Rajiv Gandhi Chair Special Publication - 2008

GLIMPSES OF AQUATIC BIODIVERSITY

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Published by

RAJIV GANDHI CHAIR IN CONTEMPORARY STUDIES
SCHOOL OF ENVIRONMENTAL STUDIES
COCHIN UNIVERSITY OF SCIENCE AND TECHNOLOGY
Kochi - 22

Glimpses of Aquatic Biodiversity - Rajiv Gandhi Chair Spl. Pub., 7 : 49 - 57 (2008).

BIODIVERSITY OF DRUG-BEARING MARINE ORGANISMS: HARVESTS, CONSERVATION CONCERNS AND SUSTAINABLE MANAGEMENT

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Nearly 75% of the populations in under- developed countries depend on plant-based medicines. According to the WHO, this dependence is due to either economic or cultural reasons. Of late, there is growing awareness of the societal benefit of effective drug therapy to treat various existing and emerging human diseases. It is expected that treatment will improve and also accessible to the world's population. This expectation is predicated on a continued and determined effort by academic scientists, researchers and private industries to discover new and improved drug therapies. Natural products have had a crucial role in identifying novel chemical entities with useful drug properties (Newman *et al.*, 2000).

The ocean covers more than 70% of the earth's surface and contains about 300,000 described species of plants and animals. The tropical marine habitats are rich sources of biological and chemical diversity. In the marine environment, the bioactive compounds, medicines, drugs, marine natural products are produced as secondary or non-primary metabolites. Natural products chemists considered them as sources of new and unusual organic substances. Synthetic chemists targeted these structures for development of new analogues. Pharmacologists considered this for treatment of human/agricultural diseases and pests. Hence marine organisms are targeted for developing new medicines.

From the relatively small number of marine plants, animals, and microbes more than 12,000 novel chemicals could be isolated. It is well known that the ocean provides several types of unique and potential environments such as extreme pressure, salinity, temperature and their combinations. Considering the vast resources, marine natural products chemists from several countries, in collaboration with both academic pharmacologists and the pharmaceutical industry, have reported several novel metabolites with useful and sensational pharmacological properties. A number of clinically useful drugs, investigational drug candidates, and pharmacological tools have already resulted from marine-product discovery programmes. Some of them are listed in Table1.

When it comes to the patented drug format, the process appears to be slower (Faulkner, 2000). It is generally argued that pharmacological research involving marine organisms is intrinsically slower. It has disadvantages compared with a program based on synthesis in spite of the number and quality of the leads generated. One of the problems is the bulk supply of source organisms. There are differences in activity due to habitat and seasonal changes are also reported. Some of the marine-derived compounds licensed for development are given in Table 2.

Drug/Biomedical Potential Offered by Marine Organisms

In order to evaluate the biomedical potential of any plant or animal, one must consider both the chemical ecology of the organism and its evolutionary history. It is probable that chemical defense mechanisms evolved with the most primitive microorganisms but have been replaced in manymore advanced organisms by physical defenses and/or the ability to run or swim away and hide. Sessile, soft-bodied marine invertebrates that lack obvious physical defenses are therefore

Table 1: *Commercially Available Marine Bioproducts (Modified from Pomponi,1999)*

Product	Application	Original Source
Pharmaceuticals		
Ara-A (acyclovir)	Antiviral drug (herpes infections)	Marine sponge, *Cryptotethya cryta*
Ara-C (cytosar-U, cytarabine)	Anticancer drug (leukemia and non-Hodgkin's lymphoma)	Marine sponge, *Cryptotethya cryta*
Molecular Probes		
Okadaic acid	Phosphatase inhibitor	Dinoflagellate
Manoalide	Phospholipase A_2 inhibitor	Marine sponge, *Luffariella variabilis*
Aequorin	Bioluminescent calcium indicator	Bioluminescent jellyfish, *Aequora victoria*
Green fluorescent protein (GFP)	Reporter gene	Bioluminescent jellyfish, *Aequora Victoria*
Enzymes		
Vent and Deep Vent DNA polymerase (New England BioLabs)	Polymerase chain reaction enzyme	Deep-sea hydrothermal vent bacterium
Nutritional Supplements		
Formulaid (Martek Biosciences)	Fatty acids used as additive in infant formula nutritional supplement	Marine microalga
Pigment		
Phycoerythrin	Conjugated antibodies used in ELISAs and flow cytometry	Red algae
Cosmetic additives		
Resilience (Estée Lauder)	"Marine extract" additive	Caribbean gorgonian, *Pseudopterogorgia elisabetha*

