Perspectives in Marine Biotechnology Research and Development in India

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Introduction

In simple terms 'Marine Biotechnology' can be described as the sustainable commercial exploitation of marine life for the benefit of mankind. Marine biotechnology has continued to develop in recent years as a field of application of modern science and engineering of critical importance to the understanding, protection and exploitation of the potential resources of the sea, for the progress of fundamental science and benefit of humanity. Oceans comprise the biggest part of the biosphere and hold the most ancient and diverse forms of life. It is recognized that the sea's resources remain largely unexplored and marine organisms represent a vast untapped resource with potential benefits in many different areas of life, including mariculture, fisheries, industry, research tools and environmental applications. The combined expertise of many fields from molecular biology to chemical and physical oceanography contribute to the development of the knowledge platform upon which marine biotechnology applications render goods and services for public benefit.

With its vast human capital, long coast line and tropical seas, marine biotechnology in India has the great potential to emerge as the major cutting edge technology next to the information technology. Post green revolution era showed glaring inadequacies in the agricultural sector alone to produce the food for the ever-increasing Indian population. Hence, we have to look to alternatives such as mariculture (growing/farming animals and plants in marine environment) for meeting increasing demand for protein source. When the global mariculture production touched about 20 million tons with a value of $ 26.2 million and marine bioprospecting is estimated at somewhere between US $ 30-100 billion, the marine biological wealth of India remains largely untapped even today. The effort of farming of marine organisms for food and exploitation of sea for other commercial use is only in the nascent stage in India, initiated by institutions like Central Marine Fisheries Research Institute (CMFRI).

Marine biotechnological research and development in India

India is bestowed with vast stretches of marine and brackishwater habitats and their bountiful living resources. Indian coasts that extend to about 5,700 km on mainland and to about 8,118 km including two groups of islands (Lakshadweep and Andaman & Nicobar) have rich diversity of corals, fishes, crustaceans, molluscs and seas of microbes. The Western coastline (covering Gujarat, Karnataka, Maharashtra, Kerala and Goa) has a wide continental shelf having an area of about 0.31 million km, which is marked by backwaters and mud flats. The East coast consists of Tamil Nadu,

Pondicherry, Andhra Pradesh, Orissa and West Bengal, is flat, deltaic and rich in mangrove forests covering an area of about 1,430 km. Asia's largest brackish water lake Chilka, Andaman & Nicobar islands, Lakshadweep, Gulf of Kutch and Khambat and Gulf of Mannar are also important biodiversity reserves.

The Government of India, in order to promote biotechnology, relevant to the needs and priorities, constituted an agency, viz. the National Biotechnology Board (NBTB) in the year 1982 under the Ministry of Science and Technology, as an apex coordinating body to identify priorities, coordinate, oversee and plan for required manpower, integrated industrial development and large scale use of biotechnology products and processes later upgraded in the year 1986 into a full fledged separate Department under the Ministry of Science and Technology, viz. Department of Biotechnology (DBT). The DBT has set up a task force on aquaculture and marine biotechnology, under which the projects are funded for utilizing marine resources for exploitation and products and processes from marine organisms utilizing biotechnology tools and techniques. Marine biotechnology in India primarily targets research and development in the areas of mariculture, prospecting for beneficial biomolecules from marine sources and development of bio-processes for ensuring food security, sound health and clean environment to its people. The progress in some of these areas and the prospects of application of marine biotechnology are presented under different headings.

Genetic improvement of mariculture stock

Success in aquaculture/mariculture ventures purely depends on the sound management and use of superior germplasm with high production potential. Though one can bring about improvement in the production performance by environmental manipulations as well as through genetic manipulations, any improvement from the former cannot be transmitted to the next generation unless and until the improvement is inherited. Though a number of modern genetic manipulations techniques like genetic engineering as well as chromosomal engineering for production of polyploid, gynogenic and androgenic populations are available for genetic improvement, the quantitative genetics is still an attractive technique for production of genetically superior brood stock.

Stock improvement through quantitative genetic tools

Quantitative genetic approach for production of genetically superior brood stock is a time tested technique. In farm animals and plants, application of quantitative genetic principles and genetic tools has lead to increased production in accordance with the demands of the nation. The wide variations among the individuals of a population provide ample scope for genetic manipulations for developing genetically superior lines and strains.

