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Biotechnology in Aquaculture

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Abstract: *Supplying food to billions of people and providing income for many millions more, aquaculture is the fastest growing area of animal-derived food production in the world. Aquaculture is the farming of fish and other animals living in water. The aquaculture industry is currently faced with solving the simultaneous problems of developing economically viable production systems, reducing the impact on the environment and improving public perception. This paper considered aquaculture as the only way to increase fish production and also discusses technical environmental and management considerations regarding the use of genetically modified organism (e.g. fish) in aquaculture.This paper discusses advantage of biotechnological research application and commercialization. Fish farmers, and the researchers who assist themselves with implementing biotechnology tools, are testing various ways of improving production. Transgenic techniques using biotechnology creates fishes with highdisease resistance, environmental tolerance,reproductive efficiency and nutritional value. Gene transfer technology is one area where great advances have been made. Triploidy is a technique for producing sterile fish. Many commercial trout and carp farmers around the world have adopted the use of triploidy and research is underway to optimize its use in salmon. While nature is feeling the increasing pressure of seven billion hungry people, aquaculture provides a sensible alternative to harvesting the rivers and oceans. When holding hands with biotechnology, it has huge potential for becoming the main protein producer of the future. Perhaps even in the near future, when you dip your sushi roll into the soy sauce, delight in the knowledge that what is about to tickle your palate is probably a triploid, genetically superior, sustainably farmed GMO (Genetically Modified Organism) - the product of biotechnology in aquaculture.*

1. INTRODUCTION”

Aquatic biotechnology has been broadly defined as any technique that uses living aquatic organisms or part of these organisms to make or modify products to improve plants or animals or to develop microorganisms for specific use. The European Federation of Biotechnology (EFB) has given a definition of biotechnology that encompasses both traditional and modern molecular biotechnology. The definition given by EFB is as follows: “The integration of natural science and organisms, cells, parts thereof, and molecular analogues for products and services”. Of late it has assumed crucial importance in the context of the imperative need to upgrade the quality of cultivated species for enhancing aquaculture productivity, conservation and management of genetic diversity in natural fish stocks. Food security will be a major issue facing mankind in the coming millennium. Aqua cultural output will need to be increased several fold in order to meet the rising demands for fish in coming years and which will be possible through biotechnology.

2. PRINCIPLES OF BIOTECHNOLOGY”

Among many, the two core techniques that enabled birth of modern biotechnology are:

1) Genetic engineering: (techniques to alter the chemistry of genetic material)
2) Maintenance of sterile ambience: (to promote the growth of only desired microbe)

Asexual reproduction preserves genetic materials, while sexual reproduction permits variation. Traditional hybridization procedures used in plants and animal breeding, very often lead to inclusion and multiplication of undesirable genes along with the desired gene. The technique of genetic engineering which include creation of recombinant DNA, use of gene cloning and gene transfer overcome this limitation and allows us to isolate and introduce only one or a set of desirable genes without introducing undesirable genes into the target organisms.

In a chromosome there is a specific DNA sequence called the origin of replication, which is responsible for initiating replication. Therefore, for the multiplication of any alien piece of DNA in an organism it needs to be a part of a chromosome(s) which has a specific sequence known as ‘origin of replication’. Thus an alien DNA is linked with the origin of replication, so that, this alien piece of DNA can replicate and multiply itself in the host organism. This can also be called as cloning or making multiple identical copies of any template DNA.

The construction of first recombinant DNA emerged from the possibility of linking a gene encoding antibiotic resistance with a native plasmid (autonomously replication circular extra chromosomal DNA) of *Salmonella typhimurium.* Stanley Cohen and Herbert Boyer accomplished this in 1972, by isolating the antibiotic resistance gene by cutting out a piece

of DNA from a plasmid which was responsible for conferring antibiotic resistance. The cutting of DNA at specific locations became possible with the discovery of restriction enzymes also called as 'molecular scissors'. The cut piece of DNA was then linked to plasmid which acts as the vector to transfer the gene to the host organism. The linking of antibiotic resistance gene with the plasmid became possible with the enzyme DNA ligase. The plasmid containing the desired gene will replicate in the host by using the enzyme DNA polymerase of the host and will produce multiple copies.

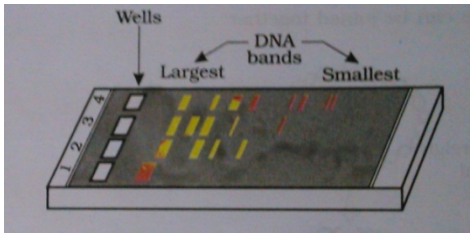
Three basic steps in genetically modifying organisms are:

1) Identification of DNA with desirable genes;
2) Introduction of the identified DNA into the host;
3) Maintenance of introduced DNA in the host and transfer of the DNA into its progeny.

