1
Introduction to Marine Actinobacteria

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1.1
Introduction

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 indicates that 67% of marine natural products were sourced from Australia, the Caribbean, the Indian Ocean, Japan, the Mediterranean, and the Western Pacific Ocean sites [1].

Marine Actinobacteria have been looked upon as potential sources of bioactive compounds, and the work done earlier has shown that these microbes are the richest sources of secondary metabolites. They hold a prominent position as targets in screening programs due to their diversity and their proven ability to produce novel metabolites and other molecules of pharmaceutical importance [2]. Since the discovery of actinomycin [3], Actinobacteria have been found to produce many commercially bioactive compounds and antitumor agents in addition to enzymes of industrial interest [4]. Approximately, two-third of the thousands of naturally occurring antibiotics have been isolated from these organisms [5]. Of them, many have been obtained from *Streptomyces* [6] and these natural products have been an extraordinary source for lead structures in the development of new drugs [7].

Although the diversity of life in the terrestrial environment is extraordinary, the greatest biodiversity is in the oceans [8]. More than 70% of our planet's surface is covered by oceans and life on Earth originated from the sea. In some marine ecosystems, such as the deep sea floor and coral reefs, experts estimate that the biological diversity is higher than that in the tropical rainforests [9]. As marine environmental conditions are extremely different from the terrestrial ones, it is surmised that marine Actinobacteria have characteristics different from those of terrestrial counterparts and, therefore, might produce different types of bioactive compounds. The living conditions to which marine Actinobacteria had to adapt during evolution range from extremely high pressures (with a maximum of 1100 atmospheres) and anaerobic conditions at temperatures just below 0 °C on the deep sea floor to high acidic conditions (pH as low as 2.8) at temperatures of over 100 °C

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near hydrothermal vents at the mid-ocean ridges. It is likely that this is reflected in the genetic and metabolic diversity of marine actinomycetes, which remain largely unknown. Indeed, the marine environment is virtually an untapped source of novel Actinobacteria diversity [10,11] and, therefore, of new metabolites [12–14].

However, the distribution of Actinobacteria in the sea is largely unexplored and the presence of indigenous marine Actinobacteria in the oceans remains elusive. This is partly caused by the insufficient effort put into exploring marine Actinobacteria, whereas terrestrial Actinobacteria have been, until recently, a successful source of novel bioactive metabolites. Furthermore, skepticism regarding the existence of indigenous populations of marine Actinobacteria arises from the fact that the terrestrial bacteria produce resistant spores that are known to be transported from land into sea, where they can remain available but dormant for many years [15–17]. In this chapter, we evaluate the current state of research on the biology and biotechnology of marine Actinobacteria. The topics covered include the abundance, diversity, novelty and biogeographic distribution of marine Actinobacteria, ecosystem function, bioprospecting, and a new approach to the exploration of actinobacterial taxonomic space.

1.2 Actinobacteria

Actinobacteria are aerobic, nonmotile, and Gram-positive bacteria with high guanosine–cytosine (GC) content in their DNA (70–80%) and are phylogenetically related to the bacteria based on the evidence of 16S ribosomal RNA cataloging studies [18]. Although originally considered an intermediate group between bacteria and fungi, they are now recognized as prokaryotic organisms. Actinomycetales is an order of Actinobacteria, which have substrate hyphae and form aerial mycelia and spores. Aerial hyphae of Actinobacteria give rise to sporophores that differ greatly in structure. The spore-bearing hyphae of the aerial mycelium have somewhat greater diameter than the substrate mycelium. The spores are resistant to desiccation and can survive in soil in a viable state for long periods. This stage of the life cycle imparts resistance to adverse environmental conditions in the soil such as low nutrients and water availability. These microorganisms are phenotypically highly diverse and found in most natural environments [18].

1.3 Origin and Distribution of Marine Actinobacteria

Actinobacteria are mostly considered as terrigenous bacteria because of their wide occurrence and abundance in soil. Their distribution in the aquatic environment remained largely undescribed for many years. Most of the workers questioned the indigenous nature of aquatic Actinobacteria because these produce resistant spores that are known to be transported from land into sea and other aquatic bodies where

they can remain dormant for many years. In fact, they were considered to originate from dormant spores that were washed from land [18].

It is now clear that specific populations of marine-adapted Actinobacteria not only exist in the marine environment but also significantly add to diversity within a broad range of *Actinomycetes taxa* [19,20]. Recent studies have also shown that Actinobacteria can be isolated from mangrove swamps, other coastal environments, and even deep ocean sediments [21,22].

Despite the fact that the selective methods used to culture Actinobacteria targeted only the mycelium-producing strains, thereby omitting the important marine groups such as the mycolate Actinobacteria [23], it can be seen that marine Actinobacteria include new phenotypes that have clearly diverged from those known to occur on land.

Although the ecological roles of marine Actinobacteria remain undefined, it is possible that like their terrestrial counterparts, they are involved in the decomposition of recalcitrant organic materials such as chitin, a biopolymer that is particularly abundant in the sea [21]. Given that Actinobacteria living in the ocean experience a dramatically different set of environmental conditions compared to their terrestrial relatives, it is not surprising that speciation has occurred and unique marine taxa are now being recognized. Not only the extent of marine actinobacterial diversity is yet to be determined, but also the adaptations of these microbes in the sea resulting in the production of secondary metabolites are to be studied.

1.4
Isolation and Identification of Marine Actinobacteria

Actinobacteria are ubiquitous in marine environment and there are several techniques for their isolation. In the conventional isolation techniques, several factors must be considered, namely, choice of screening source, selective medium, culture conditions, and recognition of candidate colonies in the primary isolation. Some of the researchers employ pretreatments of sediments by drying and heating to stimulate the isolation of rare Actinobacteria [24]. An alternative approach would be to make the isolation procedure more selective by adding chemicals such as phenol to the sediment suspension. Many media have been recommended for isolation of Actinobacteria from marine samples. Specialized growth media have been developed to isolate specific actinomycete genera with macromolecules such as casein, chitin, hair hydrolysate, and humic acid that are carbon and nitrogen sources for obtaining rare Actinobacteria. Several antibiotic molecules are also used in selective media to inhibit unwanted microbes, including fast-growing bacteria and fungi.

Strains are preliminarily indentified according to their morphological criteria, including characteristics of colonies on the plate, morphology of substrate mycelium and aerial hyphae, morphology of spores, pigments produced, cell wall chemo type, whole-cell sugar pattern, and so on, and their identification is confirmed by 16s rDNA analysis [25–32].

1.5 Indigenous Marine Actinobacteria

The indigenous deep sea Actinobacteria warrant some specific consideration because if we can define some or all of the features of the deep sea Actinobacterial physiology, this should lead to greater efficacy of isolation. Although an obligate requirement for Na+ and the 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 made. We believe that physiological understanding of this type could enable more precise ecosimulation or microcosm approaches to targeting the recovery of a greater diversity of deep sea Actinobacteria [33].

