

MARINE BIOTECHNOLOGY : OPPORTUNITIES FOR INDIA

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Biotechnology and genetic engineering offer great promises in finding novel solutions to biological problems that have challenged humanity for decades. The scientists are fully aware of this potential and in fact have ever succeeded in finding solutions to some of the existing problems. However, such attempts are limited mostly to agriculture and animal husbandry, and very little attention has been paid to harvest or utilize the biological resources of the oceans which cover approximately 71% of the earth's surface and harbour over one million species of animals, plants and microorganisms. From time immemorial, the sea has served as a constant and major source of fish and algae for human consumption; but our utilization of marine resources has been very much limited. A few countries have made substantial efforts to apply the experience, gained from agriculture, for cultivating and harvesting marine organisms. China and Japan have been at the forefront, as world leaders, in farming coastal waters.

The application of techniques of modern molecular biology and molecular genetics to marine organisms presents many unique opportunities and unlimited potential, for exploiting the genetic diversity of marine organisms, to obtain products of commercial value through marine biotechnology. The potential of marine biotechnology are two fold: the genetic potential and the crop potential. The genetic potential includes an organism's metabolic products (e.g., pharmacologically-active compounds, fine chemicals, etc.) and the processes it mediates *in situ*. The crop potential of marine organisms includes their use as food and food supplements and also, the applications of their biomass. It is true that marine organisms possess not only a remarkable phenotypic diversity but also a much more varied genotypic diversity. Compared to our knowledge about the molecular genetics of terrestrial flora and fauna, only very fragmentary information on the genetic diversity of marine life is available.

In the face of harsh conditions like high salinity, hydrostatic pressure, low temperature etc., the physiological adaptations, as offensive and

defensive capabilities of marine organisms, are in the form of bioactive metabolites (Martin and Padilla, 1973; Scheuer, 1973 and Hashimoto, 1981). These and other bioactive metabolites are excellent candidates for a variety of applications in, among others, the pharmaceutical, agrochemical and food industries (Scheuer, 1973; Baslow, 1977; Targett, 1979; Hashimoto, 1981; Faulkner, 1983; and Tachibana, 1984).

Aquaculture

One of the major applications of biotechnology in marine science is with regard to aquaculture. In any aquaculture practice the aim has always been to develop fish varieties that can grow faster, healthier, and bigger with more delicious meat.

McLean of the Southampton University was successful in introducing mammalian growth hormone (GH) genes, and in obtaining second generation transgenic fish. He was able to raise the transgenic fish upto 3 years from the second generation transgenics. Although there was evidence for the presence of the GH gene in these fish, no dramatic growth effects were so far observed. French Scientists have also made similar observations. Both groups have also reported that the mortality rate was high in transgenic fish. They have so far used the rat growth hormone gene with metallothionine promoters in the transgenic animals. Efforts are now underway to use fish metallothionine promoters and initial experiments show a 20 fold increase in the expression of growth hormones in cultured cells (rainbow trout hepatoma cells). The Japanese workers have succeeded in the integration of carp algae-globin gene (C G) into the genome of rainbow trout by means of microinjection (Oshiro *et al.*, 1989). Studies on gene transfer, expression and inheritance of rainbow trout and human growth hormone genes in carp and loach have also been carried out (Chen *et al.*, 1989). These results point to the possibility of raising fast growing fish strains for commercial purposes through manipulation of GH genes.

A major problem in aquaculture is disease control. In a recent study, Bright Singh(1986) pointed out that *Vibrio* was the causative agent for mass mortality of larval stages of *Penaeus indicus*. Application of monoclonal antibodies and DNA probes to new diagnostic methods for pathogens has become very promising. Recent advances made in the field of oyster pathology have opened the way to these new types of diagnostics for infectious diseases of molluscs (virus, rickettsia, protozoa) (Mialhe, *et al.*, 1989) Production, stabilization and delivery of

vaccines, employing both hybridoma technology and genetic engineering will definitely enhance the productivity from the egg through larval stages, presently a high-risk portion of the life cycle.

