TFR
Trends in Fisheries Research
An International Peer-Reviewed Journal
www.sciencejournal.in

Review Paper

TRANSGENIC FISH: TECHNIQUES, POTENTIAL AND PROSPECTS.

Amita Saxena and Paras Nath Jha
Department of Fishery Biology, CFSC, GBPUAT, Pantnagar 263145, India.
(E-mail: amitasaxena12@yahoo.co.in)

ABSTRACT
Genetically improved crops and animal breeds produced in the last 2-3 decades as a result of biotechnological research have steadily increased their productivity and quality of the produce. However, this has not happened in aquaculture sector because aquaculture biotechnological research is lagging behind. Only less than 1% of world fish production comes from genetically improved fish stocks (Reddy *et al.*, 1999). Focus of aquaculture research till now has been on improving fish productivity by improving management practices related rearing environment, feed feeding practices and control of diseases. Complete benefits of these improvements cannot be realized until we have genetically improved varieties which respond to such improvements. The transgenic fish production involves transfer of a new fish growth hormone gene, due to which transgenic fish grows faster i.e. reaches market size much earlier, consumes less feed and is thus more economical for the fish farmer, as the feed accounts for roughly 60-70% of total operation cost in aquaculture. The most useful application of transgenic fish production technology is in stock improvement of commercially important fish species. Till now selective breeding is done to improve the stock. This takes several generations. Moreover, there are incremental up gradations in desirable traits from generation to generation. But these take inordinately long time. The transgenic technology, on the other hand introduces genes encoding desirable traits into the genome of organisms in one generation. This is inherited by future generation resulting in a rapid development of new genetic stocks with desirable traits. Other application of this technology is in providing a model system for basic research on gene structure, function and also for the production of specific proteins in fish. Transgenic fish production technology holds great potential for aquaculture industry. The recognition of its potential for research and development is now gaining strength far and wide. It is a powerful technology for genetic enhancement. Traits like fast growth, freeze resistance; cold resistance can all be introduced producing superior or transgenic strains/varieties of fish in much shorter time. It can therefore remove our independence solely on selective breeding procedures which take generations. Transgenesis is mainly of two types. Autotransgenesis- It involves just increase in the copy of GH present in a fish and, Allotransgenesis- It involves the transfer of gene from different species.

KEY WORDS: fisheries, modified gene, transgenic.

INTRODUCTION

An organism that has a foreign or modified gene in its genome is called transgenic or GMO or LMO. A genetically modified organism (GMO) is an organism whose genetic material has been altered using genetic engineering techniques. Organisms that have been genetically modified include micro-organisms such as bacteria and yeast, insects, plants, fish, and mammals. GMOs are the source of genetically modified foods, and are also widely used in scientific research and to produce goods other than food. The term GMO is very close to the technical legal term, 'living modified organism' defined in the Cartagena Protocol on Biosafety, which regulates international trade in living GMOs (specifically, "any living organism that possesses a novel combination of genetic material obtained through the use of modern biotechnology").

Over the past decade, genetic engineering has emerged as a one of the most powerful transforming technologies known to this generation. Scientists can now transfer the beneficial traits of a particular gene from one organism to another in far less time than needed in traditional breeding and with more precision. But this technology has also triggered debate among scientists, philosophers, environmental advocates, public health officials, business leaders, and regulators over a range of issues- from environmental safety and ecological impacts to the ethics of altering genome.

The first recorded instances of production of transgenic in aquatic species are those of Maclean and Talwar (1984) in rainbow trout and Zhu *et al.* (1985) in goldfish. Since then over 35 species have been genetically engineered in research laboratories. Revolutionary progress in genetic engineering in the 1970s made it possible to isolate eukaryotic genes. Gordon *et al.* (1981) developed technique of microinjection of foreign genes into mice eggs. The first transgenic study on fish was reported by Vielkind *et al.* (1982). The first batch of transgenic fish was produced in china in 1984. This consists of fast growing common carps. In 1985-86 Zhu *et al.* reported the production of transgenic fish by GH gene

transfer. Used a metallothionien promoter ligated to human GH structural gene. First transgenic fish was produced at MKU in 1991 by Pandian. In January 1996, for the first time in history, genetically engineered salmon named **"*aqu advantage salmon*"** was grown in a commercial hatchery in Loach Fyne Scotland.In USA 2002, transgenic medaka has been produced for assessing the environmental hazards. In 2002, Sehgal *et al*. (college of animal sciences, Zhejiang University, Hangzhou) isolated, cloned and characterized growth hormone gene from grass carp. In July 2003 the world's first glowing transgenic fish, nickname 'night pearl' was displayed at a Bio Taiwan Exhibition in Taipei. The developers injected the green fluorescent gene of jellyfish into the colorless freshwater fish glowing in dark.