Early evidences supporting the existence of marine Actinobacteria came from the description of *Rhodococcus marinonascene*, the first marine actinomycete species to be characterized [34]. Further support has come from the discovery that some strains display specific marine adaptations [35], whereas others appear to be metabolically active in marine sediments [36]. However, these early findings did not generate enough excitement to stimulate the search for novel Actinobacteria in the marine environment. Recent data from culture-dependent studies have shown that indigenous marine Actinobacteria indeed exist in the oceans. These include members of the genera *Dietzia*, *Rhodococcus*, *Streptomyces*, *Salinispora*, *Marinophilus*, *Solwaraspora*, *Salinibacterium*, *Aeromicrobium marinum*, *Williamsia maris*, and *Verrucosispora* [10–12,14,37]. Among these, the most exciting finding is the discovery of the first obligate new marine Actinobacteria genus *Salinispora* (formerly known as *Salinospora*) and the demonstration of the widespread populations of this genus in ocean sediments by Fenical's research group [19,38]. Subsequently, *Salinispora* strains were also isolated from the Great Barrier Reef marine sponge *Pseudoceratina clavata* [39]. The formal description of *Salinispora*, with two types of species – *Salinispora tropica* and *Salinispora arenicola*, has recently been published [40]. Furthermore, Mincer *et al.* [38] have demonstrated that *Salinispora* strains are actively growing in some sediment samples indicating that these bacteria are metabolically active in the natural marine environment. In this context, Grossart *et al.* [41] have illustrated that actinomycetes account for ~10% of the bacteria colonizing marine organic aggregates (marine snow) [42] and that their antagonistic activity might be highly significant in maintaining their presence that affects the degradation and mineralization of organic matter. Therefore, Actinobacteria are active components of marine microbial communities. They form a stable, persistent population in various marine ecosystems. The discovery of numerous new marine actinomycetes taxa, their metabolic activity demonstrated in their natural environments, and their ability to form stable populations in different habitats clearly illustrate that indigenous marine Actinobacteria indeed exist in the oceans. Another important observation is that novel compounds with biological activities have been isolated from these marine Actinobacteria [13,14,37], indicating

that marine actinomycetes are an important source for the discovery of novel secondary metabolites.

1.6
Role of Actinobacteria in the Marine Environment

Actinobacteria have a profound role in the marine environment. The degradation and turnover of various materials are a continuous process mediated by the action of a variety of microorganisms. There is a speculation that the increase or decrease of a particular enzyme-producing microorganism may indicate the concentration of natural substrate and conditions of the environment [43]. Actinobacteria are also reported to contribute to the breakdown and recycling of organic compounds [44].

1.7
Importance of Marine Actinobacteria

1.7.1
Antibiotics

Marine Actinobacteria constitute an important and potential source of novel bioactive compounds [45]. Since environmental conditions of the sea are extremely different from the terrestrial conditions, they produce different types of antibiotics. Several antibiotics have been isolated from marine Actinobacteria by many researchers [46–56]. These isolated antibiotics are entirely new and unique compared to those isolated from the terrestrial ones [57].

The discovery of new molecules from Actinobacteria marked an epoch in antibiotic research and subsequent developments in antibiotic chemotherapy. Since the discovery of streptomycin, a large number of antibiotics, including major therapeutic agents such as amino glycosides, chloramphenicol, tetracyclines, and macrolides, and more recently β-lactam cephamycin group, have been isolated from cultures of *Streptomyces* and *Streptoverticillium* (Atlas of Actinomycetes, The Society for Actinomycetes, Japan, 1997). As more new antibiotics were discovered, the chances of finding novel antimicrobial leads among conventional Actinobacteria dwindled. The focus of industrial screening has therefore moved to markers of less exploited genera of rare Actinobacteria such as *Actinomadura*, *Actinoplanes*, *Amycolatopsis*, *Dactylosporangium*, *Kibdelosporangium*, *Microbispora*, *Micromonospora*, *Planobispora*, *Streptosporangium*, and *Planomonospora* [58].

Screening of microorganisms for the production of novel antibiotics has been intensively pursued by scientists for many years as they are used in many fields, including agriculture, veterinary, and pharmaceutical industry. Actinobacteria have the capability to synthesize many different biologically active secondary metabolites: antibiotics, herbicides, pesticides, antiparasitic substances, and enzymes such

as cellulase and xylanase that are used in waste treatment. Of these compounds, antibiotics predominate in therapeutic and commercial uses [59–64].

1.7.2 Melanins

Melanins are complex natural pigments, widely dispersed in animals, plants, and microorganisms. They have several biological functions, including photoprotection, thermoregulation, action as free radical sinks, cation chelators, and antibiotics. Plants and insects incorporate melanins as cell wall and cuticle strengtheners, respectively [65]. The function of melanin in microbes is believed to be associated with protection against environmental stress. For example, bacteria producing melanins are more resistant to antibiotics [66], and melanins in fungi are involved in fungal pathogenesis of plants [67]. In mammals, two types of melanin can be distinguished: a dark eumelanin and a yellow to red pheomelanin [65]. Eumelanin, the more ubiquitous mammalian melanin type, is found in different regions of the human body, including the skin, hair, eye, inner ear, and brain [68].

Marine Actinobacteria also synthesizes and excrete dark pigments, melanin or melanoid, which are considered to be useful criteria for taxonomical studies [69,70]. Melanin compounds are irregular, dark brown polymers that are produced by various microorganisms by the fermentative oxidation, and they have radio-protective and antioxidant properties that can effectively protect the living organisms from ultraviolet radiation. Melanins are frequently used in medicine, pharmacology, and cosmetics preparations [71].

Biosynthesis of melanin with tyrosinase transforms the tyrosine into L-DOPA (3,4-dihydroxy phenyl-L-alanine), which is further converted into dopachrome and autoxidized to indol-5,6-quinone. The latter is polymerized spontaneously into DOPA-melanin that gives a dark brown pigment until the further examination [72].

1.7.3 Enzymes

Marine Actinobacteria have a diverse range of enzyme activities and are capable of catalyzing various biochemical reactions [43].

1.7.3.1 α-Amylase

Amylases are enzymes that hydrolyze starch molecules to give diverse products, including dextrins and progressively smaller polymers composed of glucose units [73]. These enzymes are of great significance in the present-day biotechnology with applications in food, fermentation, textile, and paper industries [74]. Although amylases can be derived from several sources, including plants, animals, and microorganisms, microbial enzymes generally meet industrial demands. Today, a large number of microbial amylases are available commercially and they have almost completely replaced chemical hydrolysis of starch in starch processing industry [74].

The history of amylases begins with the discovery of first starch-degrading enzyme in 1811 by Kirchhoff. This was followed by several reports of digestive amylases and malt amylases. It was much later in 1930 that Ohlsson suggested the classification of starch digestive enzymes in malt as α- and β-amylases, according to the anomeric type of sugars produced by the enzyme reaction. α-Amylase (1,4-α-D-glucan glucanohydrolase, EC. 3.2.1.1) is a widely distributed secretary enzyme. α-Amylases of different origins have been extensively studied. Amylases can be divided into two categories: endoamylases and exoamylases. Endoamylases catalyze hydrolysis in a random manner in the interior of the starch molecule. This action causes the formation of linear and branched oligosaccharides of various chain lengths. Exoamylases hydrolyze from the nonreducing end, successively resulting in short end products. Today, a large number of enzymes are known that can hydrolyze starch molecules into different products, and a combined action of various enzymes is required to hydrolyze starch completely [75]. Occurrence of amylases in Actinobacteria is a characteristic commonly observed in *Streptomyces* [76], a genus that is considered a potential source of amylolytic enzymes.

1.7.3.2 **Proteases**

Proteases, also known as peptidyl–peptide hydrolases, are important industrial enzymes accounting for ~60% of all enzyme sales and are utilized extensively in a variety of industries, including detergents, meat tenderization, cheese-making, dehairing, baking, and brewery, in the production of digestive aids, and in the recovery of silver from photographic film. The use of these enzymes as detergent additives stimulated their commercial development and resulted in a considerable expansion of fundamental research into these enzymes [77]. In addition to detergents and food additives, alkaline proteases have substantial utilization in other industrial sectors such as leather, textile, organic synthesis, and wastewater treatment [78,79]. Consequently, alkaline proteases with novel properties have become the focus of recent researches. Alkaline proteases are generated by a wide range of organisms, including bacteria, Actinobacteria molds, yeasts, and mammalian tissues.

Several studies have been made on the proteolytic enzymes of mesophilic actinomycetes [80–84]. Recently, alkaline protease from *Nocardiopsis* sp. NCIM 5124 [85] has been purified and characterized.

