Marine actinobacteria: new opportunities for natural product search and discovery

Alan T. Bull¹ and James E.M. Stach²

¹Department of Biosciences, University of Kent, Canterbury, Kent CT2 7NJ, UK
²Institute for Research on Environment and Sustainability, University of Newcastle, Newcastle upon Tyne, NE1 7RU, UK

It is widely accepted that new drugs, especially antibiotics, are urgently required, and that the most promising source remains natural products. We argue that in exploring new sources of bioactive natural products the marine environment warrants particular attention, in view of the remarkable diversity of microorganisms and metabolic products. Recent reports of new chemical entities and first-in-class drug candidates, and confirmation of indigenous marine actinobacteria, make exciting discoveries even more likely given the unreviewed capacity of this class of bacteria to produce exploitable natural products.

The rationale for marine biodiscovery

This review embodies three essential elements: natural products; one of the largest classes of bacteria, the Actinobacteria; and the marine environment, by far the largest environment on the planet. The topics of marine actinobacteria, and their capacity for natural product synthesis, have been reviewed several times in the past few years [1–6] and it is not our intention to revisit much of this material; rather we will update certain topics and focus on ways and means of enhancing the success of biodiscovery in this group of bacteria. Much has been written about natural product search and discovery but the subject has been bedevilled in recent years by several misconceptions that have combined to reduce research interest and commercial investment (Box 1, Box 2).

The notion that the oceans are home to huge microbial populations and diversity is indisputable [7,8], and recent research has confirmed the existence of bona fide marine actinomycetes [4]. This latter point is pivotal for targeting these bacteria for marine natural product discovery programmes. Moreover, if at best only one part in 10¹² of the terrestrial surface of the earth has been screened for actinomycetes [9], imagine the potential rewards of the treasure house represented by the oceans! As the pace of research and development in marine microbiology quickens, increasing numbers of novel actinobacterial metabolites are being described [4,5,10,11], several of which are currently in clinical trials [3]; some, such as salinosporamide A, have advanced to phase 1 trials in less than 3 years after discovery [4]. It can be argued that because of the great diluting effect of seawater marine-derived bioactive compounds have evolved great potency, a fact that might severely reduce the opportunities for their use as drugs. In this context a strategy of low-dosage synergy screening could provide a means of exploiting toxic natural products. A recent report of antifungal chemotherapy illustrates the potential of such an approach [12]. We opine, therefore, that investment in marine actinobacterial natural products is entirely warranted, and the remainder of this review is devoted to reviewing what is known of marine actinobacteria and suggesting ways and means for realizing their biodiscovery potential.

Marine actinobacteria research

Marine microbiology is developing strongly in several countries with a distinct focus on bioactive compounds. Analysis of the geographical origins of compounds, extracts, bioactivities and actinobacteria up to 2003 indicate that ~67% of marine natural products were sourced from Australia, the Caribbean, Indian Ocean, Japan, the Mediterranean, and Western Pacific Ocean sites [10]. Interestingly the latest data (for 2004 [10]) reveal a dramatic rise in reports pertaining to the China Sea, reflecting growing international attention on marine natural products.

The old impression that the diversity of actinobacteria in the oceans was small and restricted has been completely dispelled by 16S phylogenetic diversity inventories and estimates, and cultivation approaches. Thus, deep-sea sediments were found to contain >1300 different actinobacterial operational taxonomic units, a great proportion of which are predicted to represent novel species and genera [8]. Complementing this strategy are intelligent approaches to sample handling and growth conditions, which have led to the recovery in culture of many new taxa. Among newly described marine genera are: Salinispora (Micromonosporineae) [13], Demequina (Micrococcinae) [14], and others awaiting formal taxonomic description: Marinispora [15], Solvaraspora [16] and Lamerjespora [17]. New species of known actinobacterial genera are being described on a regular basis [18–22]; the only serious limitation in this endeavour is the availability of qualified taxonomists.

