CHAPTER 35

ADVANCEMENT IN AQUACULTURE SYSTEM BY APPLYING BIOTECHNOLOGICAL TOOLS

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INTRODUCTION

The usage of technology has always been a desire of humans to make life easier. Seafood is in high demand all across the world. Sufficient natural harvesting does not appear to be a viable option for meeting rising demand. Several natural ocean and freshwater fisheries are being fished to their limits, according to worldwide consensus. Aquaculture might assist to fulfil rising demand and biotechnology can help to enhance aquaculture output significantly. The application of current biotechnological methods to promote production of aquatic species offers significant promise not only to satisfy demand but also to improve aquaculture. Fish farming is in high demand and biotechnology can assist to supply that need. Within the last few decades, developments in biotechnology have offered the equipment needed to manipulate genes and chromosomes in live beings artificially. The existence of transgenic fish and shellfish is a hot issue in marine research because of the promise for increased productivity that this technology may provide (Zbikowska 2003; Dunham 2004). Researchers are looking for alleles that will boost the synthesis of natural fish growth hormones and innate defensive chemicals which are utilized by sea creatures to combat microbial diseases. Gene editing is now making a major impact to coastal population growth while also posing considerable problems. It believes that current biotechnologies ought to be utilized in conjunction with traditional technologies instead of as a replacement. Genetic manipulation and bioengineering have enormous promise for improving the production of aqua cultured fish. Aquaculture is in high demand, and biotechnology can assist supply that need. Aquaculture, like other bio-engineered foods, would be heavily controlled prior to getting allowed on the market. Biotechnology in aquaculture is also environment friendly. Biotechnology may help to satisfy the demands of a rising and highly industrialized society over the next century if implemented fully with some other techniques in the field of food, farm products, and services. Only a thorough study and learning foundation in the biology, breeding, physiology, pathology, biochemistry, and genetics of the transformed organism can result in effective biotechnology implementation. But without the need for a continuous commitment to fundamental research, the benefits promised by emerging technologies will not came to realization. Use of artificial hormonal steroids in induced breeding, transgenic fish, gene banking, uniparental and polyploidy population are all possible biotechnology applications in aquaculture. This is either the prevailing biotechnology which has piqued the interest of the people because it has the potential to pose a significant impact on the global market. Genetic manipulation is a fundamental aspect of modern biotechnology. Genetic manipulation is not a biotechnology within itself, but rather a biotechnological approach that has evolved over years of fundamental study in cell and molecular biology. A gene may now be found, extracted, cut out, inserted and altered. Genetic engineering is the term for this type of genetic alteration. Food, agricultural production, forestry, fish farming, livestock farming, and horticulture are just a few of the areas where genetic modification has improved our awareness of living organisms and allowed us to adapt that expertise to our lives and activities (Opabode and Adebooye 2005; Ezeonu et al. 2012). The increased public need for fisheries and the degradation of natural resources have prompted researchers to look at how biotechnology may help increase the production of fishery, resulting in aquaculture being a burgeoning topic of research (Billington and Hebert 1991). In fisheries, biotechnology helps researcher to search and integrate good characteristics in marine species to boost productivity and profitability. Researchers are looking for genomes which might boost the

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synthesis of aquatic animal growth and natural defensive proteins which marine organisms utilize to combat microbial illnesses.

Importance of Aquaculture

Living beings have recognized the value of water as a resource since it covers 70% of the earth's surface. As a result, fish farming, primarily for the production of food, is one of the most intensively utilized sectors in terms about the use of freshwater resources, as compared to just utilizing terrestrial land. Aquaculture is the practice of raising, reproducing, and harvesting aquatic animals and plants in controlled aquatic settings such as seas, lakes, rivers, ponds, and streams. Aquaculture has the potential to significantly boost and try to guarantee worldwide supply of food in environmentally and socially responsible ways. Aquaculture is among the most resource-efficient and ecologically friendly ways to generate protein for humans when compared to rearing of land animals like cows and pigs. Aquacultured seafood can also contribute to the achievement of the United Nations' Sustainable Development Goals (SDGs). It's a lot more effective, which means the seafood producers can increase yields with less feed. Because less feed and energy are required in the manufacturing of food, the procedure is also less expensive. It conserves resources and even allows for the production of additional food, resulting in secure stockpiles and reduced environmental stress. Individuals and organizations all around the globe, benefit from aquaculture because it provides economic possibilities and quality jobs.

