



Marine bacterial diversity as a resource for novel microbial products

PR Jensen and W Fenical

Center for Marine Biotechnology and Biomedicine, Scripps Institution of Oceanography, University of California-San Diego, La Jolla, CA 92093, USA

Marine bacteria are an important and relatively unexplored resource for novel microbial products. In this review, we discuss a number of issues relevant to the industrial potential of marine microorganisms including how marine and terrestrial bacteria differ, both physiologically and taxonomically, and what constitute reasonable expectations of the biosynthetic capabilities of marine bacteria relative to terrestrial bacteria and to marine macroorganisms. Also discussed is the concept that bacterial associations with marine plants and animals, which range from casual encounters to obligate symbioses, provide unique opportunities for bacterial adaptation. It is proposed that some of these adaptations would not be selected for in the absence of environmental parameters associated with the host, and that these adaptations can include the biosynthesis of unique metabolic products.

Keywords: marine bacteria; bacterial diversity; microbial products

The marine environment covers more than 70% of the earth's surface and includes a vast array of diverse habitats ranging from tropical, shallow-water coral reefs to sub-zero, deep-ocean trenches. Within these habitats reside diverse plant and invertebrate phyla, the majority of which are uniquely marine. During the past two decades, the chemical potential of marine plants and animals has been extensively surveyed, and it is now widely recognized that these organisms represent an exceptionally rich source of structurally novel, biologically active secondary metabolites (see review [11] and references contained therein). Given the wealth and diversity of pharmacologically active organic substances discovered to date from marine organisms (at the time of this writing four compounds are in clinical trials for cancer), and the eventual limits to the numbers and types of plants and animals that can be collected for chemical study, it seems only logical to expand upon marine chemical explorations by initiating studies of resident microorganisms. Such studies are in fact underway at various academic and industrial institutions world-wide, and it is the purpose of this review to interpret the results of these studies in terms of how successfully marine bacterial diversity has been assessed as a resource for drug discovery and what should realistically be expected from this resource relative to marine macroorganisms and the more traditional study of terrestrial microorganisms.

Based on initial chemical studies, it is clear that marine bacteria, like marine plants and invertebrates, represent an emerging and relatively unexplored resource for secondary metabolite discovery. Not only are large numbers of diverse marine microbes amenable to current fermentation technologies but many display unusual morphological and physiological adaptations that exemplify strategies for survival in

the sea. Attesting to the growing awareness of marine microbial resources is the number of academic and industrial programs currently studying these microorganisms and the resulting increased rate at which novel metabolites are being discovered (*ca* three compounds per year were reported between 1989 and 1993, the rate increased to *ca* eight compounds per year for the years 1994–95). In this review, we explore the concept that bacterial adaptations to diverse marine microhabitats represent a driving force behind the selection for and maintenance of biosynthetic pathways leading to new metabolic products. We also compare the taxonomic levels at which marine and terrestrial bacteria are distinguished and comment on the implications of these differences for secondary metabolite discovery. We refer to recent reviews for ecological considerations of this subject [20] and comprehensive discussions of compounds discovered from marine microorganisms [8,12,13,23,31]. Annually-updated chemical information can be obtained from the reviews of Professor D John Faulkner [11].

Let us first consider what should realistically be expected of marine bacteria relative to chemical studies of marine plants and invertebrates. When chemical studies of marine invertebrates began in earnest two decades ago, most of the metabolites isolated were new to science, and many of these possessed new carbon skeletons. In hindsight, this is not particularly surprising considering that of 33 animal phyla described, 21 are unique to the sea [27]. The high taxonomic levels at which marine and terrestrial macroorganisms are distinguished suggests that species diverged long ago and have subsequently followed independent evolutionary paths. The evolution of marine invertebrates has generated innumerable species, many of which display morphological and physiological adaptations unparalleled by life forms outside of the ocean. These adaptations include, usually in soft-bodied organisms that lack structural defenses, the production of secondary metabolites that effectively deter natural predators, ie function as chemical defenses [33]. Thus, there is an ecological foundation as to

why marine macroorganisms are a rich source of biologically active secondary metabolites and a taxonomic rationale as to why many of these substances are chemically unique. Given the degree of invertebrate speciation and the use of chemical defense as a survival strategy, it is with little wonder that marine invertebrate secondary chemistry shows little structural similarity to natural products described from non-marine sources, where completely different environmental challenges must be addressed. The clear divergence between the secondary chemistry of marine and terrestrial plants and animals is paralleled by the unambiguous distributions of these organisms in their respective environments. Marine plants and animals seldom cross the readily perceived boundaries distinguishing land from sea, and for this reason, questions seldom arise as to the indigenous habitat of marine macroorganisms collected for chemical study.