Several issues merit reiterating and updating from the perspective of effective biodiscovery. First, the question of whether to sample shallow or deep marine sites, associated...
Box 1. Some misconceptions regarding natural product discovery

- The search for natural products is simply stamp collecting – wrong! Natural products, including drugs, are known unequivocally to occupy a larger and different chemical space than combinatorial chemicals [69]. Completely novel chemical skeletons continue to be discovered among natural products, supporting the claim that nature is the more innovative chemist: after all, microorganisms have been creating new chemical structures for at least 3.5 billion years. The term ‘natural product’ embraces a much wider range of entities than drugs, for example biocatalysts, biomaterials, nutriceuticals and biocontrol agents, but for the purposes of this review we concentrate on anti-disease and other biopharmaceutical compounds.

- Microbial systematics is yet more stamp collecting – wrong! Although the novel organism–NCE maxim is a major guide to our search and discovery activities, the anticipated returns from even reasonably well-circumscribed groups of bacteria should not be underestimated (see Box 2), particularly in the light of new search technologies. More than 900 compounds from marine microorganisms, including microorganisms, were described in 2005, the latest year for which collated information on marine natural products was reported; see the MarinLit database of the University of Canterbury (www.chem.canterbury.ac.nz/marinlit/marinlit.shtml) [10].

- Synthetic chemicals provide drug candidates superior to those of natural products – wrong! Synthetic and natural compounds violate Lipinski’s rule-of-five for druggability [1,70]. Moreover, the promise of combinatorial chemistry (including diversity-oriented synthesis) as a superior source of drug leads has not yet materialized.

- Isolation and characterization of natural product leads is laborious and slow – wrong! The availability of methods for the deconvolution of complex mixtures, for structural determination and for sensitive bioassays has largely overcome this potential log-jam in natural product research, the case being well illustrated by lyngbyatoxin [71].

Box 2. Are actinobacterial natural products exhausted?

The scale of microbial natural product diversity can be gauged from the following facts regarding bacteria:

- Currently ~80 classes of Bacteria have been defined although in only a minority of cases (~33%) have representative members been brought into laboratory culture.

- The class Actinobacteria is especially notable for containing organisms producing diverse natural products, with members of the order Actinomycetales alone accounting for ~10 000 such products.

- The genus Streptomyces produces ~70–80% of currently characterized actinomycete natural products whereas the intensively studied species S. coelicolor has been shown to contain >20 clusters of potential natural product genes. Overall the natural product diversity of this genus has been predicted to exceed 10⁵ [72].

- The metabolic versatility of Streptomyces could be mirrored by other actinomycete taxa as genome sequencing and metabolite profiling efforts intensify. For example, genome scanning (see below) of the deep-sea actinomycete Verrucosispora maris has produced an estimate of ~20 biosynthetic gene clusters in this organism [18] (I.E.M.S., unpublished).

- Although the magnitude of the oceans and their component ecosystems and habitats is widely appreciated by microbiologists [2], serious exploration of their biology, especially in the deep seas, has been possible only after innovations in marine engineering and developments in manned and robotic submersibles. The extent of ocean biodiscovery activities is detailed in the latest review of marine natural products (to December 2004 [10]), and most oceanic ecoregions have been sampled, albeit generally in a piecemeal fashion. Whereas reports of novel natural products from terrestrial microorganisms have remained almost unchanged over the past two decades, those from marine microorganisms have increased linearly in the same period [73]. Current marine biodiscovery is centred largely on anticancer and antibiotic drugs, but antiviral, anti-inflammatory, immunomodulatory and agricultural targets also are being prioritized [10].

Deep ocean convective mixing and, thereby, nutrient availability, thus drawing attention to the importance of temporal sampling. In an important paper Bouvier and del Giorgio [30] reported the potential effect of viruses on marine bacterioplankton composition and abundance. When these communities were incubated in virus-free ambient seawater ‘unexpected and dramatic increase in the relative abundance of bacterial groups that are generally undetectable in the in situ assemblages’ occurred; in open ocean regions the proportions of actinobacteria rose from zero to 35% of the bacterioplankton.