Selective breeding

Selective breeding is a potential technique can be applied to develop genetically improved strains of finfish, shellfish and live feeds for aquaculture ventures. However, an in-depth knowledge of the genetics of the species in question is a pre-requisite for the formulation of suitable techniques for their improvement. Knowledge of the genotypic and phenotypic parameters is vital for any quantitative genetic improvement programmes. Estimates of parameters such as heritability, phenotypic and genetic correlations, heterosis, genotype environment interactions etc. are essential for planning a proper breeding strategy. Scientific breeding programme could be formulated only after careful consideration of these parameters. As for example, when there is relatively larger

additive genetic variance, simple selection methods like individual/mass selection should yield good progress. On the other hand if non-additive genetic variance is predominant, special selective breeding methods are to be formulated to exploit them. When over dominance is important for a trait, reciprocal or recurrent reciprocal selection needs to be employed. If heterosis is found to be high, cross breeding programmes could be given priority. Genotype environmental interactions of high magnitude call for developing different strains to suit each of the environments.

Genetic improvement of mariculture stock and live feeds are still remains a gray area in our country and only very limited reports are available on the application of quantitative genetics for genetic modification of marine species in India. The initiative in quantitative genetic work on species of mariculture importance has come from CMFRI, India, to study the quantitative genetic parameters as well as the genetic and phenotypic response to selection in *Artemia franciscana* from Great Salt Lake, Utah using mass selection for genetically altering the naupliar size (ìm) and was found very successful. The substantial genetic gains realized from selection indicated the usefulness of selective breeding for developing genetically altered lines. No comparable work in *A. franciscana* was available.

Inbreeding and crossbreeding (hybridization)

These are two traditional breeding approaches that have been successfully used for the improvement of crops and livestock. Inbreeding is often combined with hybridization to improve the results of the crossbreeding programme. Crossbreeding is a breeding programme that tries to find mating combinations between different populations of fish which produce superior offspring for grow-out, offspring that are said to exhibit hybrid vigor. Although crossbreeding is a tried and true method of increasing yields, the results of crossbreeding programmes are impossible to be predicted (unless the mating has been made previously). Many combinations often have to be evaluated before a combination that produces offspring with hybrid vigor is discovered. Crossbreeding programmes usually involve different strains within a species (intraspecific hybridization), but different species can also be hybridized (interspecific hybridization). To date, much of the breeding work in aquaculture has been devoted to hybridization among the different species of tilapia in an attempt to produce all hybrids for grow-out. In general, crossbreeding is used to produce superior fish for grow-out, while genetic selection is used to create superior brood fish. The hybrids that are created in a crossbreeding programme are usually grown and sold as food. On the other hand, brood fish that are created in a selective breeding programme are created to produce the next generation of genetically superior fish for grow-out and their offspring can, in turn, be retained and selected to continue the process.

Chromosomal engineering for production of superior stock

In recent years, chromosome engineering research had led to the development of animals with controlled sex and reproductive characteristics. These include the induction of polyploidy, androgenesis and gynogenesis.

Ploidy manipulation

Ploidy manipulation has received much attention, mainly because of its potential advantages in *Crassostrea virginica* successfully. Thereafter, triploidy was induced in *C. gigas, C.virginica, Saccostrea glomerata* and *Ostrea edulis*. In India, CMFRI has successfully developed triploid edible oyster and reared.

Gynogenesis

Gynogenesis is a special kind of reproductive process facilitating the inheritance of maternal genetic material alone into the progenies. Gynogenesis occurs naturally in some fish species but it can be induced artificially. The research in gynogenesis may help to (i) produce all-female populations, (ii) establish isogenic and homozygous lines of fish (iii) increase higher production at least in some species and (iv) understand the genetic regulation of development and construction of linkage maps.

Androgenesis

Androgenesis is a developmental process facilitating the inheritance of paternal genetic material. Androgenesis may prove useful technique for production of (i) viable YY supermale in male-heterogametic species, (ii) inbred isogenic lines and (iii) conservation of germplasm. Androgenesis has successfully induced viable YY supermales in a few cyprinids, cichlids and salmonids.

Biotechnological manipulation of sex and reproduction

Biotechnology can be applied to enhance reproduction and early development of cultivated aquatic organisms. This is particularly important because of the difficulty to depend on the vagaries of nature for procuring spawners for the year round hatchery operation. Many important cultured marine species fail to breed in captivity. This bottleneck has been overcome by biotechnological application, e.g., hypophysation using hormones and synthetically produced analogues. The most common breeding programmes in aquaculture are the production of sex-reversed brood stock to produce monosex populations for grow-out. This is done either because one sex is superior or more desirable or to prevent reproduction during grow-out. In aquaculture a particular sex preference is usually linked with desirable traits, such as faster growth rate. In India, attempts are being made to produce tetraploids by ploidy manipulation in *Oreochromis* spp. The rationale for the induction of such ploidy with differing genomic status in a number of fish is its potential to generate genetically sterile population and rapidly inbred lines, which could ultimately benefit the aquaculture sector.