Relationship Between Aquatic Life and Ecosystem

The phrase "aquatic environment" refers to everything in the bodies of water to the coastal waters that surround them, to large lakes (especially landlocked saline open sea), to lakes and ponds and the small marshes and swamps that are frequently found nearby. Water is found in huge amounts in all living species, and existence as we know it would not be conceivable without the unique qualities of such water (Barnes and Mann 1991).

Biotechnological Application in Aquaculture

Biotechnology in Fish Breeding

According to Bhattacharya et al. (2002), GnRH (Gonadotropin-Releasing Hormone) has become the most effective biotechnological tool for triggering fish breeding. GnRH is the primary responsible and fundamental activator of the reproductive cascade in all vertebrates. It's a decapeptide that can cause the hypothalamus to release luteinizing hormone (LH) and follicle stimulating hormone (FSH). It was initially identified from pig and sheep hypothalamus (Schally et al. 1973). Since then, just one version of GnRH has been discovered as the single polypeptide responsible for both the secretion of LH and FSH in all terrestrial animals, including humans. Nevertheless, twelve GnRH variations have been already structurally characterized in non-mammalian species (excluding the guinea pig), with seven or eight distinct forms extracted from marine animals (Powell et al. 1994; King and Millar 1995; Jimenez-Liñan et al. 1997). The recent development in GnRH discovery were made by Carolsfeld et al. (2000) and Robinson et al. (2000). A variety of synthetic equivalents have been developed based on structural variants and bioactivities, one being the salmon GnRH alternative, which is widely used nowadays in fish breeding and sold publicly with the name "Ovaprim" across the globe. In fact, unless the hormone stimulates them, the majority of commercially significant culturable fish in landlocked water do not reproduce. The advancement of GnRH technology has now effectively enabled induced breeding of fish (Halder et al. 1991).

Transgenesis

Transgenesis provides a wonderful chance, enabling aquatic scientists that can change as well as improve the biological characteristics of commercially significant fishes, shellfish, and crustaceans. External gene/DNA is introduced into the host's genetic material which results among its permanent retention, propagation, and activation. The procedure has already been used to a variety of aquatic species with tremendous results. Palmiter et al. (1982) were among the first to develop transgenic mice by inserting metallothionein-in human growth hormone fusion gene (mT-hGH) in mice eggs, leading to a remarkable increase in growth. This sparked a flurry of genetic manipulation experiments in commercially significant species, notably fish. Zhu et al. (1985) in China generated the very first transgenic fish, claiming transitory transcription in presumptive transgenic organisms but provided no scientific proof for transgenic incorporation. Substantial growth increase has been shown utilizing this strategy notably in Salmonids (Devlin et al. 1994). Hew et al. (1995) revealed that rise in size is remarkable, averaging four to six times than that of the reference, with many animals as large as ten to thirty times that of the control. An improvement of fish resilience to low temperatures has become a focus of fish transgenic studies for some times (Fletcher et al. 2001). Many fishes are stressed by cold-water conditions, and just a few that are able to tolerate temperature range below 0°C. In frigid regions, this is really a big issue in aquaculture. Certain fishes generates a signal that contain large quantities of serum antifreeze proteins (AFP) or glycoproteins (AFGP), that substantially lower the subzero temps by inhibiting ice crystal development. For a long time, researchers in Canada have been studying the isolation, characterization, and control of these antifreeze proteins, notably in the Atlantic Ocean bream *Pleuronectes americanus*. As a result, the genes for winter flounder liver antifreeze protein was effectively incorporated into the genomic sequence of Atlantic Salmon, where it was linked into the germ line and subsequently transient response to the progeny F3 where it is being produced particularly in the liver (Hew et al. 1995). The production of lines with this mutation would've been extremely beneficial in aquaculture production in areas where minimum temperature frequently approach such fish' biological limitations.

