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Transgenic Fish Technologies: A Review

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تقنيات الأسماك المُعدّلة وراثيًا: مراجعة

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Abstract

The current state of transgenic fish technologies is the main subject of this review. Aquaculture and biomedical research both make extensive use of transgenic fish technologies. The effective and safe application of this technology requires the resolution of several important issues. To boost public acceptance and steer clear of sequences with bacterial or viral origins, future research should focus on fish DNA rather than mammalian DNA. Technology connected to transgenics is evolving quickly. Consumers and environmentalists, however, continue to question its safety. Accelerated research in this field is necessary to boost public trust and guarantee the safe application of transgenic technology.

Keywords: Transgenic fish, Microinjection, Electroporation, Sperm mediated gene transfer, Retroviral infection.

الملخص

تم التركيز في هذه المراجعة على تقنيات الأسماك المعدلة وراثيا المستخدمة حاليا. وتستخدم كل من البحوث الطبية الحيوية وتربية الأحياء المائية تكنولوجيات الأسماك المعدلة وراثيا بشكل كبير. يجب حل العديد من المشكلات المهمة من أجل استخدام هذه التكنولوجيا بكفاءة وأمان. يجب أن تركز الدراسات المستقبلية على تسلسلات الحمض النووي للأسماك بدلاً من تلك الخاصة بالثدييات لزيادة القبول العام وتجنب التسلسلات ذات الأصل البكتيري أو الفيروسي. تتطور التكنولوجيا المتعلقة بالجينات المحورة بسرعة. ومع ذلك، لا يزال المستهلكون ونشطاء البيئة متشككين بشأن سلامته. ويلزم تسريع وتيرة البحث في هذا المجال من أجل زيادة ثقة الجمهور وضمان التنفيذ الآمن للتكنولوجيا المعدلة وراثيا.

الكلمات المفتاحية: الأسماك المعدلة وراثيا، الحقن المجهري، الصعق الكهربائي، نقل الجينات بوساطة الحيوانات المنوية، العدوى الفيروسية الرجعية.

Introduction

Transgenic animals are those whose genetic information has been altered by adding foreign genes from another animal to affect its natural genetic composition. Gene transfer experiments have been conducted on many animal species like (mice, rabbits, chickens, cows, sheep, dogs, monkeys and mosquitoes) , and research is still ongoing in this field on other types of animals. The first transgenic fish were produced in China in 1985 (Maclean 1998), by the late-1990s the salmon was the first transgenic fish species produced for this purpose (Devlin et al., 2007). Nearly 35 different fish species, including trout, catfish, tilapia, and salmon, have undergone genetic modification. These have been genetically modified to grow quickly, build enormous muscles, and tolerate heat. Conventional techniques in this industry are being offset by increased efficiency and high output levels, especially in developing nations. The transgenic fish can reproduce in an environment free from containment with other species. Some of them are already sold in countries like China (carp), the US (salmon), and Cuba (tilapia) (Anjana and Vandana, 2018; A´lvarez, Garcia-Vazquez, 2011). Moreover, enhancing the traits of commercially available fish, using them as bioreactors to develop biomedical important proteins, using them as indicators of aquatic pollutants, creating new non-mammalian animal models, and conducting functional genomics studies are the five major uses of transgenic fish production. Until now, studies have focused on developing new strains of fish using economically advantageous characteristics via emphasis on development. improvements and refining transgenic gene deletion and gene editing procedures for fish experimentation as presented in Figure 1.