Cryopreservation of gametes and embryos

Some important cultured species, such as Seabass (protoandrous) and groupers (protogynous) are sequential hermaphrodites in which getting the inverted sex in mature condition from the wild is almost always an uphill task. Here too, modern biotechnological innovations have significant role to play for ensuring the availability of reproductive elements from both sexes of broodstock ready at the same time for spawning. The problem of lack of synchronization of maturity in male and female fishes can be overcome through establishment of sperm or seed banks. Although sperm cryopreservation is well known, research is needed for cryopreservation of ova/embryos of cultivable species. Utilization of cryopreserved gametes in aquaculture would greatly reduce the cost of maintenance of broodstock and also permit free exchange of superior quality stock between different geographical origins. The studies on cloning through embryonic stem cells are the one of the emerging alternative technology, which can be used in the field of fish biodiversity and conservation of species.

Genetic engineering

Genetic engineering is being increasingly attempted for producing superior genetic stock. Production of transgenic with desirable traits such as faster growth, disease resistance and increased environmental tolerance is an active and important research area in aquaculture. The commercial

potential of transgenic fish lies in developing transgenic broodstock lines with desired traits. This requires combining transgenic technology with traditional selective breeding program to produce superior strains of fish. Transgenic salmon has been reported to adapt itself in cold climate when a gene encoding antifreeze protein was used for transgenesis. In India preliminary success has been reported in developing gene transfer technology in zebra fish, medaka and Indian catfish.

Although genetic engineering has generated much publicity, this type of breeding programme is very expensive, highly regulated and requires trained scientists. Commercialization of transgenic technology for food production has run into problems due to the concerns of safety and ethics. The gene transfer efficiency needs to be improved further along with the lookout for more useful novel genes. Due to the ethical issues related to production of transgenic food fishes, researches have diverted into the production of genetically modified ornamental fishes for aquarium keeping. The glaring examples is the use of tiny aquarium zebra fish, a popular laboratory animal, genetically modified to produce fluorescent pigments viz. red, green, yellow and is being promoted as a household aquarium pet, the 'glofish'. Ornamental transgenics in India has a promising future with large number of freshwater and marine ornamental fishes. The recent success of CMFRI in closing the life cycle of clown fish, a popular marine ornamental fish, is an achievement in this line of research.

Marker assisted selection (MAS)

A wide array of molecular markers, such as allozymes, mitochondrial DNA, RFLP, RAPD, AFLP, microsatellite, SNP and EST's are being used for characterization of aquatic animal populations. The use of molecular markers include identification of individual animals in broodstock populations, determination of genetic diversity between randomly selected animals, identification of the broodstock parents of post larvae or juvenile progeny displaying desired traits, identification of siblings and half-siblings in a mixed parentage spawn. **Identification of genetic relatedness,** genetic diversity, pedigree determination, **molecular tagging**, tracking family and population lines, strain identification are the potential application of markers. Determination of markers linked to **Quantitative Trait Loci (QTL) of economical important traits is envisaged to have beneficial role in the development of** genetically superior broodstocks.

As most of the production traits are controlled by multiple genes and inherited as quantitative traits, analysis of their associated quantitative trait loci (QTL) is emerging as a very important part of aquaculture genetics/genomics. Marker assisted selection refers to a selection process in which future breeders are chosen based on genotypes using molecular markers. To implement MAS, researchers need to produce high-resolution linkage maps, understand the number of QTL affecting a given performance or production trait and their mode of inheritance and relative contribution, determine the linkage and potential interactions of different QTL for the trait and for other traits and estimate the economic importance of each trait. This in turn would lead to even more precise selection by gene assisted selection (GAS), in which future breeders could be chosen according to favorable genotypes based on genes directly controlling performance traits, rather than on neutral markers associated with those traits via linkage.

Molecular Taxonomy

Molecular taxonomy is the identification of specimens based on molecular rather than morphological characters. Molecular techniques have become a major tool for systematic

ichthyologists at the species level and above. These approaches may also be useful to fishery biologists for taxonomic ambiguities ranging between the species and population levels.