The advancement of embryonic stem cell (ESC) technology is definitely the most intriguing technique also for advent of transgenic fish farming. Because the cells are undeveloped and totipotent, they may be altered in laboratory and then reintroduced into embryonic cells to participate towards the recipient's genetic structure. Then it would make it easier to add or remove traits in a stable fashion (Melamed et al. 2002). Whilst great advancements have been made in multiple research centers across the globe, there are still a number of issues to be tackled until recombinant hatchlings for farming

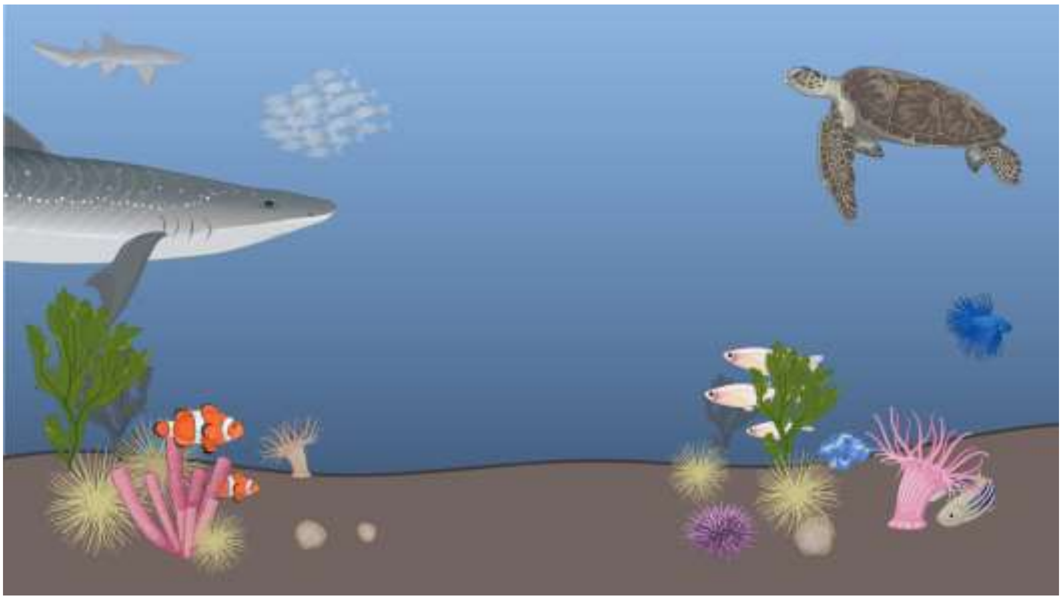

Fig. 1: The Aquatic Environment.