1.7.3.3 **Cellulases**

Cellulose, the most abundant organic source of feed, fuel, and chemicals [86], consists of glucose units linked by β-1,4-glycosidic bonds in a linear mode. The difference in the type of bond and the highly ordered crystalline forms of the compound between starch and cellulose make cellulose more resistant to digest and hydrolyze. The enzymes required for the hydrolysis of cellulose include endoglucanases, exoglucanases, and β-glucosidases [87]. While endoglucanase randomly hydrolyzes cellulose, producing oligosaccharides, cellobiose, and glucose, the exoglucanase hydrolyzes β-1,4-D-glucosidic linkages in cellulose, releasing

cellobiose from the nonreducing end. On the other hand, β-glycosidases of thermophilic origin, which have received renewed attention in the pharmaceutical industry, hydrolyze cellobiose to glucose.

In the current industrial processes, cellulolytic enzymes are employed in the color extraction from juices, detergents causing color brightening and softening, biostoning of jeans, pretreatment of biomass that contains cellulose to improve nutritional quality of forage, and pretreatment of industrial wastes [88–93]. To date, the alkaline- or alkali-tolerant cellulase producers have mainly been found in the genera *Streptomyces* and *Thermoactinomyces* [94].

1.7.3.4 **Chitinase**

Chitin, an insoluble linear β-1,4-linked polymer of *N*-acetylglucosamine (GlcNAc), is the second most abundant polymer in nature. This polysaccharide is found in the cell walls of fungi and exoskeleton of insects and the shells of crustaceans. Chitinases (EC 3.2.1.14) are produced by many organisms such as viruses, bacteria, Actinobacteria, and higher plants and animals and they play important physiological and ecological roles [95]. Chitinases hydrolyze the β-1,4 linkages in chitin, yielding predominantly *N*-*N*′-diacetylchitobiose that is further degraded by *N*-acetylglucosaminidases to the GlcNAc monomer [96].

Chitinase is involved in the process of producing mono- and oligosaccharides from chitin. Furthermore, chitinase is a potential antifungal agent because of its chitin degradation activity [97–100]. Among Actinobacteria, the genus *Streptomyces* is the best studied for chitinases [101,102] and is mainly responsible for the recycling of chitinous matter in nature.

Different chitinous substrates that have been reported in the literature for chitinase production include fungal cell walls, crab and shrimp shells, prawn wastes, and flake chitin [103–105]. Production of inexpensive chitinase will be important, if the use of chitinous wastes (shrimp shells, chitin from seafood industry, etc.) will solve environmental problems [106].

1.7.3.5 **Keratinase**

Keratinase is a specific protease, hydrolyzing keratin, which is a protein found in feathers, wool, and hair. Keratins as well as other insoluble proteins are generally not recognized as a substrate for common proteases. Hydrolysis of bacteria is however affected by specific proteases (keratinases) that have been found in some species of *Bacillus* [107,108], saprophytic and parasitic fungi [109], Actinobacteria [80,84], *Fervidobacterium pennavorans*, and some other microorganisms [110,111]. The microbial degradation of insoluble macromolecules such as cellulose, lignin, chitin, and keratin depends on the secretion of extracellular enzymes with the ability to act on compact substrate surfaces.

Feathers contain about 90% crude proteins in the form of β-keratin [112]. The poultry processing industry produces several millions of tonnes of feathers per year as by-products worldwide [113]. Hydrolysis of keratin-containing wastes by microorganisms possessing keratinolytic activity represents an attractive alternative method for efficient bioconversion and improving the nutritional value of keratin

wastes, compared to currently used methods, through the development of economical and environment-friendly technologies [114].

Keratinolytic proteinases play an important role in biotechnological applications like enzymatic improvement of feather meal and production of amino acids or peptides from high molecular weight substrates or in the leather industry [115–118]. These enzymes, keratinases, could be applied for wastewater treatment, textile, medicine, cosmetic, and feed and poultry processing industries, as well as leather industry [119].

1.7.3.6 **Xylanases**

Xylan, which is the dominating component of hemicelluloses, is one of the most abundant organic substances on Earth. It has a great application in the pulp and paper industry [120–122]. The wood used for the production of the pulp is treated at high temperatures and basic pH, which implies that the enzymatic procedures require proteins exhibiting a high thermostability and activity in a broad pH range [123]. Treatment with xylanase at elevated temperatures disrupts the cell wall structure. As a result, this facilitates lignin removal in various stages of bleaching. For such purposes, (i) xylanases must lack cellulytic activity to avoid hydrolysis of the cellulose fibers and (ii) need to be of low molecular mass to facilitate their diffusion in the pulp fibers. Most importantly, high yields of enzyme must be obtained at a very low cost [89]. Alkaliphilic and cellulase-free xylanases with an optimum temperature of 65 °C from *Thermoactinomyces thalophilus* subgroup C [124] were also reported. Thermostable xylanase were isolated from a number of Actinobacteria [125]. *Streptomyces* sp. have been reported to produce xylanases that are active at temperatures between 50 and 80 °C [125].

1.7.4
Enzyme Inhibitors

Enzyme inhibitors have received increasing attention as useful tools, not only for the study of enzyme structures and reaction mechanisms but also for potential utilization in pharmacology [126]. Marine Actinobacteria are the potential source for production of enzyme inhibitors [127,128]. Imade [127] reported different types of enzyme inhibitors: β-glucosidase, *N*-acetyl-β-D-glucosaminidase, pyroglutamyl peptidase, and α-amylase inhibitors from marine Actinobacteria.

1.7.5
Anticancer Compounds

Cancer is a term that refers to a large group of over a hundred different diseases that arise when defects in physiological regulation cause unrestrained proliferation of abnormal cells [129]. In most cases, these clonal cells accumulate and multiply, forming tumors that may compress, invade, and destroy normal tissue, weakening the vital functions of the body with devastating consequences, including loss of quality of life and mortality. Nowadays, cancer is the second cause of death in the

developed world, affecting one out of three individuals and resulting in one out of five deaths worldwide [129]. Diversified groups of marine Actinobacteria are known to produce different types of anticancer compounds. Several kinds of cytotoxic compounds have been reported from marine Actinobacteria [130–137]. The isolated compounds showed significant activity against different cancer cell lines.

1.8 Symbioses

The association of bacteria with marine sponges, bryozoans, tunicates, and holothurians has long been known, and sponge systems have attracted much attention [138–140]. Interest in such animals has been excited by their diversity of bioactive products that most probably are secondary metabolites of their bacterial partners. Actinobacteria are often components of these symbiotic communities, and because of their pedigree as natural product sources, they are increasingly targeted in biodiscovery programs. Actinobacteria are found in reef and deepwater sponges and evidence for sponge-specific symbioses exists [139]. In at least one case, Actinobacterial symbionts such as species of *Micromonospora* have been shown to produce bioactive compounds (manzamines) that have no terrestrial equivalents. Of considerable interest is the reported isolation of *Salinispora* strains (only known previously from marine sediments) from the Great Barrier Reef sponge *P. clavata*, and their activity against other bacterial symbionts [141]. These *Salinispora* isolates possess a polyketide synthase (PKS) gene that is most closely related to the rifamycin B synthase of *Amycolatopsis* [141], 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 synthesis. Reports of Actinobacterial symbionts of sponges have also appeared from Chinese groups. Sponges in the South China Sea harbor a large diversity of Actinobacteria and show evidence of host specificity [142]. The greatest Actinobacterial diversity was found in *Craniella australiensis*, with many of the strains having broad-spectrum antibacterial activities [143]. Similarly, Actinobacteria associated with the Yellow Sea sponge *Hymeniacidon perleve* showed broad taxonomic diversity and included *Actinoalloteichus*, *Micromonospora*, *Nocardia*, *Nocardiopsis*, *Pseudonocardia*, *Rhodococcus*, and *Streptomyces* [144]; 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 [145]. Second, a culture-based study of the symbionts of *Fungia scutaria* [146], a Red Sea species, revealed a large proportion (23%) of Actinobacteria in the mucus layer. Although the cultivation efficiency was low, this was the first account of Actinobacteria being isolated from corals. Continued isolation and screening of coral-associated

Actinobacteria seem entirely warranted, given the early success in discovering valuable bioactive compounds such as thiocoraline [54].