DNA sequence analysis is a powerful tool for identifying the source of samples thought to be derived from threatened or endangered species. CMFRI has generated partial DNA sequences of mitochondrial cytochrome b and control region (D- loop) of 11 species of cetaceans (marine mammals), bottlenose dolphin, spinner dolphin, spotted dolphin, common dolphin, humpbacked dolphin, Risso's dolphin, finless porpoise, sperm whale, blue whale, Bryde's whale and dugong. Till date the Institute has released a total of 63 sequences of cytochrome b gene and control region of mtDNA from 40 individuals of 11 species in the GenBank (NCBI). Gender identification is of fundamental importance in the studies of population structure, social organization, distribution, behaviour or heavy metal accumulation in marine mammals. However, distinguishing the males and females among the marine mammals is difficult due to the poor sexual dimorphism, especially during their free ranging state. With the advent of DNA-based tools, such as PCR, it is possible to even identify the sex using tissue sample. Recently CMFRI has developed sex determination technique based on the amplification of genomic DNA extracted from the skin tissues of marine mammals. Molecular sexing was standardized by the Institute in several species of dolphins, finless porpoise, whales and dugong. Molecular taxonomy could also prove to be a promising tool in mussel farming for larval identification, regardless of developmental stage and can be used for the assessment and tracking of larval dispersion as well as assist in the identification of sites of seed settlement for their optimum exploitation.

Nutrition and feed biotechnology

Nutritional biotechnology applications in livestock feed sector itself is low in India. It is lowest in the aquaculture sector and in the area of marine biotechnology is only getting initiated. Major areas were biotechnology research outputs have translated into products available in the open market are, enzymes, probiotics, prebiotics, dietary aminoacids, toxin binders and nucleotides.

Enzymes

Improvement in nutrient availability and reduction in waste outputs are the major advantages of using enzymes in feed. These enzymes are mostly from microbes, which are genetically modified or biochemically refined to produce the enzymes in large quantities with desired properties to make them economically viable. Enzymes used for the formulated feeds need to be robust to stand variations in physiochemical parameters. They need to have high temperature stability to withstand pelletization and also have a long shelf life.

One of the most promising enzyme is phytase that breaks down the indigestible phytic acid (phytate) in plant based nutrient sources such as cereals and oilseeds and releases digestible phosphorus. This reduces the use of expensive supplemental inorganic phosphorus like dicalcium phosphate in feeds. Addition of inorganic phosphorus results in excessive excretion of phosphorus in manure, posing an environmental concern, especially in areas of intensive animal production. Phytase also releases minerals (Ca, Mg, Zn and K), amino acids and proteins, which are complexed with the phytate molecule.

Seaweeds utilization

The Indian coastal line offers wide scope and suitable environment for sea weed culture. Nearly 10 – 12% of the total marine algae available in different parts of coastal areas come under the category of important seaweeds belonging to green, red, blue and brown varieties. Most of the seaweeds are rich source of bioactive compounds, trace elements, minerals and proteins which finds their extensive application in the field of food & beverages and pharmaceutical industries. The cultivation of these seaweeds in turn will augment the social and economic status of coastal people

Probiotics

Microorganisms are natural inhabitants in the digestive system of the animals. Some microbes aid beneficial and others can cause pathogenesis. Understanding the microbial ecology of the gut merits great attention due to implication of gut ecology for nutrition, feed conversion and disease control. Use of antibiotics disturbs the microbiological balance of gut flora and which leads to the elimination of the major beneficial flora. On stopping the antibiotic treatment, pathogens begin to reestablish themselves in the intestine. Overgrowth of these organisms and subsequent invasion of the system by pathogenic organisms cause inflammatory, immunological, neurological and endocrinological problems. Probiotics are "Live microorganisms which when administered in adequate amounts confer a health benefit on the host". Application of Probiotics can help build up the beneficial bacteria in the intestine and competitively exclude the pathogenic bacteria. These bacteria also release enzymes, which help in the digestion of feed. The concept of using probiotics or direct fed microbials in animal feed particularly poultry and aquaculture is slowly becoming popular. The common organisms in probiotic products are *Aspergillus oryzae, Lactobacillus acidophilus, L. bulgaricus, L. plantarium, Bifidobacterium bifidium, Streptococcus lactis* and *Saccharomyces cerevisiae*. These products can be administered through water or incorporated in the feed. Indian researchers have successfully isolated indigenous microbes from saline waters with probiotic properties with potential application in aquaculture/mariculture.

