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Gene transfer technology in aquaculture

J. A. Levy[1], L. F. Marins[1] & A. Sanchez[2]
[1]*Fundação Universidade Federal de Rio Grande, Departamento de Química, Laboratório de Bioquímica Marinha, C.P. 474, Rio Grande, RS, 96201-900 Brazil*
E-mail: dqmlevy@super.furg.br
[2]*Universitat Autônoma de Barcelona, Facultat de Veterinária, Unitat de Genética, Bellaterrra, 08193 Barcelona, Spain*
E-mail: sanchez@uab.es

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Abstract

The gene transfer technique, transgenesis, has permitted the transfer of genes from one organism to another to create new lineages of organisms with improvement in traits important to aquaculture. Genetically modified organisms (GMOs), therefore, hold promise for producing genetic improvements, such as enhanced growth rate, increased production and efficiency, disease resistance and expanded ecological ranges. The basic procedure to generate transgenic fish for aquaculture includes: (1) design and construction of transgenic DNA; (2) transfer of the gene construct into fish germ cells; (3) screening for transgenic fish; (4) determination of transgene expression and phenotype; (5) study of inheritance; and (6) selection of stable lines of transgenics.

GMOs offer economic benefits, but also pose environmental threats. Optimising the mix of benefits and risks is of fundamental importance. The potential economic benefits of transgenic technology to aquaculture are obvious. Transgenic fish production has the goal of producing food for human consumption; thus the design of genetic constructs must take into consideration the potential risks to consumer health, as well as marketing strategies and product acceptance in the market.

Introduction

Aquaculture is one of the fastest growing food-producing sectors, providing an acceptable supplement to and substitute for wild fish and other aquatic products. Regional, cultural and historic attributes have played major roles influencing both the production base and rate of expansion of aquaculture. The historic tradition of growing fish in Asia, which is well documented in countries such as China, India, Cambodia and Indonesia, has played a significant role in maintaining Asia's dominant role in aquaculture. Production of finfish, shellfish and plants from culture in Asia increased from 8.4 million metric tons (mt) in 1984 at a growth rate (APR) of 10.4 to over 25 million mt in 1995 and accounted for over 90% of world output. Much of this growth, however, specifically relates to China. When China is excluded, the APR of the sector in Asia is only 4.4, not too dissimilar to that in Europe (3.9) and North America (3.6). In Africa and Latin America, the aquaculture production base is considerably lower and although there is no tradition of aquaculture, the sector has expanded at higher rates. Data from Brazil show that, between 1996 and 1997, the aquaculture production increased from 40.5 mt yr^{-1} to 60.7 mt yr^{-1} (CNPq, 1998).

The potential of aquaculture to meet the challenges of food demand and to generate employment and foreign exchange is clearly demonstrated by the rapid expansion of this sector which has grown at an average annual rate of almost 10% since 1984 compared with 3% for livestock meat and 1.6% for capture fisheries production. In contrast, with the exception of the increase in 1993–1994 which is probably attributable to small pelagic species, data reported for capture fisheries show near zero or negative growth (Rana, 1997). In fact, in several parts of the world this activity has declined seriously. FAO (1997) evaluated 17 world

fisheries and determined that four have been commercially depleted and nine are in serious decline. Off the coast of New England, catches of cod, haddock and yellowtail flounder in 1993 were 85% lower than in the mid-1960s. In eastern South America some coastal fish stocks seem to have diminished in recent years (Haimovici, 1997). Such data suggest that the fish food supply will be affected in the near future, because of the progressive global demand stimulated by the increasing human population.

However, whilst aquaculture shows potential for expansion to meet global demand for fish for food, this activity has a slow growth rate within the low income food deficit countries (LIFDCs). This may be for a combination of reasons, including countries being landlocked, low national priority of aquaculture, small and unsuitable coastline, inadequate water supply, poor infrastructure and limited capacity of institutions and technical and financial constraints. A solution to this problem lies in increasing aquaculture production through genetic improvement, thus leading to more efficient use of facilities.

