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THE REVIEW OF RECENT ADVANCES IN FISH GENETICS AND BIOTECHNOLOGY

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ABSTRACT
Great advances have been, and are being made in our knowledge of the genetics and molecular biology (including genomics, proteomics and structural biology). Global molecular profiling technologies such as microassays using DNA or oligonucleotide chip, and protein and lipid chips are being developed. The application of such biotechnological advances are inevitable in aquaculture in the areas of improvement of aquaculture stocks where many molecular markers such as RFLPs, AFLDs and RAPD are now available for genome analysis, finger printing and genetic linkage mapping. Transgenic technology has been developed in a number of fish species and research is being pursed to produce transgenic fish carrying genes that encode antimicrobial peptides such as lysozyme thereby achieving disease resistance in fish. Also it is a short cut to achieving genetic change for fast growth and other desirable traits like early sexual maturity, temperature tolerance and feed conversion efficiency.

KEYWORDS: Fish genetics, transgenesis, monoploidy, diploidy, polyploidy,gynogenesis, androgenesis, cryopreservation.

INTRODUCTION

Great advances have been made in our knowledge of genetics and molecular biology, including genomics, proteomics and structural biology of aquatic organisms. The puffer fish (Fugu rubriges) genome has been sequence (Aparicio *et al*., 2002) and the genomic of the zebra fish and Japanese Medaka will soon follow. Global molecular profiling technologies have been developed and are being developed in the developing countries of Africa (Ude *et al*., 2006). These include microassays using DNA or Oligonucleotide chip, and protein and lipid chips are being developed, with such advances, biotechnological applications are inevitable even in aquaculture (Hulata, 2001; Hew and Fletcher, 2001; Melamed *et al*., 2002, Ude *et al*., 2006).

The several areas of aquaculture where biotechnology can be applied include production of fish of high production of fish seedling. Fishes appear to have better scope for research in genetics and breeding. Chromosomal engineering as spermiation occurs in fish eggs prior to the extrusion of the second polar body. There is a hope for activation of the egg with irradiated sperm, retention of the second polar body and inhibition of the spindle formation during the first cleaving (Thorgaard, 1983). The main vision of aquaculture biotechnology is to achieve improvements of aquacultural stock, preservation of genetic resources, disease diagnosis and control of microbial/ microalgal genetic engineering.

IMPROVEMENT OF AQUACUTURAL STOCKS

SELECTIVE BREEDING

The genetic improvement of aquacultural stocks involves selection, cross/out breeding and by hybridization. Selectively bred stocks with superior traits such as disease resistance and rapid growth have been produced and used in aquaculture (Hulata, 2001). More than one selectively bred stock/strain should be developed for each species so that cross breeding them can be done to ensure heterosis (Sumantadinata, 1995).

Hulata (2001), reported that hybridization is very useful if it result in triploid, sterile or all-male progeny.

MARKER-ASSISTED BREEDING

Many molecular markers are now available for genome analysis, finger printing and genetic linkage mapping (Kumar, 1999). These include RFLPs (restriction fragment length polymorphism), AFLPs (amplified fragment length polymorphism) and

RAPD (random amplified polymorphic DNA). There are also specific PCR marker based on target sequence primar such as short tandem repeats and simple /short sequence repeats which are also referred to as microsatellites. These markers can be employed to tag quantative trait loci (QTLs) and assist the breeding program.

TRANSGENESIS

Transgenic technology has been developed in a number of fishes (Fletcher, 2001, Melamed *et al*., 2002). It is a short cut to achieving genetic change for fast growth, disease resistance, tolerant to low level of dissolved oxygen in the water and fish resistant to freezing temperature (Ude *et al*., 2006). By microinfecting 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*., 1998; Hew and Fletcher, 2001). Adelizi (1998) reported that in order to grow fishes that are longer and heavier, one method in use is to dip the fish in a solution, containing the desired growth hormone. However, there are some problems with this technique.

Firstly, it may be difficult to determine if the fish is getting the right amount of growth hormone. Therefore, current research focus is to develop new strains or transgenic fish which naturally produce just the right amount of growth hormone to speed their growth (Ude *et al*., 2006).

The two main techniques which researchers use to transfer genetic materials in fish are:
Microinjection explained above; and electroporation that involves transferring the genetic material or DNA into fish embryos through the use of an electric current.

Research is being pursed 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. Other approaches to enhance disease resistance in fish including using antisense and ribozyme technologies against viral RNA.