Finally, there is the question of marine actinobacterial biogeography [2]. In general a large body of research supports the hypothesis of biogeographical distribution of free-living microorganisms and that such spatial variation reflects the proposition that ‘the environment selects’ [31]. Hughes et al. [32] provide an excellent analysis of the contributions of environmental and historical effects and a framework for experimental testing of microbial biogeography; they argue that such studies should involve systematic sampling and recording of data from various distances, habitats and environmental factors. Such crucial data for free-living marine actinobacteria are lacking, but a study of bacterial symbionts in the sponge Cymbastela concentrica in tropical and temperate locations of Australia [33] showed that symbiotic communities had similar compositions at temperate locations, but different communities (which included actinobacteria) occurred in
the temperate and tropical sponge populations, which might reflect endemic distribution or environmental selection, or both. The biogeography debate is not simply of academic interest because a greater understanding of distribution patterns has clear importance for biodiscovery. For example, although the three currently known species of Salinispora co-occur at six widely separated and distinct locations [34], only strains of S. tropica isolated from the Caribbean produce the potent anticancer compound salinosporamide A [35].

In conclusion, progress in isolating actinobacteria from the seas and oceans, especially from sediments, has been notable in the past few years. Figure 1 reveals the diversity of marine-derived actinomycetes isolated at the Scripps Institution of Oceanography [4], and work in our laboratories has extended the number of actinobacterial families containing members isolated from marine environments from six to fifteen [28,13]; see J.P. Euzéby (www.bacterio.cict.fr/classification.html) for the current list of actinobacterial families with standing in nomenclature.

Maximizing opportunities for biodiscovery
The success or otherwise of isolating novel actinobacteria from natural environments is dependent in large measure upon taking a holistic view of the particular environment in question; for example, what are the probable physiologies of the resident organisms, and what are their relationships with other members of the community? We examine some of these issues in this section.

Ecophysiology
The ascendancy of phylogenetic and metagenomic contributions to biodiscovery notwithstanding, organism isolation and cultivation remain essential goals. The work of Peter Janssen’s group, albeit on soil bacteria, signals much thought for the marine actinobacteriologist. Janssen opines that given the advances made in culturing soil bacteria in recent years, ‘it is probably incorrect to speak of the majority of bacterial species in soil as being unculturable’ [36]; stemming from this optimism, however, is the rejoinder that innovation, ecological insight and, especially, patience are the prerequisites for success. The dispersion-differential centrifugation pretreatment procedure exemplifies this philosophy and, for example, has yielded fivefold increases in actinobacteria isolated from fjord sediment [13].

Three cultivation approaches merit particular attention: dilution to extinction of environmental samples; low nutrient concentrations; and virus-depleted incubation conditions. A high-throughput system based on microencapsulation of single cells [37] offers special advantages...
for recovering slow-growing organisms. Moreover, the encapsulated cells can be perfused with media that simulate in situ composition (e.g. seawater, sediment or host organism extracts). In a recent study [38] encapsulated sponge symbionts were perfused with sterile sponge or sediment extracts for up to 5 weeks, and of the bacteria isolated, 42% were novel compared with 7% obtained by plating samples directly onto marine agar. It is claimed that as many as 10⁴ isolates per sample can be recovered using this method [37]. Dilution procedures involving serial pressing of dried sediment onto agar media or liquid dilution in sterile seawater were used by Gontang et al. [26] to assess the diversity of Gram-positive bacteria in marine sediments. Approximately 65% of representative isolates were found to be actinobacteria, a significant proportion (23%) of which represented new phylotypes. A notable feature of this study was the extended incubation times allowed to enable slow-growing bacteria to be detected. Although there seems to be no definitive evidence for oligotrophic marine actinobacteria (sensu Button [39]), compelling grounds for the use of media containing low concentrations and wide ranges and combinations of substrates inoculated with greatly diluted environmental samples are being established for the isolation of novel bacteria including actinobacteria [26,40,41]. Such strategies have resulted in considerable (23%) cultivation efficiencies from subsurface coastal sediments [41]. Finally, the effects of viral infection on bacterioplankton composition present additional opportunities for culturing members of supposed ‘rare’ planktonic groups such as actinobacteria. The suggestion coming from this research [30] that viral regulation might be operating at broad taxonomic rather than at individual strain levels should encourage efforts to exploit this phenomenon as a means of enhancing the isolation of planktonic actinobacteria.