Prebiotics

The concept of prebiotics in feed is fairly recent. Prebiotics are basically feed for probiotics where they are resistant to attack by endogenous enzymes and hence reach the site for proliferation of gut microflora. Some of the prebiotics, which are currently used in animal feed, are Mannan-oligosaccharides (MOS), fructo-oligosaccharide and mixed oligo-dextran. The concept of using prebiotics has not yet been accepted but the advantages of prebiotics are that it can stand high pelletizing temperatures in the feed and also have a long shelf life.

Bioremediators

Beneficial microbes have also been used in a big way as pond cleaners or bioremediators in aquaculture. Bacteria directly uptake or decompose the organic matter or toxic material and improve the quality of water. The microbial cultures can produce a variety of enzymes such as amylase, protease, lipase, xylanase and cellulase in high concentrations than the native bacteria, which help in degrading waste. The pond probiotics also have a special blend of denitrifying bacteria that remove the algal primary source of food, nitrogen from the water. This drastic reduction in nitrogen concentration makes it difficult for the algae to bloom thereby maintaining pond health. Commercial

bioremdiators such as *Detrodigest* developed by research institution such as national centre for aquatic animal health (NCAAH), Cochin, using indigenous bacterial isolates has already in use by the aquafarmers in India.

Dietary amino acids

Essential aminoacids are added as supplement to the feed to get a balanced aminoacid profile. Since the aminoacid profiles of the ingredients do not match the profile of aminoacid requirement of the specific species, supplemental essential aminoacid are added. The new trend is to formulate diets on digestible amino acid levels thereby reducing the requirement of protein. So far lysine and methionine have been used as supplements. Genetically enhanced micro-organisms are being used to produce threonine and tryptophan on a commercial basis. Using all these amino acids it is possible to lower dietary crude protein level by 2 – 3 %, which is a substantial saving for the farmer.

Toxin Binders

Feed manufacturers have been incorporating various mold inhibitors in their diets to prevent mycotoxin formations. A variety of physical, chemical and biological approaches to counteract the mycotoxin problem have been reported, but largescale, practical and cost effective means for detoxification of mycotoxins containing feed stuffs are limited. Most of them are fungistats and not fungicides that is they only inhibit growth of moulds and do not inactivate any toxins already present. Present day methods are, use of organic acids and their salts like propionic acid or adsorbents like bentonites, zeolites, and hydroxyl aluminosilicates. In the future, biotechnology based products like microbes, herbal extracts or esterified glucomannan could be used. Aqua extracts of garlic, onion, turmeric, neem have been shown to exert antifungal activity or inhibit aflatoxin production.

Nucleotide nutrition

Nucleotide nutrition research in fishes is in its infancy and many fundamental questions remain unanswered, observations so far support the contention that nucleotides are conditionally or semi-essential nutrients for fishes and further exploration of dietary supplementation of nucleotides for application in fish culture is warranted. Hypothesized reason(s) associated with these beneficial effects include dietary provision of physiologically required levels of nucleotides due to limited synthetic capacity of certain tissues (e.g. lymphoid), inadequate energetic expenditure for de novo synthesis, immunoendocrine interactions and modulation of gene expression patterns. However, currently there are numerous gaps in existing knowledge about exogenous nucleotide application to fish including various aspects of digestion, absorption, metabolism and influence on various physiological responses especially expression of immunogenes and modulation of immunoglobulin production.Encapcell®., a patented nucleotide is a new, microencapsulated feed additive that enhances growth and aids in preventing disease. Incorporation in animal diets, it gives better weight gains, improves feed conversion, aids in reducing mortalities, improves general health and dramatically increases antibody production when used with vaccines.

Nutrigenomics

Functional genomics refers to how the genome of an organism regulates homeostasis and responds to stimuli. The application of functional genomics in nutrition research is known as

nutrigenomics. Influence of feed (nutrients) on the organism at a molecular level is the simplest explanation of nutrigenomics. With the development of cutting edge tools in molecular biology monitoring the affect of a nutrient at the molecular level is reality now. The regulatory control mechanisms of these processes can be based at all levels from genetics and gene expression to the feedback of specific metabolites. Modern technology is providing a new opportunity to monitor the regulation of these processes on a system wide basis. Nutrition researchers are just beginning to utilize these tools to ask key scientific questions about diet and its effects on the organism using functional genomics. The mechanism by which nutrients specifically regulate the expression of genes in vertebrates in general is poorly understood. These technologies have significant implications for nutrition research and include aspects of genomics (polymorphism), functional genomics (gene expression), proteomics (protein expression), and bioinformatics (data storage and integrated data analysis).