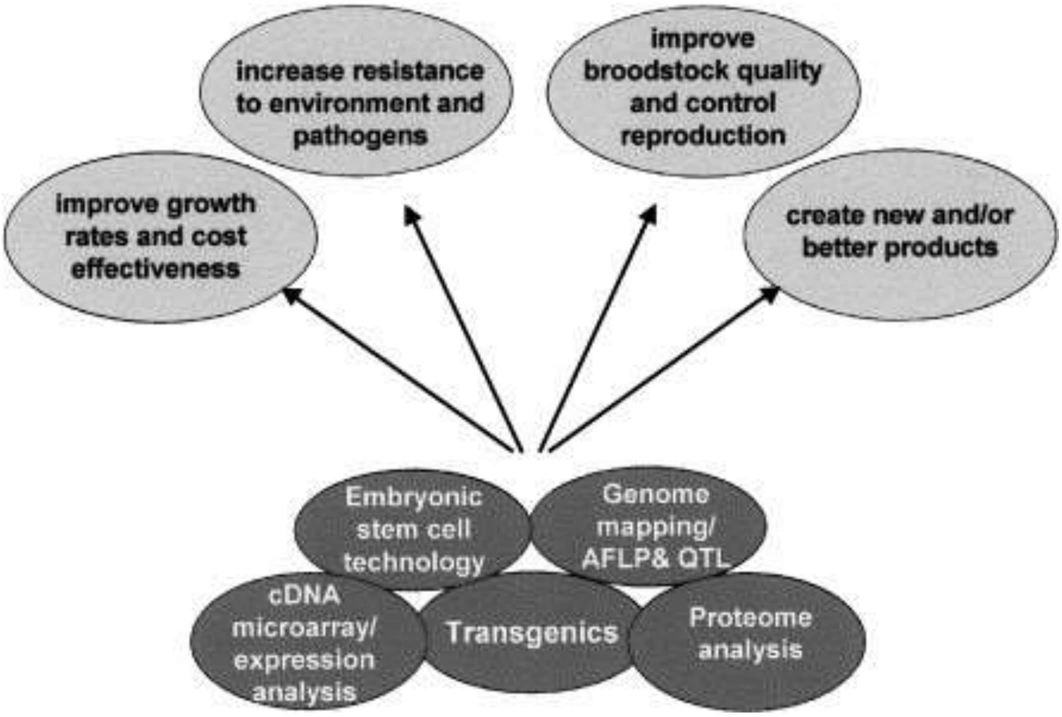

Fig. 2: An illustration of several genetic and genomic technological tools (dark-shaded) and their prospective farming uses (light-shaded).

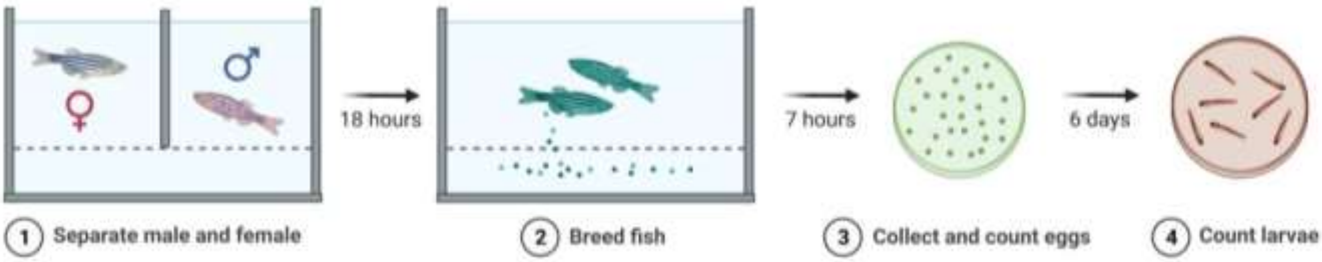

Fig. 3: Fish breeding procedure

may be successfully commercialized. Numerous significant technological discoveries are necessary to fully fulfill the capabilities of mutant fish biotechnology in aquaculture. There are (i) highly accurate bulk genetic manipulation methods (ii) focused transfection techniques such as embryonic stem cell gene transfer (iii) adequate regulators to guide gene transcription at optimum levels at the appropriate phases of growth (iv) Genetic mutations for desirable features in aquaculture and its other uses found. (v) Genetically modified fish security and ecological consequences. Employing various transgenic approaches, researchers are attempting to enhance the specific genes of farmed fish. Employing various transgenic approaches, scientists have attempted to enhance the specific genes of farmed fish. Scientists are attempting to create fish that will be fatter, mature quicker, much more effective at turning their diet into musculature, diseases resilient, can withstand lower oxygen concentration, and can withstand cold temperatures. In recent times, tremendous progress has been made in the breeding programs of Tilapia utilized in farming. For instance, by the employment of mono-sex and selective breeding procedures (Beardmore et al. 2001).