TRANSGENESIS VIA STEM CELLS AND
CELL/NUCLEAR TRANPLANTATION

This is a new approach to transgenesis that fields better transgene integration and expression compared to the direct transgenesis described in Fig 1 (Hong and Schartl; 1996; Hong *et al*., 1998a, b). With the availability of a fish embryonic stem (ES) cell line (Hong *et al.,* 2000), embryonic stem can be transfected with a particular gene construct or expression cassette and screened for homologous recombination. The transfected cell can then be trung planted into early embryos/blastulae either directly or through removal of nucleus and nuclear transplantation (Yan, 1998). The pluripotent ES cells/ nuclei can enter lineages and colonize germ line (Hong *et al.,* 1998a, b).

GENDER MANIPULATION AND STERILITY INDUCTION

Studies have shown that it is possible to achieve polyploidy and gynogenesis on a fairly routine basis (Ude *et al.,* 2006, Aria, 2001). The technologies include hormonal sex reversal, gynogenesis, androgensis and polyploidization, which may be combined to produce all- female sterile triploid fish. It may be possible to induce sterility in fish by transgenesis involving the use of antisense/ ribozyme technology to degrade mRNAs of GnRH (Gonadotropin releasing hormone or GtHIβ (Gonadotropin 1β) has been proposed by Hew and Fletcher, in 2001.

(a) Polyploidy: basically, induction of triploidy involves applying shock treatment by heat, cold, pressure or chemicals to eggs soon after sperm entry when the eggs are about to undergo the mitotic phase of meosis (Metaphase II). The stock treatment suppresses the separation of sister chromatids (anaphase II) thereby preventing the second polar body formation and inducing the diploid condition instead of maintaining the haploid. Subsequent fusion of diploid eggs result in difficulty in producing sterile individuals when it is required that all the fish are sterile. Another disadvantage is that the survival rate of triploid may be lower

than the survival rate for normal diploids at some life stage. However this problem is off set by faster growth or increased survival at other life stages (Ude *et al.,* 2006).

(b) Gynogenesis: This is the production of viable progeny with all maternal inheritance. This involves irradiating spermatozoa with gamma radition, x-ray, or ultraviolet light to destroy the genetic material without inactivating the spermatozoa, and using these dechromosomed spermatozoa to activate the eggs followed by shock and the haploid spermatozoa, triploid zygote results. (Ude *et al.,* 2006).