3. TOOLS OF RECOMBINANT DNA TECHNOLOGY"

- **Restriction enzymes** belong to a larger class of enzymes called nucleases. These are of two kinds; exonucleases and endonucleases. Exonucleases remove nucleotides from the ends of the DNA whereas endonucleases make cuts at specific positions within the DNA. Each restriction endonuclease recognizes a specific palindromic nucleotide sequence in the DNA.
- **Gel electrophoresis** is the tool used for separation of DNA fragments cut down by restriction enzymes. Since DNA fragments are negatively charged molecules they can be separated by forcing them to move towards the anode under an electric field through a medium/matrix (agarose gel). DNA fragment separate according to their size through sieving effect provided by the agarose gel. Hence the smaller the fragment size, the farther it moves.

Figure showing movement of DNA under electric field



- **Cloning vector**: A good vector should possess an ori (origin of replication), selective marker and cloning sites. Plasmids and bacteriophages are commonly used as vectors as they have the ability to replicate within the bacterial cells independent of the control of chromosomal DNA.
- **A competent host** functions mainly for transformation with the recombinant DNA. A good host should not reject the recombinant DNA (vector + desired gene), instead it should allow the vector to replicate inside. Common ways to inject recombinant DNA into the host are microinjection and biolistics or gene gun.

4. PROCESS OF RECOMBINANT DNA TECHNOLOGY"

1) Isolation of the genetic material (DNA).
2) Cutting of DNA at specific location.
3) Amplification of gene of interest using PCR.
4) Insertion of recombinant DNA into the host / cell / organisms.
5) Obtaining the foreign gene product.
6) Downstream processing.

5. TRIPLOIDY"

As the name implies, triploids have three sets of chromosomes in their somatic cells rather than the normal two sets (diploids). Although there are a few naturally occurring triploid species of fish that exist as all-female, for most species triploidy is not a natural condition. Tetraploidy has played a role in the evolution of many widespread and economically important groups of fish, including salmonids. Triploidy, on the other hand, generally leads to such severe gametogenic impairment that these individuals are sterile. Triploid organism is not a Genetically Modified Organism (GMO).

Triploids are produced by retention of the second polar body – a package of maternal chromosomes that normally leaves the egg with the completion of meiosis shortly after fertilization. Triploidy is generally induced through either thermal or hydrostatic pressure treatment of eggs within the first hour of fertilization; these treatments disrupt spindle fibers and thereby interfere with the normal movement of chromosomes during meiosis. The variables that must be controlled for the successful induction of triploidy are the time after fertilization at which to begin the treatment (which is itself temperature-dependent and therefore measured inC-min) and the duration and magnitude of the treatment itself.

The volume of cell nuclei is increased to accommodate the extra genetic material, and a corresponding increase in cellular volume typically results. However, triploid individuals are no larger than diploids because of a corresponding reduction in cell numbers. An important consequence of increased nuclear and cellular volume is the resulting decrease in the ratio of surface area to volume. This could theoretically affect processes limited by surface area, such as nutrient and metabolite exchange, passive and active ion exchange, and membrane binding of hormones and other messengers. Because of lower cell numbers, the decrease in ratio of surface area to volume applies to entire tissues and organs as well. A second important consequence is that, depending on the shape of the cell and its nucleus, internal transport and diffusion distances may be increased. This could affect processes such

as signal transduction from the cell surface to the nucleus, and resultant production and movement of RNA and proteins within and out of the nucleus and cell. Some of these potential disadvantages may be offset by the energetic advantages arising from reduced production and maintenance of cellular membranes and from the smaller relative surface area across which ionic and osmotic gradients must be maintained.

Female triploids are always sterile; at least in all species currently studied, they do not undergo gonad development; however triploid males are only functionally sterile, they develop gonads (but these gonads show a differential development compared to testis in diploid males) and undergo "partial or altered" puberty, but their sperm does not give rise to viable progeny. For these reasons it is preferable to produce all-female triploid stocks.

Among the salmonids, triploid males develop much larger gonads than triploid females and often produce functional spermatozoa, but these spermatozoa are aneuploid and therefore unable to yield viable offspring upon fertilization of eggs from diploid females.With the exception of characteristic jaw abnormalities (described later), triploid salmon are morphologically identical to diploids. All-female populations of triploids are better suitedfor aquaculture than mixed-sex populations, since females remain sexually immature. Triploid males, although sterile in the sense that they are unable to produce viable offspring, do go through the physiological processes associated with sexual maturation, exhibit normal spawning behaviour, and will mate with diploid females when placed in an appropriate spawning environment.

