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Commercial Applications of Biotechnology in Fisheries and Aquaculture

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Fisheries and aquaculture are potential beneficiaries of a variety of commercial applications of biotechnology that might enhance sustainability, disease management, and production efficiency. Here are some significant applications of biotechnology. Enhancing desirable characteristics in fish and shellfish species involves the application of biotechnology techniques such as selective breeding, genetic engineering, and marker-assisted selection. Faster growth rates, disease resistance, increased feed conversion efficiency, and greater fillet quality are a few examples of these features. Disease control and prevention in aquaculture is greatly aided by biotechnology. Pathogens can be swiftly and precisely identified with the aid of diagnostic methods based on DNA/RNA analysis. The development of biotechnology-based vaccines has reduced the need for antibiotics and other chemical therapies by enabling the prevention of several viral, bacterial, and parasite infections. Biotechnology techniques like hormone manipulation, artificial insemination, and sex reversal enable controlled reproduction and spawning of fish species. This helps in the production of large numbers of fry, selective breeding programs, and the conservation of endangered species.

Keywords
Genetic improvement, disease management, reproductive manipulation, environment monitoring, aquatic organism health.

Introduction

India has become a multi-billion-dollar industry with the help of biotechnological interventions. Today, aquaculture is regarded as a well-organized, attractive business sector that contributes significantly to the GDP of a number of countries. Some of the most important biotechnological interventions, including the creation of inducing hormones, disease diagnosis kits, probiotics, biofilters, selective breeding, and so on, have made a significant contribution to speeding up the blue revolution. Seed, feed, disease, and the environment are all critical pillars of aquaculture that can be extremely well integrated with biotechnology, resulting in a multifold increase in productivity. Aquaculture's success is mostly determined by four factors: high-quality seed, high-quality feed, disease management, and soil water quality management. Even though feeding management receives the most attention in aquaculture, the success of the industry is also dependent on the other three components. All of these components have a high prevalence of biotechnological interventions, which are discussed in depth in the following sections.

Biotechnology's Impact on Seed Production

The most significant condition for starting aquaculture is the quality and purity of the seed. The development of the semi-intensive and intensive aquaculture industries is dependent on the availability of high-quality seed with better performance in large quantities. The introduction of induced breeding techniques and selective breeding is a revolutionary development in aquaculture hatchery technology that has lit the blue revolution lamp.

Biotechnology and Induced Breeding

The method of induced breeding based on hypophysation (Chaudhuri, H., and Alikunhi, K. H., 1957) was created in the 1950s, but it is the creation of synthetic hormones that has helped to spread the technology to the ground level. Biotechnological methods were used to create synthetic super-active analogues. Biotechnological approaches such as recombinant DNA technology and protein engineering were used to create synthetic super-active analogs that induce effectiveness at a lower dose. This counterpart has a changed amino acid in position 6, resulting in increased peptidase resistance. It has also changed the GnRHa's polarity and tertiary structure, resulting in increased receptor binding affinity. The race to create a species-specific, more efficient counterpart that uses a bioinformatics method to identify the influence of amino acid substitution on receptor binding is still on. Different types of inducing chemicals, such as ovatide, ovaprime, and ovapel, are currently employed in the hatchery (Chattopadhyay, N. R., 2016). New candidate hormones, such as Kisspeptin, have been found as a significant regulator of breeding in several fishes, representing upstream in the biological route of breeding, and synthetic Kisspeptin hormone has been proven to be helpful in increasing fish reproductive performance (Ohga *et al.*, 2018)

