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Asian Biotechnology and Development Review
Vol. 10 Nos. 1 and 2, pp 89-102

Marine Biotechnology: A Natural Biorefinery Source of Marine Products and By-products for Human Welfare

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Abstract: About 70 per cent of the earth's surface is covered by oceans and seas, and four-fifth's of all life forms inhabit this ecological niche. Therefore, the importance of marine organisms as a biorefinery technology resource is overwhelming. Till recently, from the bioresources of the deep ocean, once considered to be a 'biological desert', a number of new unique organisms have been discovered. This results from the use of improved sampling techniques and of modern molecular biological tools to reveal some of the recent developments and the biotreasures of the deep oceans with an emphasis on marine bioactive compounds.

Keywords: Marine biotechnology, biorefinery, microorganisms.

Introduction

Man has been using natural products as a source of food, fragrances, pigments, insecticides and medicines for centuries. Generally, terrestrial plants have served as the major source of medicinally useful products and these have been developed from a legacy of folk medicine. Joffe and Thomas (1989) estimate that about 25 per cent of all pharmaceutical sales are drugs derived from plant natural products and an additional 12 per cent come from microbial natural products. The marine biosphere encompasses a huge thermal range from freezing temperatures in Antarctic waters to about 350° C in deep hydrothermal vents, a pressure range of 1 atmosphere at the surface to 1000 atmospheres in the deep oceans, a nutrient range from oligotrophic to eutrophic conditions and extensive photic and non-photic zones. This extensive variability

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Introduction

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has facilitated extensive speciation at all phylogenetic levels from microorganisms to mammals.

The importance of marine organisms as a biorefinery technology resource is over-whelming taking into account that about 70 per cent of the earth's surface is covered by oceans and seas, and that four-fifth's of all life forms inhabit this ecological niche. Worldwide sales of marine biotechnology-related products during 2000 were estimated to be US $100 billion. Through the application of molecular biological techniques in studying marine organisms, many basic biological principles of relevance to both marine and terrestrial organisms are clarified and understood. Much of what is known about nerve transmission has been learnt using squid and its giant nerve axon. Likewise, the mysteries of vision have been unraveled through a study of the eye of the horseshoe crab, shark and skate. And, whereas the surf clam is proving to be an excellent model to study cell cycle and its regulation, the sea urchin on the hand is a key model for understanding the molecular and cellular basis of reproduction and development.

The deep ocean often considered a 'biological desert' is a gold mine of new unique organisms that have been discovered through the use of improved sampling techniques and modern molecular biological tools such as metagenomic approach, high throughput DNA sequencing, and computational genomics. More than 1.2 million new genes have been identified in DNA extracted from about 1.5 L surface sea water off the Bermuda coast.[1] Despite the fact that the biodiversity in the marine environment far exceeds that of the terrestrial environment, research into the use of marine biomass for biofuel production and of marine natural products as a reservoir of pharmaceutical agents is still in its infancy. This may be attributed to the fact that the deep ocean has never been seriously considered as a source of biofuels and to the lack of ethnomedical history and difficulties involved in the collection of marine organisms.[2]

Lessons from Physiology of Marine Organisms

Life originated from the seas.[3] Marine organisms through evolution developed very sophisticated physiological and biochemical systems. During adaptation to the terrestrial environment and notwithstanding

several physiological changes that have taken place, the basic functions have been retained. The architecture of the shark liver is similar to that of the human liver. Also, it is interesting that the biochemical transformation that takes place in a shark's liver is similar to that occuring in a human liver.[4] Insulin from fish such as cod exerts the same hormonal activity in mammals as homologous insulin.[5]

Knowledge of the physiological and biochemical features of marine organisms might contribute to the identification of natural products of biomedical importance. For instance, a damaged optic nerve in fish begins to repair itself spontaneously whereas a rabbits injured optical nerve ceases to function and degenerates. An extract from a fish nerve will induce regeneration in a rabbit nerve.[6] Fish are found to be useful models for the study of factors involved in axon regeneration.[7] Marine metabolites, therefore, have a potential to correct abnormalities that occur in terrestrial animals.