1.9 Bioinformatics

Bioinformatics and its component "-omic" elements have created a paradigm shift in our approach to natural product discovery. Much of the relevant information is contained in Ref. [147], 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 eliaophylin, geldanamycin, nigericin, and 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) [148]. Although such patterns have not been demonstrated unequivocally in marine Actinobacteria, pursuit of the relationship is encouraged by recent chemodiversity analyses of the genus *Salinispora* [149], 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 principal coordinate space illustrates dramatically the extensive regions of unexplored Actinobacterial taxonomy [10,150]. Recently discovered marine taxa *Marinospora*, *Verrucocispora maris*, and alkalitolerant *Streptomyces* occupy distinct new regions of phylogenetic space [150] and synthesize exciting new chemical entities (NCE). The prospect of massive sequencing of 16S rRNA and other diagnostic genes (e.g., by means of the 454 pyrosequencing platform) [151] 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 Ref. [152].

1.10 Conclusions

The study of marine Actinobacteria is at an early stage, but the developments in molecular biology and genomics will greatly enhance our capacity to clarify their systematics, to understand their ecology and evolution, and to inform bioprospecting programs. Research programs will need to be focused at the levels of individuals, species, metapopulations, and those communities of which Actinobacteria are components. Furthermore, we have reiterated 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 on a fuller, but slower, understanding of marine biology. Optimism is also encouraged by a wide range of natural products and diversity of their applications (biocatalysts, biomaterials, agrichemicals, etc.); however, in this chapter, the focus has been on bioactive metabolites.

Marine actinobacterial search and discovery is one thing, development of discoveries to end 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 to "neglected" diseases. There has been much recent comments about the scarcity of new antibiotic entities, why their need has reached alarming proportions, and the reason for the withdrawal of many big pharmaceutical companies from this field. The analysis made by Projan [153] remains true, although there are encouraging signs of 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 [154] and this, as Projan has cautioned, will call for urgent changes in public and social policy, and will come at some cost!

References

1 Blunt, J.W. *et al.* (2007) Marine natural products. *Nat. Prod. Rep.*, **24**, 31–86.

2 Ellaiah, P., Ramana, T., Bapi Raju, K.V. V.S.N., Sujatha, P., and Uma Sankar, A. (2004) Investigation on marine actinomycetes from Bay of Bengal near Kakinada coast of Andhra Pradesh. *Asian J. Microbiol. Biotechnol. Environ. Sci.*, **6**, 53–56.

3 Lechevalier, H. (1982) *The Development of Applied Microbiology at Rutgers*, The State University of New Jersey, p. 3.

4 Tanaka, Y. and Omura, O. (1990) Metabolisms and products of actinomycetes: an introduction. *Actinomycetologica*, **4**, 13–14.

5 Takaizawa, M., Colwell, W., and Hill, R.T. (1993) Isolation and diversity of actinomycetes in the Chesapeake Bay. *Appl. Environ. Microbiol.*, **59**, 997–1002.

6 Goodfellow, M. and O'Donnell, A.G. (1993) Roots of bacterial systematic, in *Handbook of New Bacterial Systematics* (eds M. Goodfellow, and A.G. O'Donnell), Academic Press, London, pp. 3–54.

7 Sivakumar, K., Sahu, M.K., Thangaradjou, T., and Kannan, L. (2007) Research on marine Actinobacteria in India. *Indian J. Microbiol.*, **47**, 186–196.

8 Donia, M. and Hamann, M.T. (2003) Marine natural products and their potential applications as anti-infective agents. *Lancet Infect. Dis.*, **3**, 338–348.

9 Haefner, B. (2003) Drugs from the deep: marine natural products as drug candidates. *Drug Discov. Today*, **8**, 536–544.

10 Bull, A.T., Stach, J.E.M., Ward, A.C., and Goodfellow, M. (2005) Marine Actinobacteria: perspectives, challenges, future directions. *Antonie Van Leeuwenhoek*, **87**, 65–79.

11 Stach, J.E.M., Maldonado, L.A., Ward, A. C., Goodfellow, M., and Bull, A.T. (2003) New primers for the class Actinobacteria: application to marine and terrestrial environments. *Environ. Microbiol.*, **5**, 828–841.

12 Jensen, P.R., Gontang, E., Mafnas, C., Mincer, T.J., and Fenical, W. (2005) Culturable marine actinomycete diversity from tropical Pacific Ocean sediments. *Environ. Microbiol.*, **7**, 1039–1048.

13 Fiedler, H.P., Bruntner, C., Bull, A.T., Ward, A.C., Goodfellow, M., Potterat, O., Puder, C., and Mihm, G. (2005) Marine actinomycetes as a source of novel secondary metabolites. *Antonie Van Leeuwenhoek*, **87**, 37–42.

14 Magarvey, N.A., Keller, J.M., Bernan, V., Dworkin, M., and Sherman, D.H. (2004) Isolation and characterization of novel marine-derived actinomycete taxa rich in bioactive metabolites. *Appl. Environ. Microbiol.*, **70**, 7520–7529.

15 Bull, A.T., Ward, A.C., and Goodfellow, M. (2000) Search and discovery strategies for biotechnology: the paradigm shift. *Microbiol. Mol. Biol. Rev.*, **64**, 573–606.

16 Cross, T. (1981) Aquatic actinomycetes: a critical survey of the occurrence, growth and role of actinomycetes in aquatic habitats. *J. Appl. Bacteriol.*, **50**, 397–423.

17 Goodfellow, M. and Haynes, J.A. (1984) Actinomycetes in marine sediments, in *Biological, Biochemical, and Biomedical Aspects of Actinomycetes* (eds L. Ortiz-Ortiz, L.F. Bojalil, and V. Yakoleff), Academic Press, New York, pp. 453–472.

18 Goodfellow, M. and Williams, S.T. (1983) Ecology of actinomycetes. *Annu. Rev. Microbiol.*, **37**, 189–216.

19 Mincer, T.J., Jensen, P.R., Kauffman, C.A., and Fenical, W. (2002) Widespread and persistent populations of a major new marine actinomycetes taxon in ocean sediments. *Appl. Environ. Microbiol.*, **68**, 5005–5011.

20 Stach, J.E.M., Maldonado, L.A., Ward, A.C., Bull, A.T., and Goodfellow, M. (2004) *Williamsia maris* sp. nov., a novel actinomycete isolated from the Sea of Japan. *Int. J. Syst. Evol. Microbiol.*, **54**, 191–194.

21 Sivakumar, K. (2001) Actinomycetes of an Indian mangrove (Pichavaram) environment: an inventory, PhD Thesis, Annamalai University, India, p. 91.

22 Tae, K.K., Garson, M.J., and Fuerst, J.A. (2005) Marine actinomycetes related to the '*Salinospora*' group from the Great Barrier Reef sponge *Pseudoceratina clavata*. *Environ. Microbiol.*, **7** (4), 509–518.

23 Colquhoun, J.A., Mexson, J., Goodfellow, M., Ward, A.C., Horikoshi, K., and Bull, A.T. (1998) Novel *Rhodococci* and other mycolata actinomycetes from the deep sea. *Antonie Van Leeuwenhoek*, **74**, 27–40.