The traditional methods of genetic improvement such as selective breeding, have not always been successful in increasing the capacity and diversity of farmed fish. Genetic gains through selective breeding have been reduced because of the difficulty of separating beneficial from undesirable traits. There is a need to adopt new technology to further stimulate the growth of this industry by overcoming several of its major obstacles, including high feed costs, susceptibility of the farmed fish to diseases, as well as the limitations of culture facilities and the environment.

Today, the search for increased productivity follows two trends:

1. the search for new species with better yield; and
2. a further development and application of methods for genetic improvement.

The introduction of new methodologies based on molecular biology has lead to new research fields in this sector and new markets for aquaculture industries.

Gene transfer techniques (transgenesis) have permitted the transfer of traits from one organism to another to create new lineages of organisms with improved aquaculture performances. Genetically modified organisms (GMOs), therefore, hold promise for producing genetic improvements, such as enhanced growth rate, increased production and efficiency, disease resistance and expanded ecological ranges.

According to Beardmore (1997), in the strictest sense, ‘transgenic’ should be used to denote a individual or a cell with a DNA sequence which has been transferred and integrated, into that individual or cell, by genetic engineering techniques. The first transgenic fish was produced by Zhu et al. (1985) and considerable progress has been made since that time. The application of gene transfer technology is obviously not limited to the topics maintained above. It should be possible to generate other beneficial traits useful in aquaculture such as adaptation to salinity, pH, stress and the development of sterile fish. With the development of fish molecular biology, other candidate genes should be available for transfer.

The basic procedure to generate transgenic fish for aquaculture includes:

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The transgene is constructed, essentially, by associating an ubiquitous promoter, a structural gene coding for a desirable trait, and a termination sequence (polyadenylation site). One of the principal problems to generate transgenic fish is the choice of promoter/enhancer. The first promoters that have been used include mouse metallothionein-1 (mMt-1), Rous Sarcoma Virus and SV40. Constructs made with fish promoters, for example, anti-freeze protein (AFP) and carp B-actin are expressed more efficiently in fish than are mammalian constructs.

Microinjection is a well established method for gene transfer in fish. This method was fully described in the zebrafish by Khoo et al. (1993). Essentially the process is straightforward, but technically tedious, and there are numerous variations (review by Khoo, 1995). Other procedures of gene transfer can take advantage of the large number of eggs produced by most fish species. Electroporation, which uses short electric pulses to permeate cell membranes, has been used as an effective means of introducing foreign DNA in zebrafish, salmon, loach and others. More recently, gene transfer via electroporated sperm, subsequently used to fertilise eggs, has been reported in marine molluscs, but its efficacy is still a matter of debate in other species.

Alternative methods based on the use of pantropic retroviral vectors, which direct DNA integration, will probably be of minor impact in aquaculture, even

if they can provide a convenient stable approach to introduce and express foreign genes in fish.

Transgenesis, therefore, can be applied to genetic improvement in fish production. The use of genetically enhanced organisms may increase production efficiency through improvements in growth rates, food conversion, disease resistance and product quality. The application of biotechnology to aquaculture also may help conserve wild species and genetic resources and provide unique models for biomedical research.

Food safety of transgenic fish

The development and use of genetically modified organisms (GMOs) pose both economic benefits and risks to human health and the environment. Optimising the mix of benefits and risks is fundamental. The potential economic benefits of transgenic technology to aquaculture are obvious. Transgenic fish production has as a goal the production of food for human consumption and the design of genetic constructs must take into consideration the potential risks to consumer health, as well as marketing strategies and product acceptance in the market.

For evaluating the food safety of transgenic fish derived from common food fish stock consideration should be given to:

1. the transgene; and
2. the gene product.

The DNA used for transgenesis in fish, at the present, include genes such as the growth hormone gene associated with ubiquitous promoters (Beta-actin, Histone H3). New alternative structural genes such as insulin-like growth factor (IGF) are being tested. These genes code for mitogenic peptide hormones that play an important function in the growth and differentiation of vertebrates.

