Genetics and Molecular Biology in Aquaculture - Review -

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ABSTRACT : Genetics has played a pivotal role in increasing the world food production through revolutions in plant and animal sciences. Though the attention on fisheries has been inadequate but the growing importance of modern genetic manipulations and biotechnological innovations to aquaculture has been realized. Recent advances in fish genetics and molecular biology have provided a suite of useful techniques, which have several applications in aquaculture. This paper reviews the advancement in the applications of selection, hybridization, chromosome engineering, sex control, gene transfer and molecular technologies for enhanced aquaculture productivity. *(Asian-Aust. J. Anim. Sci. 2001. Vol. 14, No. 6 : 894-898)*

Key Words : Genetics, Molecular Biology, Aquaculture

INTRODUCTION

The application of genetics and molecular biology has come to aquaculture comparatively recently considering what has been achieved in livestock and crops. However, the nature of reproduction and high fecundity of fish makes it an excellent model for genetic manipulations. There are a number of options available for genetic improvement of aquaculture species covering traditional and modern approaches. Selective breeding, hybridization, chromosome and gene manipulations and sex control and sex reversal are now the proven genetic techniques and biotechnologies used for fish stock improvement in aquaculture. More recently, molecular markers have been used to map fish genomes and to indicate the presence of particular useful quantitative characters for breeding programmes.

SELECTIVE BREEDING

It has been demonstrated for several species that selective breeding can be used to produce better performing fish for aquaculture (Gjerde, 1993; Eknath et al., 1993).

Selection involves developing a breeding program in which individuals or families are chosen on the basis of their value for commercially important trait(s) in one generation in an effort to change the population mean in the next generation. Selection can only work if there is sufficient genetic variation for these traits of interest. If there are low levels of genetic variance in a cultured stock, it may be necessary to bring in new germplasm to increase levels of variation prior to initiating a selection program. There are several different approaches to selection including mass selection, family selection and within family selection, each have their own merits depending on the nature of the character to be selected and the resources available for the selection program. Selective breeding can become complicated if it is aimed at producing fish for diverse agro-climatic and aquaculture environment. Where some strains or genotypes of species perform better in certain conditions and others do better in another set of conditions (known as genotype-environment interaction), it may be necessary to select for strains for specific conditions.

Although selective breeding has been undertaken to produce superior fish in several species used in aquaculture including atlantic salmon, common carp, channel catfish, tilapia and rohu, but the overall contribution of selectively bred aquaculture stocks to world aquaculture production is still very low (Gjedrem, 1997). It should be possible to increase greatly the productivity of aquaculture stocks through selective breeding.

HYBRIDIZATION OR CROSSBREEDING

The aim of hybridization is to achieve an improvement in performance vis a vis the parent species. This exploits a different kind of genetic variance, termed non-additive genetic variance. Crosses between two different species (hydridization) or between distinct strains of the same species (cross-breeding) create new combinations of alleles at each gene locus (with one allele contributed by each parental species/strain). Occasionally, these new combinations of alleles can fortuitously produce certain interactions creating expression of traits that are

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superior to those of either parental species/strain. When a hybrid exhibits superior performance to both of the parental species, it is said to be exhibiting heterosis for this particular trait. Hybridization is a good breeding programme only when hybrids show heterosis.

It has been reported that most of the fish hybrids produced in the past 100 years have been found less fit than the parents (Purdom, 1993). It is only in a handful of species groups where the hybrids have proved to be of significant practical value. These include hybrids of sturgeons, cyprinids, bass, salmonids, tilapias and Asian catfish. The hybrid sturgeon produced by crossing female *Huso huso* × male *Acipenser ruthenus* remains a good example of evolving an improved breed for aquaculture. The hybrid combined the faster growth rate and freshwater tolerance of the parents. Genetic improvement in tilapias is due to their ease of hybridization. The red tilapia was produced by crossing *O. niloticus* with albino *O. mossambicus*. This came into commercial production in a rather big way. A few interspecific hybrids are used on a large scale in aquaculture today (Bartley et al., 1997). Examples are male *Clarias gariepinus* × female *C. macrocephalus* which is a major product in freshwater aquaculture in Thailand (Little et al., 1994), hybrid tilapia (*Oreochromis niloticus* × *O. aureus*) in Israel and bass (*Morone saxatilis* × *M. chrysops*) in the USA (Hallerman, 1997).

CHROMOSOME ENGINEERING

Chromosome engineering techniques of uniparental inheritance (androgenesis and gynogenesis) and induced polyploidy (triploidy and tetraploidy) have been applied to various fish and shellfishes either to genetically improve the species for aquaculture or for research purposes (Lakra and Das, 1998; Pandian and Koteswaran, 1998). Numerous reports have described the techniques to induce polyploidy (triploidy and tetraploidy) and uniparental chromosome inheritance (gynogenesis and androgenesis) in fish, as reviewed by Purdom (1993) and Pandian and Koteswaran (1998). The main rationales for the use of these techniques in fish culture are the production of inbred lines and the production of monosex or sterile populations.