Another useful application of molecular techniques is for stock assessment of migratory fish and species identification which still remain as unresolved issues in fishery management. A method recently developed, for comparison of mitochondrial DNA (mtDNA) from different individuals, offers an opportunity for mapping the mtDNA genome. When applied to marine fishes and invertebrates, analysis of mtDNA offers a valuable mechanism for assessing the population structure in marine fishes.

Marine algae may represent the greatest market potential due to diversity of products that can be obtained from them (Singleton and Kramer, 1988). Besides their use as food for man and animals, several consumer products such as toothpaste, shampoo, dairy products etc., use the algal products. Many types of marine algae, both macroscopic and microscopic, can be used in marine biotechnology industries. Borowitzka and Borowitzka (1988) have estimated that only 60 of over 22,000 microalgal strains have been screened for vitamins, pharmaceuticals or biochemicals.

The culture of *Dunaliella salina* has been perfected. Over 50% of its dry weight is known to be glycerol (Borowitzka and Borowitzka, 1988; Moss and Doty, 1987). Currently, glycerol production is petroleum-based but it is possible to supplement its production with algal products. *Dunaliella* is also known to be a rich source of β -carotene (most promising source of lipid soluble dietary natural β -carotene) (Ben-Amotz, 1989). Some strains of microalgae are known to contain as much as 72% lipid which can be converted to a high quality energy source. In *Botryococcus braunii*, a new cyclic compound of botryococcene type hydrocarbon was found (Iwamoto, 1989). Catalytic cracking was applied to crude hydrocarbon and a high yield of gasoline fraction of a high octane number, suitable for direct use in automobiles was obtained. Some strains of red microalgae yield sulfated polysaccharide dyes and pigments and polyunsaturated fatty acids (Arad, 1920). Photoproduction of hydrogen by species of *Chlamydomonas*, *Chlorella* and *Halochlorococcum* is another possibility (Greenbaum, 1989).

Metabolic products of marine algae, especially microalgae, have significant potential as pharmaceutical compounds (Baslow, 1977), which are antimicrobial to antihelminthic to cytotoxic to anticoagulant

to hypocholesterolemic. A number of polyether compounds, of dinoflagellate origins, have been isolated, their structures have been determined, and they appear to be promising tools as specific ion channel agonists or as specific enzyme inhibitor (Yasumoto *et al.*, 1989). Out of 8 species tested, 7, produced antifungals, indicating wide distribution of such compounds in phytoplankton. Antifungal polyether macrolide, 'Goniodomin', has been isolated from the dianoflagellate, *Alexandrium hiranoi* (formerly *Goniodoma pseudogoniaulax*) (Murakami and Yamaguchi, 1989). Plant growth-promoting compounds are also common in marine algae (Augier, 1978; Kingman and Moore, 1982).

All three groups of macroalgae (Chlorophyta, Phycophyta and Rhodophyta) can provide digestible proteins, essential vitamins and trace minerals when consumed by humans. The seaweeds based industry, including their uses and cultivation techniques, has been summarised recently by Tseng (1984). Alginates, carrageenans and agar - all comprise the three general classes of seaweed-based phycocolloids, mainly used as gelling agents, stabilizers and emulsifiers. According to one estimate, the wholesale value of chemicals extracted from macroalgal sources was approximately \$500 million in 1983 (Moss and Doty, 1987). It has been well predicted that these markets will expand much further in the near future.

New strains of seaweeds have been developed through hybridization of gametes from separate populations. Such intra-specific crosses of kelps have been very successful. There are reports of success in inter-specific crosses and true inter-generic hybrids (Lewis *et al.*, 1986). Experiments of this type have been performed on members of Laminariales (e.g. *Macrocystis* vs *Nereocystis*).

One of the techniques very popular recently is the somatic hybridization (protoplast fusion). Protoplasts were isolated from *Ulva pertusa* and *Ulva conglobata* and fused (Reddy and Fujita, 1989). Protoplast induction, isolation, culture and fusion in *Gracillaria tenuisipitata* var. *lieu* have been attempted (Chou and Lu, 1989). This species is a major cultivar in Taiwan earning approximately \$16 million annually.