MATERIALS AND METHODS

Identification of genes of interest i.e. chromosomal DNA or DNA copy of mDNA or cDNA.
Isolation of specific gene.
Amplification (to produce more copies).
Association of gene with promoter and poly-A sequence.
Insertion into plasmid.
Plasmid amplification in bacteria.
Cloning of construct for injection.
Transference of cloned construct in recipient tissue (usually fertilized eggs).
Gene integration in recipient genome.
Expression of gene.
Inheritance of gene through further generation.

Fish gene transfer methods

Microinjection
Electroporation
Gene gun injection
Use of Retroviral vector
Lipofection
Use of embryonic stem cells
Tissue injection
Gene targeting method

Microinjection

Microinjection method is widely used, a better way to guide people fish genes, the main procedures are (1) artificial aphrodisiac, (2) after fertilization ~ 5min, with 0.25% trypsin to remove the egg consumption of shell eggs into the bare filled with Holtfreter's medium flat dish, (3) dissolved in ST (88mMNaCl, 10mMTris-HCl, pH7.5) solution of foreign genes loaded glass micro-needle, before the implementation of the first cleavage microinjection of foreign gene surgery, each egg injection of 1-2nL DNA solution containing approximately 1 * 106 copies of the foreign gene, (4) After microinjection, eggs receptor solution in Holffreter's training and development of human embryonic development to the gastrula period, the culture medium with the storm gradually dilute gas of cold water, development to the heartbeat period, the embryos are transferred to the storm gas in cold water until the formation of fish in embryonic development, the need careful management and timely removal of dead embryos. The method is mainly applied to some of the egg shells of fresh trypsin susceptible carp species for some of the cold water of fish for salmon and trout, trypsin digestion is difficult to remove egg shells, so the development of three kinds of alternative microinjection method first hole from the DNA solution was injected into fertilized eggs, called MP method, the second is in the rainbow trout eggs just after fertilization when the egg shell has not harden direct injection, referred to as EL method, third, first with a hard metal pin in a hole on the egg shell, and then micro-injection, referred to as LI method.

Electric pulse method

Electrical pulses for gene transfer in fish eggs and microinjection first two steps the same is different trypsin digestion of naked eggs together with exogenous DNA solution to release a special electric pulse treatment tank, and then applied a certain intensity of electrical pulses of exogenous DNA in the electric pulse processing into the fertilized egg. The advantage of this method is relatively simple, can handle a large number of fertilized eggs. drawback is that the leaders of non-directional, low efficiency, for different species of fish need to establish a corresponding electrical pulse conditions, etc. of foreign genes in the electric pulse processing conditions into the fertilized eggs of the mechanism is

not very clear in the electric pulse fish have been successful gene transfer reports, are used in low voltage has been suggested that, in such a voltage, not enough to produce fertilized eggs or abnormal membrane holes occurs so that the foreign genes into the mechanisms also different from the cultured cells, suggesting that fertilization membrane is naturally present in fish some of the holes, at low voltages, the foreign gene into the fertilized eggs on these holes.

Sperm-mediated method

Sperm in the isotonic with the foreign gene in the first heat, the foreign DNA fragment can be with a human sperm cell, and then with the egg is fertilized, the foreign DNA fragments can be fertilized with a man inside. The method is simple, convenient, rely on physical role in fertilization, less damaging to the prokaryotic. External source of gene approach are quite different, but have been hybridization or PCR detection of positive results, the positive rate of 5% to 38% from the total of the experimental results, sperm carrying transgenic gene transfer, there are still positive rate, transfer rate instability.