The use of triploids for aquaculture originated with the industry's need to prevent sexual maturation of production fish before they reach market size, because maturing salmon, being chronically stressed, have reduced flesh quality and are more susceptible to disease.Induced triploidy is the only effective method currently available for mass production of reproductively sterile salmonids for aquaculture. Induced triploidy is by no means the only effective way of sterilizing fish, but it is currently the only method available by which to sterilize large (commercial-scale) numbers of salmon without the use of chemicals that would otherwise affect consumer acceptance of the fish. Some alternative methods for the production of sterile salmon, such as surgical castration, hormonal sterilization, and induced gonadal autoimmunity, have been described elsewhere.

Additional advantages of triploid populations for trout aquaculture:

- Triploid trout feed more reliably over the winter months and therefore grow more evenly.
- Evidence suggests that triploid trout do not accompany spawning fish onto the shallow areas (on their introduction into the wild for angling restocking purposes), but remain in their normal feeding areas throughout autumn and winter; they therefore cause no permanent damage to wild stock.
- Highly effective, (98%) losses of fish due to fungal infections may be reduced (e.g. trout)

Although pilot-scale aquaculture results with triploids to date have not been good, they certainly have not been disastrous. In fact, if one accepts the suggestion to treat triploids as a ''new species'' for aquaculture development, then the initial results are encouraging. Once optimum rearing conditions are determined, triploid Atlantic salmon may prove to be just as good as, if not better than, diploids as production fish. Specifically, research is needed on determining environmental tolerances and optima (temperature, oxygen, salinity, etc.), nutritional requirements (energy, micronutrients, etc.), disease resistance, and behaviour (aggression, competition with diploids, etc.).

6. GENE THERAPY"

Gene therapy is the insertion of gene into an individual's cells and tissue to treat disease, especially hereditary disease. It does so by replacing a defective mutant allele with a functional one or gene targeting which involves gene amplification. Viruses that attack their host and introduce their genetic material into the host cell as a part of their replication cycle are used as vectors to transfer healthy gene or more recently portion of genes. Cells can be removed from a patient lacking a functional gene and the missing or damaged gene can be replaced. The cells can be grown in a culture medium for a while and then transferred to patient.

7. APPLICATIONS OF BIOTECHNOLOGY IN AQUACULTURE"

7.1 For Cell Culturing

Recombinant DNA technology, an application of biotechnology is used in for culturing of cells of aquaculture species which leads to the successful farming of these species by knowing their growth, reproduction and health. Finally they can be a great source of diverse biochemical products for use in aquaculture, medicine and food industry.

7.2 DNA barcoding

DNA sequence analysis of a uniform target gene to enable species identification has been referred to as DNA barcoding. Just like UPC barcodes, the DNA sequences within each species are unique. A run of 15 nucleotides, with four options at each position, creates the possibility of 1 billion codes, a hundred fold excess over the estimated number of animal species. Of course, specific nucleotides are fixed at some positon by selection however; this constraint can be overcome by focusing on protein coding genes, where every third position is generally free to vary because of the degeneracy of

the genetic code. As a result by examining a stretch of 45 nucleotides in these genes, one has the prospect of close to 1 billion alternates.

An appropriate target gene for DNA barcoding is conserve enough to be amplified with broad range primers and divergent enough to allow species discrimination. A remarkably short DNA sequence should contain more than enough information to distinguish 10 or even 100 million species.

Why do we need a molecular taxonomy tool? : An increasingly accepted view is that traditional taxonomic practices are insuffient on their own to cope with the growing need of accurate and accessible taxonomic information. Although approximately 1.7 million species been have described and names under the Linnaean system, the total number of species on earth remains unknown and estimates vary widely ranging from 10 million to more than 100 million. The task of recognizing new species has certain urgency; the diversity of our biosphere is so large that the methodical cataloguing of new species by traditional methods is being outpaced by losses from human impacts. In the face of such mounting losses to biodiversity, the need to catalogue and describe life is greater than ever, and there is a growing realization that it will be critical to see technological assistance for a species' initial recognition and its subsequent identification. Additionally, barcoding clearly has enormous potential to relieve taxonomists of routine identifications, providing more time to focus on new taxonomic hypothesis and to concentrate rare poorly characterized and new species. The ability to quickly to put a name to an unknown specimen benefits not only conservationists, but is also a tremendous tool for an ecologists as well. The use of barcoding will readily allow the identification of small plant fragments are sterile material, eggs and larvae of marine species and forensic material which previously would have been extraordinarily difficult or impossible to identify.

7.3 Mitochondrial DNA

Mitochondrial genome of animals represents a better target for analysis than the nuclear genome because of its lack on introns, its limited exposure to recombination and its haploid mode of inheritance. As well there are robust primers that enable the recovery of specific segments of the mitochondrial genome from a broad range of animals. The mitochondrial genome includes just 13 protein coding genes that might serve as the core of a DNA based identification system.