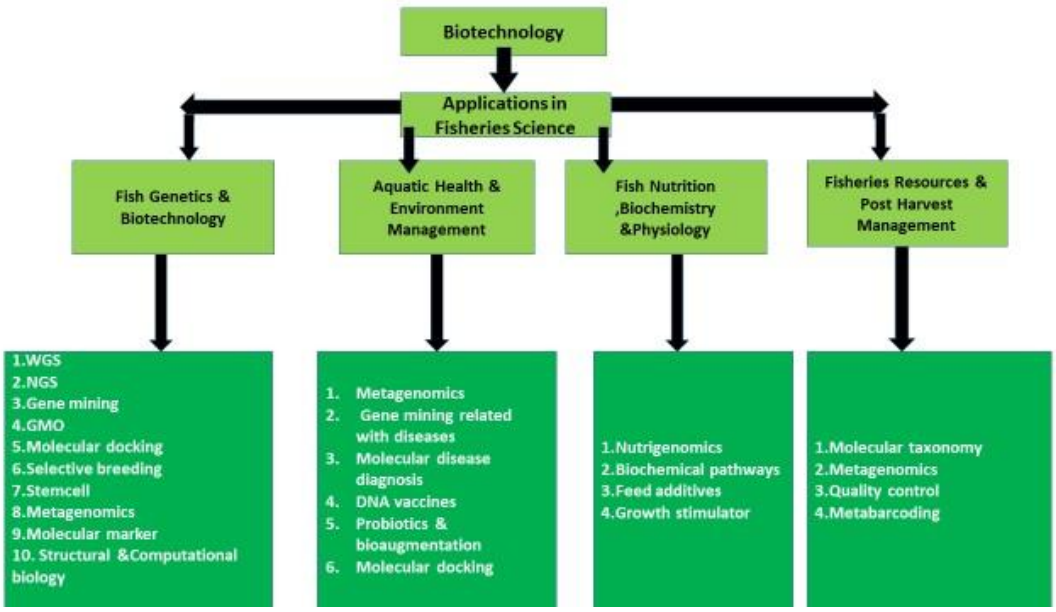

Fig: 1. Application of biotechnology in different sector of fisheries & Aquaculture

Improved Seed Production (Selective Breeding)

The present and future of hatchery technology is the production of enhanced quality seed in terms of growth performance, disease resistance, and environmental adaptability. In the hatchery, selective breeding is an important tool. Many successful examples of such species include GIFT (Genetically Improved Farmed Tilapia, Jayanti rohu, common carp, salmon, sea bass, and others. Selective breeding is the only practical way for producing pathogen-resistant seed at the moment. Around the world, efforts are being made to breed Specific Pathogen Resistant (SPR) shrimp and fish for disease-free aquaculture**.**

Selection, cross/outbreeding, and hybridization are all used to improve the genetics of a fish population. Aquaculture has generated and employed selectively bred stocks with improved features such as disease resistance and quick development. Traditional selective breeding (selecting high-performing individuals) and MAS (Marker Assisted Selection) breeding are two methods for selective breeding. The former could be utilized only for features that are visible, such as growth, whereas MAS is the greatest option for traits like disease resistance and carcass. At the DNA level, molecular markers are a direct reflection of genetic variation. To obtain molecular markers, a variety of methods have been developed, including Variable Number Tandem Repeats (VNTR), Random Amplified Polymorphic DNA (RAPD), Single Nucleotide Polymorphisms (SNP), Amplified Fragment Length Polymorphism (AFLP), simple sequence repeats (SSRs), Restriction Fragment Length Polymorphism (RFLP), and others. These markers can be used to identify quantitative trait loci (QTLs) and aid in breeding. The discovery of genes responsible for resistance or susceptibility can be aided by the association of markers with any trait (QTL). Marker Assisted Selection (MAS) is used to select based on marker data, and Gene Assisted Selection (GAS) is used when a gene is employed (GAS). SNP markers, which are abundant across the genome, are extremely useful in selective breeding, and with the development of more advanced technologies like NGS (Next Generation Sequencing), identifying these markers has never been easier or more cost-effective (Agarwal *et al.*, 2017). In 1997, CIFA launched the first batch of Jayanti Rohu with a higher growth rate, which was generated by selective breeding to commemorate India's 50th year of independence (17 percent more). CIFA also produces disease-resistant Jayanti rohu (resistant to *Aeromonas hydrophila*) (Mahapatra *et al.*, 2017).