Fish Health management

Aquaculture health management using novel diagnostic tools for the early and rapid detection of disease causing pathogens and screening of the broodstock and seeds is very important for sustainable and economic viability of an aquaculture industry. The current knowledge on pathogens affecting marine organisms has mostly come from the disease conditions reported on the maricultured finfish and shellfish, from the overseas. Majority of these diseases are listed by international disease commission (OIE), as the epizootics that can cause serious damage to the rearing species. CMFRI has recorded initial success in developing the hatchery technology for marine ornamentals, bivalves such as green mussels, edible and pearl oysters and sand lobster and grow out for these species and hatchery technology for groupers, rabbit fishes are only under experimental stages. Effort to collect research data on the pathogen profile of these selected species in wild or in captivity is a priority as the entire broodstock of finfishes and shellfishes are sourced from the wild.

Conventional methods of pathogen detection have its own limitation with reference to specificity, sensitivity and speed. The classical methods such as, clinical examination, light microscopy and histopathology, culture methods for the isolation and identification of the microbes, electron microscopy and cell culture methods failed to deliver an early diagnosis in cases of subclinical and asymptomatic disease manifestations, especially in the cases of viral etiologies.

Nucleic acid based diagnosis

Nucleic-acid based diagnostics are the first line of biotechnology that found direct application in the fast growing aquaculture/mariculture arena. The very basic of the nucleic acid based diagnosis rests in the unique genetic make up or the genetic fingerprint of each organism or pathogen in the form of DNA or RNA. Information generated on the DNA sequence, can be directly used for the development of DNA/RNA based diagnosis.

Polymerase chain reaction (PCR)

Polymerase chain reaction (PCR) has been accepted as the most sensitive and useful diagnostic tool for the pathogen detection. This simple *invitro* DNA synthesizing technique has opened up new vistas in the disease diagnosis and health management. The DNA based diagnostic methods such as PCR and gene probes have emerged as the integral part of the fish health in aquaculture/

mariculture, in quarantine, biosecure systems, specific pathogen free (SPF) stocks, hazard analysis and critical control point (HACCP) and pathogen monitoring. In India institutions such as Central Marine Fisheries Research Institute (CMFRI), Kochi, Central institute of Brackishwater aquaculture (CIBA), Chennai and College of fisheries Mangalore are the fore runners in the development and commercialization of DNA based diagnostic kits.

DNA vaccines

DNA vaccines are made of a modified form of an infectious organism's DNA. This material, when introduced into an organism, leads to the expression of the genes within this modified, foreign DNA. This gene expression ultimately leads to synthesis of infectious organism proteins inside the host system. As a result, the defense system of the host responds in a protective manner almost the same way as would occur if the organism were actually infected by the true organism, itself. Most of DNA vaccines are still at the early investigative stages and have not yet reached the level of a product in the field.

Phage therapy

Phage therapy is the therapeutic use of lytic bacteriophages to treat pathogenic bacterial infections. The treatment uses a phage virus to infect and kill specific bacteria whilst not interacting with the surrounding tissue or with other harmless bacteria. The virus replicates quickly so a single, small dose is usually sufficient. Researchers from, Mangalore Fisheries College, National centre for aquatic animal health (NCAAH), Cochin and Central Institute of Brackishwater Aquaculture (CIBA), Chennai have already isolated phages of pathogenic vibrios, and efforts are underway for the development of treatment regimes against the pathogenic vibrios causing vibriosis in fish and shellfish rearing systems.

RNAi

RNA interference (also called "RNA-mediated interference", but abbreviated RNAi) is a mechanism for RNA-guided regulation of gene expression that is common in eukaryotic cells. RNAi involves short chains of double-stranded ribonucleic acid (dsRNA) interfering with the expression of genes with sequences complementary to this dsRNA. RNA interference is a form of posttranscriptional gene silencing in which dsRNA binds to specific mRNA and induce degradation of the homologous endogenous transcript, resulting in the reduction or loss of gene activity without there being detectable effects on the expression of genes unrelated in sequence. It is possible to silence specific genes in cell cultures, by transfecting the cells with predesigned dsRNA or siRNA with strict homology to the mRNA targets. An RNAi is also a promising tool for the control of viral disease among aquacultured organisms, through blocking the mechanism of viral multiplication.

GMOs for disease resistance

A genetically modified organism (GMO) is an organism whose genetic material has been altered using techniques in genetics generally known as recombinant DNA technology. The only transgenic fish that is commercially available today is not designed to be eaten, but a zebrafish that glows (GloFish™) for aquarium keeping. Research efforts are underway to induce genetic modifications, in various fish species, to develop fish and shellfishes with better resistance to infectious diseases.