Gene gun injection

Gene gun injection of DNA by the adsorption of high-speed metal particles hit the cells, the gene into cells of the way. This method, the results of 70% of individual survival, part of the individual into a foreign gene. This method is not easy to make, but can handle more than one individual short-term benefit, but too little coverage now, pending further research.

Retrovirus infection method

The virus is an RNA virus into the host after reverse transcription from RNA into DNA, and binds to host cell chromosome, the chromosome into the viral genes present within the virus gene. If the purpose of gene combinations to virus chromosome, with the modified virus infect host cells is likely to guide people to the cells of foreign genes 17J. But because the virus has genetically modified the strict host specificity and analysis of the virus transgenic fish is very backward, for this fish method is not applicable to temporary.

Tissue injection

There are reports of foreign genes directly injected to the body tissue, the surrounding cells to exogenous, and found that foreign gene. Was the CAT gene (streptomycin transferase gene) was injected into the muscles of some fish on the side of the body from the muscle homogenate CAT activity was detected. 'On the other hand, the synthesis of melanin containing complementary DAN plasmid injected into the lateral muscle of some fish, so that the surface around the synthesis of melanin, the black surface of this method is able to very easy to guide people to the foreign gene into the cells, so the detection of foreign gene promoter is very effective.

Gene transfer in oocytes

Selection, collection, preparation and development to a certain period of oocytes, blastocysts examined under a microscope, if the eggs for injection, control injection tube, headlong into blastocysts for microinjection, will accurately inject foreign genes in the oocyte nucleus, in vitro egg to mature eggs, and skilled with the normal fertilization, the fertilized egg to make the integration of foreign genes, develop into transgenic fish.

Gene targeting method

1990s, the emergence of new technologies exogenous gene, which gene knockout (gene knock out) and gene wedge (geneknockin) technology is a knockout gene targeting (gene targeting) a method similar to the homologous recombination refers to exogenous DNA and the receptor cell gene combinations, which is an advanced transmission technology, transmission technology cannot overcome the other and eliminate the chance of blindness, with integration sites identified, accurate, high frequency of gene transfer and other advantages, but gene knockout technology cannot produce the exact mutation in the nucleotide level. These new strategies can be carried out on cells in any of the underlying nucleotide level because of the precise mutation of gene transfer technology wedge, also known as gene set (gene replace · men technique), the breaking point to double-stranded homologous sequences in the edge or homologous sequences, the result is a genetically modified to replace the endogenous exogenous DNA target sequence, although the technology has just begun human gene wedge, but it has been demonstrated in the application of research in the broad prospects for above described Several gene targeting method greatly improves the rate of integration of foreign genes, but failed to address site-specific integration of foreign genes problem. mouse ES cell gene targeting technology can be a point of a genetic transformation of homozygous individuals The technology was first changed to contain a good part of the gene or genes inserted into the vector, and the introduction of the ES cell lines from mice, ES

cells for tissue culture, and can produce any tissue cells, cell proliferation, after a period of time, selected a small number of cells homologous recombination occurs cloned and amplified and then injected with a micro-capillary cells in early mouse embryos to produce chimeras.

Transgenic Mosaicism: Embryonic development occurs at very fast rate and exogenous DNA in fishes take hours to get integrate into the host genome. By this time several round of replication should have already been over. As a result some cell type contain the transgene while others do not, this is called Transgenic Mosaicism.

Application of transgenic technology in fish biology and aquaculture

1. **Improves economics of fish culture**
   Increase growth rate
   Increase market size
   Decrease dress out percentage
   Improves feed conversion efficiency
   Utilize low cost diet
   Improve cold tolerance
   Improve freeze resistance
   Increase brood stock fecundity
   Control smolting, reproduction and sex
   Reduce aggression
   Improves disease resistance
2. **Tailor fish for market**
   External appearance
   Flesh colour, flavor and texture
   Fatty acid composition
3. **Fish as bioreactor**
4. **Basic research aimed at understanding development growth and reproduction**
   Zebra and Japanese medaka as experimental models

Some example with fish species

Salmon

Salmon belong to the Salmonidae family which also includes salmon and trout. Although the smallest species is just 13 centimeters (5.1 in) long as an adult, most are much larger, and the largest can reach 2 meters (6.6 ft). All salmonoids spawn in fresh water, but they spend most of their maturity in the sea. This life style is known as anadromous. They are considered to be predators, because they feed on small crustaceans, aquatic insects, and smaller fish. A genetically modified Atlantic salmon known as the AquAdvantage salmon has an increased growth rate and size over the wild type Atlantic salmon from which it was derived, up to doubling its weight with a reduced time of growth to maturity. Although materials have been submitted to obtain approval to grow and market the Aqu Advantage salmon, as of December 2012 the FDA had not granted approval.

