughout the three divisions of true marine algae, although our relatively limited survey of chemical defenses in this extensive group suggests that results to date represent only the tip of the iceberg. The chlorophytes are common members of tropical to temperate waters, they are often calcified, and they typically produce terpenoid compounds that are active antifeedants. Arguably the most important algal genus of tropical marine ecosystems is *Halimeda*.

In addition to their interesting chemistry, blades of *Halimeda* spp. are heavily calcified and these algae are responsible for the production of significant proportions of sand on tropical reef flats. These species produce three structurally similar metabolites, of which two (halimedatetracetate and halimediatrial) have been shown to be feeding deterrent to a variety of herbivorous fishes from the western Pacific and the Caribbean. In addition, *Halimeda macroloba* produces a diterpene that is very similar to halimedatetracetate; this is sequestered and converted by the ascoglossan specialist *Elysia halimedae* for its own defense, and for defense of its egg masses.

Figure 2. The green algae *Halimeda* spp. are important members of tropical coral reef communities; they use both chemical and morphological defense strategies.

The combined chemical and structural defenses of *Halimeda* deterred more herbivorous fishes (parrotfishes, rabbitfishes and surgeonfishes) than either defense by itself, although in the case of the parrotfish *Scarus sordidus*, the chemical defenses were apparently offset by the preference this fish exhibits towards calcified foods. Two defensive compounds, cymopol and bromoisocymobarbatol, have been isolated from the tropical Atlantic green alga *Cymopolia barbata*. These compounds have shown activity against a natural assemblage of herbivorous fishes in field assays, and against the omnivorous pinfish *Lagodon rhomboides* and the amphipod *Hyale macrodactyla* in laboratory assays. Interestingly, the compound cymopol deterred feeding by the sea urchin *Lytechinus variegatus*, but stimulated feeding by the sea urchin *Diadema antillarum.* The Caribbean green alga *Avrainvillea longicaulis* produces a brominated compound, avrainvilleol, which deters reef fish herbivory and, when sequestered by the ascoglossan specialist *Costasiella ocelliferous*, deterred predation by the omnivorous bluehead wrasse *Thalassoma bifasciatum*. Other Caribbean green algae, *Caulerpa* spp. and *Udotea* spp., produce caulerpenyne and udoteal, respectively. Caulerpenyne is deterrent to the sea urchin *Lytechinus variegates*, as well as to several species of reef fish. Udoteal also deters these two species of damselfish. There is also some evidence that a diet rich in caulerpenyne or udoteal may reduce survivorship of juvenile conchs, *Strombus gigas*, although replication was limited. In the western Pacific (Guam and the Great Barrier Reef, Australia) the green algae *Chlorodesmis fastigiata* and *Pseudochlorodesmis furcellata* produce a complex mixture of defensive metabolites that were deterrent to a variety of herbivorous reef fishes, including rabbitfishes and the surgeonfish *Zebrasoma flavescens*. Among these compounds, chlorodesmin, which was deterrent to several herbivorous fishes, acted as a feeding stimulant to the crab *Caphyra rotundifrons,* and was sequestered by the ascoglossans *Elysia* sp. and *Cyerce nigricans* for their own defenses. Finally, the calcified green alga *Neomeris annulata* produces brominated compounds that deter herbivorous fishes on the reefs surrounding Guam.

The Phaeophyta, or brown algae, have been the subject of extensive studies with respect to their bioactive metabolites. Most of these studies have occurred in subtropical to temperate clines and have focused on a unique class of compounds called “phlorotannins,” or more generally, “polyphenolics.”

Figure 3. The temperate kelp *Macrocystis pyrifera* is a representative brown alga; these algae commonly produce polyphenolic defenses.