7.4 Forensic identification of meat of endangered aquatic species – a case study

Whale shark (*Rhincodon typus*) is the largest shark in the ocean; they are highly migratory and have a broad distribution in tropical and warm temperate seas; in both deep and shallow coastal waters and the lagoon of coral, attols and reef. Due to over exploitation it was given a conservation status of 'vulnerable to extinction' as listed by World Conservation Union in the Red list of threatened species.

To enable trade in whale shark products to be adequately regulated, *Rhincodon typus* was nominated in Appendix II of convention on International Trade in Endangered Species (CITES) in April 2000. To conserve the species in Indian waters it is enlisted as one of the protected species and its fishing prohibited under schedule I of the Indian Wildlife Protection Act, 1972.

Flesh suspected as that of whale shark was seized from fisherman by the forest range officer (Government of Kerala), Kannur, Kerala. On September 29, 2008 a case was filed and the Judicial First Class Magistrate, Thalassery, Kannur, Kerala approached the NBFGR, Kochi unit to analyses the meet sample for conformation of the species using DNA markers. Based on DNA sequencing the seized meat sample was confirmed as that of endangered whale shark and the result was communicated to the court. This was the first case in India in which scientific evidence was sought to identify the meat of a fish enlisted in the wildlife Protection Act, 1972 and the DNA markers reiterated their ability to reliably identify product / meat sample of a species, thus helping in curtailing illegal trade of the endangered animals.

7.5 Genotoxic Studies

An increasing number or genotoxic chemicals that damage DNA, like pesticides, heavy metals etc. are being released into the aquatic environment, threatening not only our rich aquatic biodiversity but also human health. The tests like micro nuclei test, chromosome aberration test, sister chromatid exchange (SCE), assay and Comet Assay are implied to study genotoxic effect in fishes. The later one is very sensitive technique for quantification of DNA damage due to genotoxicants. Such studies are important to determine safe level of genotoxicants in water bodies and in planning remedial measure for conservation of aquatic biodiversity.

7.6 Biotechnology for Transgenics & Polyploidy

A major focus of research in the aquaculture industry is on the use of biotechnology to increase food availability and reduce production costs, specifically through the manipulation of the genes and chromosomes of cultivated species. Examples include transgenic fish with properties such as increased growth rates, feed conversion efficiency, disease resistance, cold tolerance, and improved metabolism of land-based plants. However, use of transgenic organisms in aquaculture is a very controversial topic due to a number of environmental and human health concerns such as escapement and introduction of genetically modified organisms into the food chain. In response, some transgenic research has also been focused on

inducing sterility to reduce the risk of transgenic organisms breeding with wild species. A method of chromosome manipulation, referred to as polyploidy, provides the option of creating sterile organisms, some of which also exhibit increased growth rates.

7.6 Biotechnology in Surrogate Production in Teleost Fishes

Surrogate production is a new technique for fish-seed production in aquaculture. Surrogate production in fish is a technique used to obtain the gametes of a certain genotype through the gonad of another genotype. It is achieved by inducing germ-line chimerism between different species during early development. Primordial germ cells (PGCs) are the key material of this technique to induce germ-line chimera. In several species, it has been reported that PGCs differentiated from the blastomeres inherited some maternally supplied mRNA located in the terminal regions of the early cleavage furrows. PGCs from donor species (or strains) are isolated and transplanted into host species to induce the germ-line chimera. Four methods for inducing germ-line chimera are described: blastomere transplantation, blastoderm-graft transplantation, transplantation of PGC from the genital ridge, and transplantation visualised PGC with GFP fluorescence.Surrogate production, however, opens the possibility of efficient fish-seed production and effective breeding and transfer of biodiversity to an aquaculture strain. Conservation and efficient utilization of genetic resources will be achieved through surrogate production combined with the cryopreservation of PGCs.

8. ACKNOWLEDGEMENTS”

Future of biotechnology research with respect to aquaculture development and fisheries management is promising notwithstanding some technical problems which stand in the way of commercialization. Fundamental research using molecular markers will lead to a better understanding of aquatic structures of wild population. This can lead to improved management and production of specific fisheries and selective breeding programs which will also lead to a greater ability to catalogue and better manage and conserve fish diversity. Annual world landings of aquatic resources have increased more than four – fold during the last fifty years. The larger share of this production came from the capture fishery sector, which has been over exploited, leading to decline of fish diversity. On the other hand, aquaculture provides greater scope for increasing fish production. Thus, biotechnology as a growing discipline will have an important role to play in the future for increasing aquaculture productivity and would help in reducing the fishing pressure on natural resources. Transgenic fish produced through gene transfer technique have demonstrated superior performance. Sex reversed or sterile transgenic aquatic organism with secure physical containment would be a boon and appear to be an assuring approach in enhancing the fish production and food security.

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