Tilapia

Tilapia is the common name for several species of cichlid fish from the tilapine cichlid tribe. Tilapia inhabits wide range fresh water habitats, including lakes, streams, ponds and rivers. Anciently, tilapia holds great significance in artisan fishing in Africa, and is paramount lately in aquaculture. Tilapia is very vulnerable to cold temperatures, and thus survives well with temperatures above 60 °F (16 °C). (See tilapia as exotic species.)

Tilapia is the fifth most important fish in fish farming, with production reaching 1,505,804 metric tons in 2000. Because of their large size, rapid growth, and palatability, tilapine cichlids are the focus of major farming efforts, specifically various species.

Zebrafish

Zebra fish are freshwater fish and are part of the Cyprinidae. They are a popular aquarium fish, commonly sold as zebra danio, and have been very vital as model organisms in research. They derive their name from the uniform horizontal stripes along the side of the body bilaterally. Males bear gold stripes within the blue stripes, while females bear silver stripes within the blue stripes. Zebra fish can mature up to 6.4 centimeters in the wild, but usually it is rare for them to mature beyond 4” in captivity.

The benefits could be taken from transgenic fish:

I. Helps in bridging large gaps- between an organisms natural characteristics and what the scientist wants.
II. Production of cold tolerant strains by producing the anti-freeze protein from winter flounder.
III. To improve input/output ratio, i.e. enhance growth or efficiency of food conversion.
IV. To increase tolerance.
V. Production of new color.
VI. Enhance flesh characteristic of fish.
VII. Control reproductive activity and/or sexual phenotype.
VIII. To increase resistance of species for pathogen/parasites.
IX. Modify behavior e.g. Aggression.
X. Control fertility/ viability.

RESEARCH USE OF TRANSGENIC ANIMALS INCLUDING FISHES:

- **Producing human therapeutics**

Within the field known as pharming, intensive research has been conducted to develop transgenic animals that produce biotherapeutics. On 6 February 2009, the U.S. Food and Drug Administration approved the first human biological drug produced from such an animal.

Production or food quality traits

Enviropig is a genetically enhanced line of Yorkshire pigs created with the capability of digesting plant phosphorus more efficiently than conventional Yorkshire pigs and dubbed them Enviropig. These pigs produce the enzyme phytase, which breaks down the indigestible phosphorus, in their saliva. The enzyme was introduced into the pig chromosome by pronuclear microinjection. With this enzyme, Enviropig is able to digest cereal grain phosphorus, so there is then no need to supplement the pigs' diet with either phosphate minerals or commercially produced phytase, and less phosphorus is lost in the manure. Enviropig would reduce feed costs because farmers would not need to purchase feed including the phytase, and it also would reduce the potential of water pollution since the Enviropig excretes from 30 to 70.7% less phosphorus in manure depending upon the age and diet. The lower concentrations of phosphorus in surface runoff reduce algal growth, because phosphorus is the limiting nutrient for algae. Because algae consume large amounts of oxygen, it can result in dead zones for fish. This would not only be advantageous for the waters surrounding the pigs, but also for the water neighboring the areas which use the manure for fertilizers. There are no current regulations or pending approvals on the Enviropig for human consumption in the United States. In February 2010, Environment Canada determined that Enviropigs are in compliance with the Canadian Environmental Protection Act and can be produced outside of the research context in controlled facilities where they are segregated from other animals.