24 Sahu, M.K., Murugan, M., Sivakumar, K., Thangaradjou, T., and Kannan, L. (2007) Occurrence and distribution of actinomycetes in marine environs and their antagonistic activity against bacteria that is pathogenic to shrimps. *Isr. J. Aquacult. Bamid.*, **59** (3), 155–161.

25 Okami, Y., Okazaki, T., Kitahara, T., and Umezawa, H. (1976) A new antibiotic apasmomycin, produced by a streptomycete isolated from shallow sea mud. *J. Anibiot.*, **28**, 176–184.

26 Hayakawa, M. and Nonomura, H. (1987a) Humic acid–vitamin agar, a new medium for the selective isolation of soil actinomycetes. *J. Ferment. Technol.*, **65**, 501–509.

27 Hayakawa, M. and Nonomura, H. (1987b) Efficacy of artificial humic acid as a selective nutrient in HV agar used for the isolation of soil actinomycetes. *J. Ferment. Technol.*, **65**, 609–616.

28 Nonomura, H. (1988) Isolation, taxonomy and ecology of soil actinomycetes. *Actinomycetologica*, **3**, 45–54.

29 Hayakawa, M., Sadaka, T., Kajiura, T., and Nonomura, H. (1991) New methods for the highly selective isolation of *Micromonospora* and *Micronbispora*. *J. Ferment. Technol.*, **72**, 320–326.

30 Cho, S.H., Hwang, C.W., Chung, H.K., and Yang, C.S. (1994) A new medium for the selective isolation of soil actinomycetes. *J. Appl. Microbiol. Biotechnol.*, **22**, 561–563.

31 Kim, C.J., Lee, K.H., Shimazu, A., Kwon, O.S., and Park, D.J. (1995) Isolation of rare actinomycetes from various types of soil. *J. Appl. Microbiol. Biotechnol.*, **23**, 36–42.

32 Nolan, R.D. and Cross, T. (1988) Isolation and screening of actinomycetes, in *Actinomycetes in Biotechnology* (eds M. Goodfellow, S.T. Willams, and M. Mordarski), Academic Press, San Diego, CA, pp. 1–32.

33 Bull, A.T. and Stach, J.E.M. (2007) Marine Actinobacteria: new opportunities for natural product search and discovery. *Trends Microbiol.*, **15** (11), 491–499.

34 Helmke, E. and Weyland, H. (1984) *Rhodococcus marinonascens* sp. nov., an actinomycete from the sea. *Int. J. Syst. Bacteriol.*, **34**, 127–138.

35 Jensen, P.R., Dwight, R., and Fenical, W. (1991) Distribution of actinomycetes in

near-shore tropical marine sediments. *Appl. Environ. Microbiol.*, **57**, 1102–1108.
36 Moran, M.A., Rutherford, L.T., and Hodson, R.E. (1995) Evidence for indigenous *Streptomyces* populations in a marine environment determined with a 16S rRNA probe. *Appl. Environ. Microbiol.*, **61**, 3695–3700.
37 Jensen, P.R., Mincer, T.J., Williams, P.G., and Fenical, W. (2005) Marine actinomycete diversity and natural product discovery. *Antonie Van Leeuwenhoek*, **87**, 43–48.
38 Mincer, T.J., Fenical, W., and Jensen, P.R. (2005) Culture-dependent and culture-independent diversity within the obligate marine actinomycete genus *Salinispora*. *Appl. Environ. Microbiol.*, **71**, 7019–7028.
39 Kim, T.K., Garson, M.J., and Fuerst, J.A. (2005) Marine actinomycetes related to the 'Salinospora' group from the Great Barrier Reef sponge *Pseudoceratina clavata*. *Environ. Microbiol.*, **7**, 509–518.
40 Maldonado, L.A., Fenical, W., Jensen, P.R., Kauffman, C.A., Mincer, T.J., Ward, A.C., Bull, A.T., and Goodfellow, M. (2005) *Salinispora arenicola* gen. nov., sp. nov. and *Salinispora tropica* sp. nov., obligate marine actinomycetes belonging to the family Micromonosporaceae. *Int. J Syst. Evol. Microbiol.*, **55**, 1759–1766.
41 Grossart, H.P., Schlingloff, A., Bernhard, M., Simon, M., and Brinkhoff, T. (2004) Antagonistic activity of bacteria isolated from organic aggregates of the German Wadden Sea. *FEMS Microbiol. Ecol.*, **47**, 387–396.
42 Alldrege, A.L. and Silver, M.W. (1988) Characteristics, dynamics and significance of marine snow. *Prog. Oceanogr.*, **20**, 41–82.
43 Das, S., Lyla, P.S., and Khan, S.A. (2006) Marine microbial diversity and ecology: importance and future perspectives. *Curr. Sci.*, **90**, 1325–1335.
44 Weyland, H. (1969) Actinomycetes in North Sea and Atlantic Ocean sediments. *Nature*, **223**, 858.
45 Colwell, R.R. and Hill, R.T. (1992) Microbial diversity, in *Diversity of Oceanic Life: An Evaluative Review* (ed. M.N.A. Peterson), The Centre for Strategic and International Studies, Washington, DC, pp. 100–106.
46 Bernan, V.S., Montenegro, D.A., Maiese, W.M., Steinberg, D.A., and Greenstein, M. (1994) Bioxalomycins, new antibiotics produced by the marine *Streptomyces* sp. LL-31F508: taxonomy and fermentation. *J. Antibiot.*, **47**, 1417–1424.
47 Biabani, M.A., Laatsch, H., Helmke, E., and Weyland, H. (1997) delta-Indomycinone: a new member of pluramycin class of antibiotics isolated from marine *Streptomyces* sp. *J. Antibiot.*, **50**, 874–877.
48 Woo, J.H., Kitamura, E., Myouga, H., and Kamei, Y. (2002) An antifungal protein from the marine bacterium *Streptomyces* sp. strain AP77 is specific for *Pythium porphyrae*, a causative agent of red rot disease in *Porphyra* spp. *Appl. Environ. Microbiol.*, **68**, 2666–2675.
49 Maskey, R.P., Helmke, E., Fiebig, H.H., and Laatsch, H. (2002) Parimycin: isolation and structure elucidation of a novel cytotoxic 2,3-dihydroquinizarin analogue of gamma-indomycinone from a marine streptomycete isolate. *J. Antbiot.*, **55**, 1031–1035.
50 Maskey, R.P., Helmke, E., and Laastsch, H. (2003) Himalomycin A and B: isolation and structure elucidation of new fridamycin type antibiotics from a marine *Streptomyces* isolate. *J. Antibiot.*, **56**, 942–949.
51 Charan, R.D., Schlingmann, G., Janso, J., Bernan, V., Feng, X., and Carter, G.T. (2004) Diazepinomicin, a new antimicrobial alkaloid from a marine *Micromonospora* sp. *J. Nat. Prod.*, **67**, 1431–1433.
52 Li, F., Maskev, R.P., Qin, S., Sattler, I., Fiebig, H.H., Maier, A., Zeeck, A., and Laatsch, H. (2005) Chinikomycins A and B: isolation, structure elucidation and biological activity of novel antibiotics from a marine *Streptomyces* sp. isolate M045. *J. Nat. Prod.*, **68**, 349–353.
53 Buchanan, G.O., Williams, P.G., Feling, R.H., Kauffman, C.A., Jensen, P.R., and Fenical, W. (2005) Sporolides A and B: structurally unprecedented halogenated macrolides from the marine actinomycete *Salinispora tropica*. *Org. Lett.*, **7**, 2731–2734.
54 Lombo, F., Velasco, A., de laCalle, F., Brana, A.F., Sanchez- Pulles, J.M.,

Mendez, C., and Salas, J.A. (2006) Deciphering the biosynthesis of the antitumor thiocoraline from a marine actinomycetes and its expression in two *Streptomyces* species. *ChemBioChem*, **7**, 366–376.

