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Bioactive Compounds from Marine Sponge Associates: Antibiotics from *Bacillus* Sp.

Lanka Sri Santhi[1*], Prasad Talluri VSSL[1,2], Nagendra SY[1] and Radha Krishna E[1]

[1]Department of Biotechnology, GITAM Institute of Science, GITAM University, Visakhapatnam-530 045, Andhra Pradesh, India
[2]Department of Biotechnology, College of Natural and Computational Science, University of Gondar, Gondar, Ethiopia

Abstract

Marine organisms like algae, invertebrates, microbes contain a wide range of bioactivity. Marine organisms associated microorganisms are bacteria, actinomycetes, fungi, yeast etc. The secondary metabolites from these organisms have recently gained importance in the pharmaceutical and pesticide industries. Reports of the marine organisms have proved that compounds isolated from them are potential in several fields particularly as new therapeutic agents for variety of diseases. These are potential in producing the bioactive compounds like antibiotics, metabolites, steroids, glycoprotein's etc; which are useful for the mankind. Marine natural products discovery includes the search for pharmaceuticals, enzymes, dietary supplements, and biopolymers. The marine environment is attractive as it holds promise as a source of entirely new bioactive compounds.

Keywords: Bioactive compounds; Marine sponges; Antibiotics; *Bacillus*; Microbes

Introduction

Bioactive compounds from marine resources

Marine natural products discovery includes the search for pharmaceuticals, enzymes, dietary supplements and biopolymers. The search for new pharmaceuticals is a US$20 billion effort per annum although only a small portion of these funds have been directed towards marine natural products discovery. The marine environment is attractive as it holds promise as a source of entirely new bioactive compounds. By isolating compounds from the marine environment, scientists may avoid the rediscovery problem that has been plaguing terrestrial natural product discovery.

Marine natural products are diverse and often difficult and expensive to synthesize. The amount of metabolite found in the source organism is rarely enough to get through clinical trials. Increasing the amount of compound by a massive harvest of the source organism is rarely a viable option because of the disastrous ecological impact. In the case of bryostatin, 13,000 kg of the bryozoan *B. neritina* had to be harvested in order to obtain only 18 g of bryostatin for clinical trials.

There is a true supply issue that needs to be overcome in order to meet the requirements of the demand for those compounds that become successful drugs. In some cases, it may be possible to chemically synthesize the compound but most of the time, the complexity of the molecules or the costs involved precludes this approach. Aquaculture is another option but it can be unreliable, and there are reports of diseases wiping out the entire production. In the event of the compound being produced by a microbe, isolation and culture of the bacteria can provide with a reliable source for the bioactive compound of interest and open a wide range of possible production improvement.

A huge number of bioactive compounds have been isolated from marine sponges and their associated microbes. The majority prolific marine producers of novel compounds are sponges, with more than 250 new metabolites reported every year [1]. The occurrence in unrelated sponges of structurally similar compounds, particularly those which were otherwise known exclusively from microorganisms, led to speculation that such compounds were of microbial origin [2-4]. Since chemical synthesis of natural products can be problematic and expensive due to their structural complexity [5-7] the realization that at least some compounds may be produced by microbes raised hopes of obtaining a sustainable, essentially unlimited supply of compounds for testing and subsequent drug production (e.g., via cultivation of the relevant bacteria) [4,8]. The possibility of convergent evolution of biosynthetic pathways among different sponges has also been raised [9].

Sponge (or microbe)-derived compounds span a wide range of chemical classes (e.g., terpenoids, alkaloids, peptides, and polyketides) with an equally wide range of biotechnologically relevant properties (e.g., anticancer, antibacterial, antifungal, antiviral, anti-inflammatory and antifouling) [1,4,10-14]. The attention of natural product chemists and pharmaceutical companies, at present, is focused firmly on anticancer drugs, with several promising sponge-derived compounds in clinical and preclinical cancer trials [15,16]. Biologically active natural products are often produced in relatively small amounts, and often by rare animals whose natural populations cannot sustain the extensive collections required for clinical trials. Alternative means for producing large amounts of metabolites are therefore required.