These compounds differ from terrestrial tannins in that they are complex polymers that originate from a phloroglucinol precursor compound. Unfortunately these compounds are not easily isolated; thus most of the research into their bioactivity has examined them as complex mixtures or, very rarely, as fractions based on molecular weight. The ecological roles of phlorotannins include herbivore deterrence, prevention of fouling, and UV absorption. Several terpenoids and acetogenins have also been isolated from brown algae, particularly from *Dictyota* spp., which are discussed extensively in Section 4. The temperate brown algae *Ascophyllum nodosum*, *Ecklonia radiata*, *Eisenia arborea*, *Fucus vesiculosus*, *Halidrys siliquosa*, and *Sargassum vestitum* all produce polyphenolics which deter a variety of generalist herbivores including snails (*Littorina littorea*, *Tegula funebralis*, *T. brunnea*, *Turbo undulata*, *T. smaragdus*, and *Cookia sulcata*) and sea urchins (*Evichinus chlorotichus* and *Tripneustes gratilla*). Geranylacetone, a feeding deterrent compound isolated from the eastern Australian brown alga *Cystophora moniliformis*, prevented herbivory by the resident sea urchin *Tripneustes gratilla*. In western Australia, the sea urchin *Tripneustes esculentus* was deterred by one of two prenylated phenols isolated from *Encyothalia cliftoni*. Another prenylated phenol, sporochnol, from the Caribbean brown alga *Sporochnus bolleanus*, deterred parrotfish herbivory, but not grazing by herbivorous invertebrates (i.e., the sea urchin *Diadema antillarum* and the amphipod *Cymadusa filosa*). The simple C_{11} hydrocarbons, dictyopterenes A and B, isolated from the Caribbean brown alga *Dictyopteris deliculata*, exhibited significant feeding deterrent activity against a variety of herbivorous fishes in the field, but not against an assemblage of herbivorous amphipods collected from this turf algal species. The temperate seaweeds *Desmarestia* spp. concentrate sulfuric acid within their tissues; this has been suggested as an example of an inorganic chemical defense against a diversity of herbivores.

To date, the Rhodophyta, or red algae, have yielded the greatest number of algal marine natural products and the greatest diversity of structural classes, both known and novel. Red algae also represent the most dramatic differences in functional morphology, ranging from delicate foliose turf species to calcified coralline crusts, and ecology (e.g., depths vary from the splash zone of the intertidal to the subtidal benthos at over 90 m).

It is therefore surprising that so few studies have examined the chemical ecology of this group. *Laurencia* spp. produces over 500 compounds, only a few of which have been tested in feeding assays. Elatol and isolaurinterol were deterrent to herbivorous reef fishes in the Caribbean, as well as to the endemic sea urchin *Diadema antillarum*. The former compound also deterred a natural assemblage of herbivorous reef fishes on Guam. Elatol, debromolaurinterol, and chlorofucin were all deterrent to the rabbitfish *Siganus doliatus* from the Great Barrier Reef, Australia. However, four related compounds were not deterrent, and one, palisidin A, was deterrent to conspecific rabbitfishes from Guam, but only at twofold natural concentrations. A halogenated monoterpene, ochtodene, has been isolated from the Caribbean red alga *Ochtodes secundiramea* and the Pacific red alga *Portieria hornemanii*. In field assays, both in the Grenadine Islands and on Guam, herbivorous reef fishes avoided food containing ochtodene, but not food containing the related metabolite chondrocole C. More recently, apakaochtodenes A and B have also been isolated from *P. hornemanii*, and these compounds deterred a natural assemblage of herbivorous fishes in the field, as well as the rabbitfish *Siganus argenteus* and the sea hare *Aplysia parvula*, in the laboratory.