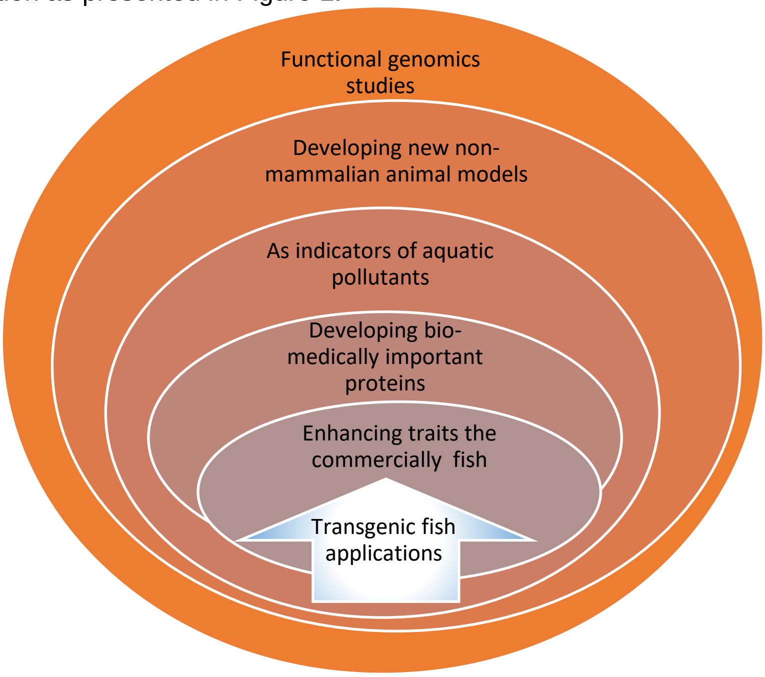

Figure 1: General applications for fish transgenic.

The transfer of growth hormone has been the subject of the majority of transgenic research. Even though relatively little study has been done specifically on improving disease resistance, major improvements have been made. Extensive functional genomics research has advanced the comprehension of gene expression during disease epizootics in fish, which may be useful in the future. The multiple impacts of various transgenes, specifically the growth hormone gene, may affect the resistance to diseases in positive as well as negative ways. The majority negative effects associated with all transgenes seem to reduced fitness attributes, which is good for biological containment. While this research is not entirely convincing, transgenic fish appear to present little environmental risk. Research on transgenic sterilization is being conducted to speed up commercialization and lower environmental risk. Concerns about ethics, the economy, the environment, the law, and society are raised by the use of genomics in aquaculture and capture fisheries. The production and use of genetically modified organisms are currently the most well-known of them. More research is required to develop new technologies that enable the safe use of transgenic technology as well as to address problems with the safety of employing transgenic fish (Zhanjiang, 2008).

Technology used to produce transgenic fish:

To develop transgenic fish by genetic engineering (gene transfer), the following essential components are needed as shown in Figure 2.

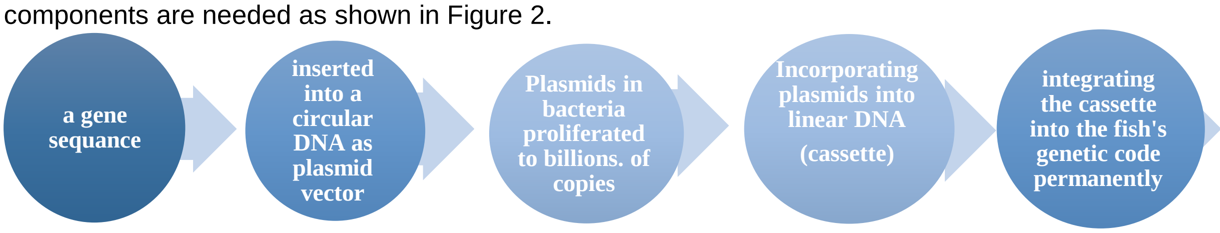

Figure 2: Essential components of transgenic fish.

Microinjection method

Because of its ease of use and security, the microinjection approach is an effective method that has been successfully utilized to produce transgenic fish. This technique was initially applied to model fish like zebrafish and medaka because those fish's egg chorions could be removed or softened before being microinjected. The use of the microinjection method results in higher survival rates for manipulated fish embryos than the electroporation method. The most established method for gene transfer in fish is microinjection. Microinjection that allows delivery of the transgene directly into the nucleus, Transgene is directly microinjected into the male pronuclei of fertilized eggs. Transgenic technology through DNA microinjection into zebra fish embryos has made great gain in the last decade. DNA inserted into the cytoplasm of fertilized zebrafish eggs has been demonstrated to have the ability to integrate into the fish genome and be passed down through the germ line. According to Daniel et al. (2003), the incidence of germline transfer of microinjected DNA can rise up to 20% in zebrafish and up to 77% in salmon.

