

Genetics & Biotechnology in Aquaculture: Status, Promises & Issues

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Introduction

Great advances have been, and are rapidly being made in our knowledge of the genetics and molecular biology (including genomics, proteomics and structural biology) of aquatic organisms. The puffer fish (*Fugu rubripes*) genome has already been sequenced (Aparicio *et al.*, 2002) and the genomes of two other species, the zebrafish and the Japanese medaka, will soon follow. Global molecular profiling technologies have been or are being developed. These include microarrays using DNA or oligonucleotide chip, and protein and lipid chips are being developed. With such advances in knowledge and platform technologies, biotechnological applications are inevitable, and aquaculture should not be left out. Indeed such biotechnological applications have been made in aquaculture (Hulata, 2001; Hew and Fletcher, 2001; Melamed *et al.*, 2002), but there are ecological, food-safety and other issues which need to be resolved before the biotechnologies could be fully integrated in commercial aquaculture. In this introductory presentation, I will focus on the following areas:-

1. Improvement of aquaculture stocks
2. Preservation of genetic resources
3. Disease diagnosis and control
4. Microbial/microalgal genetic engineering

1. Improvement of Aquaculture Stocks

1.1. Selective breeding

Conventionally, genetic improvement of aquaculture stocks involves selection, cross/out-breeding and/or hybridization. Selectively bred stocks with superior traits such as disease resistance and rapid growth have been produced and used in commercial aquaculture (Hulata, 2001). If these stocks are inbred, genetic depression and deterioration of seed quality would eventually occur. A proper breeding program should include both selection and cross/out breeding, and even hybridization. More than one selectively bred stock/strain should be developed for each species so that cross breeding among them can be done to ensure heterosis (Kirpichnikov, 1993; Sumantadinata, 1995).

Hybridization is particularly useful if it results in triploid, sterile or all-male progeny (see review by Hulata, 2001).

1.2. Marker-assisted breeding

Many molecular markers are now available for genome analysis, fingerprinting, and genetic linkage mapping (Kumar, 1999). These include RELPs (restriction fragment length polymorphisms), AFLPs (amplified fragment length polymorphisms) and RAPD

(random amplified polymorphic DNA). There are also specific PCR markers based on target sequence primers such as short tandem repeats and simple/short sequence repeats, which are also referred to as microsatellites. These markers can be employed to tag quantitative trait loci (QTLs) and assist the breeding program (Agesti *et al.*, 2000). In addition, the molecular profiling microassays mentioned earlier can be used to determine the genes that are expressed, upregulated, or downregulated in a particular desirable trait. This information may then be used to design appropriate markers for the breeding program.

1.3. Transgenesis

Transgenic technology has been developed in a number of fish species (Hew and Fletcher, 2001; Melamed *et al.*, 2002). It is a short cut to achieving genetic change for fast growth, disease resistance and other desirable traits. By just microinjecting into freshly fertilized eggs a fish-growth-hormone gene, linked to a suitable fish promoter, transgenic fish with remarkable growth rates have been obtained (Devlin *et al.*, 1994; Devlin, 1998; Hew and Fletcher, 2001). Recently, transgenic zebrafish with different body colours have also been produced by using the green or red fluorescent protein receptor gene linked to a skin- or muscle-specific promoter (Wan *et al.*, 2002). This has implications for the ornamental fish industry.

Research is being pursued to produce transgenic fish carrying genes that encode antimicrobial peptides such as lysozyme (Melamed *et al.*, 2002). This is one approach to obtain disease resistance in fish. Hew and Fletcher (2001) have tabulated other transgenic approaches to enhance disease resistance in fish including using antisense and ribozyme technologies against viral RNA.

In principle, transgenic technology can be used to induce or enhance other traits in fish once the relevant genes are known. Similarly the technology may be applied to other aquaculture organisms.

1.4. Transgenesis *via* embryonic stem cells and cell/nuclear transplantation

This is a new approach to transgenesis that yields better transgene integration and expression compared to the direct transgenesis described above (Fig.1; Hong and Scharl, 1966; Hong *et al.*, 1998a,b). With the availability of a fish embryonic stem (ES) cell line (Hong *et al.*, 2000), ES cells can be transfected with a particular gene construct or expression cassette and screened for homologous recombination (gene targeting; Hong *et al.*, 1998b). The transfected cells can then be transplanted into early embryos/blastulae, either directly or via removal of nucleus and nuclear transplantation (Yan, 1998). The pluripotent ES cells/nuclei can enter cell lineages and colonize the germ line (Hong *et al.*, 1998a,b). Thus the transgene will be properly integrated and expressed. In contrast, transgenesis by the direct injection of a gene construct to the embryos will result in a high rate of random integration and mosaicism (Fig.1).

