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Marine biotechnology: An overview

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Marine biotechnology is the creation of products and processes from marine organisms through the application of the techniques of biotechnology, molecular and cellular biology, and bioinformatics. This is a scientifically fascinating and economically expanding field of science. No ecosystems provide greater genetic diversity or possibilities for new products and processes than the world's marine environments. Marine organisms, from bacteria to eukaryotes are certainly a source of molecules of great interest in biotechnology. Today, marine biotechnology has numerous applications from the production of lifesaving drugs to better food and conservation of organic waste. This article provides a selective overview of past achievements, present scenario and future directions of marine biotechnology

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Introduction

Biotechnology can be defined as the application of living organism, system or process to develop a commercial product or service. The field of biotechnology is not new and it has been known to human beings since long time. Fermentation technology (the earlier form of biotechnology) was originated with mold-fermented foods in China and beer brewing and bread making in Egypt[1]. Cohen *et al* produced the first recombinant DNA by cloning a gene into a bacterial plasmid[2], which was the major breakthrough in the growth of commercial biotechnology. Today, biotechnology has numerous applications from the production of life saving drugs to better food and conservation of organic waste. Such tremendous applications of this subject lead us to believe that society has already shifted from the age of information to biotechnology. Biotechnology can be applied in four major segments; biomedical, agricultural, industrial and environmental. Among these, the biomedical segment is growing very rapidly. Recombinant human insulin, novel pharmaceutical drugs, different vaccines, etc. are some of the important examples of this success. In the agriculture field, introduction of genetically engineered tomatoes, soybeans, cotton, etc in market shows the impact of biotechnology. On the industrial and environmental fronts also there is a tremendous progress

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Marine Biotechnology

Marine biotechnology is defined as the application of scientific and engineering principles to the processing of materials by marine biological agents to provide goods and services[3]. Marine biotechnology explores the oceans to develop novel pharmaceutical drugs, chemical products, enzymes and other industrial products and processes. It also plays a vital role in the advancement of biomaterials, health care diagnostics, aquaculture and seafood safety, bioremediation and biofouling. The commercial success stories of other field of biotechnology are familiar. However, marine biotechnology is still in the infant stage.

Life originated in the sea and sustained itself to the present day. The oceans comprise more than 70% of the earth's surface and contain the most ancient and diverse forms of life. Then the question is, "Can these biological resources be studied in detail and explored for human benefit and fundamental biological progress?" The application of biotechnology in marine field will give positive answer to this question.

The population and human needs continue to increase, obviously the pressure on natural resources will also continue to grow. To meet these growing needs, we can turn towards marine environment, which occupies one-third portion of our planet

Applications of Marine Biotechnology

Human Health

Most of our medicines come from natural resources and scientists are still exploring the organisms of tropical rain forest for potentially valuable medical products. Historical records show that human beings were aware of the venomous nature of some sea creatures for, at least, the last 4000 years[4]. More than 2000 years ago, the extracts of marine organisms had been used as medicine. In the 19th and early 20th centuries, cod liver oil was in use as supplementary nourishment. However, only in the middle of 20th century scientists began to systematically probe oceans for medicines.

How do some delicious looking sea creatures protect themselves from predators? While answering this ecological question, scientists found that these organisms have defensive chemical weapons. In view of this, investigations were carried out to explore these chemicals for drug discovery. By the early 1950s, Ross Nigrelli of the Osborn Laboratories of the New York Aquarium (New York Zoological Society) extracted a toxin from cuvierian organs of the Bahamian sea cucumber, *Actynopyga agassizi*. He named this toxin as 'holothurin', which showed some antitumour activity in mice[5]. Although, 'holothurin' was never commercialized, the search for drugs from the sea has continued. From this humble beginning, the number of potential compounds isolated from marine realm has virtually soared and this number now exceeds 10,000, with hundreds of new compounds still being discovered every year[6]. With the combined efforts of marine natural product chemists and pharmacologists, a number of promising identified molecules are already in the market, clinical trials or in pre-clinical trials (Table 1). Interestingly, these precious natural products have been obtained from marine microorganisms as well as invertebrates such as sponges[7], molluscs, bryozoans, tunicates, etc (Fig. 1). Some of the commercialized products from marine organisms include antibiotic cephalosporin from marine fungus, cytostatic cytarabine from sponge, anthelmintic insecticide kanic acid from red alga, analgesic zincototide from mollusk, etc. Antiviral compound Ara-A (active against Herpes virus) and anti-tumour compound Ara-C (effective in acute lymphoid leukemia) were obtained from the sponge and these compounds are now in clinical use[8]. Arabinosyl Cytosine (Ara-C) is currently sold by the Pharmacia & Upjohn Company under the brand name Cytosar-UR. Apart from these, some products, such as blood-clotting compound from cone snail, anti-inflammatory ointment from sea sponge, anticancer substance and disinfectants from shark, gene therapy vehicle and adhesive from shellfish's chitosan are under development.