55 Adinarayana, G., Venkateshan, M.R., Bapiraju, V.V., Sujatha, P., Premkumar, J., Ellaiah, P., and Zeeck, A. (2006) Cytotoxic compounds from the marine actinobacterium. *Bioorg. Khim.*, **32**, 328–334.

56 Lam, S.K. (2006) Discovery of novel metabolites from marine actinomycetes. *Curr. Opin. Microbiol.*, **9**, 245–251.

57 Meiying, Z. and Zhicheng, Z. (1998) Identification of marine actinomycetes S-216 strain and its biosynthetic conditions of antifungal antibiotic. *J. Xiamen Univ. Nat. Sci.*, **37**, 109–114.

58 Lazzarini, A., Cavaletti, L., Toppo, G., and Marinelli, F. (2000) Rare genera of actinomycetes as potential producers of new antibiotics. *Antonie Van Leeuwenhoek*, **78**, 399–405.

59 Lacey, J. (1973) *Actinomycetales: Characteristics and Practical Importance, Society for Applied Bacteriology Symposium Series*, vol. 2 (eds G. Sykes and F. Skinner), Academic Press, London.

60 McCarthy, A.J. and Williams, S.T. (1990) Methods for studying the ecology of Actinomycetes, in *Methods in Microbiology*, vol. 22 (eds R. Grigorova and J.R. Norris), Academic Press, London, pp. 533–363.

61 Ouhdouch, Y., Barakate, M., and Finanse, C. (2001) Actinomycetes of Moroccan habitats: isolation and screening for antifungal activities. *Eur. J. Soil Biol.*, **37**, 69–74.

62 Saadoun, I. and Gharaibeh, R. (2003) The *Streptomyces* flora of Badia region of Jordan and its potential as a source of antibiotics active against antibiotic-reistant bacteria. *J. Arid Environ.*, **53**, 365–371.

63 Waksman, S.A. (1961) *The Actinomycetes, Classification, Identification and Description of Genera and Species*, vol. 2, The Williams and Wilkins Company, Baltimore, pp. 61–292.

64 Oskay, M., Tamer, A.U., and Azeri, C. (2004) Antibacterial activity of some actinomycetes isolated from farming soils of Turkey. *Afr. J. Biotechnol.*, **3** (9), 441–446.

65 Riley, P.A. (1997) Melanin. *Int. J. Biochem. Cell Biol.*, **29**, 1235–1239.

66 Lin, W.P., Lai, H.L., Liu, Y.L., Chiung, Y.M., Shiau, C.Y., Han, J.M., Yang, C.M., and Liu, Y.T. (2005) Effect of melanin produced by a recombinant *Escherichia coli* on antibacterial activity of antibiotics. *J. Microbiol. Immunol. Infect.*, **38**, 320–326.

67 Butler, M.J. and Day, A.W. (1998) Fungal melanins: a review. *Can. J. Microbiol.*, **44**, 1115–1136.

68 Clancy, C.M.R. and Simon, J.D. (2001) Ultrastructural organization of eumelanin from *Sepia officinalis* measured by atomic force microscopy. *Biochemistry*, **40**, 13353–13360.

69 Zonova, G.M. (1965) Melanoid pigments of Actinomycetes. *Mikrobiologiya*, **34**, 278–283.

70 Arai, T. and Mikami, Y. (1972) Choromogenecity of *Streptomyces*. *Appl. Microbiol.*, **23**, 402–406.

71 Dastager, S.G., Li, W.J., Dayanand, A., Tang, S.K., Tian, X.P., Zhi, X.Y., Xu, L.H., and Jiang, C.L. (2006) Separation, identification and analysis of pigment (melanin) production in *Streptomyces*. *Afr. J. Biotechnol.*, **5** (8), 1131–1134.

72 Mencher, J.R. and Heim, A.H. (1962) Melanin biosynthesis by *Streptomyces lavendulae*. *J. Gen. Microbiol.*, **28**, 665–670.

73 Windish, W.W. and Mhatre, N.S. (1965) Microbial amylases, in *Advances in Applied Microbiology*, vol. 7 (ed. W.U. Wayne), Academic Press, New York, pp 273–304.

74 Pandey, A., Shukla, A., and Majumdar, S.K. (2005) Utilization of carbon and nitrogen sources by *Streptomyces kanamyceticus* M 27 for the production of an antibacterial antibiotic. *Afr. J. Biotechnol.*, **4** (9), 909–910.

75 Gupta, R., Gigras, P., Mohapatra, H., Goswami, V.K., and Chauhan, B. (2003) Microbial α-amylases: a biotechnological perspective. *Process Biochem.*, **38**, 1599–1616.

76 Vigal, T., Gil, J.F., Daza, A., Garcia-Gonzalez, M.D., and Martin, J.F. (1991) Cloning characterization and expression of an alpha amylase gene from *Streptomyces griseus* IMRU 3570. *Mol. Gen. Genet.*, **225**, 278–288.

77 Germano, S., Pandey, A., Osaku, C.A., Rocha, S.N., and Soccol, C.R. (2003) Characterization and stability of protease from *Penicillium* sp. produced by solid-state fermentation. *Enzyme Microb. Technol.*, **32**, 246–251.

78 Kalisz, H.M. (1988) Microbial proteinases. *Adv. Biochem. Eng. Biotechnol.*, **36**, 1–65.

79 Kumar, C.G. and Takagi, H. (1999) Microbial alkaline proteases from a bioindustrial viewpoint. *Biotechnol. Adv.*, **17**, 561–594.

80 Noval, J.J. and Nickerson, W.J. (1958) Decomposition of native keratin by *Streptomyces fradiae*. *J. Bacteriol.*, **77**, 251–263.

81 Nakanishi, T., Matsumura, Y., Minamiura, N., and Yamamoto, T. (1973) Purification and some properties of an alkalophilic proteinase of a *Streptomyces* species. *Agric. Biol. Chem.*, **38**, 37–44.

82 Moormann, M., Schlochtermeier, A., and Schrempf, H. (1993) Biochemical characterization of a protease involved in the processing of a *Streptomyces reticuli* cellulase (Avicellase). *Appl. Environ. Microbiol.*, **59**, 1573–1578.

83 Abd El-Nasser, N.H. (1995) Proteolytic activities by some mesophilic *Streptomyces* species. *J. Agric. Sci.*, **20**, 2913–2923.

84 Bockle, B., Galunsky, B., and Muller, R. (1995) Characterization of a keratinolytic serine proteinase from *Streptomyces pactum* DSM 40530. *Appl. Environ. Microbiol.*, **61**, 3705–3710.

85 Dixit, V.S. and Pant, A. (2000) Comparative characterization of two serine endopeptidases from *Nocardiopsis* sp. NCIM 5124. *Biochim. Biophys. Acta*, **1523**, 261–268.

86 Spano, L., Medeiros, J., and Mandels, M. (1975) Enzymatic hydrolysis of cellulosic waste to glucose. *Resour. Recover. Conserv.*, **1**, 279–294.

87 Matsui, I., Sakai, Y., Matsui, E., Kikuchi, H., Kawarabayasi, Y., and Honda, K. (2000) Novel substrate specificity of a membrane-bound β-glycosidase from the hyperthermophilic archaeon *Pyrococcus horikoshi*. *FEBS Lett.*, **467**, 195–200.

88 Buchert, J., Pere, J., Oijusluoma, L., Rahkamo, L., and Viikari, L. (1997) Cellulases tools for modification of cellulosic materials, in *Niches in the World of Textiles* (eds J. Buchert, J. Pere, L. Oijusluoma, L. Rahkamo, and L. Viikari), World conference of the Textile Institute, Manchester, England, pp. 284–290.