In 2006, a pig was engineered to produce omega-3 fatty acids through the expression of a roundworm gene. Genetically modified fish have been developed with promoters driving an over-production of growth hormone for use in the aquaculture industry to increase the speed of development and potentially reduce fishing pressure on wild stocks. Aqua Bounty, a biotechnology company working on bringing a GM salmon to market, claims that their GM AquAdvantage salmon can mature in half the time it takes non-GM salmon and achieves twice the size. Aqua Bounty has applied for regulatory approval to market their GM salmon in the US. As of May 2012 the application was still pending. GM fish are used for scientific research and as pets, and are being considered for use as food and as aquatic pollution sensors.

Genetically engineered fish are widely used in basic research in genetics and development. Two species of fish, zebrafish and medaka, are most commonly modified because they have optically clear chorions (shells), rapidly develop, and the 1-cell embryo is easy to see and microinject with transgenic DNA. The GloFish is a patented brand of genetically modified (GM) fluorescent zebrafish with bright red, green, and orange fluorescent color. Although not originally developed for the ornamental fish trade, it became the first genetically modified animal to become publicly available as a pet when it was introduced for sale in 2003. They were quickly banned for sale in California. Genetically modified fish have been developed with promoters driving an over-production of "all fish" growth hormone for use in the aquaculture industry to increase the speed of development and potentially reduce fishing pressure on wild stocks. This has resulted in dramatic growth enhancement in several species, including salmon, trout and tilapia. Aqua Bounty, a biotechnology company working on bringing a GM salmon to market, claims that their GM AquAdvantage salmon can mature in half the time it takes non-GM salmon and achieves twice the size. Aqua Bounty has applied for regulatory approval to market their GM salmon in the US. As of December 2012 the application was still pending.

Several academic groups have been developing GM zebrafish to detect aquatic pollution. The lab that originated the GloFish discussed above originally developed them to change color in the presence of pollutants, to be used as environmental sensors. A lab at University of Cincinnati has been developing GM zebrafish for the same purpose, as has a lab at Tulane University.

- **Human gene therapy**

Gene therapy, uses genetically modified viruses to deliver genes that can cure disease into humans. Although gene therapy is still relatively new, it has had some successes. It has been used to treat genetic disorders such as severe combined immunodeficiency, and treatments are being developed for a range of other currently incurable diseases, such as cystic fibrosis, sickle cell anemia, Parkinson's disease and muscular dystrophy. Current gene therapy technology only targets the non-reproductive cells meaning that any changes introduced by the treatment cannot be transmitted to the next generation. Gene therapy targeting the reproductive cells so called "Germ line Gene Therapy"—is very controversial and is unlikely to be developed in the near future.

Uses of transgenic fishes: actual and potential

Research

Most genetically engineered fish are used in basic research in genetics and development. Two species of fish, zebrafish and medaka, are most commonly modified because they have optically clear chorions (shells), rapidly develop, and the 1-cell embryo is easy to see and microinject with transgenic DNA. Also, zebrafish have the capability of regenerating their organ tissues, and GM zebrafish are being explored for benefits of unlocking human organ tissue diseases and failure mysteries. For instance zebrafish are used to understand heart tissue repair and regeneration in efforts to study and discover cures for cardiovascular diseases.

Some of the examples in case of fishes:

Species	Gene(s) Introduced 1	Desired effect	Country
Atlantic Salmon	AFP-salmon GH	Cold tolerance, Increased growth and feed efficiency	Canada
Coho Salmon	AFP- chinook salmonGH	Increased growth	Canada
Chinook Salmon	AFP - salmon GH	Increased growth and feed efficiency	New Zealand
Rainbow Trout	AFP - salmon GH	Increased growth and feed efficiency	Canada
Cutthroat Trout	AFP – chinook Salmon GH	Increased growth	Canada
Tilapia	AFP - salmon GH	Increased growth and feed efficiency	Canada, UK
Tilapia	CMV- tilapia GH	Increased growth	Cuba
Tilapia	Tilapia insulin gene	Production of human insulin	Canada
Salmon	Rainbow trout lysozyme, flounder pleurocidin	Disease resistance	United States,
Striped Bass	Insect cecropin	Disease resistance	United States
Mud Loach	Loach and mouse MT - Mud loach GH	Increased growth and feed efficiency	China, Korea
Channel Catfish	RSVLTR-GH	Increased growth	United States
Common Carp	Salmon and human GH	Increased growth, disease resistance, tolerance of low dissolved oxygen	China,United States
Indian Major Carps	Human GH	Increased growth	India
Goldfish	AFP-GH	Increased growth	China
Abalone	Various promoters coho salmon GH	Increased growth	United States
Oysters	Various promoters coho salmon GH	Increased growth	United States

