Genetic Analysis of Immunological Traits in Tilapia

A. Cnaani, M. Ron, E. Seroussi and G. Hulata

Abstract

The immunological response to handling stress of four tilapia species is evaluated. Polymorphism is examined in genes known to influence immune response in fish.

Biochemical and Immunological Analyses

A comparative evaluation of a series of biochemical blood parameters and of immunological indicators in response to handling stress was conducted in four species of tilapia (*Oreochromis aureus, O. mossambicus, O. niloticus* and red *O. niloticus*). Significant differences were observed among the four tilapia species in the following biochemical blood parameters: total protein, albumin, globulin, cholesterol, triglicerides, bilirubin, b-hydroxybutiric acid, calcium, magnesium and phosphorus, and the following immunological indicators in response to stress: hematocrit, glucose and lysozyme activity. *O. aureus* differed from the other three species in all variables mentioned above, with largest differences occurring between *O. aureus* and *O. mossambicus* (Palti et al. 1999). None of these parameters differed significantly between males and females, nor was a correlation with fish size observed.

Creation of Segregating F_2 Populations

A breeding family, consisting of *O. mossambicus* females and *O. aureus* males, which participated in the above-mentioned experiment, was established to produce F_1 hybrids. F_1 hybrid progeny were obtained and reared to sexual maturity. Three breeding families, each consisting of a single F_1 male and 4-6 F_1 females were established and four F_2 progenies, each consisting of 200 or more individuals were obtained. These batches were 9 months old at the time of reporting (October 2001) and ready for blood sampling and testing immunological response to handling stress.

Screening for Informative Genetic Markers

A few F_1 fish, and their respective *O. aureus* and *O. mossambicus* parents were screened using 65 microsatellite markers. Thirty-five of those were found to be informative (i.e., heterozygous in the F_1 generation). These markers will be used to scan the genome of the F_2 generation in search for QTLs (Quantitative Trait Loci) for immune response and disease resistance.

Detecting Polymorphism and Mapping Candidate Genes

Polymorphism was examined in several genes, known to play a role in the immune system. Different genotypes of these genes are known to influence immune response in fish and avian species.

The MHC genes: The major histocompatibility complex (MHC) genes are attractive candidates for this approach. Primers for various loci of the *O. niloticus* MHC system (Mallaga-Trillo et al. 1998; Sato et al. 2000) were used for PCR (Polymerase Chain Reaction). Products were cloned into plasmids and sequenced. Two loci of MHC class II were identified, using primers flanking intron-1 and exon-3, and one of MHC class I, using primers flanking intron-3. Using polymorphism found in the MHC class I locus, a segregating family was genotyped. This locus was found to be located on linkage group #15 of the tilapia linkage map. Single nucleotide polymorphism (SNP) sites were found in a coding region (exon) of one loci of MHC class II for the two parental species, *O. aureus* and *O. mossambicus*.

Genes of the non-specific immune system: The gene sequences coding for enzymes of the innate immune system are not known in species of the Cichlidae family or in any fish from the order Perciformes. A search in the GeneBank database for known

sequences in fish species belonging to three different orders (flounder, salmon, trout, medaka and zebrafish) revealed conserved regions in several such genes. Heterologous primers were designed at the conserved regions for detection of three genes: transferrin, lysozyme and ceruloplasmin. So far, only the primers for transferrin resulted in good PCR amplification; the product obtained was cloned into a plasmid and sequenced. Comparison with known sequences in the databases confirmed that this is indeed a section of the gene for transferrin, which consists of four exons and three introns. Length polymorphism was found in one of these introns and specific primers were designed to trace the segregation of these alleles. A mapping family, consisting of 129 individuals, was genotyped for the transferrin site. The gene for transferrin was located on linkage group #18 along with nine microsatellite markers (Cnaani et al. 2002).

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Progress on Genetic Improvement of Rohu in India

The Indo-Norwegian project on "Genetic improvement of Rohu, *Labeo rohita* for growth through selective breeding" initiated at the Central Institute of Freshwater Aquaculture (CIFA) in collaboration with the Institute of Aquaculture Research (AKVAFORSK), Norway in 1992 is now in its second phase.