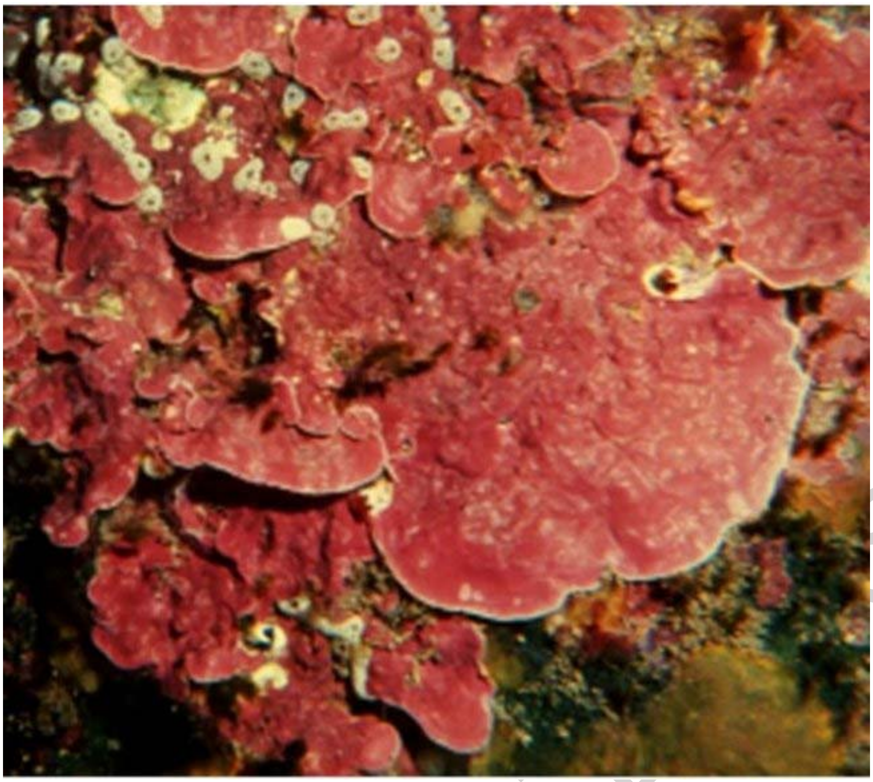

Figure 4. Coralline red algae, including *Lithothamnion* sp., often produce metabolites that serve as larval settling cues for benthic invertebrates such as reef-building corals and economically important shellfish.

Seagrasses are true flowering plants (angiosperms) and only a few representatives occur within marine environments.

Figure 5. Seagrasses, such as *Thalassia* spp., are not known to produce chemical defenses, but they serve as important nursery grounds and/or habitats.

While several phenolics and flavonoids have been isolated from seagrasses, ecological roles for these compounds have not been ascertained. Nonetheless, herbivory and fouling are clearly important factors in structuring seagrass communities, thus it would not be surprising to learn at a future date that some of these compounds are ecologically active.

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Biographical Sketches

Dr. Marc Slattery received his B.Sc. in Biology from Loyola Marymount University in 1981, his M.A. in Marine Biology from San Jose State University at Moss Landing Marine Laboratories in 1987, and his Ph.D. in Chemical Ecology from the University of Alabama at Birmingham in 1994. The focus of Dr. Slattery’s dissertation research was the population dynamics and chemical ecology of three species of Antarctic soft corals. Upon completion of his degree, Dr. Slattery was awarded a NSF Marine Biotechnology Postdoctoral Fellowship to work at the University of Guam Marine Laboratory on tropical soft coral chemical ecology and reproductive biology. In 1995 Dr. Slattery was hired as an Assistant Professor of Pharmacognosy at the University of Mississippi, and in 2001 he advanced to the rank of Associate Professor. Dr. Slattery’s current research interests are focused on the chemical ecology of marine bacteria, algae, and invertebrates. Specifically he is looking at phenotypic versus genotypic variability in defenses, and the environmental variables that effect these changes.

Dr. Deborah Gochfeld received her B.A. in Biology from Vassar College in 1988 and her Ph.D. from the University of Hawaii at Manoa in Zoology in 1997. The focus of her dissertation research was the behavioral and chemical ecology of coral–butterflyfish interactions. Following completion of her degree, Dr. Gochfeld was Resident Director of the Hofstra University Marine Laboratory in Jamaica, where she taught field courses in marine science. In 1998, she accepted a National Institutes of Health Postdoctoral Fellowship at the University of Mississippi, which enabled her to cross-train into the field of marine natural products chemistry. Dr. Gochfeld is now a Research Scientist at the National Center for Natural Products Research at the University of Mississippi. Her present research interests focus on the ecological roles of secondary metabolites in marine invertebrates, particularly as they relate to disease resistance and predator defense, and their implications for structuring marine communities.