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Biotechnology in freshwater finfish aquaculture

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Genetic aspects of fish reproduction, rearing and husbandry can be used to increase productivity and have become as important in modern aquaculture as nutrition and water quality (Tave 1986). Biotechnology provides particularly sophisticated tools for aquaculture technologies to promote this increased productivity (Adams and Thompson 2006; Tramper et al. 2003).

In the aquaculture industry, biotechnology is expected to provide high quality and safe food through environmentally friendly production methods. But rapid developments make it increasingly difficult to determine which scientific methodologies, commercial techniques and industrial applications can be reckoned among biotechnologies. Among others, the key areas are:

- reproduction manipulation (Komen and Thorgaard 2007),
- gametes quality (Bobe et al. 2006),
- sex control strategies (Pandian and Koteeswaran 1998),
- breeding programs (Wenne et al. 2007),
- nutrition (Nam et al. 2002),
- aquatic organism health (Trippel and Tsang 2004).

We would add the support and restoration of endangered aquatic species as another important field, in which biotechnology may be useful for the aquaculturists.

Genetics plays a crucial role in most of the fields mentioned above. To attempt to clarify we would define three (of the main) genetics-based methodologies, which are (or soon will be) applied in aquaculture biotechnology:

- genetic engineering,
- chromosome engineering,
- genome engineering.

Genetic engineering

Genetic engineering involves gene transfer, i.e. insertion of the gene into the DNA of a recipient organism. The purpose of the gene transfer is to improve the recipient organism. The new gene may be derived from any species and might confer, for example, disease resistance or faster growth of the recipient organism. If the transfer is successful, at least one copy of the gene is integrated into the recipient's genome and expressed. It is expected that the inserted gene will be passed to future generations through the gametes of the recipient (Chen et al. 1987).

The results of the first research on transgenic fish were published in 1985 (Zhu et al. 1985). Since then, more than 30 species of fish have been genetically engineered, including many of the major world aquaculture species. To date most effort has been targeted on enhancing growth and feed conversion efficiency through the transfer of growth hormone (GH) gene constructs. However, despite spectacular achievements (Devlin et al. 2001, 2004), commercial implementation of transgenic fish technology for food production has remained elusive. This has been due to technical challenges and regulatory requirements in addition to significant opposition to transgenic fish within the scientific, public and aquaculture-producer sectors (Aerni 2004; Devlin et al. 2006; Kapuscinski 2005; Logar and Pollock 2005).

A less controversial issue is the generation and use of transgenic biomonitor fish for expression of easily detectable phenotypes in response to different pollutants (Aleström et al. 2006; Zeng et al. 2005). For instance bright transgenic zebrafish (*Danio rerio*) may express various fluorescent proteins in skeletal muscle. This concept could potentially be exploited for designing biosensor fish for monitoring water pollution and other toxicological exposures (Nakajima et al. 2005).

Chromosome engineering

Recent developments enable the creation of fully functional chromosome vectors as a potential gene-delivery system. Engineered chromosomes are usually much smaller than normal chromosomes yet contain all the functional elements for long-term stability within cells. These include a centromere to facilitate correct mitotic segregation, at least one origin of replication for synchronized DNA replication within each cell cycle, and telomeres to stabilize the chromosome ends. Among several advantages over current vector systems, engineered chromosomes have the capacity to carry large transgenes of gene clusters (Irvine et al. 2005).

Genome engineering

A genome is defined as all of the genetic material of a cell or of an individual (Passarge 1995). In the case of a gamete, a genome refers to all of the genes carried by this single gamete

(King 1968); a set of single chromosomes of all chromosome pairs is called a haploid genome.

An organism's ploidy is the number of copies of haploid genomes that it has. Usually, two haploid gametes (a sperm and an egg) fuse to form a diploid zygote. However, it is possible for organisms to possess three (triploid), four (tetraploid) or more copies of each chromosome, in which case they are known as polyploids. Because the eggs of most fish are released into water before fertilisation it is relatively easy to access the early embryos of fish. Such access allows manipulations to create polyploid embryos, to produce diploid embryos that contain only maternal chromosomes (gynogenotes) or only paternal chromosomes (androgenotes) (Babiak et al. 2002), or to obtain interspecies diploid and polyploid hybrids (Pandian and Koteeswaran 1998), or groups of clones, etc. We call such manipulations, in which the whole haploid genomes are handled in order to obtain a variety of diploid and polyploid fish genomes, genome engineering.

Fish genome engineering started with polyploidisations: tetra- and tri-ploidisations, because the fish farmers were interested in sterile triploid animals. Sterility of fish eliminates problems related to sexual maturation, such as: decrease in growth, decline in flesh quality, increased mortality, etc. These effects significantly reduce the profitability of the large-sized fish production (Chourrout et al. 1986).

The first mass production of tetraploid rainbow trout fingerlings was accomplished by Chourrout (1980, 1982, 1984). Since then a variety of techniques have been developed and used for a number of farmed fish species. Sadly, today, new biotechnology products are widely regarded with suspicion by the general public. In order to overcome this and help to ensure product success, development of new biotechnological and product testing techniques are needed (Evans and Cox 2006).

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