Marine bioprospecting

Bioprospecting describes the systematic search for and development of new sources of chemical compounds, genes, micro and macro organisms, and other valuable products from nature and incorporates two fundamental goals (1) the sustainable use through biotechnology of biological resources and their conservation, and (2) the scientific and socioeconomic development of source countries and local communities. The potential applications offered by the screening of marine substances extend to pharmacology, agrochemistry and the environment. Marine organisms are the sources of: pharmaceuticals, enzymes, cryoprotectants, cosmaceuticals, agrichemicals, bioremediators, nutraceuticals etc. Marine drugs can be used as antioxidant, anti-fungal, anti-HIV, antibiotic, anti-cancer, anti-tuberculosis and anti-malarial.

Developing novel drugs for treating disease

Overuse of broad-spectrum antibiotics has resulted in the emergence of antibiotic-resistant pathogens, and also in dramatic (and dangerous) changes in the normal, protective microflora in humans and aquatic animals. There is a need to find new, effective antibiotic substitutes. This has prompted the constant search for and development of novel anti-infective agents.

Moreover, the use of combined approaches enhances these possibilities because marine molecules often belong to new classes without terrestrial counterparts; for example, halogenated compounds. Secondary metabolites produced by marine bacteria and invertebrates have yielded pharmaceutical products such as novel anti-inflammatory agents (e.g. pseudopterosins, topsentins, scytonemin, manoalide) anti-cancer agents (e.g. bryostatins, discodermolide, eleutherobin and sarcodictyin) and antibiotics (e.g. marinone). Melanins have a range of chromophoric properties that can be exploited for sunscreens, dyes and colouring. They also sequester different kinds of organic compounds, inducing fungicides and antibiotics, which may allow them to act as slow-release agents. The lysate from the horseshoe crab provides the major assay for bacterial endotoxin. Antioxidant peptides have been isolated from protease digests of prawn muscle, and the mycosporine amino acid (MAA) precursor, 4-deoxy-gadusol, has been isolated from seaweeds; these have applications as food additives and in cosmetics. A “living fossil”, the horseshoe crab, has furnished us with useful biochemicals and insights. Its circulating cells (amoebocytes) contain molecules that react with the lipopolysaccharide of Gram-negative bacteria, and thus have been of use in detecting early infection in humans as well as traces of LPS (pyrogens) in biotechnological products.

Enviromental biotechnology

Biosensors

Microorganisms provide the basis for development of sophisticated biosensors, and diagnostic devices for medicine, aquaculture and environmental biomonitoring. Intact cell preparations and isolated enzyme systems for bioluminescence are used as biosensors. The *lux* genes encoding these enzymes have been cloned from marine bacteria such as *Vibrio fischeri* and have since been transferred successfully to a variety of plants and other bacteria. The encoded enzymes are expressed only under defined environmental conditions, proving its use in pollution monitoring.

Environmental cleaners

Marine microorganisms, either as independent strains or as members of microbial consortia, express novel biodegradation pathways for breaking down a wide variety of organic pollutants. Marine microorganisms frequently produce environmentally friendly chemicals such as biopolymers and non-toxic biosurfactants that can also be applied in environmental waste management and treatment. Recent findings into the basis of cell-cell communication have shown that this process is involved in biofilm formation leading to environmental corrosion and plugging. This has generated a search for new bioactive molecules that prevent such communication and control subsequent fouling.

Products derived from marine ecosystems

- *Dogfish shark* have yielded squalamine, a potent antibiotic. Because squalamine works differently from today's antibiotics, it may also combat diseases that have become drug-resistant.
- *Sea squirt* produces Didemnin B, a compound that arrests cancers including leukemia, melanoma and those of the ovary, breast and kidney. It also may fight genital herpes and other viruses.
- *Moss animal* contains Bryostatin B, a cancer-fighting compound that has just entered human clinical trials and expected hit the market shortly.
- *Caribbean sponge* provides compounds from which a drug called AraC is synthesized to treat non-Hodgkin's lymphoma and acute myelocytic leukemia.
- *Gray encrusting sponge* emits manoalide, which appears to thwart the inflammation and pain caused by everything from bee stings to arthritis.
- *Bahama sponge* makes discodermolide, an effective immunosuppressive agent that could prevent tissue rejection after organ transplants.
- *Brown algae and kelp* manufacture a compound that seems to prevent duplication by the herpes and human immunodeficiency viruses (HIV). While researchers explore this, some obstetricians use sterilized strips of brown algae in place of metal instruments to dilate the cervix during invasive procedures such as IUD insertion.
- *Red algae* produce carrageenans or sulfate polysaccharides, used to treat peptic ulcers.
- *Horseshoe crab* has blue blood used to detect meningitis, septic shock and other forms of bacterially related conditions. Pharmaceutical companies routinely use the blood to screen for bacterial contamination.
- *Blue mussel* produces an adhesive that provided a model for Cell-Tak. Now used to repair corneas and retinas, it may soon work to secure dentures and dental fillings.
- *Blue crab* has an exoskeleton from which chitin, a crystalline polymer, can be extracted. Chitin is used in absorbable, nonallergenic sutures that remain stable in the alkaline environment of human intestinal and urinary tracts.