prime candidates to possess bioactive metabolites. If it is assumed that secondary metabolites, evolved from primary metabolites in a random manner, any newly produced secondary metabolite that offered an evolutionary advantage to the producing organism would contribute to the survival of the new strain. The specific evolutionary pressures that led to chemically rich organisms need not be defined but the longer the period of evolution, the more time the surviving organism has had to perfect its chemical arsenal. Sessile marine invertebrates have a very long evolutionary history and have had ample opportunity to perfect their chemical defenses. Chemical defense mechanisms cannot be directly equated with potential biomedical activity, but it is remarkable how well the two correlate in reality. This could be explained by the fact that targets of the chemical defenses, primary metabolites such as enzymes and receptors are highly conserved compared with secondary metabolite.

Among the many phyla found in the oceans, the best sources of pharmacologically active compounds are bacteria (including cyanobacteria), fungi, certain groups of algae, sponges, soft corals and gorgonians, sea hares and nudibranchs, bryozoans, and tunicates. Some marine organisms such as dinoflagellates, echinoderms and some fish, are well- know for their ability to produce potent toxins, but these are usually too toxic for medicinal use.

Marine Microbes

Microorganisms are not only the causative agents of infection, but they also produce substances that can cure infections (Example: Discovery of Penicillin in 1929). After the discovery of Penicillin, about 50,000 natural products are discovered from microorganisms. More than 10,000

Table 2: *Marine-Derived Compounds Licensed for Development.*

Marine Source	Drug	Organism	Current Status
Sponge	Discodermolide	*Discodermia dissoluta*	licensed to Novartis
	Isohomo-halichondrin B	*Lissodendoryx* sp.	Licensed to PharmaMar S.A.; in advanced preclinical trials
	Bengamide	*Jaspis* sp.	Synthetic derivative licensed to Novartis; in clinical trials
	Hemiasterlins A & B	*Cymbastella* sp.	licensed to Wyeth-Ayerst
	Girolline	*Pseudaxinyssa cantharella*	Licensed to Rhone Poulenc
Bryozoan	Bryostatin 1	*Bugula neritina*	In Phase I/II clinical trials in U.S./Europe; U.S. National Cancer Institute (NCI) sponsored trials
Sea hare	Dolastatin 10	*Dolabella auricularia*	Phase I clinical trials in U.S. NCI sponsored trials
Tunicate	Ecteinascidin 743	*Ecteinascidia turbinata*	Licensed to PharmaMar S.A.; in Phase III clinical trials in Europe and in U.S.
	Aplidine	*Aplidium albicans*	In Phase II clinical trials; licensed tc PharmaMar, S.A.
	Isogranulatimide	*Didemnum granulatum*	Licensed to Kinetik, Canada
Gastropod	Kahalalide F	*Elysia rubefescens*	In Phase I clinical trials; licensed to PharmaMar, S.A.
Actinomycete	Thiocoraline	*Micromonospora marina*	Licensed to PharmaMar, S.A.; in advanced preclinical trials

products are biologically active and more than 8,000 are antibiotics. From generations, it is known that seawater has bactericidal properties. This could be attributed to the production of antibiotics by planktonic algae and bacteria. Rosenfeld and Zobell in 1947 showed that bacteria produce antimicrobial products. Marine microorganisms encompass a complex and diverse assemblage of microscopic life forms and occur throughout the oceans. They are recorded from environments of extreme pressure, salinity and temperature. They developed unique metabolic and physiological capabilities that not only ensure survival in extreme habits, but also produce novel metabolites which are not generally encountered from terrestrial microbes.