The tendency is that both structural genes and promoters will be derived from the target species (in Beardmore's 1997 terminology 'autotransgenic'), or from fish species as closely related as possible, thus decreasing considerably the health risks and facilitating commercialisation. Furthermore, coding sequences derived from distantly related species may pose problems, not only at a transcriptional or translational level, but in terms of physiological effectiveness (Maclean, 1998).

Autotransgenic gene constructs have given impressive increases in growth rate. Hew et al. (1991) and Du et al. (1992) used growth hormone gene from the chinook salmon linked to the ocean pout antifreeze protein promoter; second generation transgenic salmon. These fish are fast growing and fully capable of entering full strength seawater as smolts almost a year earlier than non-transgenic siblings (Hew & Fletcher, 1997). The most dramatic increase of growth rate was obtained by Devlin et al. (1994) who inserted into coho salmon a gene construct containing the metallothionein promoter and the type 1 growth hormone gene, both from sockeye salmon. This gave an 11 fold average increase in growth of the transgenic coho salmon.

The evaluation of gene products and their secondary effects parallel the safety evaluations of chemicals or drugs introduced into animal tissues. Observations of the health of transgenic animals and monitoring for unexpected effects will yield critical information on safety. Genetically modified organisms or products derived from them are likely to be accepted by the public only after the demonstration, by governmental agencies, that they are safe to eat. It will be necessary to educate the public about the safety of the products. At present, the commercial use of transgenic fish has only just started, although transgenic salmon are being grown in Scotland (Martinez, 1997) and New Zealand and applications have been filed in the United States (Dunham, 1999).

The food safety of transgenic animals has been addressed internationally by a joint consultation of the FAO and WHO (World Health Organisation, 1991) and by OECD (Organisation for Economic Co-operation and Development, 1992). These groups have concluded that modern techniques of molecular biology and biotechnology do not inherently result in foods that are less safe and that these foods should be assessed by comparing them with their closest conventional counterparts. Chatakondi et al. (1995) compared the body composition of transgenic common carp containing rainbow trout growth hormone gene with non-transgenic full-siblings. The transgenic carp muscle had an increase protein content of about 7.5% and a decreased fat content of 13%. The results showed that significant, but relatively subtle, changes occurred in the flesh of transgenic common carp and were not detrimental to human health and safety and may have been beneficial.

Public acceptance of genetically modified marine organisms or products derived from them is very much influenced by the public perception of safety. The exclusive use of fish DNA in the constructs for gene transfer will probably facilitate the approval by

regulatory agencies and overcome the considerable resistance of public opinion to transgenic food and products.

Perspectives

Transgenic technology has proved successful landmarks in several aquatic species, in particular in salmon and trout, in contrast to the relative inefficiency of the technology in the generation of 'improved' transgenic farm animals. This difference can be attributed to the relative ease of availability of fish embryos for microinjection of foreign DNA, the traditional technology of gene transfer used in animals. However, embryo mortality in some cases is high, rates of integration are often low and the process of random gene insertion is subject to positional effects for transgene expression.

Despite large efforts, totipotent cell lines for use in techniques of homologous recombination have been available only to generate transgenic mice, but progress in the establishment of pluripotent cells in livestock species and preliminary results on ES-like cell cultures in the Japanese medaka and in zebrafish have been reported (Sun et al., 1995; Hong et al., 1996).

There are numerous problems to be resolved in the successful development of transgenic broodstock for aquaculture. Ecological, socio-economic as well as ethical issues have to be considered. New techniques need to be developed to control sterility and broodstock management. Nevertheless, after the arrival of 'Dolly', the first mammal to be cloned from differentiated adult cells (Wilmut et al., 1997), the advent of new transfection-mediated homologous recombination methodology in animal transgenesis, by cloning, seems more feasible and opens new horizons for research and practical applications in animal production.

Genomes of aquatic species can be analysed and characterised and quantitative trait loci identified. This can be applied to improve our understanding of the molecular basis of gene regulation and expression as well as sex determination and thereby create methods for defining species, stocks and populations. Approaches that will play a key role in the aquaculture industry in the near future include developing marker-assisted selection technologies, improving the precision and efficiency of transgenic techniques, and improving technologies for the cryo-preservation of gametes and embryos.

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