Future research needs of Indian Marine Biotechnology

- Profiling of major pathogens in candidates species identified for mariculture and DNA based diagnostics
- Use of probiotic in place of antibiotics to prevent infectious diseases
- Initiate genomics of farmed species with nutrigenomics and pathogenomics for the development of fast growing and disease resistant varieties

- Genetic and genomic studies of pearl oyster and other bivalves
- Quantitative genetic manipulations of cultured fish, shellfish and live feeds
- Development of molecular markers for MAS and taxonomical applications
- Development of ornamental transgenics
- High health formulated feed for better FCR and reduced disease problems
- Bioprospecting for novel products from biotic resources of sea
- Gene mining and metagenomics for developing novel biotech products
- Stem cell and tissue culture research
- Cell lines for marine finfsihes (vertebrates) and shellfishes (invertebrates)

Conclusion

India is yet to translate the benefits to be derived from marine organisms as sources of new products and develop viable strategies to conserve them as an investment opportunity through bioprospecting and marine biotechnology. Presently the potential bioprospecting of marine organisms is less than 1% and hence massive scale screening is essential by giving focus on few selected potential groups like microbes (bacteria and fungi), microalgae and macroalgae (seaweeds). There is urgent need to develop research and development projects with objective to study Indian Ocean flora, fauna, and microbes and to search for substances having physiological ac-tivity, which could serve as models for new drugs, antifouling, signal substances, pheromones, neurotransmitters, and antifertility compounds.

In a project jointly sponsored by the Oceanic Biology Program of the Office of Naval Research (ONR) of the United States Government, the Council of Scientific and Industrial Research (CSIR) and the Department of Science and Technology (DST) of the Government of India, four hundred and fifty extracts from marine organisms collected from the western and eastern coasts of India, Lakshadweep and the Andaman and Nicobar Islands have been evaluated for broad biological activities. Of these, 14 extracts exhibited anti implantation, 28 antiviral, 10 CNS depressant, 31 CNS stimulants, 33 diuretic, 10 hypoglycemic, 16 hypotensive, 11 oxytocic, 9 spasmogenic, and 3 spasmolytic activities**.**

Marine biotechnology is, thus, a cross-sectoral and evolving technology , a counterpart to terrestrial biotechnology and could provide fresh development momentum in several fields, including food production, pharmaceuticals, environment and even nanotechnology. The application of molecular biology can increase aquaculture/mariculture productivity, and is being sought to achieve a better balance with marine ecology. Further, with the exciting reach of genetics and genomics, global research has widened from just aquaculture applications to mainstream marine biotechnology, relates to a better understanding of human life and health. It may be humbling to accept that our nervous systems are functionally close to that of the jellyfish. But the structural simplicity of some marine animals helps explain how humans smell, see and think. Likewise, endowed with nature's largest photoreceptors, the nine-eyed horseshoe crab helps clarify the mystery of vision. Examples abound of marine organisms as biological and biomedical research models that provide insights into disease prevention and treatment.

While countries such as Japan, Norway, US, Australia are the global leaders in marine biotech, it is recognized that among the developing countries, India has considerable strengths, with its research in marine biology and ecology, marine bacteria, screening microorganisms for bioactive compounds, bioremediation and extra-cellular production by microorganisms, micro-algal production of beta carotene, proteins, etc. These lay strong foundation for acquiring global competitiveness in select biotechnological applications and products. This fascination is understandable, as Indian subcontinent bath with three oceans with vast EEZ of about 2.02 million sq km as well as its rich biological wealth remains largely untapped, even today. We cannot, however, ignore that biotechnology confers the ability to manipulate and change the ultimate blueprint of life. So the dangers of unbridled biotechnological interference should never be lost sight of. It is therefore, of paramount importance to debate safety, ethical and intellectual property issues and resolve them amicably.

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