Revolution of Transgenic Fish and its impact on Aquaculture and Biodiversity

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Date of Submission: 6th December, 2015 Date of Acceptance: 6th January, 2016

Abstract

Transgenic fish can be produced by transferring foreign DNA into developing embryos via microinjection or electroporation. Administration of mammalian or recombinant fish growth hormone (GH) to Juvenile fish resulted in significant growth enhancement. By introducing desirable genetic traits into fishes, mollusks and crustaceans, superior transgenic strains can be produced for aquaculture. The ultimate aim is to produce a better quality fish for food to benefit humans although the adverse effect of transgenic fish into native habitat is now a subject of research.
Keywords: Transgenic fish, microinjection, electroporation, GH, aquaculture.

1. Introduction

A transgenic fish is any fish which contains a gene or genes which have been introduced through recombinant DNA techniques rather than traditional mating methods. It is also known as genetically engineered fish (GE). GE applied to aquaculture and fish in particular aims at increasing growth rates and feed utilization, increasing environmental tolerance and resistance to diseases. The 1st transgenic fish were produced in China in 1985[1]. Salmon, Tilapia & Zebra fish are commonly used fish for transgenic. Transgenic fish are used in research covering five broad areas –

- Enhancing the traits of commercially available fish.
- Their use as bioreactors for the development of bio-medically important proteins.
- Their use as indicators of aquatic pollutants.
- Developing new non-mammalian animal models.
- Functional genomics studies.

2. Aim & Objective of Fish Transgenic

Research on transgenic fish is currently under development for at least 35 species of fish worldwide, as well as for a variety of mollusks, crustaceans, plants and marine microorganism for various purposes-

- Transgenic technology has been successfully used to develop fast growing super fish stocks for human consumption.
- To produce pharmaceuticals.
- To test water contamination in both developed and developing countries.
- Several Laboratories now have GM fish with increased growth performance caused by extra copies of GH genes.

List of genetically modified fishes tested for use in aquaculture.
Source-FAO,2000b

Sl. No.	Transgenic organism	Foreign gene	Desired effect	Country
1.	Atlantic Salmon	Antifreeze Protein (AFP) (Ocean Pout)	Cold tolerance	US, Canada
2.	Chinook Salmon	AFP (Ocean Pout) & GH (Salmon)	Increased growth & efficiency	New Zealand
3.	Rainbow tout	AFP (Ocean Pout) & GH (Salmon)	Increased growth & efficiency	US, Canada
4.	Tilapia	AFP (Ocean Pout) & GH (Salmon)	Increased growth & efficiency	US, Canada
5.	Tilapia	Insulin producing gene (Tilapia)	Production of human insulin	Canada, Cuba
6.	Indian Carps	GH (human)	Increased growth	India
7.	Gold fish	GH&AFP (ocean pout)	Increased growth	China
8.	Mud Loach	GH (mud loach)	Increased growth & efficiency	China, Korea
9.	Channel cat fish	GH (rainbow trout)	Increased growth	US
10.	Common Carp	GH (Salmon & human)	150% growth improvement, improved disease resistance	US, China

3. Techniques of Gene Transfer

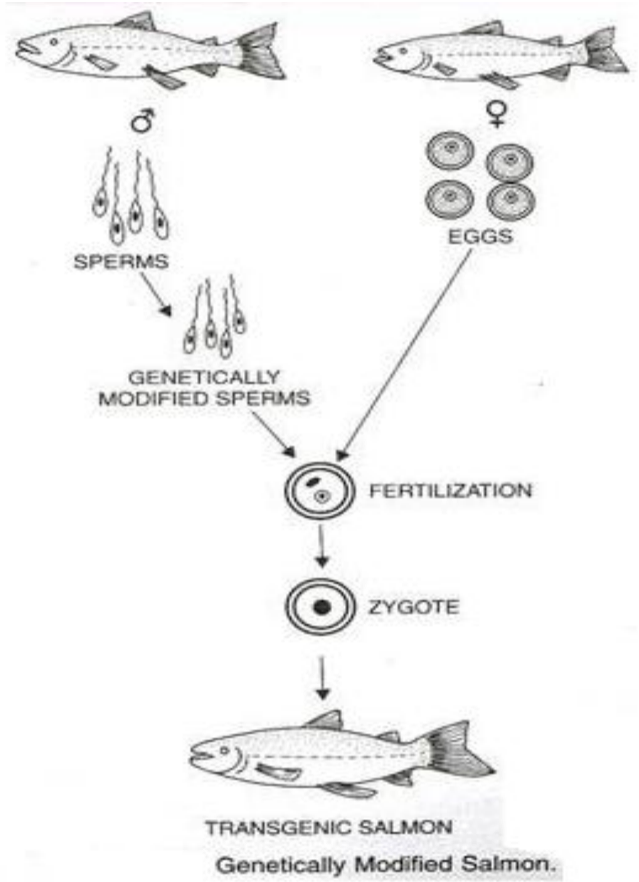
i) Microinjection :

The commonest method for introducing genes into the germ-line of fishes is microinjection.