Enzyme inhibitors have received increasing attention as useful tools in the study of enzyme structures and reaction mechanisms. They also find applications in pharmacology[9] and agriculture[10]. Recently, marine organisms are increasingly recognized as a fruitful source for potential enzymes inhibitors[11]. For example, a bryozoan, *Bugula neritina* has been the source of a family of protein kinase C (PKC) inhibitors called bryostatins, which are currently in clinical trials for cancer[11].

Table 1—Examples of marine by-products, which are currently in market or in clinical phases

Product	Source	Application area	Status
Ara-A	Marine sponge	Antiviral	Market
Ara-C	Marine sponge	Anticancer	Market
Okadaic acid	Dianoflagellate	Molecular probe	Market
Manoalide	Marine sponge	Molecular probe	Market
Vent™DNA polymerase	Deep-sea hydrothermal vent bacterium	PCR enzyme	Market
Aequorin	Bioluminescent jelly fish, *Aequro victoria*	Bioluminescent calcium indicator	Market
Green fluorescent protein (GFP)	Bioluminescent jelly fish, *Aequro victoria*	Reporter gene	Market
Phycoerythrin	Red algae	Conjugated antibodies used in ELISAs and flow cytometry	Market
Cephalosporins	*Cephalosporium* sp., marine fungi	Antibiotic	Market
Yondelis™	Sea squirt	Cancer	Clinical phase II/III
Zinconotide	Cone snail	Chronic pain	Clinical phase III
Dolastatin	Sea slug	Cancer	Clinical phase II
Bryostatin-1	Bryozone	Cancer	Clinical phase II
Squalamine lactate	Shark	Cancer	Clinical phase III
PL512602 (steroid)	Sponge	Inflammation, Asthama	Clinical phase II

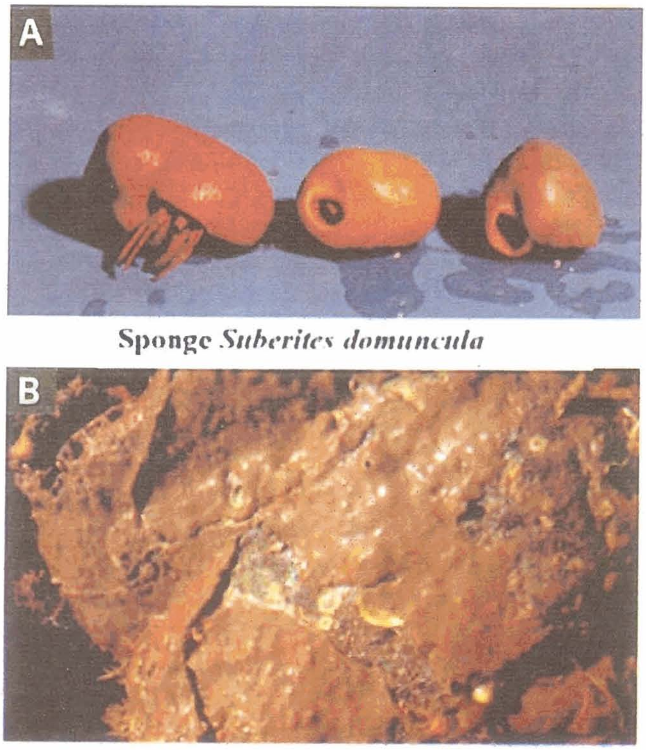

Fig. 1—More than 10,000 potential bioactive compounds have been reported from marine sources, a large proportion of which originates from the sponges (A) and ascidians (B).

(PKC) inhibitors called bryostatins, which are currently in clinical trials for cancer[11].

Marine microbes, having immense genetic and biochemical diversity, are likely to become a rich source of novel effective drugs. Marine bacteria constitute ~10% of the living biomass carbon of the biosphere[12] and they represent dramatically different environment than their terrestrial counterpart. These bacteria originate mainly in sediments, but also occur in huge numbers in open oceans and also found to be associated with the marine organisms. It was surprising to find that many bioactive compounds, reported from marine invertebrates, are produced by their microbial symbionts. Competition among microbes for space and nutrients in the marine environment is a driving force behind the production of such precious antibiotics and other useful pharmaceuticals. Interestingly, microorganisms associated with marine invertebrates are proved to be valuable candidates for drug discovery programmes[13-15]. Actinomycetes are an important group of bacteria, producing over 70% of naturally occurring antibiotics as well as other bioactive compounds. As oceans are also the titanic reservoir of novel actinomycete taxa, the efforts are underway to search antibiotics from this source. Prof. Jensen and Prof. Fenical of 'Scripps Institute of Oceanography', USA, recently reviewed the status of numerous potential drugs, isolated from marine microorganisms[16].