In contrast to the large number of uniquely marine invertebrate phyla, there are no comparable taxa of uniquely marine bacteria. In fact, it can be generalized that the differences between marine and terrestrial bacteria are largely at the species level. A cursory review of the four volumes of 'The Prokaryotes' [1] indicates that of the 535 bacterial genera representing 3058 species that have been described (genus and species numbers reported as of 1991 by Trüper [40]), there are possibly no more than seven genera that have thus far been reported exclusively from marine environments. These genera are *Oceanospirillum*, *Marinomonas*, *Planococcus*, *Listonella*, *Leucothrix*, *Photobacterium*, and *Prochloron*, the last four of which are best known for their associations with either marine algae, invertebrates, or fish, and in the case of *Listonella*, as a fish pathogen (this does not include genera inhabiting solar salterns, eg *Marinococcus*, *Salinicoccus*). To the best of our knowledge, there are no currently described bacterial taxa above the level of genus within which all of the members are strictly marine. The arguable exception would be the halophiles, eg the family Ectothiorhodospiraceae, which contains one genus *Ectothiorhodospira*, the species of which occur in marine and hypersaline environments. Before speculating as to why currently-described marine and terrestrial bacteria are largely distinguished at taxonomic levels no greater than genus, it is important to clarify that marine bacterial diversity far exceeds that encompassed by the species described to date. In addition, traditional criteria used to ascertain the indigenous habitat of bacteria obtained from the marine environment warrant discussion.

It has long been speculated that true marine bacteria may prove to be distinguishable from land forms by having a readily detectable need for sodium in the growth medium [25]. Although many marine bacteria require sodium for growth, and such information is important in describing the level of adaptation and physiological needs of a microorganism, it is ineffective from an ecological standpoint to consider as marine only those bacteria for which a specific ion requirement can be demonstrated. Restrictive views of this nature carry the potential of omitting relevant species from discussions of bacterial community ecology. All bacteria in the ocean do not have the same physiological requirements and it is therefore unreasonable to expect all marine species to require sodium for growth. Likewise, it

is misleading to conclude that bacteria isolated from marine samples are merely terrestrial contaminants based on the observation that the same species have also been reported from land. These strains may function in both environments and therefore should be considered facultatively marine unless there is evidence for metabolic inactivity or death in the marine environment. An example of such evidence would be the retrieval of thermoactinomycete spores from deep-sea marine sediments, where ambient temperatures do not achieve the minimum required for spore germination [7].

We have become accustomed to defining the habitats of plants and animals as marine or terrestrial, and in most cases these definitions are accurate. It is natural to attempt to apply these same types of habitat descriptions to bacteria, but this is probably not what should be inferred from the description of a bacterial isolate as marine. Although certain genera and species of bacteria have thus far been reported exclusively from marine environments, it is possible that some of these taxa may subsequently be found in non-marine samples. Conversely, bacteria reported to date exclusively from terrestrial environments may subsequently be found in marine samples, the point here being that it is relatively easy to conclude that an elephant occurs only in specific environments, and virtually impossible to similarly define the habitats of a microbe. Even species that require sodium or increased hydrostatic pressure for growth may subsequently be found in non-marine, high salt or deep-subsurface samples, respectively. For these reasons, the exercise of defining the habitats of bacterial taxa does not carry with it the assumption that these organisms are exclusive to those habitats. Of greater relevance is the use of the terms marine and terrestrial (and those interfacing the two, eg intertidal, estuarine) to define the habitat from which a particular organism was obtained from nature. Microbes isolated from a particular habitat can then be studied to determine if they are metabolically active in that environment and to what extent these activities have ecological significance. Metabolic activity implies adaptation to ambient environmental conditions, and we believe that the virtually unlimited number of marine microenvironments colonized by bacteria provide innumerable opportunities for bacterial adaptation. It is possible that some adaptations would not have been selected for in the absence of specific marine environmental pressures, and that these adaptations can include the selection for biosynthetic pathways leading to new metabolic products. Thus, bacteria expressing adaptations to diverse marine environments represent a potentially productive aspect of future chemical studies of marine bacteria.