Marine Microbes: As a Natural Refinery

Since the discovery of penicillin in 1929 some 10,000 and more of these natural products are reported to have biological activity inclusive of 100 microbial products that are in use today as antibiotics, antitumour agents and agrochemicals.[8]

The development of the fungal metabolite *mevinolin* for the treatment of high serum cholesterol and the bacterial metabolite FK-506 as an immunosuppressant illustrate the vast natural diversity of microbial natural products.[9] In spite of such successes in drug discovery from terrestrial microorganisms, marine microorganisms have received very little attention on account of the non-culturability of the majority (over 99 per cent) of marine bacteria.[10]

Marine toxins such as tetrodotoxin and saxitoxin are potent and specific sodium channel blockers and pharmacological studies with these toxins have played a major role in developing the concept of sodium channels in general and membrane channels in particular.[11] Recent studies have shown that these toxins may be produced by marine bacteria.[12]

There is increasing evidence that several anti-cancer compounds isolated from sponges or other marine invertebrates are actually produced by bacteria associated with these invertebrates. Bryostatins were initially

isolated from a bryozoan, *Bugula neritina*. Putative type 1 polyketide synthase genes have been found in bacteria from colonies of *B. neritina* producing bryostatin and these genes are absent from bacteria associated with colonies of *B. neritina* that do not produce bryostatin.[13] Dolastatin isolated from the Indian Ocean seahare *Dolabella auricularia* has also been isolated from marine cyanobacteria.[14] Dolastatin-like peptides have been isolated from the cyanophyte *Lyngbya majuscula*.[15]

Metabolites from Marine Microalgae

Microalgae (phytoplankton) are the major primary producers in the marine environment and are fundamental to the marine food chain converting carbon dioxide into organic compounds that can be subsequently used by invertebrates and fish. Though there are over 10,000 species of microalgae, most of the investigations have focused on the Cyanophyta (blue-green algae now classified with the prokaryotic bacteria) and the dinoflagellates.

A series of novel polyether antibiotics - the most potent antifungal agents reported to-date, have been isolated from the marine dinoflagellate *Gambierdiscus toxicus*. One of these, gambieric acid A is 2000-fold more active than the clinically useful antifungal agent amphoterecin B and has only moderate toxicity to mice and cultured mammalian cells.[16]

Novel Compounds from Seaweeds

Seaweeds in different colours–green (*Chlorophyta*), yellow-brown (*Phaeophyta*) and red (*Rhodophyta*) abundant in intertidal zones and in clear tropical waters play a significant ecological role by providing food, protection, shade and substratum for marine life. Widespread in shallow waters and easily harvested, seaweeds were the first group of marine organisms to be investigated for their natural products.

A Philippine collection of the red alga *Portieria hornemannii* has yielded a novel cytotoxic penta halogenated monoterpene, halomon which exhibited one of the most extreme examples of differential cytotoxicity in the screening conducted by the National Cancer Institute (NCI), USA.[17] The NCI screen looks for selective toxicity in a human tumour cell line panel consisting of 60 different cell lines representing eight major cancer subtypes. NCI has selected halomon for preclinical drug development since this compound shows toxicity to brain, renal

and colon tumour cell lines and preliminary *in vivo* evaluations have been encouraging.[18] Though getting sufficient quantities of the chemical from the alga has been difficult, its mode of action as an inhibitor of DNA methyltransferase has been elucidated.[19]

An Australian red algal sample *Hypnea valitiae*, a novel potent and specific inhibitor of adenosine kinase was used to study adenosine receptors, nucleotide metabolism and regulation.[20]

Many red, green and brown algae convert simple polyunsaturated fatty acids such as arachidonic acids into complex eicosanoids[21] and related oxylipins.[22] Derivatives of arachidonic acid are important in maintaining homeostasis in mammalian systems.

Metabolites from Sponges

Sponges were recognized as a source of biomedically important metabolites following the discovery of spongouridine,[23] a potent natural occurring tumour-inhibiting arabinosyl nucleoside in Caribbean sponge, *Cryptotethia crypta*.

Several bioactive compounds with anti-inflammatory, antitumor, immunosuppressive or neurosuppressive, antiviral, antimalarial, antibiotic, or antifouling activity have been discovered from sponges (Table 1). Over 5000 medically important compounds from sponges have been described from amongst the 15,000 and more from marine organisms that have been recorded.[24]

A number of metabolites from sponges are proving useful as molecular probes in biochemical studies.