Assisted Reproduction Technique

In the treatment of infertile couples, assisted reproductive techniques (ART) are now commonly used. Although this is currently being utilized in humans, it can also be used to produce high-quality spermatozoa in the hatchery. Milt recovered through stripping also contains a number of dead spermatozoa, resulting in fertilization failure. FACS (Fluorescent Activated Cell Sorting) is a flow cytometry technique that can be used to separate inactive dead spermatozoa from active spermatozoa utilizing a specific dye to stain live and motile sperm (Sousa *et al.*, 2011). It enables sorting a diverse combination of cells into two or more containers, one cell at a time, based on each cell's precise light scattering and fluorescence properties. The separated cells (live and motile sperms) might be collected and employed in breeding. However, this technology is still in its infancy, but it has enormous potential, particularly for endangered or threatened species or species with less fecundity (Agarwal *et al.*, 2017)

Surrogate Brood Stock Technology

This approach has enormous potential for aquaculture and conservation of species that are difficult to breed in captivity. Lacerda *et al*., 2013; Yoshizaki, G., and Yazawa, R., 2019) proposed the concept of Tuna from Mackerel or Trout from Salmon (Yoshizaki, G., and Yazawa, R., 2019), which has sparked interest in hatchery seed production of various key species for conservation and aquaculture.

Surrogate breeding is a new aquaculture technology in which both sperm and eggs of a species are produced within a closely related and easily cultivable species. Another fish species is utilized to produce spermatozoa and eggs via surrogate technology. It is essential to understand the mechanisms involved in gonad development in order to have an effective surrogate technology. Gonads develop from a type of cell known as a Primordial Germ cell (PGC), which can produce either spermatogonia or oogonia (future male testis or female ovaries). The PGC of one fish (donor) is implanted into the embryo of another fish (receiver), and after maturation, the recipient fish produces the donor fish's gametes. This approach provides a ray of hope for obtaining large fish from little fish that are difficult to reproduce in captivity, such as Tuna from Mackerel which saves time, space, and money due to the fact that small fish require less space, food, and maturation time.

Transgenic fish

DNA is thought to be the blueprint for life. The transgenic or genetically modified organism is created by applying the genetic engineering approach of gene transfer to make some alterations in the genome. The two GMOs that are commercially accessible for ornamental and consumption purposes are Glofish (GMO zebrafish) and Aqua-advantage salmon (Gillund, 2011). FDA's definition of "genetically engineered (GE) animals" as those animals altered by rDNA techniques, including all offspring that cover the modification." In the fields of developmental biology, animal husbandry, and aquaculture, the generation of transgenic animals produced through the transfer of foreign genes into fertilized eggs has become a valuable tool for studying gene expression in living animals. Investigators are working to improve the genetic properties of commercial aquaculture fish utilizing a variety of transgenic approaches. Scientists are working to develop fish that are disease resistant, larger and grow more quickly, can endure low oxygen levels in the water, and can withstand cold temperatures (Agarwal et al., 2017). For example, AquaBounty Technologies' AquaAdvantage salmon is the trade name for genetically modified Atlantic salmon. In case of AquaAdvantage salmon, where a growth hormone regulating gene from a Pacific Chinook salmon and an antifreeze promoter from an ocean trout were added to the Atlantic salmon genome to create the AquAdvantage salmon. Normal salmon does not grow during the winter, but with these genes, it can grow all year. The changes are intended to accelerate the fish's growth without changing its final size or other characteristics. This fish achieves commercial size in 16 to 18 months rather than 3 years. This technique is rapidly advancing, and it is already possible to transfer genes between animals that are distantly related. Just like Glo fish is also available with distant varieties and widespread amongst the ornamental fish keepers.