This is not to imply that bacterial taxonomic diversity is not of fundamental importance to the search for new secondary metabolites (see review [5]). As already mentioned, the current taxonomic differences between marine and terrestrial bacteria are largely at the species level, and these differences, although not great, are certainly of biosynthetic significance. It also must be considered that our discussion has been restricted to currently-described taxa and there is now clear evidence that marine prokaryotic diversity far exceeds that reflected by the species described to date. This evidence comes largely from analyses of community bac-

terio plankton small subunit rRNA [15], the results of which indicate the presence of broadly diverse microbial assemblages with the common presence of heretofore phylogenetically-undescribed groups [14]. Since it has been estimated that the true number of bacterial species may be between 2 and 3 million [40], it is likely that many of these previously undescribed sequences represent new taxa. Without question, gaining insight into the culture requirements of these microorganisms is an important component of future chemical studies of marine bacteria. Obtaining these microorganisms in culture will also provide type specimens that can be categorized and assigned genus and species names using the binomial nomenclature that has proven so effective as a method of cataloguing and discussing life on earth. New taxa may also be created as phylogenetic information is used to revise bacterial systematics. This was recently suggested by Kita-Tsukamoto *et al* [22] for certain marine Vibrionaceae and *Pseudomonas* species that appear, based on rRNA sequence data, to be sufficiently distinct from terrestrial species to warrant separate grouping. In the future, as our ability to apply the species concept to bacteria becomes better refined [6], it is possible that taxa above the level of genus will be described and that these taxa will reside exclusively in marine environments.

Given our current understanding of marine bacteria, why is it that we often encounter the same genera of bacteria on land (including fresh-water habitats) and in the sea, but this phenomenon is much less common with higher organisms? One possible explanation is the inherent and relatively rapid ability of prokaryotes to adapt to diverse environmental conditions. Prokaryotes are unrivaled in their ability to perform diverse metabolic processes [10], and this diversity provides a useful survival strategy by affording bacteria the opportunity not only to exploit a variety of resources for carbon and energy but to do so within a range of environmental conditions. The ability to adapt to diverse environmental conditions may explain why some prokaryotic taxa are unwilling to recognize the strict marine/terrestrial interface to which most higher life forms adhere. That species found within the sea often belong to genera that also occur on land may be the result of a combination of interacting factors. First, the transmission of bacteria from land to sea far exceeds movement in the reverse direction and as a result terrestrial species have frequent opportunities to adapt to marine conditions. Second, bacteria have short generation times and it is possible that new species evolve relatively rapidly following introduction to land or sea. These new species may not remain exclusive to either the marine or terrestrial environment, i.e. remain genetically distinct, for periods of time sufficient for the evolution of taxa above the level of genus or species. In other words, bacterial metabolic diversity, short generation times, survival adaptations, dispersal methods, and genetic exchange may, in combination, enable new species and even genera to evolve, but reduce the chances of specific marine populations being exclusive to the marine environment for periods of time sufficient for the divergence of taxonomic groups above the level of genus. The likelihood of higher taxonomic groups evolving however, may be increased for bacteria that have developed highly special-

ized associations with marine plants and animals. Symbiosis will be discussed in more detail later, however we would like to point out again that four of the seven genera that thus far appear to reside exclusively in marine environments form well developed associations with higher marine organisms.