Indigenicity

The specific issue of indigenous deep-sea actinobacteria warrants some consideration because, if we can define some or all of the features of deep-sea actinobacterial physiology, this should lead to greater efficacy of isolation. Although an obligate requirement for Na⁺, and either obligate requirements or tolerance of oligotrophic substrate concentrations, low temperatures and elevated pressures for growth would provide prima facie evidence of indigenicity, to our knowledge no systematic testing of this hypothesis with respect to deep-sea actinobacteria has been made. In addition, demonstration of growth or metabolic activity in situ should be established. We believe that physiological understanding of this type could inform more precise ecosimulation or microcosm approaches to targeting the recovery of a greater diversity of deep-sea actinobacteria. In passing, it would be interesting to screen oligotrophic marine actinobacteria (if they exist!) for natural products given the reported disparity between the genome sizes of bacterioplankton (1.9 Mb [39]) and secondary metabolite-producing actinomycetes (~8 Mb) (see Box 3).

Symbioses

The association of bacteria with marine sponges, bryozoans, tunicates and holothurians has long been known, and sponge systems have attracted much attention [42–44]. Interest in such animals has been excited by their diversity of bioactive products, which most probably are secondary metabolites of their bacterial partners. Actinobacteria are frequent components of these symbiotic communities, and because of their pedigree as natural product sources they are increasingly targeted in biodiscovery programmes. Actinobacteria are found in reef and deep-water sponges, and evidence for sponge-specific symbioses exists [43]. In at least one case actinobacterial symbionts such as species of Micromonospora have been shown to produce bioactive compounds (manzamines) that have no known structural homologues in terrestrial actinobacteria. Genomic data from marine actinobacteria have significant implications for biodiscovery, and in resolving such questions as: does Salinispora reflect other filamentous marine actinobacteria in terms of their biosynthetic potential? Do marine bacteria produce compounds that are not found in terrestrial relatives? Is the dilution factor responsible for the apparent absence of secondary metabolite biosynthetic genes in pelagic actinobacteria, and the great activity and specificity in species isolated from marine sediments? Are there specific marine genetic markers that will aid in the definition of environments that harbour obligate marine actinobacteria? Clarification of these issues will guide future rational search and discovery programmes.

Box 3. Genome sequencing of marine actinomycetes

The first complete genome of a marine actinomycete, Salinispora tropica CNB-440, released in April 2007 on the website of the Joint Genome Institute (http://genome.jgi-psf.org/finished_microbes/saltr/saltr.download.html), is 5.2 Mbp in size, ~3 Mbp smaller than those of terrestrial Streptomyces species. A further strain of S. tropica (ATCC BAA-916T) and two strains of Salinispora arenicola (CNS205 and ATCC BAA-917) are currently being sequenced. Genome sequencing of Agreia sp. PHSC20c1 in progress, the strain having been isolated from Antarctic surface waters on oligotrophic media. The release of genomes from marine actinobacteria will enable comparative genomics studies to address questions concerning the evolution and ecology of marine actinobacteria. For instance, did marine actinobacteria evolve (through gene loss) from terrestrial counterparts, or vice versa (gene acquisition), or is there a genetic continuum between deep-sea, coastal and terrestrial species? What are the specific genotypes that lead to the marine obligate phenotype? Gram-negative marine bacteria are known to have membrane transport proteins with obligate requirements for sodium, and it is likely that evolutionary pressure (sodium ions are ~25 times more abundant than potassium ions in seawater) is responsible for this sodium specificity in these substrate-importing permeases. Is there evidence for evolutionary selection of processes that use sodium in favour of other cations? These permeases bring substrates into the cell and they are not functional when sodium is replaced by potassium (whereas sodium is not obligately required for permease transport in terrestrial strains). Thus, evolution has probably led to transporter, and perhaps other, proteins having a sodium dependence. Salinispora has proven ability to produce secondary metabolites (a survey of isolates identified over 90 that produced anticancer compounds). To date 15 secondary metabolite gene clusters have been identified in S. tropica, including the biosynthetic gene cluster for salinosporamide A, a potent anticancer agent. Analysis of the S. tropica genome reveals the presence of type I and II polyketide synthases (PKS), nonribosomal peptide synthetases (NRPS) and mixed PKS and NRPS biosynthetic gene clusters; ~10% of the genome is devoted to secondary metabolite biosynthesis [74], a proportion double that of Streptomyces coelicolor.