89 Niehaus, F., Bertoldo, C., Kahler, M., and Antranikian, G. (1999) Extremophiles as a source of novel enzymes for industrial applications. *Appl. Microbiol. Biotechnol.*, **51**, 711–729.

90 Bhat, M. (2000) Cellulases and related enzymes in biotechnology. *Biotechnol. Adv.*, **18**, 355–383.

91 Nakamura, H., Kubota, H., Kono, T., Isogai, A., and Onabe, F. (2001) Modification of pulp properties by cellulase treatment and application of cellulase to wastepaper deinking and mechanical pulp refining. Proceedings of the 68th Pulp and Paper Research Conference, 18-19/06, Japan, pp. 2–5.

92 Vanwyk, J., Mogale, A., and Seseng, T. (2001) Bioconversion of wastepaper to sugars by cellulase from *Aspergillus niger, Trichoderma viride* and *Penicillium funiculosum*. *J. Solid Waste Technol. Manage.*, **27**, 82–86.

93 Zhou, L., Yeung, K., and Yuen, C. (2001) Combined cellulase and wrinkle free treatment on cotton fabric. *J. Dong Hua Univ.*, **18**, 11–15.

94 Techapun, C., Poosaran, N., Watanabe, M., and Sasaki, K. (2003) Thermostable and alkaline-tolerant microbial cellulase free xylanases produced from agricultural wastes and the properties required for use in pulp bleaching bioprocess: a review. *Process Biochem.*, **38**, 1327–1340.

95 Gooday, G.W. (1990) The ecology of chitin decomposition. *Adv. Microb. Ecol.*, **11**, 387–430.

96 Tsujibo, H., Kubota, T., Yamamoto, M., Miyamoto, K., and Inamori, Y. (2003) Characterization of chitinase genes from an alkaliphilic actinomycete, *Nocardiopsis prasina* OPC-131. *Appl. Environ. Microbiol.*, **69** (2), 894–900.

97 Kunz, C., Ludwig, A., Bertheau, Y., and Boller, T. (1992) Evaluation of the antifungal activity of the purified chitinase I from the filamentous fungus *Aphanocladium album*. *FEMS Microbiol. Lett.*, **90**, 105–109.

98 Mathivanan, N., Kabilan, V., and Murugesan, K. (1998) Purification,

characterization, and antifungal activity of chitinase from *Fusarium chlamydosporum*, a mycoparasite to groundnut rust, *Puccina arachidis*. *Can. J. Microbiol.*, **44**, 646–651.

99 Ordenlich, A., Elad, Y., and Chet, I. (1988) The role of chitinase of *Serratia marcescens* in biocontrol of *Sclerotium rolfsii*. *Phytophathology*, **78**, 84–88.

100 Roberts, W.K. and Selitrennikoff, C.P. (1988) Plant and bacterial chitinases differ in antifungal activity. *J. Gen. Microbiol.*, **134**, 169–176.

101 Robbins, P.W., Albright, C., and Benfield, B. (1988) Cloning and expression of a *Streptomyces plicatus* chitinase (Chitinase-63) in *Escherichia coli*. *J. Biol. Chem.*, **263**, 443–447.

102 Miyashita, K., Fujii, T., and Sawada, Y. (1991) Molecular cloning and characterization of chitinase genes from *Streptomyces lividans* 66. *J. Gen. Microbiol.*, **137**, 2065–2072.

103 Dahiya, N., Tewari, R., Tiwari, R.P., and Hoondal, G.S. (2005) Chitinase from *Enterobacter* sp. NRG4: its purification, characterization and reaction pattern. *Electron. J. Biotechnol.*, **8** (2), 134–145.

104 Chang, W.T., Chen, C.S., and Wang, S.L. (2003) An antifungal chitinase produced by *Bacillus cereus* with shrimp and crab shell powder as carbon source. *Curr. Microbiol.*, **47**, 102–108.

105 Singh, G., Dahiya, N., and Hoondal, G.S. (2005) Optimization of chitinase production by *Serratia marcescens* GG5. *Asian J. Microbiol. Biotechnol. Environ. Sci.*, **7**, 383–385.

106 Mukherjee, G. and Sen, S.K. (2006) Characterization and identification of chitinase producing *Streptomyces venezuelae* P10. *Indian J. Exp. Biol.*, **42**, 541–544.

107 Williams, C.M., Richter, C.S., MacKenzie, J.M., and Shih, J.C.H. (1990) Isolation, identification, and characterization of a feather-degrading bacterium. *Appl. Environ. Microbiol.*, **56**, 1509–1515.

108 Kim, J.M., Lim, W.J., and Suh, H.J. (2001) Feather-degrading *Bacillus* species from poultry waste. *Process Biochem.*, **37**, 287–291.

109 Dozie, I.N.S., Okeke, C.N., and Unaeze, N.C. (1994) A thermostable alkaline active keratinolytic proteinase from *Crysosporium keratinophylum*. *World J. Microbiol. Biotechnol.*, **10**, 563–567.

110 Wawrzkiewicz, K., Wolsky, T., and Lobarzewsky, J. (1991) Screening the keratinolytic activity of dermatophytes *in vitro*. *Mycopathologia*, **14**, 1–8.

111 Friedrich, A.B. and Antranikian, G. (1996) Keratin degradation by *Fervidobacterium pennavorans*, a novel thermophilic anaerobic species of the order Thermatogales. *Appl. Environ. Microbiol.*, **62**, 2875–2882.

112 Lee, H., Suh, D.B., Hwang, J.H., and Suh, H.J. (2002) Characterization of a keratinolytic metalloprotease from *Bacillus* sp. SCB-3. *Appl. Biochem. Biotechnol.*, **97**, 123–133.

113 Williams, C.M., Lee, C.G., Garlich, J.D., and Shih, J.C.H. (1991) Evaluation of bacterial feather fermentation product, feather-lysate as a feed protein. *Poult. Sci.*, **70**, 85–94.

114 Bertsch, A. and Coello, N. (2005) A biotechnological process for treatment and recycling poultry feathers as a feed ingredient. *Biores. Technol.*, **96**, 1703–1708.

115 Chandrasekaran, S. and Dhar, S.C. (1986) Utilization of a multiple proteinase concentrate to improve the nutritive value of chicken feather meal. *J. Leather Res.*, **4**, 23–30.

116 Dhar, S.C. and Sreenivasulu, S. (1984) Studies on the use of dehairing enzyme for its suitability in the preparation of improved animal feed. *Leather Sci.*, **31**, 261–267.

117 Mukhopadhyay, R.P. and Chandra, A.L. (1990) Keratinase of a *Streptomycete*. *Indian J. Exp. Biol.*, **28**, 575–577.

118 Pfleiderer, E. and Reiner, R. (1988) Microorganisms in processing of leather, in *Biotechnology, Special Microbial Processes*, vol. 6b (ed. H.J. Rehm), Wiley-VCH Verlag GmbH, Weinheim, Germany, pp. 730–739.

119 Mukhopadhyay, R.P. and Chandra, A.L. (1993) Protease of keratinolytic *Streptomycetes* to unhair goat skin. *Indian J. Exp. Biol.*, **31**, 557–558.

120 Dekker, R. and Linder, W. (1979) Bioconversion of hemicellulose: aspects of hemicellulase production by *Trichoderma reesi* QM9414 and enzymic

saccharification of hemicellulose. *S. Afr. J. Sci.*, **75**, 65–71.

121 Chen, C., Adolphson, R., Dean, F., Eriksson, K., Adamas, M., and Westpheling, J. (1997) Release of lignin from kraft pulp by a hyper thermophilic xylanase from *Thermotoga maritima*. *Enzyme Microb. Technol.*, **20**, 39–45.