According to Gerwick *et al.* (2001) the greatest untapped source of novel bioproducts is marine microorganisms. Even with new techniques, it is noted that less than 1.0% of the total marine microbial species diversity can be cultured with the common methods. This means chemicals produced by the rest of 99.0 % of the microorganisms in the ocean have not yet been studied for potential commercial applications. Thus, these organisms constitute an enormous un-tapped resource and opportunity for discovery of new bioproducts with applications in medicine, industry, and agriculture. In research and education special emphasis has to be given to develop solutions for the identification, culture, and analysis of uncultured marine microorganisms.

Bacteria: Highly brominated pyrrole antibiotic was the first documented bioactive metabolite isolated from bacteria in 1966. The genera *Alteromonas* was found to be the producer (formerly: *Pseudomonas bromoutilis*) and was isolated from the sea grass (*Thalassia* sp). The compound was highly active against Gram positive, but not against Gram negative bacteria with MIC ranging from 0.0063 to 0.2 μ/ml. Antibiotic-producing purple pigmented Chromobacterium (*Alteromonas* sp.) was isolated from North Pacific in 1977 and since then several antimicrobial compounds such as pyrrole, tetra bromo pyrrole, hexa bromo 2-2 bipyrrole and phenolics were isolated. The bioactive metabolite producing bacteria evolved the ability to concentrate the bromide ion from seawater and through oxidative pathways, this is incorporated into organic compounds. This results in bromination of metabolites with enhanced bioactivities.

Symbiotic bacteria: Marine bacteria form highly specific, symbiotic relationship with marine plants and animals. The Paralytic Shellfish Poison (PSP), or Saxitoxin (STX) is found to be produced by some strains of *Moraxella* sp. In Trivandrum, Kerala during 1997 eight people died due to PSP. Metabolites from marine sponges could also be correlated with the symbiotic bacterial association.

Marine macroalgae: Basic information on the availability of different algae along the coasts of India with their secondary metabolite capabilities are being investigated in the author's research group. The responses of the fish/shellfish towards macroalgal extracts were tested in CMFRI laboratory (Marine Biotech Lab, Vizhinjam, Trivandrum) and now in the Centre for Marine Science & Technology, M.S. University. Some of the salient findings are given in Table 3.

Seagrasses: The first documented identification of a bioactive marine bacterial metabolite was the highly brominated pyrrole antibiotic, isolated by Burkholder and co-workers from a bacterium obtained from the surface of the Caribbean Sea grass. The sea grass ecosystems provide good habitat for several biota.

Marine sponges: In sponges, the secondary metabolites are synthesized to protect themselves and to maintain homeostasis. The wider biosynthetic capability of sponges could be attributed to their biological association with other symbionts. About 38% of the sponge body comprises of microorganisms. Antitumor compounds Spongothymidine (arabinosyl thymine; ara T and spongouridine (arabinosyl uracil; ara U) Isolated from Caribbean sponge *Tethya crypta* led to synthesis of arabinosyl nucleoside analogs. The ara-C (arabinosyl cytosine)displayed in vivo antileukemic activity. This is used in treating acute myelocytic leukemia and non-Hodgkin's lymphoma. A major problem with the early studies on sponge-microbe symbiosis was that most microorganisms were uncultivable, so descriptions of symbioses usually relied either on morphology of symbionts or chemical measurements of nutrient transfer. The ecological relevance of symbiosis could not be determined. In the author's laboratory, a few studies reveled the potentials of utilizing them against fish pathogenic bacteria (Table 4).

Sea Fans / Gorgonids

History reminds us about the "Nautical gold rush" in 1970's to fish out the sea fans due to the availability of prostaglandin. Pseudopterosins (Diterpene ribosides A and E) are isolated from the sea fans *Pseudopterogorgia bipinata* and *P. elisabethae*. Pseudopterosin A possessed potent anti-inflammatory and analgesic activities. It was shown to inhibit pancreatic PLA2 and to effect both the cyclooxygenase and lipoxygenase pathways. Pseudopterosin E exhibited superior anti-inflammatory agents that, unlike pseudopterosin A, and were nontoxic at 300 mg/kg in mice. A partially purified extract of *P. elisabethae* is used as an additive in cosmetic products by Estee Lauder. The Lophotoxin (diterperne lactone) was isolated from gorgonian coral, *Lophogorgia* sp. This paralytic toxin produces an irreversible postsynaptic blockage at neuromuscular junctions.