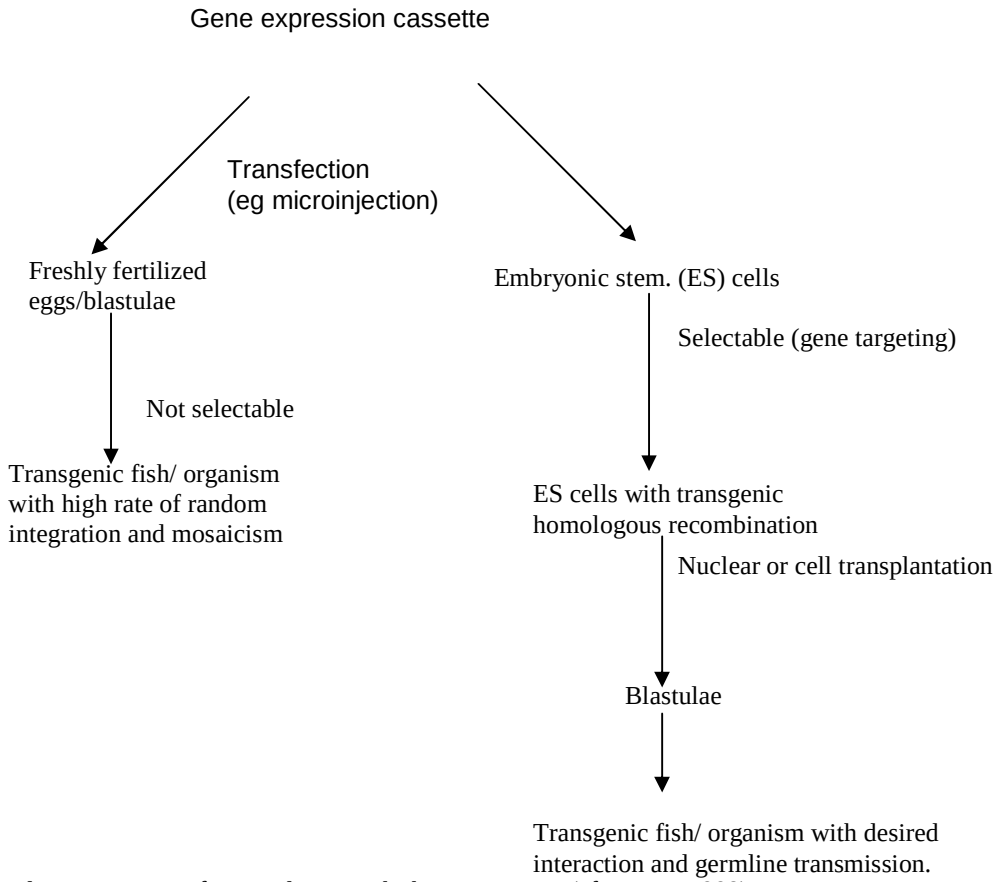

Fig1: Rapid improvement of aquaculture stocks by tansgenesis (after Yan, 1998)

Induction of tetraploidy involves applying the shock treatment soon after zygote formation. This serves to prevent the first mitotic cleavage thereby inducing the tetraploid condition, similarly triploid fish may be obtained by mating tetraploid males and diploid female (Chourrout *et al.,* 1986; Blanc *et al;* 1987). Tetraploid fish may also be obtained by shock treatment following the mixing of ovulated eggs from a diploid female with spermatozoa from tetraploid male. The advantages of triploid fish are:

(i)They are all sterile and are useful for stocking natural water bodies where population control is desired forweed and forage fish.

(ii) They grow faster at and after sexual maturity

(iii) Triploid hybrids may be more viable.

Hybridization between species is a useful tool for improving stock characteristics in aquaculture but the hybrids are often not viable, triploidy may then be resorted to promoting viability in the hybrids. Unlike triploid, tetraploid may reach sexual maturity (Blanc *et al;* 1987).

The tetraploids may therefore be used to propagate triploids by mating them with diploids. Tetraploids may also confer viability to inter- specific hybrids. It has been discovered that not all the fish produced using

these technique are sterile, some fertile diploid are produced as well. Similarly, separation of the fertile treatment to prevent second polar body formation thereby producing xx condition (Fig 2). Thus all female progeny is assured. To further separate the female stocks, some of the newly hatched larvae may be treated with androgens to produce xx males, which upon reaching sexual maturity can be crossed with normal female to produce all female fish (Fig 2). Ude *et al;* (2006) reported that by this method production of female stock can be achieved for monosex culture.

This is very useful for species where the female grow faster than the males or where the female has a better meat quality than the male.