Transgenic fishes under development for use in aquaculture (FAO, 2000)

Pets

The GloFish is a patented brand of genetically modified (GM) fluorescent zebrafish with bright red, green, and orange fluorescent color. Although not originally developed for the ornamental fish trade, it became the first genetically modified animal to become publicly available as a pet when it was introduced for sale in 2003. They were quickly banned for sale in California.

Detecting aquatic pollution

Several academic groups have been developing GM zebrafish to detect aquatic pollution. The lab that originated the GloFish discussed above originally developed them to change color in the presence of pollutants, to be used as environmental sensors. A lab at University of Cincinnati has been developing GM zebrafish for the same purpose, as has a lab at Tulane University.

FATE OF TRANSGENE

i. Difficult to predict.
ii. Either degraded by nucleases enzyme present in host cell or persist.
iii. If persist may or may not be able to integrate in to host genome.
iv. If integrate, occur before cleavage division or after one or several round of cell division.
v. 98% dietary DNA from fish including GMO in degraded by digestive enzyme quickly.
vi. Use of virus as a vector increase the risk factor as these organism are adopted to integrating into host genome and cause cancer induction.
vii. Another risk is production of transgene (Autotransgenic) such molecule could be inimical to health (through allergies).

Transgenic DNA into genome of resident gut microflora

Transgenic DNA (incorporation in genome) can alter the genetic constitute of resident gut microflora which leads to change in pathogen spectrum Leading to hosting new pathogen Which may be human pathogen.

Essential element to successful use of transgenic fish for food

The end product must be safe for environment as well as for human consumption. End product must be believed to be safe, and thus acceptable to the public at large. Use of genes and promoters derived from fish rather than other organism. We should have better understanding of transmission, expression and stability of transgene. Avoidance from escaping. GMO and environment interaction should be taken into consideration. Then also we should follow the Regulation of transgenic by FDA.

Risks involved with transgenic fish

Risk assessment of transgenic fish

Environmental concerns- it includes Competition with wild population. Movement of transgene into wild gene pool. Ecological disruption due to change in prey and other niche requirement in the transgenic variety versus wild population. Dilution of wild fish genetic pool. Where food is abundant transgenic fish can quickly grow and have the potential to displace the natural one. On the other hand transgenic fishes are more willing to take risks when feeding.

Strategies to overcome the genetic contamination

Production of sterile lines of fish. Inhibition of GnRH the neuropeptide responsible for the control of gonadotropin synthesis and release from the brain and essential for sexual maturation. Sterility can be reversed under controlled condition by GnRH treatment. Fast growing transgenic fish can revolutionise commercial fish farming and relieve the pressure on overexploited fish stocks. But what happens in the natural environment if transgenic fish escape? By furnishing fish with genes from other organisms, so-called transgenes, researchers have succeeded in producing fish that grow considerably faster or are more resistant to diseases. Fish can also be modified to cope better with cold, which facilitates breeding in colder conditions.

Genetic modification

Genetic engineering processes are becoming increasingly common and are being applied to a widening variety of organisms. Genetic modification involves identifying genes scientists hope will express the desired traits when introduced into fish. These new genes can come from other species of animals, plants, bacterium, and even humans.

There are several processes used to insert "new" DNA into fish, ranging from inserting genetic material directly into eggs to subjecting fish eggs to electrical pulses, which form pores and allow foreign DNA to access the eggs. The precise location where the new genetic material has attached to the original DNA is unknown and may vary between individual fish so scientists need to check to ensure the inserted gene is present and determine if it functions as expected. Once scientists have determined that the genes have been inserted, the fish are raised like other farmedfish. Although this article is focused on transgenic fish, other transgenic aquatic organisms, including marine and freshwater plants and shellfish, are being fast-tracked for commercialization.