Corals

Coral reefs are among the most diverse and valuable ecosystems on earth. The benthic coral reef invertebrates contain a number of unusual, biologically active metabolites with important medical, agricultural, and industrial uses-including recent applications in bone grafting, skin-care products, and bioremediation projects-as insecticides and as potential treatments for cancer and microbial infections. Although many compounds of value have already been identified, it is estimated that less than 10% of reef biodiversity is known, and only a small fraction of these species have

Table 3: *Marine macroalgale and their potential in inhibiting the growth of fish pathogens*

Species	Group	*Aeromonas*	*Vibrio*
Chaetomorpha antennina	Green	+	++
Ulva fasciata	Green	+++	+++
Caulerpa peltata	Green	+	-
Caulerpa scalpelliformis	Green	+++	-
Valoniopsis pachyrema	Green	++	-
Laurencia gracilis	Red	+	++
Hypnea musciformis	Red	+++	+++
Gracilaria corticata	Red	++	-
Gracilaria fergusonii	Red	++	-
Gracilaria foliifera	Red	++	++
Laurencia poitei	Red	+++	-
Asparagopsis taxiformis	Red	+	+
Chnoospora maxima	Brown	++	-
Stoechospermum marginatum	Brown	+++	-
Sargassum ilicifolium	Brown	+	-
Sargassum wightii	Brown	+	++
Padina tetrastromatica	Brown	-	++

.been explored as a source of biomedical compounds. New avenues for the commercial development of marine-derived compounds may further enhance the use of coral reef resources and contribute to the global economy.

Concerns on harvest and sustainable management

Increased harvest pressure is being placed on some of the marine drug bearing resources to supply growing international demand for Bioprospecting for novel products and novel genes. Although several species have yielded potential therapeutic agents, concern about adequate supply for preclinical and clinical studies is a critical issue in the development of new biomedical products. Many of the suitable species have a limited distribution or occur at a low biomass. Also, individuals often contain only trace amounts of the desired compounds; the low yield requires the harvest of substantial biomass, which may lead to depletion of natural populations. Many species extinctions are predicted in the coming decades in response to increasing pressure from human activities and natural disturbances, and the pharmacological potential of such organisms including coral reefs may be lost. The continued, largely unregulated, and unsustainable extraction of such species may have consequences that extend far beyond the overexploitation of these organisms, as their removal may also affect associated species and communities, ecological processes, and even entire ecosystems that are critical to the overall health of the oceans.

With the enormous potential for discovery, development, and marketing of novel marine byproducts, there is obligation to also to devise methods for supplying these products without disrupting the ecosystem or depleting the resource. Supply is a major limitation in the development of marine bioproducts. After the specific activity screening for unique or potentially useful biologicals, the source materials are to be collected in bulk to isolate and characterize the active compounds. For example, in the eighties, several truck loads of gorgonids were removed from the Southern coasts of India and exported. Specific large scale exploitation of sacred chanks (*Xacncus* = *Turbinella pyrum*) and sea horses (*Hippocampus sp*) were reported by the author (Lipton and Thangaran, 2002; Lipton *et al.*,1993; 1996).