Plant cell culture in bioreactors for the production of natural products has become a reality. Successful isolation of 20 cell lines capable of accumulating secondary metabolites, at levels exceeding that occurring in the plant, represents substantial advance. Monoclonal antibodies have been used to identify the redtide algae *Chaetonnella marina* and *Chaetonnella antiqua* (Nagasaki *et al.*, 1989). For better exploitation of

the potentials of marine algae, the information base on their genetics, chemistry, biology and ecology should be collected.

Industries

Biopolymers

Use of marine polysaccharides has yielded large profits and has opened the door to many basic and applied opportunities (Colwell *et al.*, 1985). One of the most remarkable developments in fluid mechanics, in recent times, has been the discovery that certain long-chain molecules, dissolved in quite small concentrations (mg/l), can lower the turbulent friction of the solvent by very significant amounts. However convincing explanations are not available for this property. The polymers of molecular weight exceeding 50,000 of a generally linear structure and soluble in the liquid of interest can reduce the turbulent friction, by up to 70% to 80%. Many polysaccharides fall under this category, and some exploratory work with marine polysaccharides shows that there are numerous candidates with high potential for drag reduction in turbulent flow. Among the marine polysaccharides, carrageenan and sodium alginate are effective drag reducers. A number of polysaccharides, isolated from bacteria and microalgae, also show this property. An important application of this might be its use to reduce the resistance of ocean-going ships, which means a saving of millions of dollars.

The polysaccharides show greater promise in enhanced oil recovery (EOR) processes from oil wells. Upon depletion of natural pressure after certain years of pumping oil, only 20-30% of the original oil will be recovered. At this point, the primary production generally stops and water flooding is the most common next step. Substantial quantities of oil remain trapped in the pore space of reservoir rock even after water flooding. Therefore a number of EOR methods are under study including thermal, carbon-di-oxide and chemical flooding. Chemical flooding techniques include the use of surfactants, miscible hydrocarbons, alcohols, alkali and polymers. A major problem encountered in simple water flooding is related to "fingering" of the injected water through the mobile fluid front, resulting in premature break through to the production wells. To reduce fingering, high molecular weight water soluble polymers may be added to the injection water to increase its viscosity. 'Xanthan gum' produced by *Xanthomonas campestris* has been successfully tested for this purpose. However many more poly-

saccharides for such possible applications (surface active and chelating agents; stabilizers in chemical reactions as enzyme immobilizers; in medicine, as anti-tumour agents and anti-coagulants; in food processing industries etc.) have been reported from the marine environment.

The microbial colonization of a surface appears to depend on the ability of microorganisms to adhere to the surface with the aid of polysaccharides or lectin-like polymers, found on microbial surfaces. Once such potential strains are isolated, the product yield can be increased with recombinant DNA techniques:

- specific genes can be isolated and identified and the concentration of gene products per cell can be raised as a result of increased gene number per cell.
- more important, basic questions as to how genes are controlled, how they are switched on and off and their products interact with and are regulated by metabolite of the cell can be answered as a result of our ability to isolate specific genes.

Preparation of monoclonal antibodies to seaweed carbohydrates has become possible and are found useful in a number of situations:

- localization of carbohydrate antigens on cell surfaces or on tissue sections.
- locating the part of the plant or the stage of its life cycle which is enriched with an antigen.
- rapid screening of algal strains, mutants and hybrid cells or plants for increased (decreased) content.
- monoclonal antibodies can serve as specific markers in conjunction with genetic engineering applications of modifying carbohydrate production pathways.
- biomedical application: *Pseudomonas alginate* (which cross-reacts with anti-algal alginate antibodies) in cystic fibrosis pulmonary infection.

Chitin, from which 'chitosan' is derived, is the most widely distributed and one of the most abundant biopolymers in the world. The amount of chitin synthesized by marine copepods alone is estimated to be one billion tons per year. Potential commercial sources of chitin are the shells of crabs, shrimps, lobsters, krill, clams, oysters and squid.