Like bacteria, marine fungi are also reported to be a potential source of bioactive substances[6]. In the course of study, Sorbicilactone-A, a novel-type alkaloid, was reported from sponge (*Ircinia fasciculata*) associated fungus, *Penicillium chrysogenum*. This compound showed promising activities in several mammalian and viral systems and qualified for therapeutic human trials[17]

Polyketide synthases (PKSs) are a class of enzymes that are involved in the biosynthesis of secondary metabolites, such as erythromycin, rapamycin, tetracycline, lovastatin and resveratrol[18]. Polyketide biosynthetic genes from bacteria and fungi have been cloned, sequenced and expressed in heterologous hosts. Some marine sponge associated bacteria with antimicrobial assets are also detected to have polyketide synthases gene clusters and investigation is underway to explore them.

In the field of marine biotechnology one cannot neglect the importance of living fossil, the horse-shoe crab. Researches highlighted the significance of its amoebocytes, which react with bacterial endotoxins and thus detect early infections in humans as well as traces of LPS (pyrogen) in biotechnological products.

Many marine invertebrates, because of their simple cellular structures, provided rich source of new information and serve as desirable non-mammalian models for research. A major area of emphasis is on genetic control of normal development and of tumour formation. In addition, many models threw light on the mechanism of nerve cells in marine invertebrates, which have direct implications in human and other mammalian researches. Sea urchins provided new information to the scientists on fertilization; a fundamental biological process. Thus, marine model systems could provide new insight in to basic biological principles that will benefit further developments in medicine and industry

Aquaculture and Fishery

Marine aquaculture is now a mature and highly successful example of progress in marine biotechnology. Fish is one of the most important protein supplies of the human nourishment in the world. The history of fishery goes back to thousand of years. Earlier, fishery was limited to inland water and coastal zones, but due to growing population fishing activity has also expanded. It is observed that due to over-fishing and changes in global environment, this important food resource seems to vanish slowly. In view of this, biotechnology can help in some vital measures, like use of molecular markers to discriminate individuals, populations, stocks and sister species of commercially important and endangered species.

In past, aquaculture was traditionally done in fishponds. However, due to recent industrialization of this sector, it now supplies high-quality food in a sustainable way. Biotechnological research to improve aquaculture procedure is focused on species diversification, optimum food and feeding, health of cultured organisms and disease resistance, as well as minimum environmental impact. By using recombinant technology, efforts are underway to develop genetically modified organisms (GMOs) with particularly useful features, such as fast growth, resistance to pathogens, temperature and salinity tolerance, etc. Production of transgenic fish through electroporation has also been successfully carried out since 1980. Furthermore, molecular biological methods have resulted in invention of new feed stocks and vaccines for aquaculture to increase its productivity. Use of marine microorganisms as probiotics in aquaculture is a gift of biotechnological research. These probiotics improve fish health and production.

Environmental Biotechnology

Degradation of hazardous material is an important issue worldwide. It has been found that marine microorganisms express novel biodegradation pathways for breaking down a variety of organic pollutants. Several such groups have been described and many others are being explored. Extensive development of such bioremediation processes will be an important area of environmental biotechnology. For example, *Pseudomonas chlororaphis* produces pyoverdin, which catalyzes the degradation of organotin compounds in seawater. Studies were carried out using immobilized cells of the above bacterium in 2% alginate beads and the results suggested that immobilized cells could be applied to *in situ* bioremediation of organotin[19]. Organic solvent tolerant bacteria and crude oil degrading marine cyanobacteria are also reported for their possible implications in environmental bioremediation[20,21].

In addition to this, marine microorganisms frequently produce eco-friendly chemicals, such as biopolymers and biosurfactants that can also be applied in environmental waste management and treatment. Researches are also on track to study the interaction of marine microbes with toxic heavy metals and suggested their use in various biosorption, bioprecipitation and biocrystallization applications for the treatment of contaminated water systems[22,23].

Biosensors are widely used for the assessment of environmental parameters of biological relevance, such as inorganic and organic nutrients, toxic products of marine organisms and harmful pollutants[24]. Marine microorganisms provide the basis for the development of sophisticated biosensors and diagnostic devices for medicine, aquaculture and environmental bio-monitoring. Some bioluminescent proteins from marine organisms are currently under study in order to produce gene probes that can be employed to detect human pathogens in food or fish pathogens in aquaculture system[25].