Supply issues notwithstanding, the pharmacological potential of marine sponges and other sessile invertebrates (e.g., corals, bryozoans, and ascidians) are enormous. Although progress toward the commercial product stage has been slow, it is highly likely that at least one of the several compounds now in clinical trials (or a synthetic analog) will be commercialized within the next few years.

A combination of improved chemical synthesis methods with the various approaches outlined in the following section should ensure a bright future for this field, with sponge-derived natural products being utilized either in their natural form or as inspiration for new, laboratory-generated compounds (e.g., via chemical proteomics) [17]. Marine sponges are rich sources of a diverse array of bioactive compounds with great potential for development as drugs. More than 10,000 metabolites

***Corresponding author:** Lanka Sri Santhi, Department of Biotechnology, GITAM Institute of Science, GITAM University, Visakhapatnam-530 045, Andhra Pradesh, India, Tel: +918019155333; Fax: +918912790399; E-mail: srishanthichowdary@gmail.com

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have been recovered from marine sources, the large majority of which originate from sponges. Sponges are soft-bodied and sessile with no apparent physical defensive mechanism thus having come to utilize/ produce secondary metabolites to overcome predation and competition. Sponges have provided not only the best source of novel compounds but also the greatest occurrence of potential pharmaceuticals. Sponges harbor microorganisms that include bacteria, cyanobacteria, Yeast and fungi. These micro organisms growing on the surfaces of the sponges as epibionts (which live in a highly competitive environment (or) growing inside the body of sponges as endobionts may also produce secondary metabolites which inhibit the settlement of potential competitors, such as invertebrate lame, and can antagonize other bacteria. It has been suggested that growth of these useful microorganisms may be under the control of the sponge host. Sponge associated microorganisms are therefore attracting attention as a source of new natural products. There is accumulating evidence that demonstrates the involvement of associated microorganisms in the second metabolism that was originally attributed to the sponge host.

Sponges and associated microbes as sources of bioactive compounds

Sponges have long been familiar organisms and were used commonly during antiquity by Romans and Greeks. They used *Spongia officinalis adriatica* essentially for bathing purposes but ancient Greek soldiers also used sponges as lining for their armor and helmets. There are records of sponges being used by Arabic Physicians as early as 932 A.D. Sponges soaked with narcotic drugs were placed over the patient's nose to provide a state of anesthesia.

A sponge can be defined as a sedentary, filter-feeder metazoan that has neither organs nor true tissues. Sponges are organized around a system of pores, Ostia, canals and chambers that are used to canalize the large flow of water that is pumped through sponges. The water enters the sponge through the inhalant canals and exits by the Oscules. It is thought that the ancestors of sponges included Protozoan's such as Choanoflagellates, amoeboid cells and siliceous- or calcareous-producing organisms that provided the skeletal material. Colonies of these protozoan ancestors would have exchanged their genetic material to eventually result in the first Precambrian sponge.

Sponge microbiology

Sponges have developed a complex association with a very diverse range of microbes including bacteria, cyanobacteria, dinoflagellates, diatoms and archaea. Although the proportion of sponge-associated bacteria can vary dramatically from sponge to sponge, bacteria can occupy up to 60% of the sponge volume, being densely packed in the intercellular and intracellular matrix. In the sponge *Lamellodysidea herbacea*, *Oscillatoria spongelliae*, a cyanobacterial symbiont, accounts for 50% of the sponge cellular volume and in a *Xestospongia* sp. Sponge from the Indian Ocean, eubacteria represented as much as 56% of the sponge biomass. The large number of bacteria present in the sponges often exceeds the amount of bacteria present in the water column by one to three orders of magnitude [18].