The global annual estimate of commercial chitin production from these sources is approximately 1,50,000 tons. Chitosan is used as adhesive, chelating agent, coagulant, drug carrier, membrane, paper and textile additives, in photographic products and as surgical adjuncts, and textile finishers.

Recently a biodegradable polymer, 'BIOPOL' was discovered in Australia. 'BIOPOL' is a polyhydroxybutyrate co-valerate polymer produced by bacteria in a fermentation reaction. 'BIOPOL' is even considered as the polymer of the future. This can be:

- used to extrude and mould like polypropylene
- used as a package material
- degraded in waste water, seawater and sewage
- compatible with animal tissues (microspheres prepared with this polymer can be impregnated with active ingredients such as vaccines, steroids, growth hormones, cytotoxic drugs for cancer treatment and either injected or placed subcutaneously for steady release as the polymer gets bioabsorbed)
- used in bone repairs
- used for progressive release of insecticides as microspheres biodegrade in soil.

Eventhough this polymer is four times costlier than traditional synthetic polymers, advancement in technologies and discovery of new sources for similar polymers in the marine environment can possibly bring down the cost to acceptable levels in the near future.

Antibiotics

Eventhough many marine organisms are reported to produce antibiotics, the pharmaceutical industry has been slow in making use of them. In one of the earliest reports (De Glaxa, 1889), the presence of antagonistic bacteria in seawater, which inhibited the causative agents of cholera and anthrax, was noticed. However, 60 years passed before we could see the next report in 1947. Rosenfield and Zobell (1947) described the production of antibiotics by marine bacteria. Then a proliferation of research resulted in numerous publications, starting from 1966. These works not only revealed the new antibiotics but also their novel chemical structures (Burkholder *et al.*, 1966). The antibiotic isolated from bacteria

(*Pseudomonas* sp.) associated with *Thalassia* sp. (turtle grass) was found to be 2,3,4-tribromo-5 (1'-hydroxy-2', 4'-dibromophenyl)-pyrrole. Within a decade of this work, another inhibitory bromopyrrole was discovered in marine bacteria (Anderson *et al.*, 1974). The significance of brominated antibiotics increased, as a result of work by Gauthier and Flateau (1976), who succeeded in isolating polyanionic polysaccharides as well as two brominated antibacterial compounds from *Alteromonas luteoviolaceus*. A marine pseudomonad (*Alteromonas*?) which was inhibitory to *Candida albicans*, *Staphylococcus aureus*, *Vibrio anguillarum* and *V. harveyi*, produced two novel antibiotics namely 2-n-pentyl-4-quinolinol and 2-n-heptyl-4-quinolinol (Wratten *et al.*, 1977).

A low molecular weight (<2000 daltons) inhibitory compound was isolated from another *Alteromonas* sp., associated with seaweeds (Lemos *et al.*, 1985). Imada *et al.* (1986) also recovered a low molecular weight (1500-1700 daltons) peptide, termed 'Marinostatins' from *Alteromonas*. 'Aplasmomycins' isolated from a marine strain of *Streptomyces griseus* indicated pronounced activity against mycobacteria and also against *Plasmodium berghei* (the causative agent of malaria) (Okami, 1986). He also described another antibiotic, 'Istamycin' from a marine actinomycete. Although majority of these studies highlighted antibacterial agents, reports dealing with the producing of antiviral compounds by marine microorganisms (Katznelson, 1978; Toranzo *et al.*, 1982) are very few.

Okami (1986) reported the isolation of a polysaccharide, with marked activity in mice against Sarcoma-180 solid tumour virus (S-180), from a marine flavobacterium associated with seaweed. The compound named 'Marinactan' (containing 69.7% glucose, 19.8% mannose, 10.8% fucose), produced complete regression of tumours.