Terpenoides[25] involved in inhibiting inflammatory response have been isolated from *Jaspis splendens* and *Suberea* spp.[26] For example, discodermolide from the Caribbean sponge *Discodermia dissoluta* is 100-1000 times more potent than the drug cyclosporine A *in vivo* and other clinically useful immunosuppressive agents.[27] A number of receptor antagonists[28] with potential as biochemical tools lead to the development of therapeutics such as xestobergsterol from the Okinawan sponge *Xestospongia bergquista*.[29] Other significant sponge-derived therapeutics are leucettamine from the marine sponge *Leucetta microraphis*.[30] Several sponge-derived antimalarial compounds have been discovered for treating malaria caused by the chloroquinone-resistant malarial parasite.[31] Menzamines are most promising antimalarial compounds discovered from sponges.[32]

Table 1: Examples of Some Biomedically Important Metabolites from Marine Organisms

Organisms	Metabolite	Potential uses
Marine bacteria	Mevinolin FK-506	Treatment of high serum cholesterol Immunosuppressant
	Macrolactin A	Antitumour activity, protection against HIV, Herpes simplex virus types I and II.
	Tetrodotoxin	Study of sodium channel
Lyngbya majuscula	Lyngbyatoxin A, debromoaplysiatoxin	Study of Proteinase kinase C, signal transduction pathways, development of tumour suppressing compounds
	Microcolin A & B, curacin A	Immunosuppressant anti-tumour activity
Marine algae *Alexandrium tamarense*	Saxitoxin	Study of sodium channel, neurophysiological and neuro-pharmacological studies
Ptychocentrum brevis	Brevitoxin	Molecular probe in studies on sodium channel.
	Gambieric acid A	Antifungal compound
Prorocentrum sp.	Okadaic acid	Study of regulatory phenomenon and signal transduction pathways
Green algae	Spingosine derivative	Antiviral activity
Brown algae	Stypoldione	Antitumour activity
Red algae	Halomon	Antitumour activity
Sponges	Spongouridine	Antitumour activity
	Dercitin	Antitumour activity
	Halichondrin B	Antitumour activity
	Theopederin	Antitumour activity
	Cribrostatin 1	Antitumour activity
	Spongistatin	Antitumour activity
	Calyculin A	Antitumour activity
	Purealin	Studies on actin polymerization and actin organization
	Manoalide	Study of structure and conformation of myosins.
	Latrunculin A	Study on the role of phospholipase A_2 in inflammation
	Discodermolide	Immunosuppression
	Xestobergsterol A	Inhibition of lgE mediated histamine release from mast cells
	Leucettamine A	Anti-inflammatory activity
	Batzelladine A& B	Therapy of HIV

Table 1 continued

Table 1 continued

Organisms	Metabolite	Potential uses
Cnidarians [i]	Palytoxin	Cellular recognition process.
	Lophotoxin	Neuropharmacological probe.
	Pseudopterosin E	Anti-inflammatory activity
	Fucoside A	Anti-inflammatory activity
Bryozoans [ii]	Bryostatin	Antitumour activity
	Convolutamide A	Antitumour activity
	Flustramine E	Antimicrobial activity
Molluscs [iii]	Conotoxins	Study of ion channel
	Dolastatin	Antimicrobial activity
	Chromodorolide A	Antimicrobial and cytotoxic activity
Ascidians [iv]	Didemnin B	Antitumour activity
	Ecteinascidin 743	Antitumour activity
	Eudistomin C	Antiviral activity
	Staurosporine derivatives	Study of cellular differentiation & proliferation.

***Source*:** Jha, R. K. and Zi-rong, X. (2004).

Molecular Probes from Cnidarians

The discovery of prostaglandins from corals in the late 1960s contributed greatly to the rapid developments in the field of marine natural products.[37] Palytoxin, one of the most potent non-protein toxins known and obtained from the cnidarian *Palythoa sp.*, is a useful tool for probing cellular recognition processes such as sodium and potassium ATPase binding and opening of the ion channel. Cosmetic companies have incorporated extracts from *P. elizabethae* into skin, hand and eye creams.[38]

Drugs from Bryozoans

The drug from the marine environment that is closest to the market may be bryostatin, a potent anticancer compound obtained from *Bugula neritina*,[39] while the New Zealand bryozoan *Cribricellina cribraria* has yielded a b-carboline alkaloid that has cytotoxic, antibacterial, antifungal and antiviral activities.[40] Biochemical entities from the Canadian water bryozoan *Flustra foliacea* have shown strong antimicrobial activity.[41] Novel cytotoxic quinones and alkaloids have been isolated from the bryozoans *Caulibugala intermis* and *Pterocella vesiculosa* respectively.[42]

Biochemical Probes from Molluscs

The predatory *Conus sp*. such as *C. geographus* have evolved deadly nerve toxins. These conotoxins are valuable probes in physiological and pharmacological studies.[43] Dolastatins are compounds obtained from the sea hare, *Dolabella auricularia*.[44] Carnivorous molluscs concentrate secondary metabolites from their selective diet of sponges, bryozoans, ascidians and coelenterates. Ulapualide A from *Hexabranchus sanguineus* exhibits cytotoxic activity against murine leukaemia cells and antifungal activity.[45]