Based on the observation that the taxonomic differences between bacteria inhabiting land and sea are not as great as those between marine and terrestrial plants and animals, it is logical to expect that differences in the secondary chemistry of marine and terrestrial bacteria will also not be of an equal magnitude. It is not uncommon, nor should it be unexpected, to isolate from marine bacteria compounds previously described from non-marine microorganisms. It is also not surprising that some of the novel compounds discovered from marine bacteria are of the same structural types as compounds produced by terrestrial isolates. Given that the genera of marine bacteria from which novel metabolites have been reported include *Pseudomonas* [29], *Alteromonas* [34], *Vibrio* [30], *Bacillus* [39], and *Streptomyces* [36], all of which encompass species common to non-marine environments, a relatively high rate of known compound isolation should be expected. Taxonomic similarities suggest that certain marine and terrestrial isolates share similar metabolic processes including biosynthetic capabilities. We expect that the rate at which known compounds are isolated from marine bacteria is a function of: 1) the degree to which close taxonomic relatives of the producing organism have been studied; 2) the fermentation conditions (eg traditional media vs marine-based media); and 3) how adaptations to life in the sea affect secondary metabolic pathways. Following this line of reasoning, it would be advantageous to target for chemical study taxa that have only been reported from marine environments or that have not traditionally been a focus of study. It would also be beneficial to work with organisms that are clearly adapted to life in the sea, for example, those that require seawater for growth or those that have well-established associations with marine plants and animals. The rational design of marine-based isolation and fermentation media is also an important and often overlooked variable affecting the diversity of bacteria obtained in culture and the likelihood that cultured microorganisms will express their true biosynthetic capability.

To assess the biosynthetic potential of marine bacteria, we should consider in more detail how adaptations to life in the sea affect secondary metabolite production. One group for which this question can be addressed is the actinomycetes. We know that actinomycetes show a high degree of halotolerance [32,38], can be readily isolated from shallow-water marine sediments (although the rate of occurrence does not rival that of most terrestrial soils), and that marine species have been described [18]. Some marine-derived actinomycetes are capable of growth in the absence of seawater, and therefore similar strains may occur in non-marine environments. Given that ca 40% of all described antibiotics (as summarized by Berdy [3]) come from non-marine actinomycetes, it could be predicted that the study of marine actinomycetes would lead to a relatively high rate of known compound isolation. This has been our experience. However, novel compounds are being reported

at an increasing frequency from marine actinomycetes, including common soil genera such as *Streptomyces*. So we can now ask the following questions: Are common soil actinomycete taxa, when isolated from marine samples, metabolically active in the marine environment and is this activity the result of specific adaptations to the sea? And if so, do these adaptations increase the likelihood that secondary metabolites produced by these bacteria will be new to science?

Although questions of this nature are difficult to answer experimentally, a number of recent studies provide some insight. Probably the most significant new information was recently provided by Moran *et al* [28] who showed evidence that *Streptomyces* isolated from marine sediments were actively growing when collected. This contrasts previous suggestions that actinomycetes exist in marine environments largely as metabolically inactive spores and that only under certain exceptional conditions do these spores germinate and grow [16]. Other evidence suggesting that actinomycetes are in fact metabolically active in the marine environment comes from distributional studies in which certain taxonomic groups of actinomycetes were recovered in higher numbers as water depths increased and members of these groups failed to grow when seawater was replaced with deionized water in the culture medium [19]. In terms of how adaptations to life in the sea affect actinomycete secondary chemistry, of four isolates that produced novel metabolites in our laboratory, all four grew to some extent when seawater was replaced with deionized water in a complex fermentation medium; however, only one of the four produced the metabolite of interest in the absence of seawater. This result suggests that in at least three of four cases some component of seawater was required for metabolite production and that adaptations to the sea, which imply growth in the sea, may lead to the production of secondary metabolites that would not be encountered in the absence of certain marine environmental parameters.

If environmental adaptations affect secondary metabolite production, then it is logical to build a microbial library based on bacteria obtained from diverse marine environments. We believe that bacteria associated with the surfaces, tissues, and internal spaces of marine plants and animals experience diverse, uniquely marine microenvironments and therefore have tremendous potential as a source of novel secondary metabolites. Some of the environmental variables experienced by these bacteria include pH, grazing, nutrient availability, and surface texture and wettability. For example, algal tissues can be basic due to a photosynthetically induced shift in the inorganic carbon equilibrium [4] and can be assumed oxygen-rich due to oxygenic photosynthesis. Conversely, the tunic fluids of certain marine ascidians are known to be highly acidic (pH 0–2, [35]) and can be assumed relatively oxygen-poor due to oxidative respiration. Additional host-associated variables include the release of biologically-active substances from marine sponges [37] and chemoattractive photosynthetic products from marine algae [2]. The numerous physical, chemical, and biological factors that can vary both between and within individual plant and animal species contribute to the creation of innumerable, highly specific, marine microen-

vironments. These microenvironments select for microbial populations that can successfully compete within a given set of environmental parameters. As bacteria become more highly specialized to utilize the resources associated with a specific host microenvironment, the bacterial-host association becomes more complex and it can be proposed that the likelihood of finding microbes expressing similar adaptations outside of this association is reduced.