Gynogenesis is the process of animal development with exclusive maternal inheritance. The production of gynogenetic individuals is of particular interest to fish breeders because a high level of inbreeding can be induced in single generation. Gynogenesis may also be used to produce all-female populations in species with female homogamety and to reveal the sex determination mechanisms in fish. It is convenient to use all female gynogenetic progenies (instead of normal bisexual progenies) for sex inversion experiments. Methodologies combining use of induced gynogenesis with hormonal sex inversion have been developed for several aquaculture species (Gomelsky et al., 2000).

Androgenesis is the process by which a progeny is produced by the male parent with no genetic contribution from female. Induction of androgenesis can produce all male population in fish which would have commercial applications in aquaculture. It can also be used in generating homozygous lines of fish and in the recovery of lost genotypes from the crypreserved sperms. Androgenetic individuals have been produced in a few species of cyprinids, cichlids and salmonids (Bongers et al., 1994).

Induced polyploidy involves the production of individuals with extra sets of chromosomes. Polyploids in fish are produced by treating the zygote with either hydrostatic pressure, temperature shock or chemical treatment shortly after fertilization to restore the second polar body (triploid induction) or shortly before the first mitotic division to cause endomitosis (tetraploid induction). The production of triploid fish is of interest to aquaculturists and fisheries managers because triploids are normally sterile. Chromosome manipulation for triploid production was first attempted by Swarup (1959). Since then the technology has been applied to several cultivable and commercially important fish and shellfish species. Induction of triploidy has been effective in producing sterile fish as has been reported in several species (Lakra and Das 1998; Pandian and Koteswaran, 1998).

Aquatic molluscs usually undergo growth reduction during their reproductive season. Over the last more than 15 years, research has also been undertaken on commercially important shellfish to develop efficient techniques to sterilize them (Beaumont and Fairbrother, 1991). Presently, there is considerable interest in commercial production of triploid oysters in Australia, China and Europe (Benoit et al., 2000). The development of tetraploid brood stock to cross with diploids for production of sterile triploids has been attempted (Thorgaard, 1992; Guo et al., 1996). If the viability of tetraploids could be increased, then this would be a better method of generating triploid fish and shellfish on a commercial scale.

SEX CONTROL

The use of sex control techniques to influence characteristics of economically desirable teleost species is becoming an important management tool to increase aquaculture production. Techniques that allow production of monosex population by sex manipulation are potentially useful in species where one sex is more useful than the other. There are basically two ways of sex manipulation i.e. hormonal and genetic. The

hormonal or endocrine control involves the treatment of fish with sex steroids during the early phase of life before sex differentiation starts. The process of sex differentiation in teleost is protracted and labile rending the hormonal induction of sex reversal possible in gonochoristic and hermaphroditic species. The induction involves administration of an optimum dose of sex steroid during the labile period which reverses the phenotypic expression of a genetic female into a male but the genetic male remains a male. Presently, protocols for hormonal sex reversal have been described for 44 species of gonochores and hermaphrodites using one of the 31 steroids (Pandian and Sheela, 1995). The genetic approach to sex manipulation for production of all male, all female or all sterile populations is through the induction of ploidy.

In teleosts some species have fully developed sex chromosomes. In others, a pair of autosomes act as sex chromosomes but their morphology is unspecialized. As a consequence the genders are difficult to recognize karyotypically although sex identification can be achieved using molecular genetic methods (Griffiths et al., 2000).

The phenotypic sex of gonochoristic fish is determined essentially by sex chromosomes but it can also be influenced by environmental factors (Baroiller et al., 1999). The most pervasive environmental factor governing sex determination in fish based on current knowledge is temperature. Indeed a complete change from strictly monosex male to strictly monosex female progenies (or vice versa) has never been observed except for the atherinid *Basilichthys bonariensis* Val. In cichlids (*Oreochromis* spp) monosex or almost monosex populations can be obtained after exposing juveniles to temperatures of 37℃ (Nile tilapia) or 35℃ (Blue tilapia O. aureaus) over 28 days after yolk sac resorption (Baras et al., 2000).

TRANSGENIC FISH

Animals into which segment of foreign DNA has been introduced and stably integrated into the host genome are called transgenic. Originally, DNA sequences from a variety of organisms, viruses, bacteria, animals, birds, fish and humans were transferred to fish. But fish researchers have now chosen to focus on transfer of fish DNA constructs into fish to increase the likelihood of social acceptance of transgenic fish and because of the belief that fish based constructs could be more effective in terms of integration and expression.