Disadvantage

This procedure is limited by the physiology of fish eggs and is time-consuming and laborious for animals such as fish that lay a lot of eggs. Transgenes are usually injected into the cytoplasm of the egg, the chorion is what hardens rapidly after fertilization, and the pronuclei of fertilized eggs are undetectable in many fish species. Fish eggs have tiny nuclei that are difficult to see. However, a major drawback of this approach is still the low efficiency of transgenics production. Transgenic selection is more effective when genetic markers are co-injected with the transgene to monitor for altered zygotes. Green Fluorescent Protein (GFP) from jellyfish (Aequorea victoria) was experimentally injected into zebrafish for this purpose.

Electroporation method:

Since electroporation is capable of treating plenty of fertilized eggs rapidly, it has been demonstrated to be the most successful method of gene transfer in fish. The transgene is introduced into the cytoplasm by electroporation, which uses a series of brief electric pulses to penetrate the cell membrane and create temporary holes on the target cells' surface. The cellular machinery then transports the transgene to the nucleus. Due to its effectiveness, simplicity, and speed, electroporation has been the method of choice for gene transfer in fish systems in many labs.

Disadvantages

Fish eggs with a tough chorion coating surrounding them are less efficient, and removing the chorion is a laborious process that puts more stress on recently fertilized eggs. Either the equipment employed in previous electroporation techniques is no longer commercially accessible, or transfer levels have been modest with few research demonstrating germ-line transmission and expression.

Sperm mediated gene transfer method

It has been investigated to use sperm-mediated gene transfer (SMGT) to create genetically altered animals by the transfection of foreign genes (Lavitrano et al., 1992). This method is based on the fact that during fertilization, sperm cells have the capacity to spontaneously attach external DNA and transfer it into an egg. Nevertheless, this method has a number of disadvantages, chief among them being the difficulty of DNA binding and subsequent sperm cell absorption. Numerous research has attempted to improve the DNA absorption of sperm cells since they are thought to be difficult to transfect. Furthermore, the findings of these experiments varied; some were able to successfully create transgenic mice (Campos et al., 2011) while others were unable to do so. These outcomes differ depending on the species and the work environment.

Disadvantages:

Because the presence of a high molecular weight gene insert does not prove integration, as concatemers of the gene insert may also produce these high molecular weight forms, the majority of fish produced were mosaic, and true genomic integration of exogenous DNA has not yet been demonstrated (Chong and Vielkind, 1989). According to Patil et al., 1994, extrachromosomal DNA transmission and episomal integration have been demonstrated and frequently reported in other species, including zebrafish, therefore germ-line transmission is likewise not a guarantee that the gene has been integrated.

Retroviral infection method

The successful use of Retroviruses for gene delivery in fish has been reported. The class of enveloped viruses known as retroviruses uses the enzyme reverse transcriptase (RT) to convert their RNA genome into complementary DNA, which is then incorporated into the genome of the host cell. There are currently 13 proliferative fish disease that have been linked to retroviruses. The detection of retrovirus-like particles and the existence of RT activity in neoplastic tissues are the primary determinants of the etiologic link between retroviruses and these diseases. For a few of the disorders, experimental transmission has been accomplished. Six fish retroviruses have been found to be fully sequenced. A number of these fish proliferative disorders, as well as virus expression, vary seasonally and are probably brought on by environmental and host factors. Fish with viral-related neoplastic conditions offer special models for examining the emergence and progression of cancer (Quackenbush, 2016).