Bioactive Molecules from Ascidians

Didemnin-B from the Caribbean tunicate *Tridemnum solidum* was the first marine compound to enter human cancer clinical trials as a purified natural product[46] which provided important structural clues for a variety of antiviral, anticancer and immunosuppressant activities.[47]

Metabolites from Echinoderms and Fish

Physiological active saponins have been studied extensively from echinoderms such as seastars and sea cucumbers.[48] However, they have not been useful as biochemical tools or drugs because of their tendency to cause cell lysis.[49] Squalamine, a member of the new class of water soluble broadspectrum antibiotics, has been isolated from the stomach extracts of dogfish shark, *Squalus acanthias*. This active entity is an aminosterol, which has shown promise as a part of combination with standard agents in clinical trials for non-responding solid tumours and in primary treatment for advanced ovarian cancer.[50]

Problems and Future Prospects

The vast potential of marine natural products that contribute to the development of biomedically important compounds has been well recognized. Trials with several compounds have progressed rapidly as a result of collaborative efforts between marine chemists, cell biologists and molecular pharmacologists. The development of automated high throughput screening methods allows researchers to test up to thousand compounds a day. However, a number of problems remain. Microorganisms maintained in artificial media may lose the ability to produce biologically important molecules.[51]

Biofuels from the Oceans

Several Asian countries are engaged in pursuing biotechnological options to lessen their dependence on imported fossil-fuel energy sources to help conserve valuable foreign-exchange resources, minimize environmental pollution and degradation of the quality of human, animal and plant life. Alternative sources such as agro-industrial residues and non-edible plants are being bioconverted into biofuels either as biogas, bioethanol or biodiesel. Marine biomass, hitherto untapped for such bioproducts now constitutes a new bioresource. "Man-made nutrient pollution" that harms the marine ecosystem gives rise to massive algal blooms that are being harvested to produce ethanol - a clean and non-polluting biofuel.[52]

Gao and McKinley (1994) view biomass production from macroalgae for CO_2 bioremediation and providing economically feasible technologies as alternatives to fossil-derived fuels. Several countries are moving into the area of using clean and green technologies from biomass feedstocks obtained from marine seaweeds and saline agriculture.[53]

The potential of using macroalgae from the Adriatic and Jonian seas for energy and biofuel production has been assessed.[54] Furthermore, a fully integrated process for biodiesel production from microalgae in saline water aims to demonstrate the technical and economic feasibility of using microalgal oils as a feedstock for biofuel.[55]

Conclusion

The discovery that many of the bioactive compounds isolated from invertebrates are produced by bacteria raises the hope that technology can be developed to produce these compounds in fermenters. However, culturablity of some of the sponge associated bacteria has been a problem. Another promising approach towards producing biomolecules is recombinant DNA technology. Recently, genes involved in the synthesis of pederin, a polyketide amide present in beetle, *Paederus fuscipes* has been cloned.[56] Interestingly, pederin is closely related to mycalamide A from the marine sponge *Mycale* sp. Such approaches are expected to yield results soon and it can be expected that a number of useful drugs would come from the marine environment.

With the advances in diving technology and the availability of remotely-operated vehicles and submersibles, samples of marine organisms from depths of even 1000 m and beyond have been obtained. Many of the organisms are unique and this has provided an additional

stimulus to the search for marine bioactive compounds and perhaps of biofuels from marine biomass. During 1977-1987, a wide variety of marine organisms yielded some 2500 new metabolites.[57] During 2003-2004, the structure and initial bioactivity profiles of more than 150 marine natural products with antitumour and cytotoxic activity have been described.[58] Many of these compounds are novel belonging to diverse structural classes including polyketides, terpenes, steroids and peptides.

Endnotes

1 Venter et al. (2004).
2 Faulkner (1992).
3 Where did life originate? (http://evolution.berkeley.edu/evosite/evo101/IIE2aOriginoflife.shtml)
4 Ballatori and Villalobos (2002).
5 Reinecke and Collet (1998).
6 Halevy (1990).
7 Schwalb et al. (1995).
8 Carte (1996).
9 Carte (1996).
10 Hugenholtz and Pace (1996).
11 Camacho et al. (2007).
12 Kodama et al. (1990), Simidu et al., (1990).
13 Davidson et al. (2001).
14 Luesch et al. (2001).
15 Newman and Cragg, (2004).
16 Nagai et al. (1992).
17 Carte (1996).
18 Carte (1996).
19 Andrianasolo et al. (2006).
20 Ireland et al. (1993).
21 Eicosanoids (eicosa in Greek means twenty) are oxygenated derivatives of 20 carbon essential fatty acids. There are four families of eicosanoids, prostaglandins, prostacyclins, thromboxanes and leukotrines.
22 Oxylipins are oxygenated compounds biosynthesized from fatty acids by the action of dioxygenase catalysed oxygenation.
23 Terpenoids: terpenes are hydrocarbons with combination of several 5 carbon isoprene units, terpenoids are modified terpenes, where methyl groups are moved or removed and oxygen atoms are added.
24 Sipkema et al. (2005).
25 Spongouridine, is an antiviral nucleotide derived from Cryptotethia crypta.
26 Carroll et al. (2001).
27 Longley et al. (1991).
28 Receptor antagonists: Agonist is a substance that binds to a specific receptor and triggers response in a cell. Antagonist blocks the binding of the agonist to the receptor.
29 Shoji et al,(1992).
30 Chan et al. (1993).
31 Sipkema et al., (2005).
32 Yousaf et al., (2002).