Remodeling of Chromosomes

In farmed fish species, chromosomal sex modification methods were used widely to create polyploidy (triploidy and tetraploidy) and autosomal chromosomal transmission (gynogenesis and androgenesis) (Lakra and Das 1998; Pandian and Koteeswaran 1998). These procedures are significant for improving fish progeny because they give a quick method for pubertal ablation, sex selection, mutant survival enhancement, and cloning. Many animals are heterozygous, which means their somatic cells have two full chromosomal sets. For aquaculture marine species, chromosomal sex modification methods have been utilized frequently to create polyploidy (i.e. triplody and tatraploidy) and uniparent chromosome transmission (gynogenesis and androgenesis) (Baroiller et al. 1999). Such procedures are significant for improving fish reproduction because they give a quick method for gonadal sterility, sex selection, offspring survival enhancement, and cloning. The most successful approach for creating infertile salmon for farming and marine control is artificial triploidy (Bongers et al. 1994). Animals with polyploidy have one or perhaps more extra chromosomal pairs, raising the overall the number of ploidy to triple in triploids, quadruple in tetraploids, and so forth. The much more successful approach for creating barren fish for farming and fishery control is artificial triploidy. Tetraploid mating strains might help fisheries by making things easier to create massive groups of sterile tetraploid fish using straightforward interploidy combinations among tetraploids with diploids (Chourrout et al. 1986; Guo et al. 1996). Whilst tetraploidy has indeed been generated in a variety of freshwater fish species genera, tetraploid survival has already been limited in most cases (Rothbard et al. 1997). Strict regulations and a lack of customer acceptability of hormone-treated fish items may hinder the adoption of synthetic hormones. Triploidy can really be generated by subjecting eggs to physical or chemical treatment soon after conception to prevent the second polar body from extruding, since sister chromatids fail to attach appropriately throughout the first stage of meiosis, triploid fish are likely to be infertile (Purdom 1983; Thorgaard 1983; Ihssen et al. 1990). Thermal stress (warm or cold), surface tension shock, or drugs such as ancolchicines, cytochalasin-B, or nitrous oxide are all used to induce triploidy in egg cells. Teraploids and diploids could also be crossed to generate triploids. Tetraploid initiation is achieved by fusing oocytes containing healthy sperm and treating the diploid zygote to thermochemical intervention in order to prevent the first mitotic phase. Gynogenesis is the stage of animal growth in which only feminine chromosomes are passed along. Fish breeders are particularly interested inside the creation of gynogenetic swimmers since a substantial rate of inbreeding may be achieved in a couple of generations. In fishes exhibiting feminine homogamety, gynogenesis can also be employed to establish overwhelmingly female colonies and expose the sex - selective processes in fish. Regarding gender reversal research, it is more practical to employ entirely female gynogenetic inbred lines (rather than regular hetero descendants) (Gomelsky et al. 2000). Androgenesis is a technique that might be used in fisheries for economic purposes. It could also be used to create homozygous fish strains and to regain lost genomes from cryopreserved male gametes.