The culture-based studies from environmental samples are known for their limitations because of "the great plate count anomaly" [19]. The colonies that are easily grown on plates usually represent less than 1% of all microbial cells present in the sample. Though for sponges there are some rare examples, like in the case of the sponge *Ceratoporella nicholsoni*, where 3 to 11% of the total bacteria were cultivable, cultured sponge-associated bacteria generally follow the norm by representing less than 1% of the total sponge microbial cells being recovered as culturable colonies [20]. This last decade saw molecular techniques being applied to the exploration and understanding of sponge-associated microbial communities. The use of community analysis 16S rRNA gene sequencing has overcome limitations associated with culture-based community studies and provided a detailed analysis of the sponge microbial community, revealing the presence of bacterial species never suspected before. The first use of this culture independent technique on the marine sponge *Discodermia* spp. revealed the presence of novel gamma-proteobacteria and other uncultivated strains. Another pioneer study using a similar approach was done on the Great Barrier Reef sponge *Rhopaloeides odorabile* and showed the remarkable microbial diversity in this sponge.

Members of several bacterial classes constitute the microbial community of *R. odorabile* among which beta-proteobacteria, gamma-proteobacteria, *Cytophaga/ Flavobacterium*, *Actinobacteria* as well as euryarcheotas and crenarchaeotes [20]. A comparative study of the microbial communities of the geographically distant sponges *Aplysina aerophoba*, *Theonella swinhoei* and *R. odorabile* suggested that there is a uniform microbial community among sponges from different oceans. It is important to emphasize that sponge-associated microbial communities have been shown to be very diverse and very different from those of the surrounding water column.

Microbial sources of antibiotics

Majority of the antibiotics isolated were obtained from bacteria and fungi. But, during the years 1950-1960 about 75% were isolated from actinomycetes [21]. A review made by Rao et al. [22], lists 1,719 antibiotics of which 1,160 are produced by bacteria. The number of these antibiotics rapidly increased in subsequent years. A large number of antibiotics have been reported in the literature. The number of known antibiotics reported till 2016 from different microbial sources is shown in the Table 1.

Screening of substrates for the isolation of microorganisms

There are several natural substrates available as ideal sources for the isolation of *Bacillus subtilis* such as 1) Soils, comprising virgin and cultivated, garden, field and forest soils as well as drained peat bogs 2) Sea sediments and seawater 3) Fresh water basins, comprising lake and river waters and bottoms 4) Manures and composts 5) The atmosphere 6) Food products including milk 7) The bodies of plants: some find in and upon the plant as temporary or permanent habitat; others are able to cause diseases of plants, 8) The bodies of man and animals, especially the digestive system, and 9) Geological formations.

Bacillus subtilis: *Bacillus subtilis* are Gram-positive that are rod-shaped bacteria with rigid cell wall [23]. *B. subtilis* has been classified as srticlty obligate aerobe, but recent studies have confirmed that this is not strictly right [24]. The rigid cell wall is used for to maintain shape of the cell and protects the cell from high internal turgor pressure

Producing Organism	Approximate number	Percentage
Bacteria	850	9
Actinomycetes	4200	45
Fungi	1450	16
Higher Organisms		
Lichens	100	1
Algae	100	1
Higher Plants	2100	22
Animal organism	500	5
Total	**9400**	**100**

Table 1: Sources and the number of known antibiotic compounds.

[25]. Neither it does not hydrolyze phospholipids nor casein; it does hydrolyze triglycerides. It produces citrate permease and cytochrome c.

Bacillus subtilis bacteria use their flagella for a swarming motility. This motility occurs on surfaces, for example on agar plates, rather than in liquids. Many strains produce spores with brown pigments. Depletion of carbon, nitrogen, or phosphorous causes the process of sporulation to begin, however, the process needs to start before the entire exhaustion of nutrients [23]. Otherwise, the spore formation cannot be completed due to the fact that the nutrients are too low for the energy-requiring sporulation process. This allows the cells to avoid being stuck in a vulnerable position.