Food Safety Issues

There are major benefits for commercial fish farming as transgenic fish are expected to deliver higher production and better yields. However, transgenic fish can also entail risks and undesirable effects on the natural environment. For example, genes inserted to promote disease resistance may cause transgenic fish to absorb toxic substances (like mercury) at a higher rate and pass these toxic substances on to consumers.
The majority of transgenic fish have been inserted with growth genes. There are also misgivings that the large doses of growth hormones may pose health risks if consumed in raw and uncooked foods like sushi. Roughly 90 percent of food allergies can be attributed to consumption of eggs, fish, shellfish, milk, peanuts, soybeans, tree nuts, and wheat.
If proteins used in the production of transgenic species originate from one of these eight sources, there may be potential for allergic reactions among consumers. Researchers at the University of Gothenburg have therefore been commissioned by the EU to study the environmental effects of genetically modified organisms (GMO) within fish farming. The results of the studies show that the genetically modified fish should be treated with great care.

Simulated escapes

Millions of farmed fish escape from open water facilities each year and contaminate native populations and it is inevitable that transgenic fish will escape from aquaculture pens or field trial parameters. Therefore Sundström has studied transgenic salmon and rainbow trout to ascertain what ecological risks they might constitute for the natural environment. The study, which simulated escapes in a laboratory environment, shows that transgenic fish have a considerably greater effect on the natural environment than hatchery-reared non-transgenic fish when they escape. Genetic alterations in transgenic fish may give them competitive advantages over native species.
For example, genetically modified fish survive better when there is a shortage of food, and benefit more than non-transgenic fish from increasing water temperatures. By using growth hormone genes, researchers have been able to increase growth rates 2 to 11 times faster than the normal rate. Faster development leads to earlier sexual maturity and potentially more breeding opportunities than their native counterparts.

Natural breeds are under threat

If transgenic fish are genetically enabled to breed earlier and at a faster rate, transgenic genes are more likely to be spread throughout native populations. This would reduce the genetic diversity of the native population. Transgenic fish may have similar effects on natural ecosystems as exotic species. An increased growth rate is often accompanied by a voracious appetite, and transgenic fish may out-compete native species for resources, destroy plants and sensitive habitat, and/or alter the food chain in an ecosystem. However, conducting studies in a laboratory environment that imitates nature is complicated, which makes it difficult to predict how escaped transgenic fish affect the natural environment. Sundström's conclusion is that international consensus is required before commercial farming can be permitted, and that a precautionary principle must be applied. "One option is to farm the transgenic fish on land, which would make escape impossible. At least fertile fish should be kept in a closed system," says Sundström. As of yet no country has permitted commercial farming of transgenic fish, but several applications for such operations are under consideration by authorities in both the USA and the EU. Neither other genetically engineered food animals have been approved for sale, although numerous animal species have been cloned (but not sold for food) and transgenic animals are producing commercial, nonfood items such as spider silk (by goats).

Other problems

The existing transgenic fish for mammalian gene are basically from the BH gene. According to their biological characteristics, macromolecules, especially proteins on both the species specificity. Thus, in transgenic research should be considered in donor and recipient genetic relationship between the fish, strengthen the main farmed fish stress resistance, disease resistance genes from working. zygote leaders of foreign genes, its integration rate and genetic manipulation techniques, whereas the rate determines the level of integration of foreign genes can be stably inherited. In addition, because of the possibility of exogenous tomb receptor cells highly expressed promoter and enhancer play a

large role, its specific gene promoter function of tissue-specific, a species-specific enhancer if the genome of fish from isolated suitable promoter, may be more effective expression. Gene transfer in fish than in other methods of foreign genes into higher consolidation ratios, but the workload is very large, the seasonal spawning of fish, injection of a limited number of eggs, embryos, and many died in the survival of fry in , a considerable part of the integration cannot occur for the follow-up screening inconvenience. Electric pulse method, efficient method of sperm carrying transgenic methods, integration and very low rate, but also to be further improved. Copy due to integration of exogenous detection rate generally use spot and Southern blot hybridization. This method requires very expensive biological drugs and enzymes, and the use of radiolabelled, costly, time-consuming and not conducive to human health, such as to form with the early stages of fish, color, etc. Mark separation of transgenic fish, early elimination of non-transgenic fish, transgenic fish will be effective research methods.