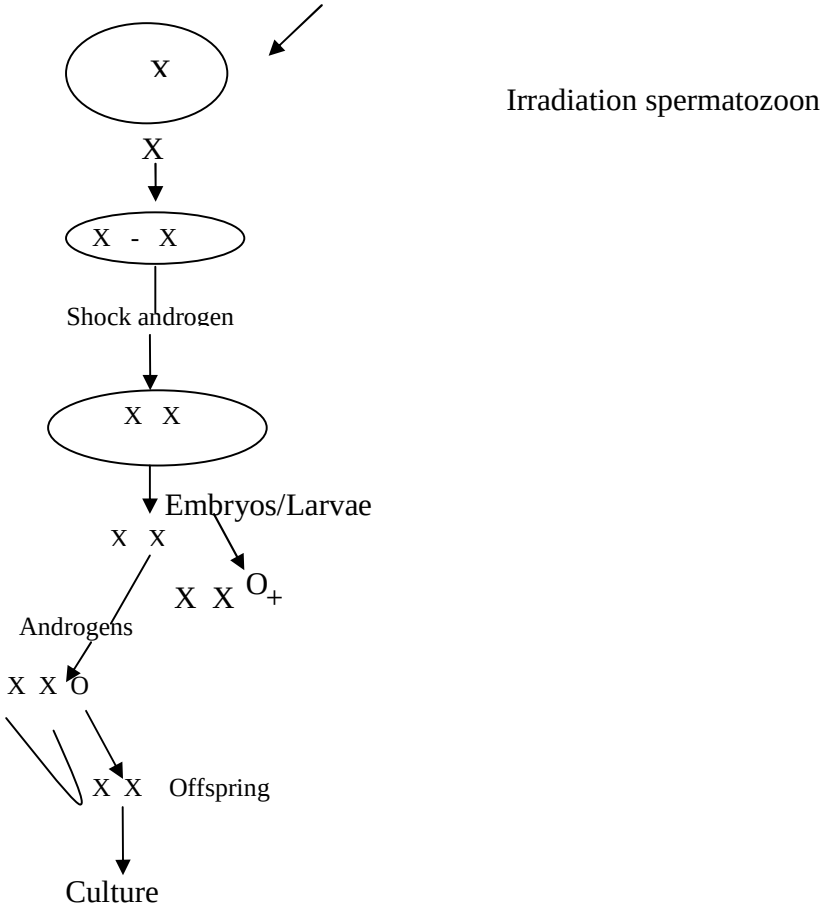

Fig 2. Hybridization in fish (after Ude *et al.*, 2006)

C. Androgenesis

This is the production of viable progeny with all paternal inheritance. In the case the DNA of the egg is inactivated by radiation. This method is used to produce sterile tripoids. In this case, eggs are irradiated instead of spermatozoa (Fig 3). The resulting YY males, if viable and fertile can be crossed with normal females to produce all male progeny.

Diploid androgenesis occurs when the eggs do not contribute DNA to the embryo; all the genetic material in the embryo comes from the sperm (Ude *et al*, 2006).

Person and Torgaard (1985) outlined the advantages of androgenesis as (1) less times required to produce inbred lines, for species in which males mature earlier than females.

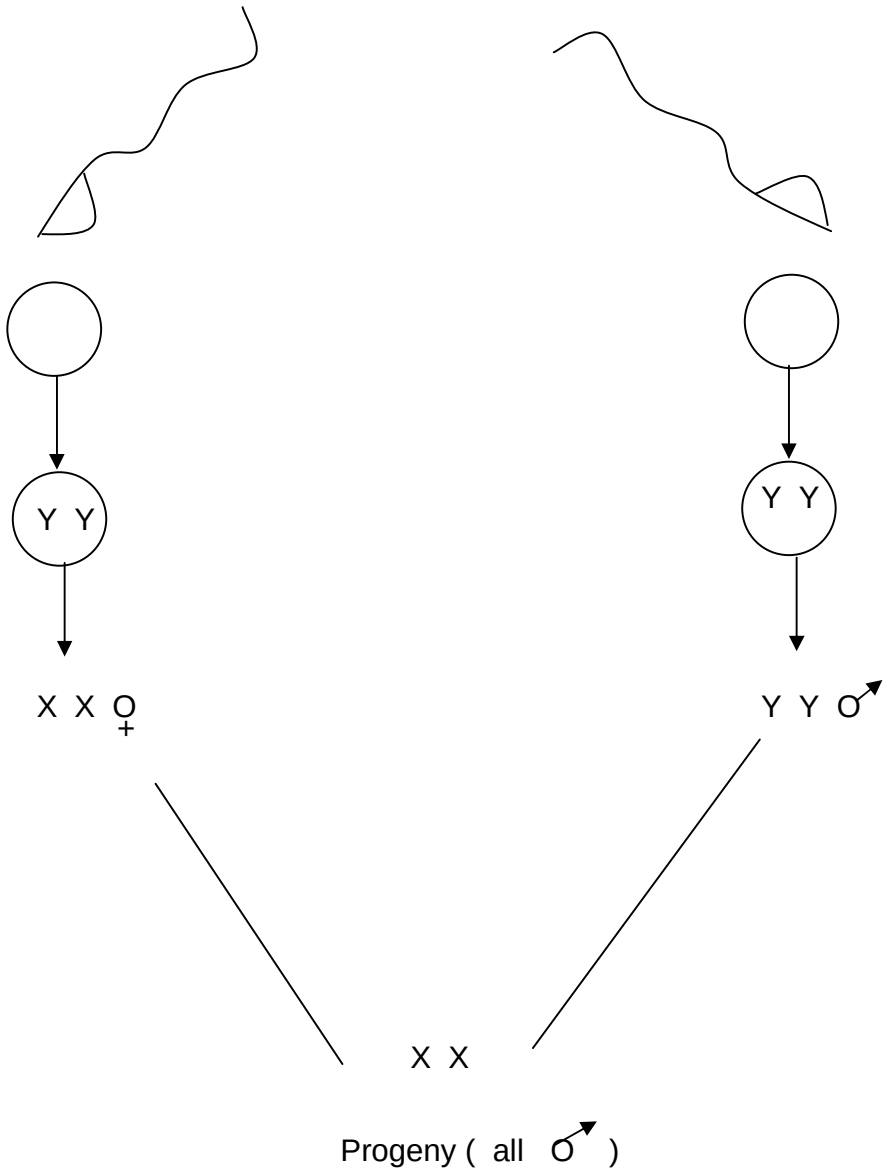

Fig 3: Androgenesis in fish (after Lawson 1997)

The shock treatment may be thermal, pressure or chemical (Table 1) and the eggs may be irradiated by gamma radiation, x- ray or ultraviolet light.