The project started with the collection of base population from 5 rivers (Ganga, the Yamuna, Gomati, Brahmaputra and the Sutlej) along with the CIFA farm stock. Growth and survival were considered as traits for selection. Combined selection method was adopted for breeding value estimation. During the execution of the project many methods and protocols for selective breeding were standardized (i.e. production of full-sib groups, rearing of full-sib groups in nursery as well as in laboratory, tagging with PIT tag, rearing of fingerlings in the communal ponds and data analysis using SAS package for breeding value estimation and other analysis). The major findings of the project are: (i) large differences in growth observed between fullsib families within stocks both in mono- and polyculture systems; (ii) substantial additive genetic variation for growth performance; (iii) highly consistent ranking of stocks and full-sib groups for growth in mono and polyculture system indicating that genotype by environmental interaction is low for growth trait in present production systems.; (iv) diallel cross of five stocks indicated negative heterosis effect; hence pure breeding may be suitable method of selection; (v) average 34.5% realized selection response at its second generation of selection.

Multilocational field-testing of improved strain (3rd generation) is in-progress at three sites (Jallandhar, Punjab; Vijayawada, Andra Pradesh; and Rahara, West Bengal).

Survey of carp hatcheries in Orissa State undertaken by CIFA to determine the rate of inbreeding revealed high rate of inbreeding (i.e. 1.67-12.16%) for different carp species in these hatcheries. The rate of inbreeding was observed to be higher in the case of exotic carps than in indigenous carps. Guidelines were prepared for the hatcheries to improve the genetic status of carp stocks.

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New Development in Tilapia Research

The Freshwater Fisheries Research Center (FFRC) of the Chinese Academy of Fisheries Sciences has succeeded in hybridization of *Tilapia* sp (Family Cichlidae) with *Siniperca chuatsi* (Family Serranidae).

FFRC has optimized the best conditions for hybridization and the treatment of fertilized eggs. According to FFRC, the success would provide data on mechanisms of hybridization on the one hand and could breed a new fish species better than their parents on the other. As is well known, tilapia grows fast with omnivorous feeding habit and strong resistance to diseases but they cannot endure low temperature. *S. chuatsi* has quality meat and is cold resistant with great economic value but they subsist on live feeds throughout their life cycle. The new hybrid is expected to have desirable traits of parents.

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Patented Genetically Modified Salmon

The European Patent Office has approved the first patent for a genetically modified Atlantic salmon. The modified fish which is owned by a Canadian company, Genesis Group Inc and Massachussetts-based Aqua Bounty Farms carries an additional gene for faster growth. The fish has been reported to grow eight times bigger than usual. Strong concerns have been expressed however by some groups calling for a ban to release the genetically modified fish in the commercial market.

Source: INFOFISH International 6/2001 (November/December).

Improved Strain of Freshwater Prawn

A private company in Thailand, Charoen Pokhphand Foods, has developed a new breed of giant freshwater prawn (*Macrobrachium rosenbergii*), which has higher meat yield and size uniformity than the traditionally farmed strain. The improved strain has a meat content of as high as 60%, twice of the undeveloped strain and is harvestable after four months. A hatchery, with an initial capacity of 50 million post-larvae a month, is being built in Ayuthaya. In view of this positive development, the new breed is seen as a potential substitute for the black tiger shrimp (*Penaeus monodon*), after a ban was imposed on the farming of this species in inland areas of Thailand following complaints that it was causing salinization of paddy fields and fruit orchards.

Source: INFOFISH International 6/2001 (November/December).

Publication of Interest *(in Chinese)*

Book on biodiversity and conservation of major fishes in Yangtze River, China

The Yangtze River is the major habitat of important fish resources and cradle of freshwater aquaculture in China. The river basin has produced 60% of freshwater fish production (by weight) in the country. However, the stress on biodiversity especially on aquatic animals is high in this river. The book entitled *A Study on Biodiversity and Conservation of Major Fishes in the Yangtze River* (in Chinese) by Li Sifa describes the author's extensive work on genetic resources and sustainable development of fisheries in the Yangtze River. The 170-page book focuses on fish biodiversity, particularly on genetic diversity of important and/or endangered economic fish species.

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