Bacillus subtilis has single circular chromosome, total size of the entire DNA is 4,214,814 bp (4.2 Mbp). A large section of the genome corresponds application of carbon source applications [26]. Out of 4,100 genes 192 are considered essential, and an additional 79 are thought to be necessary. Most of the important genes are involved in metabolism. 4% of these important genes, functions that are not known [27]. Five signal peptidase genes are responsible for secreting antibiotics in large amount to the exterior of the cell of *Bacillus subtilis* [26,28]. *Bacillus subtilis* bacteria secrete enzymes, "such as amylase, protease, pullulanase, chitinase, xylanase, lipase, among others. These enzymes are produced commercially and this enzyme production represents about 60% of the commercially produced industrial enzymes" [29].

Glycerol is the best carbon source as well as L-glutamic acid is the most selective source of nitrogen. The bacitracin antibiotic was found to be affective on Gram-positive bacteria [30]. Other antibiotics that *Bacillus subtilis* form are polymyxin, difficidin, subtilin, and mycobacillin. Polymyxin is affective against Gram-negative bacteria, whereas difficidin has a broader spectrum.

Antibiotics are mostly poly peptides [31,32]. Several peptide antibiotics produced by *Bacillus* are found to be active against Gram positive bacteria [33]. The clinically important antibiotics from the Bacillus species are shown in Table 2. Industrial enzymes produced by *Bacillus* are subtilisins, cellulases and amylases which are widely used in detergents and leather industries [34]. Some enzymes isolated from *B. subtilis* widely used in dairy products by neutral proteases [35]. *B. subtilis* is also used for production of amino acids [36].

Antibiotics

The antibiotics are the most prescribed drugs in human medicine, are also widely used in agriculture, animal husbandry and the food industry. The discovery of antibiotics and other bioactive secondary metabolites produced by microorganisms revolutionized the field of medicine. Several of the most efficacious agents are also antibiotics.

The universal use of antibiotics to treat infectious diseases has resulted in an enormous improvement in human health and has saved millions of lives. The word "antibiosis" was termed in 1889 by Paul Vuillemin to describe a type of association in which one living creature was destroyed by another in order to sustain its own life. The term 'antibiotic' was derived from antibiosis, a phenomenon of antagonism among living organisms [37]. Though this phenomenon was noticed by Pasteur and Joubert in 1877, it was Waksman who introduced the word 'antibiotic' in 1942 and defined it as "a chemical substance derived from microorganisms which has the capacity of inhibiting growth, or even destroying other microorganisms in dilute solutions". Mascherpa proposed that "Antibiotics are substances spontaneously produced by living organisms or synthetically obtained, but with analogous structure to that of natural products, endowed with selective antibacterial action through antimetabolic mechanisms". Umezawa suggested the inclusion of substances not only of microbial origin but also those produced by higher forms of life and action should not be limited to microbes, but also include tumors. Different ecological functions like, competitive interactions, morphological differentiation and selective advantage to the producer organism [38] have also been attributed to the antibiotic substances. More recently, Behal defined antibiotics as microbial products that inhibit the growth of other microorganisms [39]. In general, antibiotics are characterized by the following two properties: (i) they are biologically active against organisms sensitive to them i.e., they have high physiological effect even in low concentrations and (ii) their action is selective, which means that each antibiotic is potent biologically only against certain organisms, or groups of organisms, without affecting appreciably other living organisms.

Since the discovery of penicillin in 1929 by Alexander Fleming, more than 6000 antibiotics with different specificities and modes of action have been isolated and find applications in different fields of medicine, animal husbandry, agriculture and in molecular biology.

The antibiotics show a wide variety of physical, chemical and antimicrobial activities and also vary in their toxicity to different animals. Though it is extremely difficult to classify them based on the above activities, three major classifications were proposed basing on the following features:

i) Biological systems (the organisms that form antibiotics)

ii) Antimicrobial profile

iii) Chemical system.

On the basis of the general similarity of their structure, antibiotics were classified into 8 classes [40-42]. According to this classification, clinically useful antibiotics may be grouped as under:

1. Penicillin and related antibiotics: These antibiotics have a β-lactum ring in their chemical structure.

e.g., penicillin's, semi synthetic penicillin's, cephalosporin's.

2. Aminoglycoside antibiotics: These antibiotics have amino sugars in glycosidic linkage.

e.g., streptomycin's, neomycin, kanamycin, paromomycin, gentamycin, tobramycin, amikamycin.