Table 4: *Potentials of sponge extracts towards fish pathogenic bacteria*

Sponge species	Fish pathogenic bacterial strains			
	Aeromonas hydrophila	*Pseudomonas aeruginosa*	*Vibrio fischeri*	*Vibrio* Isolate(S-1)
Dendrilla nigra	++	++	++	++
Axinella donnani	+++	++	++	+
Clathria gorgonoides	-	-	-	-

As given earlier, the purified extract of the sea fan *P. elisabethae* is used as an additive in cosmetic products by Estee Lauder. This commercial development resulted in the harvesting of more than 4.54 tonnes of gorgonians, has provided the first real recorded test of large scale harvesting of a marine invertebrate. The gorgonians, which occurred between 15 and 25 meters depth, were pruned by hand along an approximately 60-mile length of the Bahamas coastline. Diving is limited to about 20 meters allowing the deep water specimens to provide a reservoir of breeding stock. Studies indicate that re growth occurs in about 18 months and the test population of gorgonians was pruned for several times. Investigators are cautiously optimistic that the *P. elisabethae* populations can be sustained indefinitely with careful harvesting.

Some other options for sustainable use of marine resources are:

1. Chemical synthesis,
2. Aquaculture of the source organism,
3. Cell culture of the macroorganism or microorganism source, and
4. Molecular cloning and biosynthesis in a surrogate organism.

Each option has advantages and limitations. However, understanding the fundamental biochemical pathways by which bioproducts are synthesized is the key to almost all the techniques. For example, the Molecular approaches offer promising alternatives not only to the supply of known natural products (e.g., through the identification, isolation, cloning, and heterologous expression of genes involved in the production of the chemicals) but also to the discovery of novel sources of molecular diversity (e.g., through the identification of genes and biosynthetic pathways from uncultured microorganisms) (Bull *et al.*, 2000). Manipulation of heterologously expressed secondary metabolite biosynthetic genes to produce novel compounds having potential pharmaceutical utility is at the forefront of current scientific achievements and has tremendous potential for creation of novel chemical entities (Xue a & Sherman, 2001). Efforts are needed to clone useful secondary metabolite biosynthetic pathways from natural assemblages of marine microorganisms in a similar pattern used for terrestrial soil. These approaches will offer solutions to natural-product supply and resupply problems.

Losses of marine biodiversity are largely the result of conflicting uses of, in particular, coastal habitats, which lead to degradation, fragmentation and losses of habitats. The needs of a burgeoning population for housing, disposal of human and industrial waste, forestry, fisheries, development of harbours, industrial sites and tourist complexes are combined with effects from activities such as forestry or mining up watersheds kilometres away to degrade and destroy coastal habitats. The habitat alterations, fragmentations and destructions in the coastal zone contribute to loss of marine biodiversity.

The greatest levels of marine biodiversity are found in tropical countries which are developing. Being poorer than their developed country counterparts in general they have fewer facilities, equipment, trained staff and resources available to devote to marine biodiversity conservation. In addition, it is natural that their priorities focus more on food production and development than on conserving biodiversity. There is a need to explore the economic and other practical benefits of conservation of biodiversity, so that policy decisions are made in the full knowledge of the benefits that can be gained from biodiversity conservation.

Species and genetic diversity of deep sea benthic communities and microbial communities in general are largely unknown and these communities are likely to be rich sources of genetic diversity. The pelagic system has lower species diversity than benthic systems and as yet there are few known threats to biodiversity of the pelagic system of the open ocean, but atmospheric inputs of contaminants need careful evaluation. Coastal areas have a greater variety of habitats than the open ocean and coastal habitats are known to be highly diverse and yet the greatest threats posed are to these systems.

Genomics and Proteomics Applications for Marine Biodiversity Conservation Issues

Genomics is the sequencing, annotating, and interpreting of information contained within the genome of an organism. Genome sequences of microorganisms have led to a better understanding of the biology of the organisms sequenced (Nierman *et al.*, 2000). Microorganisms have been the focus of genomic research, mainly because of their smaller genomes. Representations of the entire metabolic potential of microorganisms derived from the application of bioinformatics have indicated the presence of hitherto unsuspected metabolic pathways in even some well-characterized bacteria. Such genomic information provides a new basis for understanding physiological processes like: responses of indicator species to environmental changes, stimuli that cause an organism to synthesize a product of potential human benefit, or discover new gene targets for drug therapy (Read *et al.*, 2001). The pharmaceutical industry has taken advantage of microbial genomics to search for novel vaccine targets in pathogenic microorganisms, in order to reduce the time and cost of drug target discovery (Pizza *et al.*, 2000). Genomic methods and integration with other technologies are essential for further advancements in the marine biomedical sciences as suggested by Cary & Chisholm (2000).