1.5. Gender manipulation and sterility induction

Chromosome-set and gender manipulations in fish have been reviewed several times, more recently by Arai (2001), Beardmore *et al.* (2001) and Hulata (2001). The

al., 1996). One approach to minimizing the risk is to produce sterile GMO (Devlin and Donaldson, 1992).

There are other GMO concerns. First and foremost is the food safety concern of the public. Allergic and other undesirable reactions to the novel encoded protein are one such concern. This concern appears to have entered the public psyche and it is difficult to reassure the public to accept GMO as food without much additional information. Thus food safety evaluation of transgenic organisms should be done (Guillen *et al.*, 1999). It is mainly due to the above concerns that the commercialization of transgenic Atlantic salmon, though technically ready, is impeded (Hulata, 2001).

There are also the concern of possible unexpected consequences of GMO (pleiotropic effects) and the issue of intellectual property marginalizing small-scale farmers unable to afford the technology. The latter is particularly relevant to the developing countries where many small-scale aquaculture farmers exist.

2. Preservation of Genetic Resources

Besides wild stock conservation, genetic resources may be preserved through gamete cryopreservation. While sperm cryopreservation is well established (Lahnsteiner, 2000; Suquet *et al.*, 2000), that for ova/embryos/early larvae are still experimental except for some invertebrates like mollusks (Chao and Liao, 2001). The establishment of sperm or seed banks for aquaculture will facilitate preservation and dissemination of stocks, and hence, breeding programs.

Cell lines, particularly ES cell lines, offer another means of genetic conservation. Biodiversity can be rescued from cells by cloning through nuclear or cell transplantation (Fig.2; Hong and Scharl, 1996).

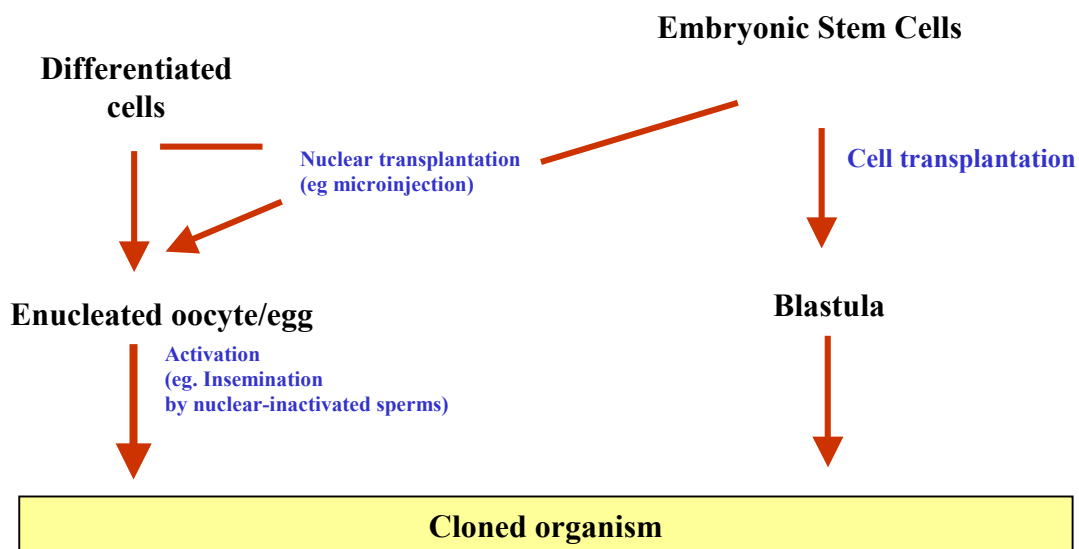


Fig. 2. Rescue of Fish Biodiversity by Cloning

3. Disease Diagnosis and Control

This is an area where molecular technologies would find fruitful applications. Monoclonal antibodies and PCR may be used to develop rapid diagnostics of pathogens (Nicholson, 1993), while DNA or DNA recombinant vaccines (Heppell *et al.*, 1996; Tighe *et al.*, 1998; Lorenzen, 1999) may be produced for protection against diseases (Fig.3). Additionally, transgenesis may be sought to confer or enhance disease resistance as mentioned earlier (1.3).