Hybrid Identification

Hybrid seeds have been a big worry for aqua culturists in recent years, since many hatcheries are now generating hybrids that are sold under the names of original species, such as hybrids of IMC, hybrids of Magur and *Clarius gariepinus*, and so on. At the spawn and fry phases, it's nearly hard to tell the difference between the original and hybrid seed using the external characters. For farmers, hybrid identification technology would be extremely useful in the screening of high-quality seeds. Molecular techniques could be used to fix this problem of getting mixed seed to a sufficient level. Some private labs and government institutes have developed simple and reliable kits that are commercially available and show good results in the identification of hybrids with the wild one. These are PCR-only kits that can detect a hybrid seed in just two steps using genomic DNA as a starting material. CIFA has developed a kit for identifying *Labeo rohita*, *Catla catla*, and their hybrids in the early stages of their lives in just a few hours (Swain, 2020). To amplify the signal, three sets of primers are utilized to amplify three diverse set of microsatellite markers from the genomic DNA isolated from the pectoral fins by using PCR. The 'hybrid–Rohu' is distinguished from wild types by PCR results employing all three primer sets. With all three primer combinations, the hybrid–Rohu DNA produces distinct PCR results. Only two PCR products are obtained from wild-type Catla DNA (by primer sets 1 and 2) or wild-type Rohu DNA (by primer sets 3 and 4). (by primer sets 1 and 3). This type of PCR-based hybrid identification can be established for others too (Jayasankar *et al.*, 2016).

Biotechnological interventions in Health management in aquaculture

The disease is always a nightmare and a major concern in aquaculture, and it is estimated to cost the industry over USD 6 billion per year (Leung and Bates, 2013). Managing the disease is never easy, but biotechnological interventions at various stages, such as diagnosis, prophylaxis, and therapy, are a big help for the aquaculturists. The aquaculture industry requires fast-growing disease-resistant species, quick and accurate disease testing tools, as well as affordable vaccines, probiotics, and cell lines.

Diagnosis of disease

Prevention is always preferable to cure, and the same is true in aquaculture. Monitoring pathogens at various stages of aquaculture can aid in the protection of the farm's strength in terms of biosecurity. The development of a simple, sensitive, reliable, and fast method for detecting infections is an important demand in this environment. Molecular techniques for pathogen detection based on biochemical features, serology, and histology may be faster and more sensitive than older methods. The science of diagnostics has been transformed by PCR technology.

Different PCR versions exist that can identify even 1 copy number of the pathogen, which would otherwise go unnoticed by other methods. A number of diseases that cause problems in aquaculture have been identified using PCR-based diagnostic methods.

Molecular approaches have been increasingly used to diagnosis fish diseases over the last 15 years or so. Practically, for all of the OIE-listed pathogens, PCR-based diagnosis techniques are available. The testing ofSPF seed is done with the help of PCR techniques.

Bioaugmentation and Probiotics

In aquaculture, the use of bacteria as gut probiotics and water probiotics to limit pathogen proliferation and maintain healthy gut microbiota is gaining popularity. Water probiotics are used in aquaculture to reduce organic load and the conversion of harmful compounds like ammonia into benign forms, as the health of the pond bottom is vital. In today's shrimp aquaculture, the bioaugmentation procedure is widely used. The creation of a vaccine is one of the other aspects of aquaculture health management. The creation of a vaccine based on recombinant DNA technology has been the subject of a variety of studies. Only one DNA vaccine for infectious hematopoietic necrosis virus (IHNV) is currently available in Canada. Although RNA infarace (RNAi)-based technology has proved to provide pathogen protection, it is still not commercially available. Even if such insights are not utilized on a large scale as required, future aquaculture must embrace this to avoid disease outbreaks.

Metagenomics

Microbial research in aquaculture aims to better understand the beneficial and detrimental effects of microorganisms on culture animals, whether they can be fish or other culture groups such as crustaceans and mollusks. By employing the genetic component of a sample from an organism or ecosystem, metagenomics can provide a better knowledge of microbial interaction with the animal in various situations such as healthy or diseased, stunted or fast-growing, and so on. The purpose of this section is to understand the important potential applications of metagenomics in aquaculture.