The genome of Agreia sp. PHSC20c1 (2.8 Mbp) is not completely sequenced, but there is scant evidence of secondary metabolite biosynthetic gene clusters (cf. above [41]). This strain is involved in the mineralization of recalcitrant carbon compounds. Genomic data from marine actinobacteria have significant implications for biodiscovery, and in resolving such questions as: does Salinispora reflect other filamentous marine actinobacteria in terms of their biosynthetic potential? Do marine bacteria produce compounds that are not found in terrestrial relatives? Is the dilution factor responsible for the apparent absence of secondary metabolite biosynthetic genes in pelagic actinobacteria, and the great activity and specificity in species isolated from marine sediments? Are there specific marine genetic markers that will aid in the definition of environments that harbour obligate marine actinobacteria? Clarification of these issues will guide future rational search and discovery programmes.

www.sciencedirect.com
terrestrial equivalents [45]. Of considerable interest is the reported isolation of Salinispora strains (only known previously from marine sediments) from the Great Barrier Reef sponge Pseudoceratina clavata and their activity against other bacterial symbionts [46]. These Salinispora isolates possess a polyketide synthase (PKS) gene that is most closely related to the rifamycin B synthase of Amycolatopsis [47], and hence might provide a novel marine source of this antibiotic. Based on the greatly conserved but nevertheless distinctive PKS genes for rifamycin found in these two actinomycete genera, the authors consider the system to be a propitious one for recombinant antibiotic discovery. Reports of actinobacterial symbionts of sponges have also appeared from Chinese groups. Sponges in the South China Sea harbour a large diversity of actinobacteria and show evidence of host specificity [48]. The greatest actinobacterial diversity was found in Cranilla australiensis, with many of the strains having broad-spectrum antibacterial activities [49]. Similarly actinobacteria associated with the Yellow Sea sponge Hymeniacidon perleve showed broad taxonomic diversity, and included Actinoalloteichus, Micromonospora, Nocardia, Nocardiopsis, Pseudonocardia, Rhodococcus and Streptomyces [50]; the value of deploying a wide range of isolation media was again emphasized by this study. Rather less research has been focused on coral-associated actinobacteria but two recent reports have alerted our interest. First, a culture-independent study of the recently discovered deep-water Mediterranean corals revealed several abundant bacterial phylotypes, one of which was the Actinobacteria [51]. And second, a culture-based study of the symbionts of Fungia scutaria [52], a Red Sea species, revealed a large proportion (23%) of actinobacteria in the mucus layer. Although the cultivation efficiency was low, this is the first account of actinobacteria being isolated from corals. Continued isolation and screening of coral-associated actinobacteria seems entirely warranted given the early success in discovering valuable bioactive compounds, such as thicoraline [53].

Technology change

In this section, we discuss how developments in a number of fields are enhancing scientists’ ability to find novel natural products in marine actinobacteria.

Bioinformatics

Bioinformatics and its component ‘-omics’ elements has created a paradigm shift in our approach to natural product discovery. Much of the relevant information is contained in Ref. [54] so here we refer only to recent developments that have implications for marine actinobacteria. Taxonomic databases could provide predictive road maps to chemical diversity, and there is some support for such a relationship at coarse (order Actinomycetales), intermediate (family, e.g. Streptomycetaceae) and fine (genus, e.g. the Streptomyces violaceusniger clade) taxonomic ranks within the Actinobacteria; all members of the latter clade produce eliaophilin, geldanamycin, nigericin and a polyene. In some microbial groups the relationship between taxonomy and the ability to synthesize particular types of natural product is stringent (e.g. in terverticillate penicillia [55]). Although such patterns have not been demonstrated unequivocally in marine actinobacteria, pursuit of the relationship is encouraged by recent chemo-diversity analyses of the genus Salinispora [4], which returns us to the need for further charting of marine actinobacterial phylogenetic or taxonomic space. Display of 16S rDNA phylogenetic distances in three-dimensional (3D) principal coordinate space illustrates dramatically the extensive regions of unexplored actinobacterial taxon-my [2,6]. Recently discovered marine taxa ‘Marinospora’, ‘Verrucosicapsa maris’ and alkali-tolerant Streptomyces occupy distinct new regions of phylogenetic space [6] and synthesize exciting new chemical entities (NCEs). The prospect of massive sequencing of 16S rDNA and other diagnostic genes (e.g. by means of the 454 pyrosequencing platform [56]) will enable a more representative inventory of marine actinobacteria to be achieved. Such capacity is crucial for discovering low-abundance marine organisms, including actinobacteria. Elegant support for this approach has come from the work of Sogin et al. [7].