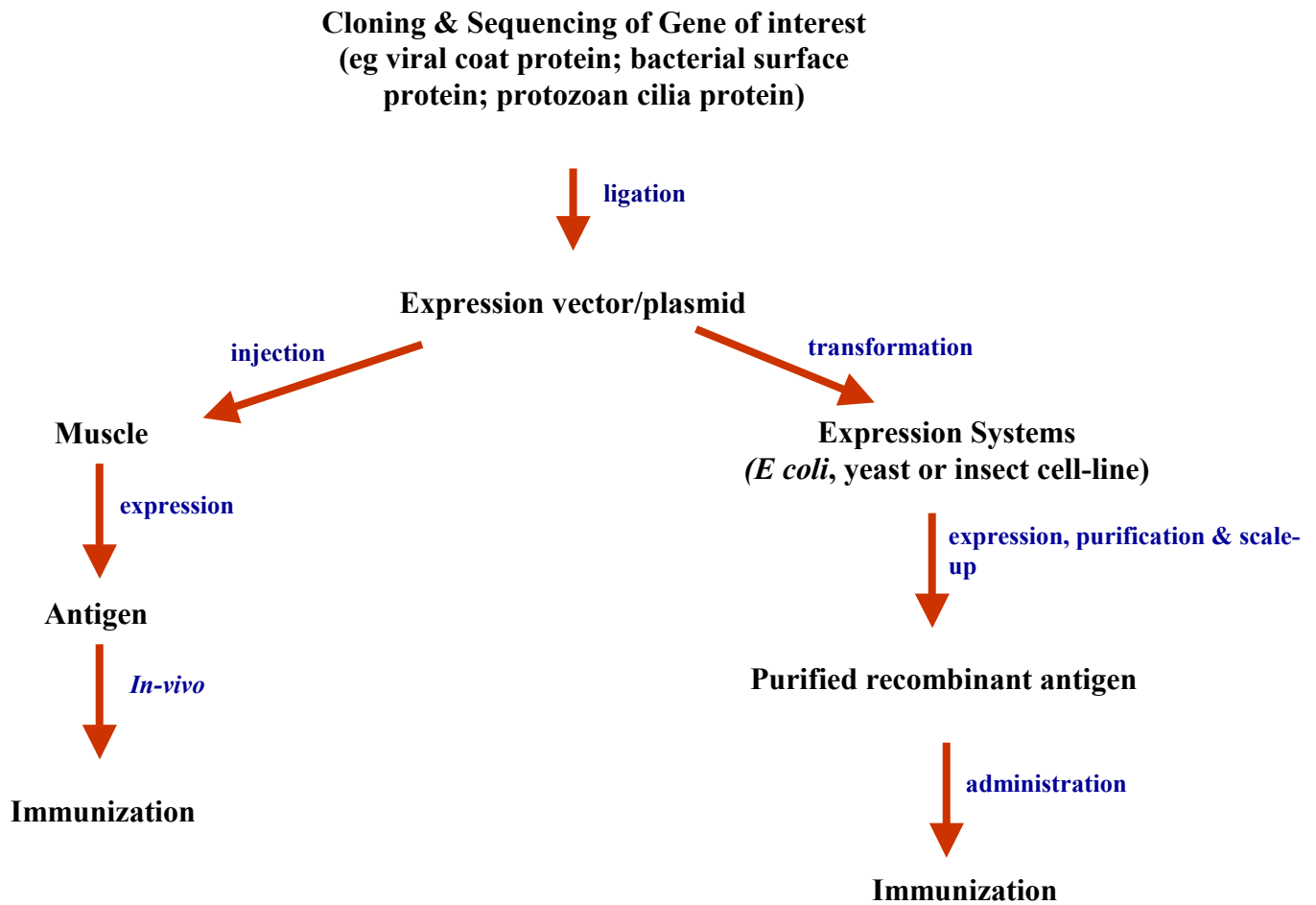


Fig. 3 Production of DNA and DNA recombinant vaccines

4. Microbial/Microalgal Genetic Engineering

Water quality management is an important issue in aquaculture. Here the role of bacteria is well recognized. These bacteria could be genetically engineered to enhance their efficiency in waste/nutrient recycling and in bioremediation.

Other beneficial bacteria like those in the gut (probiotics) that aid digestion and crowd out pathogenic bacteria (competitive exclusion), may also be genetically engineered to improve their value and efficiency.

Similarly, genetic engineering may be applied to microalgae to enhance their nutritional quality as health supplements not only for fish/shellfish but also for humans. Microalgae are known to be a good source of nutrients such as n₃ highly unsaturated fatty acids and antioxidants, and also immunostimulants and antimicrobials.

Concluding Remarks

The tide of molecular and information sciences (genomics, proteomics, structural biology, molecular and cell biology, and bioinformatics) is sweeping various fields of human endeavour. Aquaculture cannot and should not be an exception. However, adverse public perception is putting up some barricades against the tide. The barricade is particularly strong in the area of GMO as food, but in other areas the barricade is weak or absent. These later areas, which include marker-assisted breeding program, preservation of genetic resources, and disease diagnosis and control, should feel the impact of the tide. Even in the area of GMO, the barricades may not hold for long as the tide gathers strength and the risks are minimized.

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