[33] Cnidarians, all armed with stinging cells called nematocysts, are diverse in form, as evidenced by colonial siphonophores, massive medusae and corals, feathery hydroids and box jelly's with complex eyes. The name Cnidaria comes from the Greek word "cnidos" that means stinging nettle. There are four major groups of cnidarians:
— Anthozoa which includes true corals, anemones, and sea pens;
— Cubozoa, the box jellies with complex eyes and potent toxins;
— Hydrozoa, the most diverse group with siphonophores, hydroids, fire corals and many medusae; and
— Scyphozoa, the true jellyfish. (http://www.ucmp.berkeley.edu/cnidaria/cnidaria.html)

[34] Bryozoans a group with a fossil record extending back to the upper Cambrian [500,000,000 years ago] known also as moss animals are tiny colonial animals that generally build stony skeletons of calcium carbonate. Except for the class Phylactolaemata which is found exclusively in fresh water the majority of bryozoans are marine (several thousand species).
1] http://www.bio.umass.edu/biology/conn.river/bryozoa.html
2] http://paleo.cortland.edu/tutorial/Bryozoans/bryozoans.html

[35] Molluscs, a variety of animals with decorative shells that vary in form and colour are well-known as seafood and which range from tiny snails, clams, and abalone to larger organisms such as squid, cuttlefish and the octopus. Malacology is the scientific study of molluscs. The vast majority of molluscs live in marine environments mainly on the coastal plains. Two groups, viz the bivalves and the gastropods contain freshwater species. The gastropods are the only group that have snails and slugs as representatives living on land. (http://www.mesa.edu.au/friends/seashores/molluscs.html)

[36] Ascidiacea, commonly known as ascidians or sea squirts. The common name sea squirtarises from their habit of squirting a jet of water when stamped upon or when they are uncovered at low tide. Ascidians, soft and boneless, possessing a sac-like marine filter feeder and a tough outer "tunic" made of the polysaccharide tunicin are found all over the world usually and firmly attached to substrata such as rocks and shells. (http://www.seaslugforum.net/factsheet.cfm?base=ascidian)

[37] Carte (1996).

[38] Liles (1996).

[39] Liles (1996).

[40] Prinsep et al., (1991).

[41] Holst et al., (1994).

[42] Yao et al., (2003); Milanowski et al., (2004).

[43] Myers et at., (1993).

[44] Newman and Cragg, (2004).

[45] Rorsener and Scheuer (1986).

[46] Carte (1996).

[47] Sakai et al., (1995).

[48] Miyamoto et al., (1990).

[49] Carte, (1996).

[50] Newman and Cragg, (2004).

[51] Gustafson et al., (1989).

[52] Harvesting algae blooms from the open ocean, Thursday, March 1, 2007, (http://biopact.com/2007:03/Harvesting.Algae.Blooms;from.Ocean.html)

[53] Sakurai and Masukawa, (2007).

[54] Aresta et al, (2005).

[55] Project ID: RDG-07-26 Application for AP6 Project Inclusion in Renewable Energy and Distributed Generation Task Force of the Asia Pacific Partnership on Clean Development and Climate (www.asiapacificpartnership.org/APP%20Projects/

REDG/Project%20Proposal%20-%20AUS%20-%20Microalgae%20_3_.pdf) The Asia Pacific Partnership on Clean Development and Climate (AP6) is a breakthrough approach involving key developed and new industrializing countries in practical, pro-growth, technology-driven efforts in clean development and climate change through deployment of clean technologies. The partnership of the six countries - Australia, China, India, Japan, Republic of Korea and the United States was established in January 2006 in Sydney, Australia

[56] Piel, (2002).

[57] Ireland et al., (1993).

[58] Mayer and Gustafson, (2006).

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