Genetically Modified Fish in Aquaculture: Technical, Environmental and Management Considerations

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ABSTRACT

Genetically modified (GM) fish offer new possibilities for the improvement of production in aquaculture. It allows the introduction of novel traits or the improvement of old ones, in such a way that is out of reach for classical selection breeding. Examples of genes with commercial potential are among those which control growth, disease resistance, freeze tolerance, sexual maturation, food quality and food preservation parameters. Consumption of GM fish does not represent a health risk in principle. The safety of GM food is dependent on the character of the transgene, the transgene product and the new phenotype. Ethics and animal protection concerns demand the development of healthy fish only. Environmental safety calls for efficient biological containment in order to minimize possible effects caused by released farm animals. Improvements of disease control will support both production economy and the environment, in case of escapes. Since aquaculture includes both marine and fresh water species, it can be developed as new food production strategies in most countries all over the world. To avoid large-scale technology transfer failures, it is important to adapt to the regional and local needs. This calls for international research collaboration aiming at regional and local competence development sufficient for the technology implementation.

Keywords: aquaculture, fish, safety, transgenic

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RESUMEN

Los peces modificados genéticamente (MG) brindan nuevas posibilidades para el desarrollo de la producción acuícola. Esta tecnología permite la introducción y modificación de caracteres genéticos de una forma mucho más rápida y eficiente que los procesos clásicos de selección. Entre los genes con posibilidades comerciales, se encuentran aquellos que controlan el crecimiento, la resistencia a enfermedades, la tolerancia al frío, la maduración sexual, la calidad de la carne, etc. El consumo de carne de animales MG no implica ningún riesgo para la salud humana. La seguridad de los alimentos MG depende del transgén y su producto, así como del fenotipo resultante. Los aspectos éticos y de protección animal demandan la generación de peces sanos. La seguridad ambiental requiere de medidas eficientes de control biológico para minimizar los posibles daños al ecosistema por la liberación de peces MG. Los nuevos desarrollos en el campo de la resistencia a enfermedades, serán beneficiosos para la producción y el medio ambiente en caso de escape de peces MG. Debido a que la acuicultura se aplica tanto a especies de agua dulce como marinas, es posible su desarrollo en la mayor parte de los países del mundo. Para evitar fracasos en las transferencias tecnológicas, es importante adaptarse a las condiciones regionales y locales, mediante la elaboración de proyectos de colaboración capaces de desarrollar la capacidad de implementación de la tecnología.

Palabras claves: acuicultura, peces, seguridad, transgénico

Introduction

The FAO statistics for worldwide sea food production shows that fish farming represents about 15% of the worldwide catch from fisheries and is estimated to pass 20% by the year 2000 [1]. Currently, the maximal sustainable catch of wild fish has already been reached, while an increase in seafood production can be only gained through aquaculture. Increased production efficiency in aquaculture will be acquired through genetic improvement of the production species by selection breeding and, potentially, gene transfer technology.

Fish farming in ponds and dams is known from China for 5000 years and from Europe, for around 1000 years. The first examples of trout farming came just before the World War I and the Norwegian salmon farming in net pens came in the seventies. A program for selection breeding coordinated at the national level and developed approximately ten years later was the breakthrough [2]. Six generations of selection breeding have resulted in a Norwegian salmon more than 50% bigger than its ancestors in the rivers of Norway. The Institute for Aquacultural Research (AKVAFORSK) has successfully implemented the Norwegian salmon breeding program to tilapia farming in the Philippines and carp farming in India. The history of farmed salmon makes it one of the latest domesticated species together with other few farm fish species, although fish culture as a general phenomenon has been known for thousands of years.

A lot of effort is now made to domesticate novel species for the benefit of aquaculture and the global demand for an increased food production. Some 200 species are being cultivated world wide, including 110 fish species, 25 crustaceans, 50 mollusks and 8 algae, but 80% of the world aquacultural production is derived from a dozen species [1]. In order to achieve maximal improvement in new breeding programs for aquatic organisms, genetic engineering and genetically

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modified organisms (GMO) offer new possibilities. The objective of this paper is to discuss technical, environmental and management considerations of using GMOs in aquaculture.

Technical aspects

Genetic improvement of fish by gene transfer

The development of recombinant DNA technology has allowed to move DNA from one species to another, regardless of the category of organism. Thus, a plant, an animal or microorganisms like bacteria or even a virus can be either donor or recipient of genetic information encoded in the genome.

An organism receiving a new gene into its genome is called a GMO or transgenic organism. The transgene can originate from a phylogenitically distant organism or from the same species. Nowadays this technological development makes possible the improvement of fish and other aquatic organisms [3-8].