Metabolite profiling

Another issue is how to maximize gene expression to discover novel metabolites. It is clear that a more systematic, eco-driven study of natural product synthesis by marine actinobacteria is necessary. In the past we, and others, have successfully used high pressure liquid chromatography with diode array detection (HPLC-DAD) screening for metabolite profiling [11], but profiling technologies with greater resolving power are needed for interrogating microbial metabolomes. Profiling technologies of choice include mass spectrometry and nuclear magnetic resonance (NMR), the latter providing greater structural information. An important advance in the detection of novel chemotypes has come recently from differential analysis of two-dimensional (2D) NMR spectra arrays [57]. This procedure when tested against a small library of extracts prepared from an entomopathogenic fungus revealed two novel compounds and, as expected, the composition of the metabolome was influenced significantly by growth conditions.

Genomics

The application of genomic technologies to NCE discovery in actinobacteria has done much to dispel the notion that these organisms are exhausted as producers of novel exploitable compounds. The genomic sequencing of Streptomyces coelicolor and S. avermitilis [58,59] revealed that both harbour over 20 biosynthetic gene clusters for secondary metabolites; before sequencing, these species were known to produce four and three secondary metabolites respectively. Thus, the first obvious area in which genomics will accelerate discovery is the identification of cryptic (or orphan) biosynthetic gene clusters that are phenotypically silent but capable of activation by nonstandard fermentation or genetic manipulation, or both (see below). The realization that the majority of streptomycete biosynthetic gene clusters are cryptic has initiated the field of genome mining [60]. The utility of genome scanning can be clearly demonstrated by comparison with fermentation studies: when 60 actinomycete species were screened by traditional fermentation procedures, 65 natural products...
were detected, whereas genome scanning of the same species revealed ~700 natural product biosynthetic gene clusters including the biosynthetic genes responsible for the production of the 65 compounds revealed by fermentation [61].

The introduction of robotic platforms and automated screening provides technology complementary to genome mining. Automated colony picking and isolation coupled to high-throughput genomic dereplication will enable large libraries of taxonomically unique actinomycetes to be produced. Such libraries when combined with metadata (environment descriptions, taxonomy, screening hits, genome scanning) can promote rationally guided biodiscovery. The application of microarray technology enables rapid screening of tens of thousands of strains for genes of interest. Library-on-a-slide methods [62] generate sublibraries of strains with the desired genotype before fermentation screening. In our laboratories, we screened a library of marine and terrestrial actinomycetes for the presence of enediyne-like iterative type I PKS genes using a library-on-a-slide format; ~15% of the strains were positive, and PCR and sequencing enabled the identification of novel and known PKS genes (Allenby, N.E.E. et al. (2006), unpublished). Thus, only strains that have the genetic potential to produce the compounds of interest and are likely to produce chemical novelty, are taken forwards to fermentation. Library-on-a-slide screening could, to a certain degree, obviate the need for strain improvement programmes: strains with natural products homologous to the candidate can be rapidly identified, including any that have more desirable properties in terms of yield, cost of fermentation, activity, pharmacology and toxicology. Initial data on genome sequencing of marine actinomycetes is presented in Box 3.