Disadvantage

Retroviral particle preparation, incorporating the transgene of interest, is a very time-consuming, expensive, and technologically demanding procedure as illustrated in Table1.

Table 1: Advantages and disadvantages of transgenic fish technologies.

Method	Advantages	Disadvantages
Microinjection method	▪ It is the most widely used technique for transferring fish genes. Direct delivery of transgenes to the nucleus by microinjection	▪ This method is limited by the biology of fish eggs. The chorion instantly hardens after fertilization, making it impossible to see the pronuclei of fertilized eggs because fish eggs have tiny, hard-to-see pronuclei.
Electroporation method	▪ utilized to break through cell membranes. This makes it possible for the transgene to enter the cytoplasm quickly and easily.	▪ Fish eggs have a hard chorion coating, which limits efficiency, makes removal laborious, and puts additional strain on newly fertilized eggs.
Sperm mediated gene transfer method	▪ Spermatozoa have the ability to bind DNA and transfer it into an egg. Long-term storage of fish spermatozoa in seminal plasma can be done with little loss of viability. This approach has a very high likelihood of transferring genes to fish.	▪ No control of integration site
Retroviral infection method	▪ Retroviruses have been successfully used to transmit genes to fish, according to reports.	▪ Technologically complex, time-consuming, and extremely expensive is the process of creating retroviral particles with the right transgene.

Importance of transgenic fish

Genetic engineering is now being used more frequently for business purposes as the field has grown. By altering aquatic organisms, aquaculture production is increased. Transgenic fish's growth rate can be raised by 400% to 600% while feed intake can be decreased by up to 25% per unit of production, leading to better food. conversion ratios (Devlin et al, 1994). In addition to producing transgenic fish and visually appealing fish, aquaculture has also developed experimental models for biological research, environmental monitoring, and human treatments. In addition to potential environmental advantages like less feed waste and fish farm effluent, growth-enhanced transgenic fish with increased feed conversion efficiency have been profitable. The transgenic strains offer valuable model systems for investigating the genetic, physiological, and ecological effects of growth enhancement. Since aquatic animal production methods rely so significantly on growth rate, this trait has received the greatest attention. Compared to non-transgenic fish, there have been reports of two to three times as many tilapias, Atlantic salmon, and common carp (Wakchaure et al., 2015).

A suitable model for quickly studying GH-transgenes before choosing which construct to apply to economically significant species is zebra fish. One-third less time is needed for advantage salmon, a modified Atlantic salmon, to achieve commercial size than nontransgenic salmon. In addition to saving money on the amount of food produced per pound of meat, faster generational growth can result in more production per unit of time. Using transgenic fish as bioreactors to produce rare human medicinal proteins or new foods for certain nutritional needs on a big scale. tilapia transgenic lines that produce human clotting factor VII, which is used to treat wounds and perform liver transplants. The significance of an enzyme called UDP-glucose dehydrogenase in the embryonic development of the aortic valve has been recognized thanks to zebra fish that have been genetically altered to exhibit a faulty aortic valve development. It has been suggested that transgenesis could be used to eliminate allergens from shellfish. Fish are known to be test organisms that offer unique and superior advantages in terms of shedding light on disease processes.

One of the most significant developments in the study of various disease processes is the creation of transgenic animal models. Among the many traits of Medaka fish that make them particularly well-suited for environmental toxicity is their well-characterized histopathology. Chemical hazard testing, carcinogenesis bioassays, and germ cell mutagenesis investigations have all made substantial use of them. One of the most significant developments in the study of various disease processes is the creation of transgenic animal models. Among the many traits of Medaka fish that make them particularly well-suited for environmental toxicity is their well-characterized histopathology. Chemical hazard testing, carcinogenesis bioassays, and germ cell mutagenesis investigations have all made substantial use of them.