3. Macrolide antibiotics: These antibiotics have a macrocyclic lactone ring to which sugars are linked.

e.g., Erythromycin, oleandomycin, spiramycin.

4. Tetracycline antibiotics: These antibiotics are derivatives of the polycyclic naphthacene carboxamide.

e.g., Tetracycline, chlortetracycline, oxytetracycline, demeclocycline and minocycline.

5. Peptide antibiotics: These antibiotics have peptide linked amino acids which include both D-and L-forms.

e.g., Bacitracin, gramicidin and polymyxins.

6. Antifungal antibiotics: this group has been divided into two main sub-groups.

i) Polyenes containing a large ring with a conjugated double bond system. e.g., Nystatin and amphotericin B.

ii) Other antifungal antibiotics. e.g., 5-fluorocytosine, clotrimazole and griseofluvin.

7. Chloramphenicol: Nitrobenzene derivative of dichloro acetic acid.

S No	Antibiotic	Producer Organism	Sensitive organisms	Mode of action
1	Polymyxin, Difficidin, Subtilin, Mycobacillin, Bacitracin	*Bacillus subtilis*	Gram positive bacteria	Cell wall synthesis
2	Cerexin, Zwittermicin	*Bacillus cereus*	Gram positive bacteria	Cell wall synthesis
3	Gramicidin, Tyrothricin	*Bacillus brevis*	Gram positive bacteria	Ribosome biosynthesis
4	Circulin	*Bacillus circulans*	Gram negative bacteria	Cell wall synthesis
5	Laterosporin	*Bacillus Laterosporus*	Gram positive bacteria	Cell wall synthesis
6	Bacitracin	*Bacillus Licheniformis*	Gram positive bacteria	Blocks peptidoglycan synthesis
7	Polymyxin, Colistin	*Bacillus polymyxa*	Gram negative bacteria	Cell membrane
8	Pumulin	*Bacillus pumilus*	Gram positive bacteria	Cell wall synthesis
9	Bacillomycin, Mycobacillin, Fungistatin	*Bacillus amyloliquefaciens*	Molds and Yeast	Protein synthesis

Table 2: Some clinically important antibiotics from *Bacillus* species.

8. Unclassified antibiotics: These have varied structures and have not been grouped among the main groups listed above. e.g., Cycloserine, fusidic acid, novobiocin, prasinomycin, spectinomycin and vancomycin.

Conclusion

Various microorganisms are capable of producing antibiotics but, bacteria hold an important position due to their diversity and capability to produce novel antibiotics for pharmaceutical significance. The majority of the antibiotics in clinical use today are produced by bacteria. There is a lacuna of appropriate antibiotics for treatments of several pathogenic infections. Additionally, the increased development of resistant pathogens results in searching of new secondary metabolites with new structures, new organisms and new resources in the regular screening protocols.

Ocean remains as an unexploited source for many drugs and pharmacologically active substances. It is now known firmly that marine microorganisms are capable of producing unusual natural products. Many of these compounds have antibiotic and other useful activities and it is obvious that the systematic study of marine microorganisms as sources of bioactive substances can be potentially promising.

Very few reports are available concerning the presence of bacteria in marine sediments and marine water. One of the most successful approaches to obtain new types of useful microbial metabolites is investigation of rare, slowly growing or until now neglected microorganisms from marine sediments etc., where the different ecological conditions and requirements may have produced different types of activities in contrast to organisms, occurring in terrestrial environment. During the past 20 to 30 years, several novel compounds have been isolated from marine organisms and many of these have been reported to have biological activities, some of which are of interest from the point of view of potential drug development. So far, more than 300 new unique antimicrobial and antitumor compounds have been isolated from marine microorganisms.

Discovery of efficient microbial associates capable of producing biotherapeutic molecules has opened up a new era in marine biotechnology. Adopting different cultivation strategies and metagenomic approaches would be the need of the hour in discovering new genes, enzymes and natural products and in enhancing the commercial production of marine drugs.

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