The establishment of associations between bacteria and marine plants and animals is facilitated by the fundamental differences in bacterial dispersal in the sea versus on land. Because bacteria occur in seawater at concentrations of approximately one million cells per milliliter, marine plants and animals are constantly exposed to extraordinarily high concentrations of bacteria relative to their terrestrial counterparts. Many of these bacteria are motile, chemotactic, opportunistically pathogenic, and readily attach when provided an appropriate surface. We know very little about how marine organisms respond to these bacteria and how bacteria respond to the physiochemical environment of a potential host, however bacterial responses to host chemical cues appear to explain some of the epibacterial distributions observed in nature [41]. Considering the number of plant and animal species that inhabit the world's oceans, coupled with their diverse physiologies, physical and chemical peculiarities, and interactions with each other and the environment, the potential numbers and types of bacterial-host associations are virtually unlimited.

Although the bacteria associated with most marine plants and animals have not been described, there is mounting evidence that many of these populations are distinct. In addition to the more obvious symbioses, eg that between *Prochloron* and certain didemnid ascidians, there is evidence that the bacteria associated with marine algae [24] and sponges [42] are distinct from those in the surrounding seawater. Given sufficient scrutiny, it may become evident that all marine organisms possess specific bacterial associates. Many of these associations undoubtedly represent what could be considered a snapshot in time of what begins as a casual interaction and in some cases develops into a relationship of such interdependence that it is required for the survival of both host and symbiont. This range, from casual interaction to obligate mutualistic symbiosis, is accompanied by a corresponding spectrum of bacterial/host adaptation, and these adaptations may include the selection for and maintenance of unusual secondary metabolites.

That symbiosis is an important agent of evolutionary innovation has been discussed in detail [26], and is supported by the high levels of adaptation displayed by both bacterial symbionts and their hosts. Solid evidence that the associations between marine animals and bacteria foster speciation comes from studies of the chemoautotrophic, sulfur-oxidizing bacterial endosymbionts best known from deep-sea hydrothermal vents. These bacteria form a distinct phylogenetic group and appear to be unique to and invariant within the host species [9]. The prediction that symbionts co-speciate with their hosts is further supported by phylogenetic studies of the bioluminescent symbiont (*Vibrio* spp) of flashlight fishes (family Anomalopidae [17]). Evidence of symbiont co-speciation with the host supports the belief that the study of bacteria from diverse marine plants and

animals would lead not only to strains expressing unusual environmental adaptations but also to taxa that would not be obtained from other marine sources. The observation that four of seven bacterial genera reported exclusively from marine environments form associations with marine plants and animals supports this view.

Since we are only beginning to unravel the true extent of prokaryotic diversity, it is easy to conclude that the chemical potential of marine bacteria has not been effectively assessed. This is likely the case, however it must be considered that adequate comparisons between cultured and characterized microorganisms have not yet been made. For example, we routinely isolate marine prokaryotes from diverse microniches including the surfaces and internal tissues of marine plants and animals. The growth of some of these microorganisms is inhibited by traditional media components such as yeast extract and peptone [21]. Given that these nutrients have been a traditional component of marine bacteriological media (eg Marine Agar, Difco Labs, Detroit, MI, USA), sensitive strains may include species that have not previously been characterized. These microorganisms need to be classified and sequenced so that effective comparisons can be made between culturable microorganisms and those that can be detected in nature using molecular techniques. Until this gap is bridged, it will remain difficult to assess the thoroughness of efforts aimed at evaluating the industrial potential of marine prokaryotes. None-the-less, chemical studies of marine bacteria are proving productive, and as our understanding of the habitats and culture requirements of these microorganisms improves, so will their industrial potential.

Acknowledgements

We thank M. Haygood and two anonymous reviewers for their critique of this manuscript.

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