PRESERVATION OF GENETIC RESOURCES

Beside wilde stock conservation, genetic resources may be preserved through gamete cryopreservation. While sperm cryopreservation is well established (Lahnsteiner, 2000, Suquet *et al;* 2000), the cyopreservation of ova/embrayo/early larvae is still experimental except for some invertebrates like moluscs (Chao and Liao, 2001).

The establishment of sperm or seed banks for aquaculture will facilitate preservation and dissemination of stock and hence, breeding programs.

Cell lines particularly embryonic stem (ES) cell lines, offer another means of genetic conversation. Biodiversity can be rescued from cells by cloning through nuclear or cell transplantation (Fig4; Hong and Scharti 1996).

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(3) DISEASE DIAGNOSIS AND CONTROL

This is the area where molecular technologies would find fruitful application. Monoclonal antibodies and PCR may be used to develop rapid diagnostics of pathogens (Nicholson, 1993), while DNA and DNA

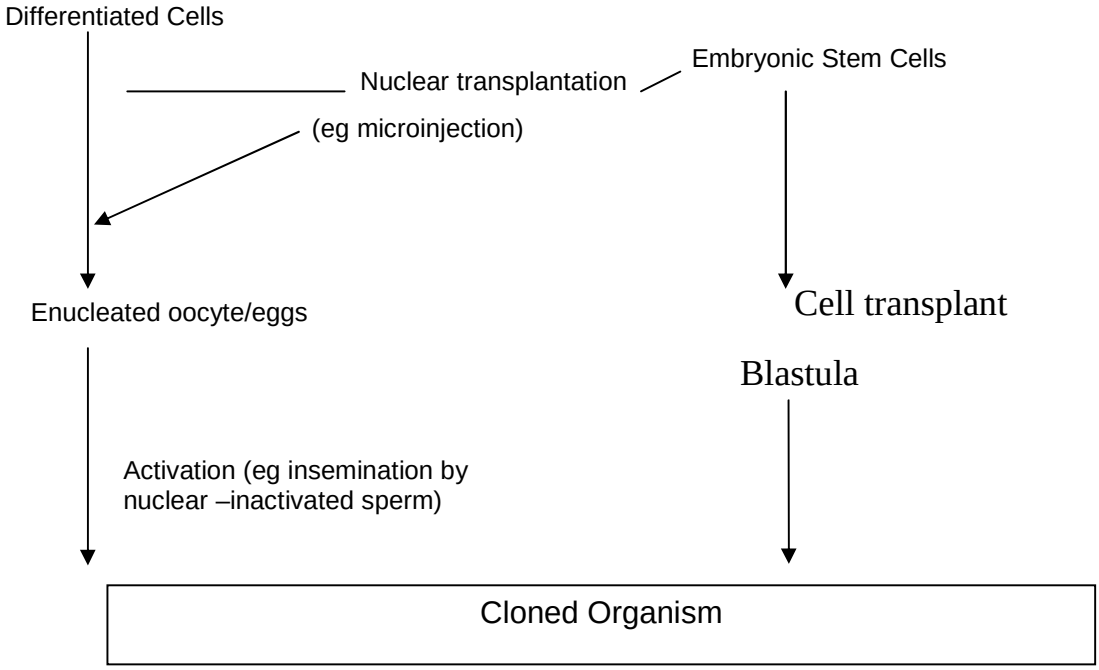

Fig 4: Rescue of fish biodiversity by cloning (after Hong and Schart, 1996)

recombinant vaccines (Heppell *et al;* 1998; Tighe *et al* 1998; Lorenzen 1999, may be produced for protection against disease (Fig 5).

Additionally, transgenesis may be sought to confer or enhance disease resistance.