Many marine eukaryotic organisms (e.g., corals, sponges, and tube worms) maintain large and diverse populations of microbial symbionts. The complete genome sequences of these consortia will not only lead to unprecedented understanding of the interactions between host and symbiont, but will also expedite the discovery of novel metabolites, such as drugs and fine chemicals, that are the products of such consortia. Molecular tools such as Metagenomics could be best in minimizing the sample requirements. For example Sharp *et al.* (2007) isolated DNA from sponge and analysed using FISH. The 16SrRNA gene sequence analyses were performed in *Rhopaloeides odorabile* and phylogenetic analysis were done by Webster *et al.* (2001) and also by Taylor *et al.*(2004). Using single strand conformational polymorphism, the novel candidate phylum *Poribacteri* in marine sponges was discovered in a sponge by Fieseler *et al.* (2003). Bacterial community diversity associated with a few marine sponges from the South China Sea was studied by Li *et al.* (2005) using 16S rDNA-DGGE fingerprinting. Molecular techniques such as RFLP, sequencing and phylogenetic analysis (BLAST Analysis) were also employed to detect the phylogenetic diversity of heterotrophic microbes associated with *Discodermia* spp. (Porifera: Demospongiae) as reported by Fieseler *et al.* (2003).

Sustaining Resources

The successful development of marine biotechnology is intimately connected with ocean biodiversity. Hence, it is essential to ensure that biodiversity is protected. Partnerships must be developed to protect marine resources in tropical areas in particular, to ensure a positive economic outcome and the long-term protection of the resource. As sustainability is one of the central challenges in further development of marine biotechnology, it must be addressed before large-scale marine harvests can begin. Innovative approaches to partnerships between stakeholders will help to support access to marine resources and to ensure as sustainable assets. It is essential to train and educate the local populations so as to ensure long-term resource sustainability.

To avoid the public's misunderstandings, it is essential that scientists partner with the public to provide information that addresses both the promise and possible problems of marine biotechnology. It is necessary to connect individuals from science, education, business, and media to address the public's formal and informal educational needs. An ecosystem approach to manage the collection of benthic reef species for biomedical research presents numerous challenges, as available information is inadequate on the biology, ecology, and population dynamics of most reef invertebrates. Through the development of partnerships among government agencies, commercial pharmaceutical companies, academia, and local communities, research in identification andscreening of bioactive compounds can expand, with concurrent efforts directed toward sustainable management approaches.

Possible conservation strategies include the development of a system of marine protected areas and studies to catalogue the diversity and status of the resources contained within these areas. Basic research is also needed on the biology of the target species, linkages among other organisms / coral reefs organisms, and ecosystem processes controlling the distribution and abundance. Mass production of the target organism through captive-breeding or mariculture may provide a consistent alternative supply without requiring sophisticated equipment for harvest or harvest techniques that are suitable for environmentally sensitive environments. Species in demand for the aquarium trade, live reef food-fish markets, and other seafood, and a variety of invertebrates as sources of bioactive compounds, are promising new species for intensive farming. Selective husbandry and other well-defined mariculture protocols may provide a new tool to improve the yield and quality of bioactive compounds, further reducing the number of individuals needed to provide large quantities of metabolites.

References

Bull, A.T., Ward, A.C. & Goodfellow, M. 2000. Search and discovery strategies for biotechnology: the paradigm shift. *Microbiology and Molecular Biology Reviews,* 64: 573-606.

Cary, S.C. & Chisholm, S.W. 2000. Ecological Genomics: The Application of Genomic Sciences to Understanding the Structure and Function of Marine Ecosystems. Report of a Workshop on Marine Microbial Genomics to Develop Recommendations for the National Science Foundation. National Science Foundation, Arlington.