The 1st report of micro injection is the fertilized eggs of gold fish[2].

Soon after fertilization the gene is micro injected into the cytoplasm since the egg nucleus is not visible in the fishes. Higher amount of DNA is used for cytoplasmic gene transfer than when it is injected to the pronucleus. Small volume of the solution 1-2 ml of DNA containing > 10^7 copies should be injected. The rate of survival and integration of the transgene after micro injection varies widely in different species of fishes and in different batches of the same species.

This process is a tedious and slow[3] and can result in high egg mortality[4].



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ii) Electroporation : It utilizes a series of short electrical pulses to make the membrane porous and permeable to DNA incorporation. Electroporation can be more efficient than microinjection with integration rates sometimes as high as 30-100%[5].

Hatching rates were higher for electroporated embryos than for micro injected channel cat fish embryos[6].

The gene transfer efficiency and integration rate do not differ much between electroporation and microinjection method.

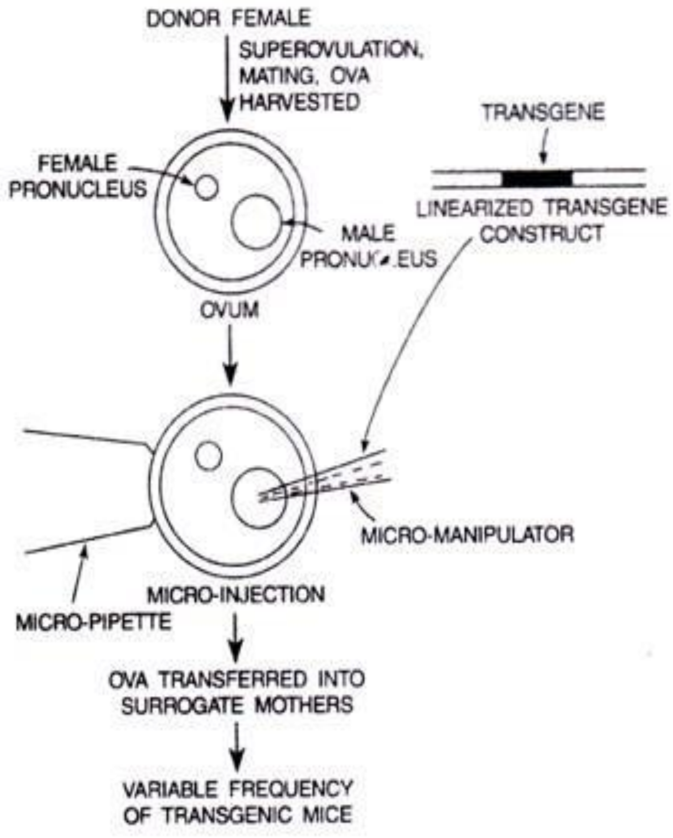

Fig-Schematic representation of the microinjection technique for producing transgenic animal
Source-www.yourarticlelibrary.com/biotechnology/animals/…../33169/

iii) Retroviral integration :

Retrovival vectors containing the envelope protein of vesicular stomatitis virus have been developed[7] and used to produce transgenic fish[8-10]. Retroviral construct was used to produce transgenic cray fish and top minnows[11].

4. Results of Gene Transfer Experiments

Reporter genes : Reporter genes have been utilized to develop gene transfer technology, to facilitate screening of transgenic individuals and to study gene expression. Transgenic fish containing bacterial genes, b-galactosidase[12], neomycin resistance, gold fish[13] hydromycin resistance[14] and Chloramphenicol transacetylase (Tilapia)[15] have been utilized to examine various research issues. Currently green fluorescent protein gene and other fluorescent pigmentation genes are widely studied to effectively study development and gene expression[16,17].

Performance of transgenic fish growth :
Positive biological effects have been obtained by transferring transgenes to fish in same cases, but not all cases. Due to the lack of transgenic available piscine gene sequences transgenic fish research employed existing mammalian GH gene constructs and growth enhancement was reported for some fish species[18]. Mammalian gene constructs failed to effect growth of salmonids[19].

Then gene constructs containing fish GH sequences driven by non-piscine promoters elicited growth enhancement in transgenic carp, cat fish, zebra fish and Tilapia[20].