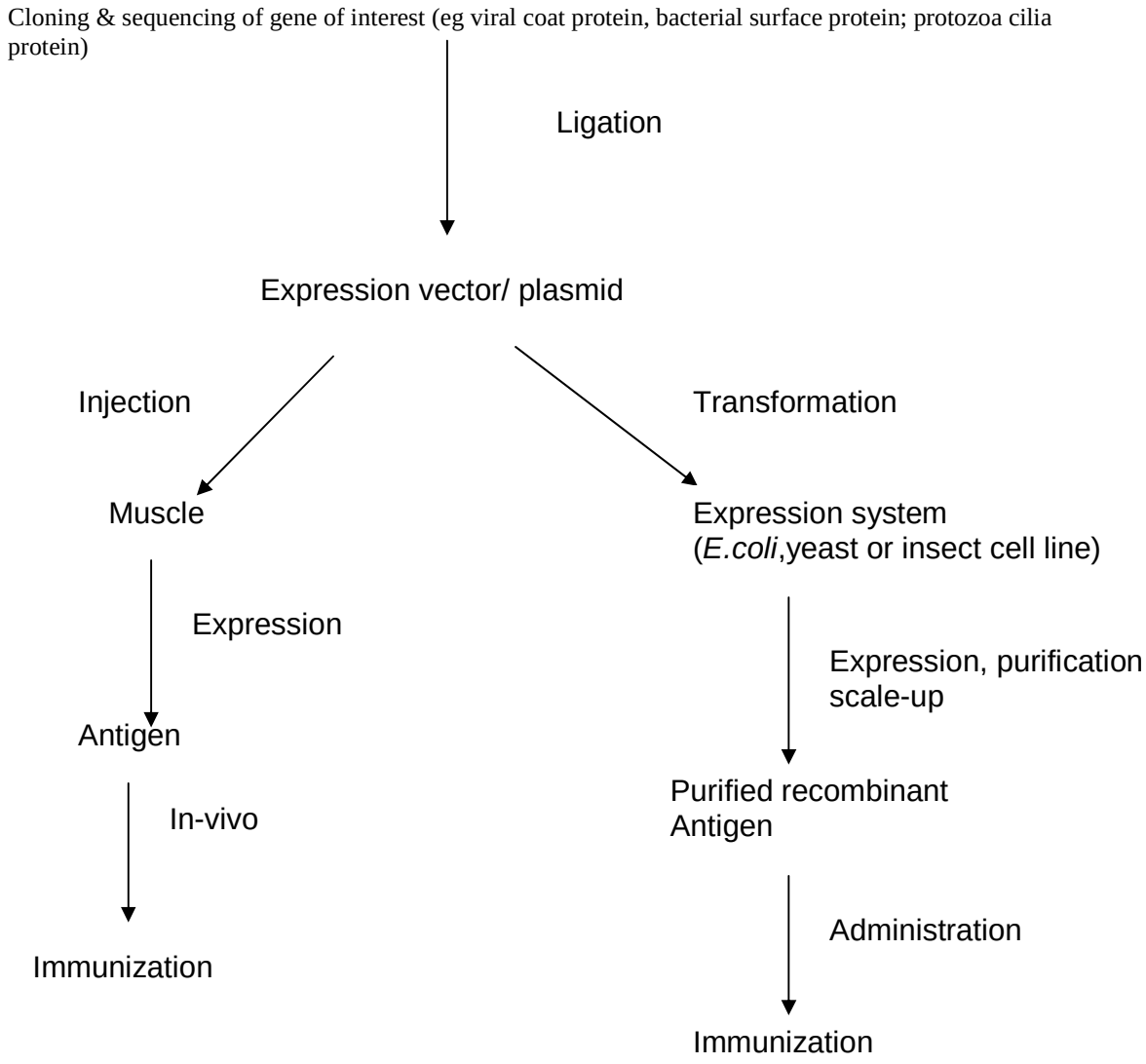

Fig 5: Production of DNA and DNA recombinant vaccines

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 values and efficiency. Similarly, genetic engineering may be applied to microalgae to

enhance their nutritional quality as health supplements not only for fish and shellfish but also for humans.

Microalgae are known to be a good sources of nutrients such as n_3 highly unsaturated fatty acids and antioxidants, and also immunostimulants and antimicrobials.

CONCLUSION

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 should not be an exception.

It may be necessary to establish a bureau of fish genetic resource whose responsible include establishment of quarantine department and control all export and import of live fish materials.

- Discover a list of endangered fish species and recommend their conservation and management.
- To identify and classify traits of cultivable and cultivated fresh water brackish water and marine fishes in order wild forms mainly through electrophonetic, spectrophotometric, genetic, morphometric, anatomical, physiological and biochemical studies either of its own or culled data from elsewhere.

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