Improved Disease Tolerance in Fish

A variety of vaccines targeting viruses and bacteria have indeed been formed for commercial fish farming. Some of them were traditional vaccinations made from dead microorganisms, however a new generation of immunizations made up of protein subunit vaccines, genetically modified organisms, and DNA vaccines is now being developed. Biotechnological technologies including molecular screening techniques, vaccinations, and immunostimulants are rapidly growing for enhancing disease resistance in marine species throughout worldwide. When it comes to viral illnesses, pathogen prevention is crucial. In this situation, a quick approach for detecting the pathogen is required. Therefore in this field, scientific technologies like genetic markers and polymerase chain reaction (PCR) have a big future. For a variety of infections that afflict fish and crustacean, gene probes and PCR-based detection approaches have been devised (Karunasagar and Karunasagar 1999). Vaccination against diseases is a typical technique for prevention. Although the immune response of shrimp is yet underdeveloped, scientific technologies can aid in the synthesis of molecules that can boost such responses. Latest research has demonstrated some bacterial substances like lipopolysacharides, peptidoglycans, and glucans which can trigger the generic defense mechanism (Itami et al. 1998). Glucan and levamisole are two immunostimulants that have been shown to improve phagocytic activity and specific immune reactivity in fish (Sakai 1999). Health issues are indeed a serious roadblock to aquaculture growth. Vaccines targeting bacterial and viral pathogens have now been obtained in the commercial fish aquaculture sector. Traditional screening for immune function and biochemical tests of microorganisms for characterization and identification are both employed to enhance animal wellness using genetic biotechnologies. DNA-based technologies are increasingly being employed to describe various pathogen's genera and types. Genomic probes may be designed to test for particular infections in tissues, entire individual, and perhaps even water and soil samples after the culprit has been identified. In several regions, such approaches are really being utilized to identify viral illnesses in marine shrimp as well as bacterial and fungal pathogens in fisheries (Subasinghe and Bondad-Reantaso 2006; Subasinghe 2009). The successful prevention as well as cure of farmed fish illness necessitates the availability of quick, accurate, and extremely susceptible diagnostic assays. To address these issues, bioassays and DNA-based screening approaches, as well as polymerase chain reaction (PCR) amplification approaches, have been developed.

Vaccines

Vaccines and immunostimulants can be given as feed supplements, dissolved in water, or injectable within the case of relatively larger cultured animals such as fish and poultry. To prevent animals from infections, genetically modified vaccines are indeed being investigated. Inside the realm of vaccinations and immunostimulants for farmed fish, technological advancements are really useful. These make it possible to perform disease-prevention strategies such as immunization or immune defense strengthening. Fish vaccines became an accepted, verified, and economical strategy for managing various contagious illnesses in farmed animals across the globe within last 2 - 3 decades (Subasinghe 2009). Several vaccines are being widely marketed for fish infections, such as for furunculosis (Aeromonas salmonicida), and many others in progress, such as viral hemorrhagic septicemia (VHS), are presently available. Vaccines not only lower the illness intensity but also minimize the use of antibiotics, leaving no remnants in the product or ecosystem, and therefore do not cause microbial resistance (Subasinghe and Bondad-Reantaso 2006).

Gamete Cryopreservation or Gene Banking

It is characterized by the fact that a really low temperature slows or incapacitates a cell's physiological and metabolic activity, allowing it to survive for an extended length of time. Livestock farming have also adopted that cryopreservation of fish spermatozoa (milt) technique. Cryopreservation is a process that involves the long-term preservation and retention of biomaterial at extremely low temperatures, typically -196 °C (liquid nitrogen temperature). Blaxter (1953) documented the very first accomplishment in keeping fish sperm at low temperatures by fertilizing Herring (Clupea herengus) oocytes using frozen thawed sperm cells. Mostly all farmed fish species' sperm cells have now been cryogenically preserved (Lakra 1993). Cryopreservation fixes the issue of male maturation prior to female maturation, allowing for selective breeding and stock management, and provides for survival (Harvey 1996). The gene pool stocks are used to create the majority of plant types. The marine gene bank, on the other hand, suffered from the reality that only the male gametes of finfishes can be cryopreserved now at the moment, with no feasible method to preserve female oocytes and embryos. Nevertheless, previous findings by Diwan and Kandasami (1997) and Subramonium and Newton (1993) upon this chilling of shrimp embryos appear encouraging. As a result, it is important that gene banking of cultured and cultivable aquatic species be completed as soon as possible.