Biofouling refers to the assemblage of marine organisms on man-made structures and devices submerged in the sea. It causes deterioration and heavy economic penalties to marine industries[26]. Several attempts are made to control biofouling with the application of physical, chemical and biological measures but results, to the greater extent, are achieved with the use of antifouling paint coatings. Though the life span of these effective coatings is longer, they have toxic proposition and excessive leaching rate of them cause abnormality in non-target organisms. Environmental concerns about the use of such toxic antifoulants increased the interest in the development of non-toxic alternatives. Efforts in this area have proved that marine natural products could be a good source of eco-friendly antifouling compounds. The sessile marine organisms, which do not allow other organisms to come and settle on their surfaces, may provide key to control biofouling. Recent biotechnological findings into the basis of cell-cell communication have described the mechanism involved in biofilm formation, leading to environmental corrosion and plugging[27]. Also, the knowledge about adhesion properties of marine bacteria has implications for understanding pathogen interactions in human[25].

Biomaterial and Bioprocessing

Marine organisms synthesize chemicals with bioactive properties, such as metabolites, proteins, enzymes, polysaccharides and lipids, which have lead to new industrial processes. A natural 'soap' (biosurfactant), produced by oil-eating marine bacterium, *Acinetobacter* is a gift of biotechnology[28]. Improved technology, allowing to sample organisms from ocean floor, has explored different group of organisms (extremophiles). These organisms have evolved to live and thrive in extreme conditions. Uniquely adapted enzymes (and other proteins), with extra stable chemical bonds, help these organisms to survive in these conditions. Few such enzymes have led to the breakthrough processes of biotechnology and some others will surely bring new advances to medicine and industry in future. For instance, thermo-stable polymerase, such as 'Taq' and 'Vent' from aquatic extremophiles, *Thermus aquaticus* and *Pyrococus furiosus*, are commercially available enzymes used in molecular biology. The best-known commercial success of thermostable enzymes is the Taq DNA polymerase, obtained from *T. aquaticus* (Yellowstone hot spring). Marine *Thermococcales* have been an important source of high fidelity thermostable DNA polymerases (Pfu,Vent, Pab, etc.), accounting for 30% of total sales[29]. In addition, the high structural conservation and complementation of DNA replication proteins between euryarchaeal *Pyrococcus* and human make hyperthermophilic archaea, a model of choice to study eukaryotic DNA replication[30].

Deep-sea hydrothermal vent microorganisms are reported to produce unusual microbial polysaccharides with interesting chemical properties. Among these polymers, poly-β-hydroxyalkanoates (PHAs) are of special interest. In the same range of high molecular weight, biopolymers, chitin and chitosan are found to be associated with crustacean shells and fungi. These natural, non-toxic, biodegradable polymers have applications in food and pharma as well as cosmetics. Seaweeds are abundant source of natural polysaccharides, many of which have commercial uses. Algal products, such as agar and agarose, have been used in the laboratory for many years as nutrient media and gels for electrophoresis. Carrageenan, another algal derivative, is used as a thickener in processed food. Algae are also sources of vitamins, other nutrients, iodine, animal feed additives, fertilizer and pharmaceuticals[4]. Some other marine natural products include enzymatic hydrolysate, having antioxidant property, from fish, mollusk or shellfish. Fish oils are sources of polyunsaturated fatty acids (PUFA) and are of interest due to their physiological effects, like prevention of atherosclerosis, their role in anti-aging and in brain development in premature infants.

Marine Molecular Biotechnology

Understanding the properties and functions of genome is a fundamental task in modern bioscience. Molecular biology has a major role in many aspects of marine biotechnology. Genome studies of different commercially important fish are related to fishery. Genome analysis of marine microorganisms facilitates the use of genes for cell factories and bioindicator strains as well as identification of new drug targets. Marine sponge is a primitive organism in animal kingdom and is described as living fossil. Hence, the genome analysis of such a primitive organism is of special interest in molecular evolution. Efforts in this area have also proved that the application of molecular biological techniques in ecological studies will be helpful to explore molecular biodiversity[31], symbiosis[32,33] and defense mechanism[34]. In summary, 'marine molecular biotechnology', a new field of science is emerging out

Conclusions and Outlook

For more than 25 years, we are familiar with red, green and white biotechnology to describe biotech approaches for pharma, agriculture and microbial products. Blue biotechnology is a newcomer in this quartet, standing primarily for marine biotechnology. As described in this review, the rich diversity of marine biota and their unique physiological adaptations to the harsh marine environment has coupled with new developments in biotechnology. It has opened up new and exciting vista for the exploration of life-saving drugs, novel industrial products and processes, and environmental monitoring devices

India is blessed with a more than 8000 km of coastline, possessing over 2 million sq km of exclusive economic zone (EEZ). However, the potential of this domain as the basis for new biotechnologies remains largely unexplored. International research institutes have recognized the importance of establishing interdisciplinary research centers focusing on marine biotechnology. Similar efforts should be made in India in order to explore biotechnological potential of India's untapped marine biodiversity

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