Environmental issues

Transgenic fish into the nature of the ecological balance will result in different degrees. Many scientists believe that the phenotype of transgenic fish may have to change three areas, namely, physiological rhythm, environmental tolerance and behaviour of these phenotypic changes will destroy the ecological balance of the current benign addition, the use of transgenic fish promoter mostly mMT, the promoter in many sports organizations have induced the accumulation of heavy metal ions, such a person if the long-term consumption of transgenic fish will cause heavy metal poisoning , which must be paid.

Screening of transgenic fish strains to establish

Switch to the tomb because the fish study was to obtain high economic value of the transgenic fish lines for production applications. Transgenic fish through the DNA level, protein level and the macro-growth comparisons and biological experimental observation is confirmed, the need for a rapid establishment of improved strains of the method , usually using a single method of sexual development in theory, after second-generation single-sexual development, superior genetic traits that can be fixed, the formation of new lines, but the actual work is not so simple, because the fish of exogenous genes in germ cell receptor transfer, subject to chemical modification, closed gene expression, and the foreign gene into the receptor cells of methylation problems, will affect the creation of transgenic fish lines.

CONCLUSION

Critics have objected to use of genetic engineering per se on several grounds, including ethical concerns, ecological concerns (especially about gene flow, and economic concerns raised by the fact GM techniques and GM organisms are subject to intellectual property law. GMOs also are involved in controversies over GM food with respect to whether using GM fish as safe is safe, whether it would exacerbate or cause fish allergies, whether it should be labeled, and whether GM fish and crops are needed to address the world's food needs. These controversies have led to litigation, international trade disputes, and protests, and to restrictive regulation of commercial products in most countries. See the genetically modified food controversies article for discussion of issues about GM fish and GM food. Adequacy of risk management tool should be there for management. Developers must know the safety of a product before it is allowed in market. FDA can restrict the use of product with levels, condition of use and post approval monitoring These three elements are backbone of public confidence. The enormous potential benefits of transgenic fish technology in research and the aquaculture industry will not be achieved without effective isolation of genetically modified fish from the wild fish genetic pool. The possibility of transmission of transgenes to wild fish or of transgenic fish establishing themselves as permanent residents of an environmental ecosystem is the single most important negative consideration in applying this technology. Current approaches to genetic isolation involve sterility by triploidisation. However, an effective means of inducing controlled reversible sterility is the complete and specific blockage of the reproductive system at the level of the brain.

REFERENCES

Chen T. T., Lu J.K., Dhuham R.A. and Powers D.A. (1993). Transfer of growth hormone and insulin- like growth factor genes into carps, catfish and medaka. *Abstract S12, Mol. Endocrinol.* Fish May 23-25, 1993, Toronto, Canada, 1993 b.

Chen T. T. and Fans R. (1998). Transgenic fish technology and it's application in fish production. *Aquacultural Biotech.* 527-527.

Gordon J.W. and Scangos G.A. (1981). Genetic transformation of mouse embryos by microinjection of purified DNA. *Proc. Natl. Acad. Sci. USA* **77:** 7380- 7384.

Fletcher G.L. and Devis P.L. (1991). Transgenic fish for aquaculture, *in : J.K. setlow (ed) Genetic Engineering.* Vol 13. Plenum, New York. Pp. 331-370.
Khoo H.W. (1995). Transgenic and its application in aquaculture. *Asian fish Sci.* 8: 1-25.
Maclean N. and Penman D (1990). The application of gene manipulation in aquaculture. *Aquaculture.* 85: 1-20.
Pandian T.J. D. and Marian L.A. (1994). Problema and prospect of transgenic fish production. *Curr. Sci.* 66: 639-649.
Zhu Z., Li G., He L. and Chen S. (1985). Novel gene transfer into the fertilised eggs of gold fish. *A angew. Ichthyology*. 1: 31-34.
Zhu Z., Xu K., Li G., Xie. Y. and He L. (1986). Biological effects of human growth hormone gene microinjection into the fertilised eggs of loach. *Misgurnus anguillicaudatus. Kexue Tongbao Academia Sinica*. 31: 988-990.