Bioinformatics

The use of digital technology to the administration of biological data is known as bioinformatics. Biological and genetic data is collected, stored, analyzed, and integrated using computers. Bioinformatics has brought several advances to the biomedical sciences, including algorithms for creating, maintaining, and accessing sequence databases. Bioinformatics aims to create an extensive list of genomes and nucleotide sequences. Proteomics, in opposition to genomics, aims to investigate the proteins that are described. Proteins are involved in both physiological and pathological processes in a cell or organism, and proteomics describes the whole catalogue of proteins in relation to in vivo factors. Proteomics is a method for studying biosciences that complements genomics.

Bioinformatics Tools

Bioinformatics tools are software applications that are used to store, retrieve, and analyze datasets in order to collect required data. These tools come in a variety of shapes and sizes.

Tools for Homology and Similarity

The concept homology refers to a parallel evolutionary development between two attributes. Homologous genes are those that have diverged from either a single ancestor and are similar. This methodology could be used to make comparisons between unique reference sequences with uncertain structure and function and database sequences with defined structure and function.

Protein structural Analysis

This package of tools helps you to measure structures to databases of known structures. Since structurally homologs happen to associate functionalities, a protein's activity is much more entirely a result of its own form than that of its sequencing.

Protein sequence Analysis

This software suite enables you to perform more in-depth research on any known sequences, such as phylogenetic analysis, genotyping detection, hydropathy areas, CpG islands, and configurational errors.

Recent Biotechnological Advancement in Aquaculture

Surrogate Broodstock Technology

Surrogate broodstock technique entails the creation of donor-derived gametes in foster parents, as well as the transplantation of donor animals' spermatozoa into sterile receivers (surrogates) of the same or similar species (Yoshizaki and Yazawa 2019). While using this approach, Okutsu et al. (2007) and Takeuchi et al. (2004) were able to efficiently form masu salmon (Oncorhynchus masou) producing rainbow trout (Oncorhynchus) mykiss reproductive cells. There are two key phases in surrogate broodstock technology: (i) Collection and fortification of germline stem cells (GSCs), the progenitors of gametes, and (ii) implantation of GSC into barren receivers (Jin et al. 2021).

By use of surrogate bloodstock technology has significant prospects to expand the breadth and effectiveness of genetic modification studies in farmed fish. The development of CRISPR/Cas genome editing tools has advanced gene and genome function studies through the production of animals and cell lines containing exact focused mutations. In farmed fish, this has often been achieved by pronuclear inoculation of genome editing agents in early-stage embryos, which has been effective in attaining gene deletion inside the founding (F0) individuals (Jin et al. 2021).

Further Perspectives

It is essential to urge individuals globally to create and adopt suitable techniques and hazard assessment standards for food biotechnology research and to assure the healthiness and quality of food chain. Appropriate bio-safety rules, hazard evaluation of biotechnological products, method and techniques for monitoring usage and observance are essential to assure that there would be no detrimental impacts on the surroundings or even for humans. Significant ecological risks from emerging technologies of biotechnology, notably including genetically modified organisms (GMOs), have created worries that in the lack of sufficient laws, multinational businesses in affluent nations may exploit underdeveloped countries as test ground for their goods. A few of the important ecological dangers involve plant - parasitic nematodes (Altman 1999). Improved fertility in physiologically acceptable wild species can come via genetic leakage from genetically modified organisms.

Conclusion

Biotechnological research and development is rapidly expanding. Biotechnology has been increasingly important for the overall growth of aquaculture, agribusiness, especially changed people's life in past few years. Biotechnology has given us technological skills and incredible capacity to develop genetic variants and genetic mutations in crops, livestock and in aquaculture. The use of biotechnology in the fishing industry is indeed a fairly new activity. Nonetheless, it appears to be a good place for increasing seafood output. In addition to stimulating in protecting the natural environment, the use of biotechnological techniques has the potential to change modern farming practices. This chapter highlights the present state of transgenesis, chromosomal editing, the use of synthetic hormones in fish breeding, biotechnology in health management, and gene banking.

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