Fieseler, L., Horn, M., Wagner, M. & Hentschel, U. 2004. Discovery of the novel candidate phylum"*Poribacteria*" in marine sponges. *Appl. Environ. Microbiol.,* 70: 3724-3732.

Faulkner, D.J. 2000. *Marine pharmacology.* Antonie Van Leeuwenhoek, 77: 135-145.

Gerwick, W.H., Tan, L.T. & Sitachitta, N. 2001. Nitrogen-containing metabolites from marine cyanobacteria, 75-184 in The Alkaloids, G.Cordell (Ed.), Academic Press, San Diego.

Li, Z.Y., Li-Ming, He., Jie, Wu. & Qun, J. 2005. Bacterial community diversity associated with four marine sponges from the South China Sea based on 16S rDNA-DGGE fingerprinting. (online)

Lipton, A.P., Marichamy, R., Ganapathy. & Ramalingam, J.R. 1993. Large-scale exploitation of seahorse (*Hippocapus kuda*) along the Palk Bay coast of Tamilnadu. *Mar. Fish. Inf. Ser. T & E. Ser.,*119: 17-20.

Lipton, A.P., Bose, M., Ramalingam, J. R. & Thillairajan, P. 1996. Specific large scale exploitation of sacred chank, *Xancus pyrum* using modified trawl net (chanku madi) along Rameswaram coast, Tamil Nadu. *Mar. Fish. Inf. Ser. T & E Ser.*, 143: 17-19.

Lipton, A. P. & Thangaraj, M. 2002. Present status of seahorse fishing along the palk bay coast of Tamilnadu. *Mar. Fish. Infor. Ser., T & E Ser.*, 174: 5-7.

Newman, D.J., Cragg, G.M. & Snader, K.M. 2000. The influence of natural products upon drug discovery. *Natural Product Reports,* 17: 215-234.

Nierman, W., Eisen, J.A. & Fraser, C.M. 2000. Microbial genome sequencing 2000: new insights into physiology, evolution and expression analysis. *Research Microbiology,* 15: 79-84.

Pizza, M., Scarlato, V., Masignani, V., Giuliani, M. M., Arico, B., Comanducci, M., Jennings, G. T., Baldi, L., Bartolini, E., Capecchi, B., Galeotti, C. L., Luzzi, E., Manetti, R., Marchetti, E., Mora, M., Nuti, S., Ratti, G., Santini, L., Savino, S., Scarselli, M., Storni, E., Zuo, P., Broeker, M., Hundt, E., Knapp, B., Blair, E., Mason, T., Tettelin, H., Hood, D. W., Jeffries, A.C., Saunders, N.J., Granoff, D.M., Venter, J.C., Moxon, E.R., Grandi, G. & Rappuoli, R. 2000. Identification of vaccine candidates against serogroup B meningococcus by whole-genome sequencing,Science, 10(287):1816-1820.

Pomponi, S.A. 1999. The bioprocess-technological potential of the sea. *J. Biotech.,* 70: 5-13.

Read, T.D., Gill, S.R., Tettelin, H. & Dougherty. B. A. 2001. Finding drug targets in microbial genomes. *Drug Discovery Today,* 6: 887-892.

Sharp, K.H., Eam, B., Faulkner, D.J. & Haygood, M.G. 2007. Vertical transmission of diverse microbes in the tropical sponge *Corticium* sp. *Appl. Environ. Microbiol.,* 73: 622-629.

Taylor, M.W., Schupp, P.J., Dahllof, I., Kjelleberg, S. & Steinberg, P.D. 2004. Host specificity in marine sponge-associated bacteria, and potential implications for marine microbial diversity. *Environ. Microbiol.,* 6: 121-130.

Webster, N.S., Wilson, K.J., Blackall, L.L. & Hill, R.T. 2001. Phylogenetic diversity of bacteria associated with the marine sponge *Rhopaloeides odorabile. Appl. Environ. Microbiol.,* 67: 434- 444.

Xue, Y. & Sherman, D. 2001. Biosynthesis and combinatorial biosynthesis of pikromycin-related macrolides in *Streptomyces venezuelae. Metabolic Engineering,* 3: 15-26.