

WINTER SCHOOL
ON
Recent Advances in
Mariculture Genetics
and Biotechnology

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Course Manual



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PERSPECTIVES IN MARINE BIOTECHNOLOGY

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Introduction

The Convention on Biological Diversity (CBD) defines Biotechnology as: "any technological application that uses biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific use". According to Dr. Rita Colwell, Director, National Science Foundation, USA, the apt definition is "making money from Biology". Biotechnology involves application of scientific and engineering principles to provide goods and services through mediation of biological agents. Traditional application includes antibiotic production through fermentation, microbial sewage treatment and water purification. Modern molecular biotechnology involves gene manipulation and gene transfer, DNA typing, bacterial cloning for production of biomolecules etc.

In 1992, an Organization of Economic Co-operation and Development report on biotechnology defined marine biotechnology as "the application of scientific and engineering principles to the processing of materials by marine biological agents to provide goods and services." This would include biotechnology-based methods for aquaculture, fisheries, and marine natural products. Marine biotechnology is gaining momentum due to its vast potential application in augmenting seafood production, safeguarding human health, protecting and restoring aquatic environment (bioremediation), reducing fouling and corrosion, exploiting marine natural products for human benefit, conserving biodiversity, managing fish and shellfish resources and in clarifying fish and shellfish species status.

The gene pool and biological processes of large majority of marine organisms including microorganisms are not known well enough. However, exploratory research shows the potential for exploiting the biochemical capabilities of marine organism to provide models for new classes of pharmaceuticals, polymers, enzymes, other chemical products, industrial processes as well as vaccines, diagnostic and analytical reagents, genetically altered organisms for aquaculture and seafood industry.

Biotechnological Interventions in Aquaculture:

Aquaculture is receiving importance as extreme focus sector for development in view of its role in augmenting production of nutritious food besides a large variety of natural products. Recent researches have amply proved the need for biotechnological interventions in sustaining aquaculture production. Significant growth enhancement has been achieved through hormonal intervention and gene manipulation. Success stories are documented on biotechnological interventions in induction and control of maturation and spawning, sex

control (gynogenesis and androgenesis), sex inversion in protandrous species like sea bass and protogynous species like the grouper, genetic improvement (production of triploids and tetraploids and transgenic species), in disease diagnosis (molecular and immunodiagnostic kits) and management (probiotics, vaccines, immunostimulants, gene therapy), cell and tissue culture (in vitro pearl production), conservation of germ plasm (cryopreservation of gametes and embryos)

Biotechnological interventions in aquafeed production include bioencapsulation of live feed, nutrient enrichment of raw materials (fermentation), reducing antinutrients and crude fibre levels, production of enzymes - α -amylase, β -amylase, cellulase / hemicellulase & phytase) amino acids (L-lysine, threonine & monosodium glutamate), microbial polysaccharides, vitamins (B 2, B 12, Biotin), organic acids (lactic acid), flavours, growth promoters, carotenoids (*Haematococcus*, *Dunaliella*), essential fatty acids (bacteria, fungi & *thraustochytrids*)

Genomic and genetic improvement

The main thrust area in genetic improvement of farmed fish and shellfish is to enhance desirable traits (phenotypic character) of high economic importance – growth rate, size at first maturity, egg number, egg size, egg weight, egg survival, larval survival, disease resistance, behaviour, resistance to environmental factors, dressing weight, carcass quality, fat content, protein content, food conversion, anatomic modification, colour and others.

Genomics will help identify and select with precision useful genes in fish, invertebrates, and algae for aquaculture. Genome mapping is being carried out in a number of economically important aquaculture species.

Production of transgenic fish by inserting desirable genes through electroporation and microinjection has been successfully carried out since the mid-1980s. In a number of cases, fish growth hormone genes, along with a reporter gene, which will tell whether the transformation is successful, are inserted into the fish. There are reports of better growth in transgenic tilapia, catfish, and rainbow trout than non-transgenic individuals.

The primary goal of genomics is to identify genetic diversity within stocks and identify genes that can be transferred into fish or invertebrate eggs and result in transgenic animals with improved safety and economic benefit such as better and faster growth rates, disease resistance, greater toleration for environmental change (e.g., cold water tolerance), increased fecundity, and more edible meat and to produce pharmaceuticals.

Biotechnology methods have resulted in production of new, genetically engineered vaccines for aquaculture, such as the vaccine against infectious hematopoietic necrosis (IHN) virus, which is responsible for the death of trout and other salmonid stock.

Bioactive compounds and biomolecules

The marine bioactive compounds or marine natural products (MNPs) offer avenues for developing cost-effective, safe and potent new drugs and other useful products. MNPs are organic compounds produced by microbes, sponges, seaweeds, and other marine organisms. The host organism biosynthesizes these compounds as non-primary or secondary metabolites to protect themselves and to maintain homeostasis in their environment. In the decade from 1977 to 1987, around 2500 new metabolites (MNPs) were reported from marine organisms ranging from microbes to fish. . Perusal of literature indicates that even the seawater has bactericidal properties, which is primarily attributed to the production of antibiotics by planktonic algae and bacteria respectively. Researches carried out globally resulted in identification of hundreds of free-living and symbiotic marine microorganisms and numerous marine invertebrates that have the potential to produce new pharmaceutical compounds cosmetics and nutraceuticals.