There are various methods for achieving gene transfer to fish. The most common and so far the most successful is microinjection. For microinjection of DNA, a capillary pipette with a tip 0.005 mm in diameter is used for injecting 0.00000025 mL of a DNA solution into fertilized fish eggs. The development of embryonic stem cells and somatic cell cloning will allow qualitative and quantitative predetermined gene insertion through homologous recombination (today, researchers work only on mammals, but recent reports suggest the introduction to other species including fish in a near future). Another improvement regarding gene transfer efficiency to fish involves complexing transgenes with synthetic NLS peptides mediating active nuclear import [9].

Most recent reports on transgenic production of fish species describe the transfer of "all fish" gene constructs, with a general concern of utilizing DNA sequences derived from the same, or closely related species. In such cases, truly new gene sequences are not added to the genome, but the copy number of the already existing genes is rather increased. However, changes are often made to obtain an altered control of gene expression by fusing a non-typical promoter to the coding region of the gene.

This technology however still has several major constrains: (i) low efficiency generation of transgenics in some species, (ii) need for species of close life cycle in the laboratory (iii) low precision regarding copy numbers and integration site, and (iv) need to identify, isolate and characterize the genes to transfer.

What species and what traits?

A number of species are in focus for gene transfer experiments and can be divided into two main groups: animals used in aquaculture [3-8] and model fish used in basic research [7, 10]. Among the major food fish species are carp *(Cyprinus* sp.), tilapia *(Oreochromis* sp.), salmon *(Salmo* sp., *Onchorryncus* sp.) and channel catfish *(Ictalurus punctatus)*, while zebrafish *(Danio rerio)*, medaka *(Oryzias latipes)* and goldfish *(Carassius auratus)* are used in basic research. In addition to food production and research, carp and goldfish are widely used as ornamental fish. Other fish sea food species important in aquaculture such as, mollusks, shellfish and shrimps are now getting into the focus for research on gene transfer-mediated improvements [11].

In the classical breeding programs, economically important traits like growth rate and food conversion efficiency have been top-ranked, aiming at an increased food production. Several other traits of economical importance have not been possible to improve with selection breeding. The pink color of salmon is still achieved by adding synthetic carotenoids to the food pellets. The lack of disease resistance, which made the heavy use of antibiotics necessary until just a few years ago is now combated through the development of new vaccination regimes.

Gene transfer technology currently applies only to single copy, or few copy gene traits. Secondly, the candidate genes must be characterized at the molecular level. Only a limited number of gene traits fulfills both criteria and thus is applicable to genetic engineering for breeding purposes, which makes necessary the performance of further studies on genes controlling disease resistance, aggression, flesh color, etc.

Growth control mediated by growth hormone (GH; somatotropin) belongs to the best understood area of vertebrate physiology. The success in the early eighties with the transfer of human and rat GH genes to fertilized mouse eggs, which resulted in fast-growing transgenic mice has since then stimulated many groups to try to achieve genetic improvements of different farm animals by genetic engineering. Several laboratories now have GM fish with increased growth performance caused by extra copies of GH gene [8]. Transgenics with up to 30 times higher growth performance compared to the average of nontransgenic siblings have been reported [6, 12]. However, to efficiently manipulate the process of growth, it is necessary to characterize at the molecular level the mechanisms involved in growth control to determine which genes to manipulate and the expression levels to achieve growth acceleration for each specie.

Freeze resistance achieved through antifreeze protein (AFP) is another trait in focus of research efforts. AFP genes have been transferred from the genome of the ocean pout *(Macrozoarces americanus)* to salmon and goldfish with the aim of introducing freeze resistance [6]. Recently, fish AFP genes have also been transferred to plant species in order to make them resistant to freezing.

There are two reasons for developing a sterile fish. Onset of sexual maturation with gonad development results in loss of both weight and meat quality in salmons. Secondly, sterile escaped farm fish cannot reproduce in the wild, and thus represents an efficient biological containment. Sterile fish is today achieved through chromosome manipulation giving rise to triploid, often monosex, fish. An attempt in making transgenic sterile fish is to antagonize the production of the chief sex gonadotropin-releasing hormone (GnRH) by the transfer of special anti-GnRH genes [13]. Other ideas of introducing biological barriers against uncontrolled breeding are discussed below.

Recent applications of gene transfer technology in fish have focused on the development of "living biomarkers" for detecting water pollutants and other environmental xenobiotics [7].

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One special case of GM animals is that of bioreactors because the animals are used exclusively for the production of a certain protein. Fish have been suggested to be a candidate of this category of organisms along with mammals and plants [7].

Environmental issues

Are GM fish safe to eat?

GM fish as such do not represent any health hazard. Of major importance for health risk evaluation is the character of the gene, the protein it encodes and the resulting phenotype. In addition, it is important to assure that the insertion of a novel gene does not affect an endogenous gene or has no other pleiotropic effects [14].