During the recent past, two major investigations were carried out on the ecology and activity of marine antagonistic *Streptomyces* spp. in India (near Porto Novo, east coast of India) (Lakshmanaperumalsamy, 1978; Vanaja Kumar, 1979). Nearly 75% of the streptomycete cultures isolated from molluscs exhibited antagonistic properties (Vanaja Kumar, 1979) which is very high compared to those from sediments (27%) (Lakshmanaperumalsamy, 1978). This observation indicates a very strong possibility of obtaining new and potent antibiotics from the microflora associated with marine organisms. The antibiotics isolated in the above studies are yet to be characterized.

Enzymes

The industrial use of enzymes has increased substantially in recent years. The uses of lactases (confectionery) and proteases (detergent) are well documented. Many marine bacteria are known to produce proteases, alginate lyases and chitinases. Bacteria isolated from deep-sea thermal vent areas may prove to be useful sources of heat-stable enzymes. *Bacillus circulans* isolated from marine mud is known to produce glucan hydrolyzing enzyme (Okami, 1986). This has significance in dental cases where insoluble glucan is produced by *Streptococcus mutans*.

L-Asparaginase (L-asparagine amidohydrolase EC 3.5.1.1.) is used in the treatment of acute lymphatic leukaemia of human beings with 60-80% remissions. The commercially available preparation is obtained from *Escherichia coli*. A number of marine microorganisms are also known to produce the enzyme. However, according to Selvakumar (1979), the preparation he obtained from a marine *Vibrio* sp., isolated from Porto Novo coastal area is superior to that of *E. coli*. Interestingly this enzyme was also found to be present in marine fungi (Nair *et al.*, 1977) and as free enzyme in marine sediments (Selvakumar *et al.*, 1977).

Surfactants

A marine isolate of *Actinobacter* was found to produce a surfactant which was later commercialized (Patel and Hou, 1983). The surfactant polymer (named as 'Emulsan') comprised of a polysaccharide moiety of N-acetyl-D-galactosamine, N-acetylaminoauronic acid and an amino sugar. - and - hydroxydodecanoic acid, were found to be esterified to sugars (Zosin *et al.*, 1982). This product was successfully marketed as a chelating agent for oil tankers and oil storage tanks. Another property of this compound is its ability to complex with uranium which may find application in recovery of uranium from waste material (Zosin *et al.*, 1982).

Others

Production of other polymers such as polyhydroxy-acid of commercial value (Stanley and Stanley, 1986), and vitamins, by marine bacteria, is well known. Bacterial polymers have also been implicated in inducing settlement of marine invertebrate larvae (Bonar *et al.*, 1986) which may have useful applications in aquaculture practices. The marine yeast *Candida maris* has potential for the production of SCP (Phaff,

1986). The marine bioluminescent bacteria can be advantageously used for pollution monitoring of our seas. Detailed studies on their occurrence, distribution and physiology with special reference to light production have already been conducted (Ramaiah, 1989).

The foregoing account clearly highlights the great promise offered by marine microbes. With appropriate technology, the strains can be improved to meet most of our demands. Now that many marine bacteria are known to harbour plasmids, they could be of immense value to molecular biologists.

Pollution control

The disposal of xenobiotic halogenated hydrocarbons, inorganics and heavy metals into the marine environment has become a serious problem especially in recent times. This has resulted in the formulation and implementation of special Environmental Protection Acts by various countries. So the industries all over the world are now more keen to dispose of their hazardous wastes in a safe manner, so that they can avoid environmental pollution which attracts heavy penalties. Many waste treatment companies developed biological methods to convert the hazardous waste (i) into harmless waste through biodegradation, (ii) into useful energy sources and (iii) to recover useful components from such wastes, if any. All these processes need appropriate technology using naturally selected or genetically engineered microbial strains. In fact biological waste treatment has developed as a 'bioremediation industry'. Laboratory scale experiments have shown that some bacteria are capable of even breaking down the highly toxic polychlorinated biphenyls (PCB's), strongly resistant to degradation.