A large variety of marine micro and macro organisms have been screened and some of them are found to contain anti-viral, anti-microbial and anti-inflammatory compounds. Micro algae are rich sources of bioactive compounds. Cyanobacteria contain natural compounds that also serve as models for drug development. By determining metabolic control mechanisms and restrictions, as well as optimizing culture media for production of secondary metabolites and methods for the isolation and identification of genes controlling secondary metabolism could help in large scale culture of important species.

Bugula neritina, a slow growing marine bryozoan is the source of a potential drug, which is active against leukemia. Prostaglandins isolated from marine organisms like gorgonids are found to be active against chemotherapeutically resistant ovarian cancer. Anti-inflammatory drugs could also be isolated from marine algae, by developing proper culture techniques.

Marine organisms, many of which can be mass cultured, are sources of commercial products, among which are food additives such as carrageenan, agar, and chitin; commercial glues; enzymes for detergents and for biological reagents; and other products.

Nutraceuticals, such as food-based enhancers, and cosmetics also can be produced from marine sources. The soft coral, *Pseudopterogorgia elisabethae*, is the source of pseudopterosin that is used as a cosmetic additive.

In sponges, the secondary metabolites are synthesized to protect themselves and to maintain homeostasis. The wider biosynthetic capability of sponges could be attributed to their biological association with other symbionts. A wide variety of secondary metabolites were isolated from sponges and these have been associated with antibacterial,

antimicrobial, antiviral, antifouling, HIV protease inhibitory, HIV reverse transcriptase inhibitory, immunosuppressant and cytotoxic activities. In addition to potential anticancer applications, the MNPs of sponges have a myriad of activities ranging from antibiotic activity including anticoagulant, antithrombin, anti-inflammatory, as well as immunomodulatory activities.

Many marine organisms have also been documented to be sources of prostaglandins-PG1, PG2, and PG3, and fatty acids like docosahexaenoic, eicosapentaenoic and arachidonic acids, which are receiving importance in human and animal nutrition and health care. The sea is also the richest source of carotenoids – beta carotene, astaxanthin.

A thermo stable alpha-galactosidase, which can hydrolyse melibiose oligomers in soya and other bean products, is isolated from the bacterium, *Thermostoga neapolitana* from oceanic black smokers. Enzymes extracted from *Archaea thermococcales* (DNA polymerase having 5 times more half life) will increase efficiency in biotechnological process requiring replication of DNA. Phytases are produced by a variety of bacteria and fungi.

New mineral organic materials could be developed from certain marine organisms, which consist of diatom produced biosilicate and chitin fibres. These materials are biodegradable and exhibit a remarkably high strength to weight ratio and are superior to synthetic materials used in industry. Industrial surfactants could be manufactured from fishmeal processing wastes.

Environmental bioremediation

Microbes have been successfully used in bioconversion for waste management in controlling pollution, detoxification of industrial organics such as PCBs, PAHs, and creosote, and heavy metals copper, chromium and arsenic to harmless products. Transgenic Cyanobacterium, *Synechococcus*, with the gene for mammalian metallothionein, increase its heavy metal resistance and accumulation.

Cyanobacteria *Synechococcus* and green alga *Chlamydomonas reihardtu* have been effectively used against heavy metal (cadmium) contamination by its genetic modification by inserting a gene for increasing its heavy metal resistance and accumulation.

Solving fishery related problems

Biotechnological tools can be applied in stock assessment of fish and shellfish, for studying genetic diversity of different marine organisms; ratification of taxonomic status of fish and shellfish and for identification of distinct stocks of fish and shellfish species and the degree to which they inter-mix.

Biosafety and environmental issues

Biosafety concerns raised against commercial production of transgenic fish or shellfish must be considered on a case-by-case basis, focusing on the species, culture system, and ecosystem and quality of the products derived. Other major concerns are possible impact if the transgenic animals escape into the environment and the parental stocks that are genetically modified but not sterile.

Conclusion

Marine Biotechnology is just beginning to revolutionize our ability to better use marine resources... In the US 190 patents were granted from 1983 to 1995, 30 marine products targeting cancer, inflammation and AIDs; the market value of 5 of the products is valued at 2 billion US\$. One anti-inflammatory agent *Pseudoterosin* derived from soft corals has projected sales of 100 million US\$. Japan has set up a network of Marine Biotechnology Laboratories investing more than 1 billion US \$ to harness the enormous potential. In India Shanta Marine Biotech and EID Parry have already set up industries to produce astaxanthin from *Haematococcus* and beta carotene from *Dunaliella*.

In the future advances in such areas as bioconversion, biomaterials, pharmaceuticals and diagnostics, aquaculture, seafood safety, bioremediation, and biofilms and corrosion would play vital roles in sustainable development programmes. Currently a multibillion dollars industry worldwide we can expect greater leaps in Marine Biotechnology in the future