Most of the existing GM fish prototypes have received extra copies of their GH genes, resulting in only moderately elevated levels of circulating GH. This hormone is a protein that is degraded along with all other food proteins. Meat from fish transgenic for GH is regarded as fully safe for human consumption. Using the principle of "substantial equivalence", recent experiments with GM fast-growing tilapia demonstrated that eating GM meat is safe [15]. In addition, food quality control concerns taste, appearance, color, texture and other parameters important for a commercial success, but not necessarily for health safety. Finally, consumers decide if they accept GM food or not.

Natural ecosystems

It is generally accepted that undeliberated release of farmed fish into natural ecosystems should be prevented, especially if the fish is genetically modified. Physical containment of fish farms has proven to be efficient only in land-based plants. Net pens in the sea are more prone to accidents because of extreme weather conditions, and in several occasions accidental release of a large number of farmed salmon has been reported. In the present, farmed salmons of many norwegian rivers reproduce and compete with the local strains, although they are generally less adapted (Hindar, 1996).

Transgenic fast-growing tilapia (line F70) retain many characteristics of the parental *O. hornorum* hybrid, including disease resistance and behavioral parameters [15-17]. This transgenic line growths 60-80% faster when compared to non-transgenic lines depending on culture conditions [17]. Metabolic and food conversion efficiency is also better in transgenic tilapia [16]. Transgenic tilapia require more energy production to sustain a higher growth speed and this process is more efficient than in wild-type tilapia with a food conversion efficiency 3 times higher than that of wild-type tilapia [16]. After completing the evaluations required by Cuban authorities, these tilapia are considered as safe for culture under controlled conditions [18]. The introduction of this line into national aquaculture has been estimated to produce important savings for the industry (R Martínez, 1998).

Management: biosafety and risk assessment

It is generally accepted that time from basic research to commercial applications is short in modern biotechnology. Supporting this statement, there already exists a commercial transgenic fast-growing AquAdvantage™ Atlantic salmon that in about two years will be marketed worldwide by the US Company A/F Protein Inc. (Aqua Bounty Farms, Waltham, MA, USA). Plans to introduce it in Scottish fish farms have recently caused debate in Europe. The establishment of a homozygous line of GM Atlantic salmon takes a minimum of 13 years from the microinjection event until the GM fish can be introduced into a breeding program [19]. For species like tilapia and carp, which have shorter life cycles, this period is also shorter. Finally, as previously discussed, before production can begin, the approval from the authorities with regards to environmental and health safety is necessary. In Cuba, transgenic fast-growing tilapia are commercially available for local aquaculture and have been incorporated into the national tilapia genetic program by the Ministry of Fisheries.

In addition to practical restrictions and limitations, general ethical and animal protection concern demand animal health effects of the transgene and its product to be adequately addressed before any commercial fish farming can be initiated. If animal health is not negatively affected by the transgene or its product, it can be argued as an indication that GM fish is not hazardous for human consumption [20]. In the case of fast-growing GH-transformed fish, symptoms similar to acromegaly are seen in some of the animals showing the highest levels of growth enhancement although the general impression at present is that the majority of transgenics are healthy.

Risk assessment of GMOs is given priority both by national and international bodies engaged in biotechnology development [19, 21-25]. In August 1994, the First International Symposium on Sustainable Fish Farming was held in Oslo, Norway [5]. During this conference "The Holmenkollen guidelines for sustainable industrial fish farming" were discussed and approved. Recommendation Nr. 14 stated that "the farming of transgenic or other genetically manipulated fish should not be undertaken until safety and ethical criteria be established". The definition of GMO by the International Council for Exploration of the Sea (ICES) includes both transgenics and fish with modified chromosome sets, but not fish improved by selection breeding. In the discussions, biological containment regimes are suggested as an important complement to the physical barriers.

In Cuba, to conduct the studies outside the laboratory, an *ad hoc* committee meeting devoted to the analysis of the conditions to release transgenic tilapia with accelerated growth was held [18]. This committee concluded that, under the conditions found in Cuba, little or no effect on natural populations would occur as a result of accidental escape of these transgenic tilapia. Most of the fresh water fish species currently found in the country have been introduced and natural populations are represented only by few species already in contact with introduced exotic tilapia species. As evaluated by González-Sansón and Aguilar [26], for example, the principal effect of the introduction in the Cuban coastal lagoons of tilapia *(O. aureus)* was to accelerate the fluxes of energy and matter. Tilapia consumes algae which excrete partially digested therefore "pelletizing" the algae mass of the lagoon and accelerating its incorporation into the detritus. Because the most abundant species in the lagoon have different feeding habits, the situation in the lagoon could be very similar to the situation in polyculture.