Considerable success has been achieved in removing the heavy metal ions from industrial effluents. The metallothioneins are small cysteine-rich proteins, with very high affinity for heavy metal ions such as zinc, cadmium, copper and mercury. Studies have been conducted to isolate metal-binding protein genes from potential organisms and cloning them into species of marine plants that could be strategically grown in harbours and coastal waters. If large quantities of pure metallothionein proteins can be made by rDNA methods, then it should become possible to produce affinity column resins, containing these proteins, bound to solid supports. Such resins could be used to treat the sewage and industrial effluents.

Another important problem is the safe disposal of wastes at low temperature (around 0°C). The psychrophilic bacteria, abundant in the permanently cold marine environment, exhibit very useful characteristics for biodegradation at low temperatures. To prove the efficiency of marine psychrophiles in key processes of organic matter degradation at high latitudes, the enzymatic degradation of scleroprotein and chitin was investigated in the cultures of Antarctic marine psychrophiles (Reichardt, 1989). Marine psychrophilic bacteria also offer great promise for application in waste disposal in colder and high altitude regions.

Basic studies

Biofouling

Fouling of surfaces (both micro- and macrofouling) is a serious problem in the marine environment causing extensive economic loss. For the last several decades attempts have been made to control biofouling but with little success. The crux of the problem lies in a basic understanding of the processes. The ability of bacteria to find, attach, adhere and elaborate specific film-forming substances (such as mucopolysaccharides) and to regulate expression of all these functions is fundamental to the fouling processes. Now it is possible to analyse the processes much more in detail, even at molecular levels, using the modern tools of biotechnology. Once we recognize and understand the specific genetic elements responsible for specific polysaccharides and polypeptides, it is possible to intervene these processes more effectively and arrive at methods to control biofouling. In fact, some of the marine organisms such as corals, are known to avoid successfully any fouling of their body surfaces. The Caribbean Sclerosponge, *Ceratoporella nicholsoni*, which contains an abundant and complex microbial community, is resistant to fouling (Colwell and Santavy, 1989). Our greater understanding of such potentials will definitely lead to duplicate these mechanisms for our benefit.

Bioactive compounds

Besides various antibiotics and anticancer compounds, a number of marine bacteria possess the ability as 'puffer-fish toxin'. Kogure *et al.* (1989) hypothesized that this toxin is produced solely by bacteria and subsequently transferred to higher organisms through the food web in the

marine environment. Similarly, compounds showing antineoplastic activity in both lymphocytic leukemia and nasopharyngeal cancer cell lines, found in *Ceratoporella nicholsoni* (Colwell and Santavy, 1989) are considered to be products of microbial community associated with it. There may be several such animal-microbe interactions in the marine environment. Marine bacteria also produce medically important enzyme inhibitors. Protease inhibitors have been isolated from *Alteromonas* sp. (Imada, *et al.*, 1989).

The ability of marine plants to withstand the osmotic pressure gradients, imposed by the concentration of salt in the sea, shows the possibility of developing new strains of terrestrial plants which can be cultivated using seawater. Infact a successful attempt has already been made in developing a salt tolerant tomato plant (Colwell, *et al.*, 1984).

Information outlined here gives us an overall picture of potentialities that exist in the sea for biotechnological applications. India has a very long coast line and an ever increasing population with its associated demands and it should make use of the marine potential by proper planning, investment and management strategies. India can concentrate on four areas viz. aquaculture, industrially important chemicals, including bioactive compounds, pollution monitoring and control, and waste utilization. In order to coordinate the entire developmental activities in the country in the field of marine biotechnology, a national central facility and germ plasm bank for marine organisms need be set up immediately. The Government of India has already formulated the guidelines for the manufacture and use of genetically engineered microorganisms under the Environment Protection Act, 1986 (Gazette of India : Extraordinary Part II-Sec.3(i), Dec., 5, 1989). Obviously our approaches in solving any problem, either in aquaculture or in waste disposal should be based on careful planning and time targeted goals, with the creation of trained manpower in marine biotechnology. We need a detailed plan on our future strategies for an integrated development through marine biotechnology.

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