Cold tolerance :

The purpose of transfer of Anti freeze protein(AFP) gene of the winter flounder was to produce Salmon that could be farmed under arctic Condition, but expression levels obtained have been inadequate for increasing cold tolerance of Salmon.

Disease resistance :

Expression of viral coat protein genes or antisense of viral early genes may improve virus resistance[21].

Bacterial disease resistance may be improved up to 3-4 fold through gene transfer. Insertion of lytic peptide ceropin B construct enhanced resistance to bacterial diseases 2-4 fold in Channel Cat fish[22].

5. Application of Transgenic Fish

1) Developmental Biology Analyses :

The transgenic technology is becoming increasingly popular as a powerful experimental tool in developmental biology of model fish, notably the Zebra fish (*Danio rerio*) and medaka (*Oryzias latipes*).

GFP (green fluorescent protein) transgenic fish system is actively used to investigate gene expression patterns, tissue / organ development, tissue specific promoters / enhances, all lineage and migration, upstream regulatory genes, mutagenesis screening and characterization, promoter / enhancer, chimeric embryos and fish cloning by nuclear transplantation[23].

2) Ornamental fish :

In aquaculture the most successful application of the transgenic technology should be the generation of growth enhanced GH transgenic fish. The benefits from such transgenic fish are obvious e.g. increase of yield, shortening of the production time, decrease of operation cost, potentially lowering the market price for consumers benefit etc.

However because of concerns in food safety and ecology none of these growth hormone transgenic fish has so far been approved for commercialization. The fluorescent transgenic fish are being marketed in US with the trade mark Glo Fish TM as the 1st transgenic animal product.

3) Biomonitoring transgenic fish :

Currently transgenic fish has developed to monitor environment. Glofish originally developed in Singapore as a way to monitor water pollution. It was produced by integrating a fluorescent protein gene from jelly fish into embryo of fish.

4) Bio reactor fish :

At present the most promising application of transgenic technology in animals is to use transgenic animals as a bio reactor to produce useful proteins and compounds in medicine and other areas.

The advantages of the transgenic fish bio reactor include the speed of generation of transgenic fish, low cost and low risk of transmitting animal pathogens etc.

5) Trangenic onco fish :

In the past few years the zebra fish have been advocated as a new Cancer model[24] because the fish as a member of vertebrate have basically the same set of genes as human does and because the molecular mechanism of gene regulation including carcinogenesis are assumed to be more or less identical between fish and human.

Recently it has also been demonstrated that tumors can also been generated by transgenic expression of on co-genes in zebra fish[25].

6. Impact on Aquaculture & Biodiversity

The adverse effects of introducing transgenic organism into native habitats include the extinction of indigenous species, the dispersal of transgenes to non-engineered organism and in same some areas a negative impact on biodiversity. So programme of monitoring must be carried out before release of GM, into aquaculture. Currently the US food and Drug Administration (FDA) is evaluating an application from A/F protein for a genetically modified Salmon that will grow faster and consume less food than its wild relatives.

Transgenic fish, if mixed with wild types will lower the variation of natural populations because transgenic lines being cultured will probably have been subjected to at least three generation of breeding.

Some transgenic fishes have a lower fitness than normal fishes. Growth hormone increases metabolic demand which in turn elevates rate of feeding, thereby increasing an animal's propensity to risk exposure to predation during feeding. This risk does not exist in aquaculture conditions, but could determine a decrease in fitness in the wild.

Furthermore, the swimming ability of some GH transgenic Salmon is reduced compared with non-transgenic Salmon, thereby making the transgenic Salmon more vulnerable to predation[26].

7. Future Perspectives

Currently there is not a great amount of research on transgenic fish throughout the world. However, there is a tremendous worldwide investment in genomics research. In general as disease are the greatest problem facing aquaculture and damaging its profitability so one of the greatest future potential benefits of gene transfer in fish will be enhancement of disease resistance in fish.

Additionally this should be an animal welfare issue. Transgenic fish with enhanced disease resistance would increase profitability production, efficiency and the welfare of the cultured fish.

Data to date indicates that transgenic fish have inferior fitness traits needed for successful establishment if accidentally introduced to the natural environment.

The future success and application of transgenic fish will be dictated by successful demonstration of a lack or potential lack of environmental risk, food safety, appropriate government regulation and labeling, public education and development of genetic sterilization of transgenic fish.

8. Conclusion

Transgenic fish technology has great potential in the aquaculture industry. There is no doubt that transgenic fish research will continue to contribute to our understanding of fish genetics, especially development and genetics. The ultimate aim will be to produce a better quality fish for food to benefit humans. However, the question of whether or not transgenic fish will eventually reach practical utilization will depend on public confidence on the results of performance testes and ecological risk analysis.

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