Transgenic fish technology has great potential in the aquaculture industry. By introducing desirable genetic traits into fishes, mollusks, and crustaceans, superior transgenic strains can be produced for aquaculture. These traits include faster growth rates, improved food conversion efficiency, resistance to some known diseases, tolerance to low oxygen concentration and tolerance to extreme temperatures. Out of these traits, growth rate has attracted the much attention and fish research on transgenic fish with the potential for practical aquaculture application involves growth hormone gene transfer. Gene transfer involves the introduction of multiple copies of novel genes into the newly fertilized eggs with the intention that some of the introduced DNA will be incorporated into the genome of the developing embryo. Gene transfer research in fish has a history of about 15 years. Ever since the first successful transfer of recombinant DNA into fish in 1985 (Zhu et al., 1985), the technique has been successfully applied to several finfish species. Most of the workers have used gene constructs of mammalian origin. Recently, various piscine genes have been isolated and cloned and used in transgenesis. Evidence of introgression and expression of the novel genes have been demonstrated in number of species and transgenic strains of tilapia, salmonids, carp and catfish already exist with occasional spectacular results in term of growth rate. The transgenic technology has shown that the growth rate of farmed fish can be increased by 400% to 600% (Devlin et al., 1994) while simultaneously reducing feed input by up to 25% per unit of output, thereby improving food conversion ratios (Hew and Fletcher, 1997). Research has also been conducted on gene transfer in other groups of aquatic organisms e.g. crustaceans (Bachere et al., 1997) microalgae (Matsunaga, 1995) and seaweeds (Qin et al., 1994). Recent developments in molecular biology e.g. polymerase chain reaction, automatic sequencing, genome mapping projects are likely to facilitate further developments in gene transfer in aquatic organisms.

The commercial application of transgenic fish has been initiated. Transgenic salmon are being grown in Scotland and New Zealand (Dunham, 1999). However, several socioeconomic issues need to be addressed before any application of transgenic fish. There include safety issues, socio-economic impact and environmental risk. Whether or not transgenic fish will have a significant impact on the environment is debatable and it is difficult to predict. However, development of biological containment of transgenic fish by induced sterility should be considered as a priority when considering the development of genetically modified fish for commercial exploitation.

MOLECULAR BIOLOGY

Recent advances in molecular biology have provided unlimited number of genetic markers which have multiple applications in aquaculture and fisheries.

Molecular genetic approaches began to be used in fisheries in the 1950s. Their use in aquaculture and fisheries has increased dramatically over the past few years. The genetic identification of aquaculture stocks is a fundamental requirement in any culture programme. Mitochondrial DNA has provided a wealth of genetic markers to answer questions on the phylogeny, evolution and population structure of fishes. Mitochondrial DNA has an effective population size one quarter that of nuclear genes and thus might be expected to show greater population divergence than nuclear genes. Billington and Hebert (1991) reviewed patterns of mtDNA variation in 40 fish species when considerable divergence of local populations was reported. Among the DNA markers, multi-locus VNTR analysis (DNA fingerprinting) can be used to assess the amount of inbreeding in cultured populations. Marker based approaches can be used to increase the efficiency of breeding programmes based on biometrical methods. Genetic markers can be used to identify individuals and family groups so that they can be reared together thus simplifying experimental designs. One very powerful application of the new DNA based technologies is to identify marker loci which are associated with nuclear loci that control economically important traits (quantitative trait loci or QTLs). Once such markers have been identified they can be used in selection programmes. An approach towards this marker assisted selection (MAS) in fish has been made in rainbow trout by Herbinger et al. (1995).

During the past few years efforts have been devoted to the development of microsatellite markers for a variety of aquaculture species (Ward and Grewe, 1994). Highly polymorphic microsatellites allow the parents of superior progeny to be identified in mixed family rearing environments, thus enabling selective breeding to occur on commercial fish farms (Wright and Bentzen, 1994). Microsatellite markers are based on length variation of tandem repeats of usually 2-5 base pairs. They have a number of advantages in aquaculture and fisheries research over other molecular markers. They are abundant in the genome, thus the number of markers is potentially unlimited. Microsatellite loci display varying levels of polymorphism. The highly polymorphic loci are of use in parentage studies, the less variable loci are more useful in discriminating populations. The assay of microsatellite variation is based on the PCR technique, thus only small amounts of tissue, for example from fish scales, are needed as a source of DNA. A number of recent studies have assessed the utility of microsatillite markers in aquaculture genetics (Herbinger et al., 1999).

Genetic markers are also used as powerful tools in confirming the successes of chromosome manipulation. Another important application of molecular biology to aquaculture relates to the production of transgenic fish. The assessment of integration, expression and germline transmission of the introduced gene is completely dependent on DNA based technologies.

CONCLUSION

Though several advances have been made in recent years in the development of genetic technologies in aquaculture but aquaculture genetics is still in its infancy especially in the tropics. Hence, there is huge potential for rapid advancement in culture performance of key aquacultural species. The properties of fish reproduction make themselves better suited to the application of genetic technologies including a number of techniques not possible in higher organisms. It is predicted that rapid improvements will be made in the culture performance of domesticated fish stocks using genetic and biotechnological tools in the coming years leading to significant increases in aquaculture productivity.

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