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Nevertheless, the committee recommended to follow the final draft of the "Performance Standards for Safely Conducting Research with Genetically Modified Fish and Shellfish" (documents Nr. 95-01 and 95-02) prepared and released by The US Department of Agriculture, the Agricultural Biotechnology Research Advisory Committee, and the Working Group on Aquatic Biotechnology and Environmental Safety. These standards are accompanied by flowcharts that guide the assessment pathway in a way that allows to consider and implement the necessary measures to safely conduct the experiments with transgenic fish for accumulating the data needed to fully characterize these new fish strains.

Biological containment is well known from biosafety restrictions of microorganisms used in recombinant DNA research and industrial applications. These often include mutations in genes of metabolic pathways, making the organism depend on the missing metabolite supplemented in the growth medium. Biological containment in the context of aquaculture includes sterile triploid fish or sterile transgenic fish carrying anti-fertility genes tailor-made into their genomes [13, 27]. In addition to the establishment and reproduction in wild biotopes, escaped salmons have spread disease to wild populations in several cases. This problem is not avoided by fish sterility. Therefore, why introduction of suicide genes that allow survival only in capture has been suggested. Triploid fish technology is a simple cost-effective method used in many countries to make sterile fish. The disadvantage is that every egg must be treated and that the extra set of chromosomes also represents a cellular content of nucleic acids 30% higher when compared to normal fish. One EU Biotechnology research project, "Biological containment of transgenic fish and risk assessment of inter-species gene transfer" aims at making GM sterile fish by expressing anti-fertility genes antagonizing the production and action of the chief sex GnRH [13]. The advantage would be that sterility becomes a stable inheritable trait, and only selected brood stock animals are rendered fertile through hormone therapy. These GM sterile fish would also carry the normal chromosome number and DNA content.

Risk assessment in cases of deliberate or accidental release of transgenic fish depends on a number of factors: (i) the species released and the biotope it is released into, (ii) the character of the transgene and the new phenotype, (iii) the general fitness of the GMOs versus wild populations, and finally, (iv) the number of released GM fish, which is an important factor. There exists quite a wide experience from the introduction of exotic species in the environment, which is helpful for making risk assessment of the GMOs. Every new GMO could be regarded as a novel exotic species. However, it does not prevent that a simple rule be followed. Since there is no information about the new performance qualities of GMO and since this knowledge is important to get, it is advised to gain it with the "precautions principle" in mind. This means a "case by case & step by step" procedure, starting with physically contained testing in laboratories and moving to field tests via small scale and intermediate scale to large scale. "Case by case" represents the pre-calculation that can be made from evaluating the aforementioned factors i-iv. Evaluation at each step will tell if moving to next is acceptable.

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International research collaboration and sustainable development

According to Agenda 21 chapter 16, it will be of great importance a broad international research collaboration at all levels of research, from molecular characterization of genes of quantitative trait loci (QTL) to environmental risk assessment experiments. This will allow biotechnology to be used in support of a sustainable development, giving the necessary concern for the environment. Recent identification and application of polymorphic fish microsatellites have proved valuable genetic markers with regard to gene mapping, population genetic analysis and individual identification [28].

In several areas ranging from fish culture economy to basic research in biology and medicine, transgenic fish technology can be foreseen to play an important role in the future (Table).

Table. What are the potentials for transgenic fish? (modified from reference nr. 3).

I. Improve economy of fish culture
- increase growth rate
- increase overall size
- increase food conversion efficiencies
- utilize low cost diets (carbohydrates *versus* protein)
- improve cold tolerance/freeze resistance
- improve disease resistance
- increase brood stock fecundity
- control smolting and reproduction
- reduce aggression
II. Tailor-made fish for the market
- flesh color, flavor and texture
- fatty acid composition
III. Fish as bioreactors
- production of commercially useful compounds
IV. Basic research

Up to now, improvement of growth in GM fish is the most successful way of genetically modifying an economical trait, achieved in several fish species in laboratories from both developed and developing countries. A growing concern to avoid farmed fish (both GM and non-GM) to escape and compete out wild strains now calls for sterile fish unable to reproduce and interbreed with wild populations. Only a few years ago disease problems forced the Norwegian salmon farming industry to use large amounts of antibiotics. The development of new fish vaccines has largely solved the problem at present. The risk of disease together with the cost of vaccination makes the development of disease resistance through gene transfer a high priority research strategy. Using transgenesis for an increased food production and a sustainable development, a lot of investment must be made in the necessary basic and applied research needed for the understanding of how complex traits are controlled by single genes. In addition, this research must be carried out based on a true international collaboration so that basic competence can be developed in many countries to use the technology, and the technology can be developed according to the needs and socio-economical situation of the user nation or region.

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