The growth rate of tilapia that express extra copies of the trout growth hormone gene is significantly higher than that of their non-transgenic counterparts that do not. In addition to improving cold resistance, growth rate, and feed utilization, transgenic fish have been utilized to investigate the regulation of genes during development. Fast-growing transgenic tilapia, rainbow trout, medaka, and coho salmon with new growth hormone genes attained sexual maturity earlier than their unaltered counterparts. By producing transgenic fish with a gene construct expressing growth hormone, finfish can also have their growth and feed conversion efficiency improved.

Disadvantages of transgenic fish

According to Campbell et al. (2015), transgenesis can induce a phenomenon known as transgenic mosaicism, in which not all embryonic cells have the desired transgene and cells with different genotypes can combine to form a single individual. Species richness decline and the loss of genetic diversity and biodiversity are the main ecological issues with the use of transgenic fish. Fish that automatically escape into the environment are known as transgenic fish. Concerns range from interbreeding with native fish populations to ecosystem and effects resulting from heightened competition for food and prey species. The sterilization of transgenic fish will help in reducing the interbreeding risks associated with the escape of transgenic fish. Introduction of transgenic fish into natural communities is a major ecological concern. Reduced sperm production in the GH transgenic Nile tilapia, Oreochromis Nilotic's.

Transgenic salmon and loach that grow incredibly quickly have poor fitness and perish inefficiently. Approximately one in every 100 microinjected eggs will successfully integrate the recombinant DNA sequence into its genome and pass the transgene on to its offspring. Since DNA material would not be inserted into transgenic animals without human assistance, transgenesis is unnatural. Transgenic fish typically exhibit decreased swimming ability and fertility rates when compared to non-transgenic fish, and they may create new or modified proteins that could be hazardous to humans. Additionally, when feeding, the transgenic fish are more aggressive and energetic, and they are more willing to take the chance of being eaten. Transgenic fish have shown alterations to the structure and function of the brain or cognitive ability. Because the transgenic salmon are not designed for their natural habitats, they represent severe ecological risks to native populations. The total amount of copies and the location of early integration can be variable as a result of transgenesis. Certain biological changes caused by the genetic engineering process have raised concerns among some scientists because they may enable transgenic fish to better withstand higher doses of previously identified hazardous substances or to absorb chemicals that normal fish cannot.

Transgenic Fish Application in Environmental Toxicology

In addition to bolstering the evidence for the importance of environmental risk assessment, the models of transgenic animals provide an essential chance to expand research on the genetic and molecular origins of disease. Transgenic fish research can use less resources and animals while producing accurate and uniform results through the use of well-defined toxicological endpoints. Using fish's receptiveness to a range of manipulations, such as microinjection of embryos, sediment-associated contaminant exposure (Walker et al. 1996), and static-renewal and flow through chronic exposures (Kane et al. 1996), transgenic fish can improve the estimation of realistic risks associated with exposure to waterborne and sediment-associated contaminants.

Production of transgenic fish around the world

Instead of transferring genes from one species to another, like all transgenesis does, Indian scientists are mainly working on producing transgenic fish by auto-transgenesis, which only involves increasing the quantity of growth hormone genes that are already present in a fish. There is more meat when the growth hormone gene is expressed more. To clarified, China is developing genetically modified (GM) fish particularly to improve feed-conversion productivity or rate of growth. There is currently no commercially approved transgenic fish for human consumption (Fu et al., 2005). Despite the creation of transgenic fish that could be advantageous to the aquaculture industry, regulatory agencies have not yet approved their commercialization (Dunham, 2014).

Conclusion

Applications of transgenic fish technology in aquaculture and biomedical research are numerous, and its safe and effective use requires the resolution of a number of significant issues. Instead of using DNA sequences from mammals, additional research should concentrate on fish DNA sequences to increase public acceptance and steer clear of bacterial or viral sequences. Technology related to transgenics is evolving quickly. Consumers and environmentalists, however, are still wary of its usability. Transgenic technology must be used safely, which requires research to boost public confidence. The aquaculture industry may gain from the production of transgenic fish, but regulatory bodies have not yet given their approval for commercial use.

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