A. Development of suitable probiotic

The comparative microbiome analysis of a healthy and diseased animal can be used to identify candidate probiotics. The results of a metagenomics investigation disclose the detailed community makeup of healthy and diseased animals, as well as possible potential probiotic bacteria from the microbiome of healthy animals. Some of the studies mentioned above have demonstrated that particular bacterial genera predominate in healthy animal samples and may be candidates for probiotics. By comparing the metagenome of different water bodies with excellent and bad water quality, a similar approach can be used to detect water probiotics.

B. In depth screening of Antibiotics resistance gene from the aquaculture system.

Aquaculture is one of the food-producing industries that is regarded as one of the reasons for resistance development owing to indiscriminate antibiotic usage, which is a big worry for the entire world. The aquatic system is also thought to be an environmental hotspot for horizontal gene transfer because it receives all types of antimicrobials along with runoff water. Because the traditional approach of studying Anti-Microbial Resistance (AMR) can only be used with cultivable bacteria, it can only provide limited information on the prevalence of antibiotic resistance. Shotgun metagenomics allows in-depth testing for the presence of antibiotic resistance of genes and it represents a better picture of AMR in aquaculture.

C. Bioprospecting of the novel protein coding gene

Marine ecology supports a wide range of microbial variety, ranging from viruses to bacteria and fungi, among others. All of these can be a great source of critical life-saving chemicals including enzymes, antibiotics, and immunostimulants, among other things. A typical method of microbiological study cannot be performed since the majority of the microbial population is non-cultivable. Shotgun metagenomics can be utilized to gather comprehensive genome information for the entire community, which can then be used to bio-prospect essential gene coding for the novel valuable proteins.

D. The microbial makeup of pond soil can be characterized using amplicon metagenomics. It is a targeted metagenomics or amplicon sequencing, a molecular biology technique used to analyze the microbial diversity within a complex sample, such as environmental samples or microbiomes. It involves the selective amplification and sequencing of specific DNA regions, typically using polymerase chain reaction (PCR) targeting conserved regions of genes.

E. It can be employed as part of a disease surveillance program to simultaneously target all pathogens.

F. Biofloc and periphyton microbial composition analysis.

Nutrigenomics

Feed is a significant cost in aquaculture, and the price of feed is rising at a considerably faster rate than the growth rate of the industry. In this scenario, other sources of protein, such as plant protein or poultry feather meal, might be used instead of fish meal. The metabolic effects of these substitutions should be investigated. Nutrigenomics is the most effective way to examine these effects, resulting in a piece of greater knowledge. When determining how organisms react to nutrients from various sources, maximizing dietary nutrient utilization, and creating diets, it is helpful to understand the biochemical and metabolic pathways involved in the use of dietary macro and micronutrients, as well as the energy provided by feeds. Because the biochemical pathway or metabolism of nutrients is regulated by one or more enzymes, it is vital to research nutrition using a molecular approach in order to comprehend the effect of nutrients or the interaction between nutrients and genes. Nutrigenomics is an example of such a method. Nutritional genomics, or "nutrigenomics," is the study of how genes and gene products interact with dietary signals to influence phenotype, as well as how genes and their products metabolize nutrients. The advancement of molecular tools such as quantitative PCR, Droplet Digital PCR (ddPCR), and next- generation sequencing has aided nutrigenomics research. Nutrigenomics can aid in the design of precisefeeds for aquaculture.

Conclusion

Aquaculture is seen as a way to provide nutritional and economic security to a growing population. However, this cannot be achieved without intensification. Biotechnology has a lot of potential for integrating with aquaculture for a better future and speeding up the blue revolution. Biotechnology has a lot to offer aquaculture in terms of solving problems like quality seed, disease, and balanced feed, to name a few. For the advancement of aquaculture, more extensive applied biotechnological research is required in the current period.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work, and approved it for publication.

Conflict of interest

The authors declare that the manuscript was formulated in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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