Identification of cryptic biosynthetic gene clusters greatly enhances the ability to isolate their products. Several techniques can be employed, including physicochemically informed (PCI) screening of multiple fermentations for compounds that match the predicted product of the gene clusters; gene knockouts and comparative metabolic profiling; heterologous expression; in vitro reconstitution; and genomisotopic approaches [63]. An example of PCI isolation is the discovery of the novel antibacterial compound ECO-501. The genome of Amycolatopsis orientalis ATCC 43491 was mined by genome scanning [64] and its vancomycin biosynthetic gene cluster was detected along with 12 additional clusters, one of which was predicted to synthesize a novel glycosidic polyketide. Structural prediction of the putative polyketide enabled the physicochemical properties (mass, polarity and ultraviolet spectrum) to guide purification of the polyketide. Heterologous expression has been achieved of entire biosynthetic gene clusters from actinomycetes where the product is known [65], and it is likely that heterologous expression of cryptic gene clusters will have been achieved by the time this review goes to press. Heterologous expression potentially circumvents the need to conduct numerous fermentations to isolate the compound of interest. Gross et al. [66] predicted the substrate specificity of a nonribosomal peptide synthetase (NRPS) thought to be involved in the production of a novel leucine-containing lipopeptide. Feeding [15N]leucine to the fermentation combined with 1H-15N HMBC NMR (heteronuclear multiple-bond correlation nuclear magnetic resonance) guided the purification of the lipopeptide. This approach can be limited by the ability to predict substrate specificity (less easy in modular PKSs) but such genomisotopic methods could aid the identification of cryptic gene products by feeding labelled compounds that are implicated in the final ‘tailoring’ of natural products [63].

**Box 4. Technical issues affecting marine biodiscovery**

**Biodiversity**
- Large amplicon libraries are needed to detect low-abundance (‘rare’) actinobacteria.
- Common phylotypes of soil actinobacteria belong to the Actinobacteridae, Acidimicrobidae and Rubrobacteridae subclasses [36]. Is the taxonomic distribution of actinobacteria in marine environments similar, or are there unique marine actinobacterial signatures?
- Isolates from a breadth of taxonomic ranks are needed to assess the genetic and chemical diversity of marine actinobacteria.
- Indigeneity mechanisms need to be characterized from proteome and genome analyses.

**Culture-dependent, culture-independent relationships**
- Recovery of uncultured phylotypes needs innovative microbiology, such as high-throughput procedures and the isolation of marine communities, in addition to pure cultures and a greater understanding of physiology.
- Use of inadequate primer sequences and biases associated with phylotyping can lead to major underestimation of marine actinobacterial diversity [8, 26, 75].

**Screening**
- Discovery of first-in-class drug candidates such as abyssomicin C [18] will be greatly stimulated by but not solely driven by genomics.
- In silico chemoinformatics is promising for the generation of new bioactive compounds. The occurrence of novel PKS genes in marine actinobacteria [47], for example, is noteworthy given recent success in designing virtual libraries of novel macrolides and computer-aided prediction of their activities [76].

**Heterologous gene expression**
- More tractable host species could be required for the production of some marine actinobacterial natural products.
- Heterologous expression provides a means of discovering new secondary metabolite gene clusters and validating the involvement of particular genes in biosynthesis, for example thiocoraline [53].
- Heterologous DNA technology can be used to generate new ‘unnatural’ natural products [77].

**Genomics**
- There are likely to be thousands of new species awaiting isolation [8, 75].
- Novel species will probably contain unique compounds (the evolution of novel secondary metabolites acts as a driver of bacterial speciation [78]).
- Actinomycetes producing NRPS and PKS-derived compounds have multiple biosynthetic gene clusters (an average of 12 clusters per genome [61]).
- Modular PKS and NRPS biosynthetic gene clusters evolve through recombination [79].
- Combinatorial biosynthesis produces novel bioactive compounds based on processes that mimic the evolution of novel bacterially derived compounds (see Ref. [80] for review).
Box 5. Nontechnical concerns impacting on marine biodiscovery

**Trained personnel**
- Biodiscovery will be hampered by a shortage of professional systematists, particularly those with expertise in actinobacterial taxonomy, and of natural product chemists. A similar decline in the study of microbial physiology gives cause for further concern in the post-genomics era.