122 (a) Lee, Y., Lowe, S., and Zeikus, J. (1998) Molecular biology and biochemistry of xylan degradation by thermoanaerobes. *Prog. Biotechnol*, **7**, 275–288; (b) Kalegoris, E., Christakopoulos, D., Kekos, D., Macris, B. (1998) Studies on the solid-state production of thermostable endoxylanases from *Thermoascus aurantiacus*: characterization of two isoenzymes. *J. Biotechnol.*, **60**, 155–163.

123 Jacques, G., Frederic, D.L., Joste, L.B., Viviane, B., Bart, D., Fabrizio, G., Benoit, G., and Jean-marie, F. (2000) An additional aromatic interaction improves the thermostability and thermophilicity of a mesophilic family 11 xylanase: structural basis and molecular study. *Protein Sci.*, **9**, 466–475.

124 Kohilu, U., Nigam, P., Singh, D., and Chaudhary, K. (2001) Thermostable, alkaliphilic and cellulase free xylanases production by *Thermoactinomyces thalophilus* subgroup C. *Enzyme Microb. Technol.*, **28**, 606–610.

125 Bode, W. and Huber, R. (1992) Natural protein proteinase inhibitors and their interaction with proteinases. *Eur. J. Biochem.*, **204**, 433–451.

126 Imade, C. (2004) Enzyme inhibitors of marine microbial origin with pharmaceutical importance. *Mar. Biotechnol.*, **6**, 193–198.

127 Imade, C. (2005) Enzyme inhibitors and other bioactive compounds from marine actinomycetes. *Antonie Van Leeuwenhoek*, **587**, 59–63.

128 Fernandez, L.F.G., Reyes, F., and Puelles, J.M.S. (2002) The marine pharmacy: new antitumor compounds from the sea. *Pharmaceut. News*, **9**, 495–501.

129 Capon, R.J., Skene, C., Lacey, E., Gill, J.H., Wicker, J., Heiland, K., and Friedel, T. (2000) Lorneamides A and B: two new aromatic amides from a southern Australian marine actinomycete. *J. Nat. Prod.*, **63**, 1682–1683.

130 Maskey, R.P., Halmke, E., Kayser, O., Fiebig, H.H., Maier, A., Busche, A., and Laatsch, H. (2004) Anticancer and antibacterial trioxacarcins with antimalaria activity from a marine streptomycete and their absolute stereochemistry. *J. Nat. Prod.*, **57**, 771–779.

131 Georis, J., Giannotta, F., DeBuyl, E., Granier, B., and Frere, J. (2000) Purification and properties of three *endo*-beta-1,4-xylanases produced by *Streptomyces* sp. strain S38 which differ in their capacity to enhance the bleaching of kraft pulp. *Enzyme Microb. Technol.*, **26**, 177–183.

132 Stritzke, K., Schulz, S., Laatsch, H., Helmke, E., and Beil, W. (2004) Novel caprolactones from a marine streptomycete. *J. Nat. Prod.*, **67**, 395–401.

133 Lang, S., Beli, W., Tokuda, H., Wicke, C., and Lurtz, V. (2004) Improved production of bioactive glycosylmannosyl-glycerolipid by sponge-associated *Microbacterium* species. *Mar Biotechnol.*, **6**, 152–156.

134 Liu, R., Cui, C.B., Duan, L., Gu, Q.Q., and Zhu, W.M. (2005) Potent *in vitro* anticancer activity of metacycloprodigiosin and undecyprodigiosin from a sponge-derived actinomycete *Saccharopolyspora* sp. nov. *Arch. Pharm. Res.*, **28**, 1641–1344.

135 Manam, R.R., Teisan, S., White, D.J., Nicholson, B., Grodberg, J., Neuteboom, S.T., Lam, K.S., Mosca, D.A., Lloyd, G.K., and Potts, B.C. (2005) Lajollamycin, a nitro-tetraene spiro-beta-lactonegamma-lactam antibiotic from the marine actinomycetes *Streptomyces nodosus*. *J. Nat. Prod.*, **68**, 240–243.

136 Soria-Mercado, I.E., Prieto-Davo, A., Jensen, P.R., and Fenical, W. (2005) Antibiotic terpenoid chloro-dihydroquinones from a new marine actinomycete. *J. Nat. Prod.*, **68**, 904–910.

137 Jeong, S.Y., Shin, H.J., Kim, T.S., Lee, H.S., Park, S.K., and Kim, H.M. (2006) Streptokordin, a new cytotoxic compound of the methylpyridine class from a marine-derived *Streptomyces* sp. KORDI-3238. *J. Antibiot. (Tokyo)*, **59**, 234–240.

138 Montalvo, N.F. *et al.* (2005) Novel Actinobacteria from marine sponges. *Antonie Van Leeuwenhoek*, **87**, 229–236.

139 Hentschel, U. *et al.* (2006) Marine sponges as microbial fermenters. *FEMS Microb. Ecol.*, **55**, 167–177.

140 Piel, J. (2006) Bacterial symbionts: prospects for the sustainable production of invertebrate-derived pharmaceuticals. *Curr. Med. Chem.*, **13**, 39–50.

141 Kim, T.K. *et al.* (2006) Discovery of a new source of rifamycin antibiotics in marine sponge Actinobacteria by phylogenetic prediction. *Appl. Environ. Microbiol.*, **72**, 2118–2125.

142 Li, Z.Y. *et al.* (2006) Bacterial community diversity associated with four marine sponges from the south China Sea based on 16S rDNA-DGGE fingerprinting. *J. Exp. Mar. Biol. Ecol.*, **329**, 75–85.

143 Li, Z.Y. and Liu, Y. (2006) Marine sponge *Craniella australiensis* associated bacterial diversity revelation based on 16S rDNA library and biologically active actinomycetes screening, phylogenetic analysis. *Lett. Appl. Microbiol.*, **43**, 410–416.

144 Zhang, H. *et al.* (2006) Culturable Actinobacteria from the marine sponge *Hymeniacidon perleve*: isolation and phylogenetic diversity by 16S rRNA gene-RFLP analysis. *Antonie Van Leeuwenhoek*, **90**, 159–169.

145 Yakimov, M.M. *et al.* (2006) Phylogenetic survey of metabolically active communities associated with the deep-sea coral *Lophelia pertusa* from the *Apulian plateau*, Central Mediterranean Sea. *Deep-sea Res.*, **53**, 62–75.

146 Lampert, Y. *et al.* (2006) Diversity of culturable bacteria in the mucus of the Red Sea coral *Fungia scutaria*. *FEMS Microb. Ecol.*, **58**, 99–108.

147 Bull, A.T. (ed.) (2004) *Microbial Diversity and Bioprospecting*, ASM Press.

148 Smedsgaard, J. and Nielsen, J. (2005) Metabolite profiling of fungi and yeast: from phenotype to metabolome by MS and informatics. *J. Exp. Bot.*, **56**, 273–286.

149 Fenical, W. and Jensen, P.R. (2006) Developing a new resource for drug discovery: marine actinomycete bacteria. *Nat. Chem. Biol.*, **2**, 666–673.

150 Ward, A.C. and Bora, N. (2006) Diversity and biogeography of marine Actinobacteria. *Curr. Opin. Microbiol.*, **9**, 1–8.

151 Goldberg, S.M.D. *et al.* (2006) A Sanger/pyrosequencing hybrid approach to the generation of high-quality draft assemblies of marine microbial genomes. *Proc. Natl. Acad. Sci. USA*, **103**, 11240–11245.

152 Sogin, M.L. *et al.* (2006) Microbial diversity in the deep sea and the underexplored "rare biosphere". *Proc. Natl. Acad. Sci. USA*, **103**, 12115–12120.

153 Projan, S.J. (2003) Why is big pharma getting out of antibacterial drug discovery? *Curr. Opin. Microbiol.*, **6**, 427–430.

154 Fox, J.L. (2006) The business of developing antibacterials. *Nat. Biotechnol.*, **24**, 1521–1528.