**Investment**
- A treat rather than cure position adopted by many pharmaceutical companies has contributed to a major decline in natural product drug discovery (notably of antibiotics), further confounded by short commercial half-lives for such products, regulatory confusion and problems surrounding patent rules.
- Inadequate training in crucial underpinning disciplines (microbial systematics and physiology, natural product chemistry) is a dangerous threat to research and development in this field.

**Convention on biological diversity**
- Guidelines for regulating the prospecting of genetic resources in the deep seas [81] will have implications for marine natural product discovery [1].

In summary, the advent of genomics has revolutionized the process of discovering actinobacterial NCEs. When estimating the degree of novelty awaiting discovery in marine actinomycetes the facts presented in Box 3 are encouraging. Richard Baltz reports that antibiotic biosynthetic pathways are distributed at frequencies ranging from a single antibiotic (streptothricin) at 1 in 10 to ~1000 different antibiotics at ~1 in 10^7, a frequency distribution suggesting that only a fraction of existing antibiotics have been discovered [9]. The advance of genomics approaches should significantly reduce the effort required to access novel compounds at the lower end of the frequency distribution.

**Realizing the opportunities**
Based upon the foregoing discussion we have identified several technical issues and nontechnical concerns – not mutually exclusive – that will affect not only marine actinobacteria but the whole field of natural product discovery and development.

The technical issues raised in Box 4 are not an exhaustive or prescriptive agenda but, that said, they emphasize the multidisciplinary effort needed to advance marine actinobacterial natural products from the level of novel discovery to bioactive lead compounds and drugs. The nontechnical matters that are included in Box 5 are those that have exercised our concern in particular, and doubtless the reader will readily add others to this list.

**Conclusions and future perspectives**
We commented earlier on the global development of marine microbiology, and the renaissance taking place in natural product research. Furthermore, we have reiter-ated our belief that natural product search and discovery in marine actinobacteria shows exceptional promise. Such optimism is based on the spectacular technological armamentarium that is now available and a fuller but more slowing developing understanding of marine biology.

Optimism also is encouraged by the wide range of natural products and diversity of their applications (biocatalysts, biomaterials, agrichemicals, etc.), albeit in this review the focus has been on bioactive metabolites.

However, marine actinobacterial search and discovery is one thing, development of discoveries to end-point products is another. We conclude with a few reflections on this dilemma and although in this context they relate to antibiotics, almost identical arguments are apposite to orphan drugs in general, and ‘neglected’ diseases. There has been much recent comment about the scarcity of new antibiotic entities, why their need has reached alarming proportions and the reason for the withdrawal of many big pharma companies from this field. The analysis made by Steve Projan in 2003 [67] remains true although there are encouraging signs of the newer biotechnology companies filling the innovation gap and, in some cases, focusing on marine organisms. Ultimately medical necessity as much as business opportunity could be the driver for investment in natural product drugs [68] and this, as Projan has cautioned, will call for urgent changes in public and social policy, and will come at some cost!

**References**

www.sciencedirect.com


Have you contributed to an Elsevier publication? Did you know that you are entitled to a 30% discount on books?

A 30% discount is available to all Elsevier book and journal contributors when ordering books or stand-alone CD-ROMs directly from us.

To take advantage of your discount:

1. Choose your book(s) from www.elsevier.com or www.books.elsevier.com

2. Place your order

   Americas:
   Phone: +1 800 782 4927 for US customers
   Phone: +1 800 460 3110 for Canada, South and Central America customers
   Fax: +1 314 453 4898
   author.contributor@elsevier.com

   All other countries:
   Phone: +44 (0)1865 474 010
   Fax: +44 (0)1865 474 011
directorders@elsevier.com

   You’ll need to provide the name of the Elsevier book or journal to which you have contributed. Shipping is free on prepaid orders within the US.

   If you are faxing your order, please enclose a copy of this page.

3. Make your payment

   This discount is only available on prepaid orders. Please note that this offer does not apply to multi-volume reference works or Elsevier Health Sciences products.

For more information, visit www.books.elsevier.com