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Discovery of Performance Traits-Linked Microsatellite Markers in Channel Catfish (*Ictalurus punctatus*)

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Genomics research has two ultimate applied goals: to isolate and clone genes of economic importance for biotechnology and gene-assisted selection (GAS), and to locate and use markers for marker-assisted selection (MAS) in selective breeding programs. To this end, we have identified linked markers for feed conversion efficiency, growth rate, and disease resistance to enteric septicemia of catfish (ESC). Three microsatellite markers, Ip266, Ip384, and Ip607 were identified to be linked to feed conversion efficiency. Similarly, one marker each was identified to be linked to growth rate (Ip607) and disease resistance to ESC (Ip477). Ip607 marker linked to both growth rate and feed conversion efficiency, indicating that the QTL for both growth rate and feed conversion efficiency may either be the same or located in the same chromosomal region in the catfish genome. On phenotypic evaluation, certain traits such as growth rate can be accurately evaluated by body weight evaluation, while other traits such as disease resistance can be quite complex. The linked DNA markers will be highly useful for MAS programs and for directing further efforts of genomic mapping for important quantitative traits.

Keywords: Channel Catfish, Microsatellite, Performance, Selection marker

Marker assisted selection (MAS) aims to substitute selection at the DNA level for selection on the basis of phenotype. Thus, marker assisted selection is needed to facilitate selection program. MAS is the use of markers to follow the inheritance of genes, particularly those genes which cannot be readily identified, and allow selection for the presence of a gene in a new progeny. The catalogue of traits and situations, where the intensity and accuracy of phenotypic selection are limited, is long and varied. Several requirements must be met before MAS can be applied in a selection program. First, it requires development of large numbers of polymorphic molecular marker. Second, a proper mapping population (segregating population in terms of polymorphic markers) should be available. Third, determination of genetic linkage between the markers and the performance traits has to be made. Fourth, a gene map would be very helpful for application of MAS even though it is not required.

Although several types of efficient molecular marker systems such as random amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP) have been used in catfish (Liu et al., 1998a; Liu et al., 1998b) and rainbow trout (Young et al., 1998), microsatellite markers offer several advantages (Ozaki et al., 2001). They are abundant, codominant, small in size, and highly polymorphic among highly related individuals, which provides tools for ecological studies of closely related populations (Hughes and Queller, 1993). Such population genetic analysis, when conducted in proper families established for genetic linkage and quantitative trait loci (QTL) analysis, would make it possible to localize genes and construct genetic linkage and QTL maps.

Despite the development of the aquaculture and catfish industry in the U.S., a large trade deficit, 4.5 billion dollars, exists for aquaculture for products. Additionally, several production problems still face the catfish industry related to disease resistance, growth, feed conversion, resistance to poor water quality, harvesting, carcass yield, and reproduction. Catfish production needs to be increased and more efficiently to increase farm profitability, provide production for increased exports to reduce out trade deficit, reduce pressure on natural fisheries which have reached maximum sustainable yield. Genetic improvement of broodstock is a proven method of addressing these problems. Little is known about

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population structures and genetic resources variations of catfishes at the molecular level. Such reality demands QTL analyses of complex traits because of its potential to facilitate the manipulation of important traits in fish breeding. Many ecological issues involving genetics need to be addressed for catfishes. For instance, channel catfish is the most important cultured fish in the United States because of its importance as a food and game fish. However, its genetic resource variations are not known.

We previously reported high levels of evolutionary conservation of microsatellite flanking sequences among several catfish species (Liu et al., 1999; Tan et al., 1999). We present here the discovery of 3 polymorphic microsatellite markers which associate with feed conversion efficiency, growth rate, and disease resistance to enteric septicemia of catfish (ESC).

Channel catfish were selected the top (best)/bottom (worst) 5% of population for the quantitative traits, feed conversion efficiency, growth rate, and disease resistance to ESC six months after stocking. 10 fish of the top and bottom 5% were randomly selected and their blood for the preparation of genomic DNA was drawn for further analysis of genotyping using microsatellite markers. The growth rate was measured by total length and weight each fish, feed conversion efficiency by the formula of feed conversion ratio, and disease resistance to ESC by exposing to ESC virus up to LD80.

One hundred pairs of primer were used for genotyping the catfish selected. The primers used were previously described by Liu et al (1999) and Tan et al (1999). Approximately 100 ng of catfish genomic DNA was amplified in PCR reactions of 25 μl containing 2.5 μl of 10x *Taq* buffer (1.5 mM $MgCl_2$ final concentration), 0.5 μl dNTPs (final concentration 0.2 mM each), 1 μl of each primer (4 ng/ml final concentration), 0.5 μl (2.5 Units) of *Taq* DNA Polymerase (Display Systems Biotech., USA) and 18.5 μl of nuclease free water. The PCR started with one cycle consisting of denaturing (94°C) for 4 min, annealing (55°C) for 1 min, and extension (72°C) for 1 min. The first cycle was followed by 30 cycles consisting of 1 min intervals of denaturing at 94°C followed by annealing at 55°C followed by extension at 72°C. These cycles were followed by a final 10 min extension step (72°C). For the primers that did not efficiently amplify the expected products at 55°C, they were tested at 45°C, 50°C, or 60°C. The products were separated by gel electrophoresis (2% agarose) and stained with ethidium bromide to determine the sizes of the amplicons.

Among 100 microsatellite loci tested, 4 microsatellite markers showed a distinct polymorphism between the best and the worst individuals (Table 1). Especially, three microsatellite markers, Ip266, Ip384, and Ip607 were identified to be linked to feed conversion efficiency since the best and worst feed converters exhibited drastically different genotypes at three loci of 100 microsatellite loci tested (Fig. 1). Ip266 microsatellite locus revealed heterozygous genotype in the best converter and homozygous genotype in the worst converter. Ip266 showed 3 alleles in the best converter due to the duplication of the primer-binding site in the target locus. For the Ip607 microsatellite locus, the genotype for both converters was reverse pattern of Ip266. Ip384 microsatellite marker produced heterozygous pattern for both converters. Similarly, one marker each was identified to be linked to growth rate and resistance to ESC. The microsatellite locus, Ip477, linked to disease resistance to ESC showed homozygous type in resistant individuals and heterozygous type in the susceptible individuals. Interestingly, the marker, Ip607, linked to growth rate is one of the three markers linked to feed

Table 1. Characterization of performance traits-linked microsatellite markers.

Locus names	Accession No.	Repeat Units	Primer sequences	Annealing temp (°C)	PCR product (bp)
Ip266	AF114781	$(GT)_{13}T_4(GT)_4$	Sense: 5' TGCATTCACTTTGTCTGTCGAT 3' Antisense: 5' AACACACTACAGAGTCCCATGCT 3'	55	210
Ip384	AF114771	$(AAAT)_{12}$	Sense: 5' CATCTGTAACACCTGCCAGCC 3' Antisense: 5' TAGGCATGTCCAGAAGTAAGGG 3'	50	189
Ip477	AF114766	$(CA)_{25}$	Sense: 5' GCAATAATTACACCAGAATG 3' Antisense: 5' GGAACCAGCTGTTAAAACTG 3'	55	114
Ip607	AF114758	$(GA)_{24}$	Sense: 5' TCAGGCACAAATCTTGTGATGG 3' Antisense: 5' TTGTAGTTCTGCTGCCTCTAACCGC 3'	50	174

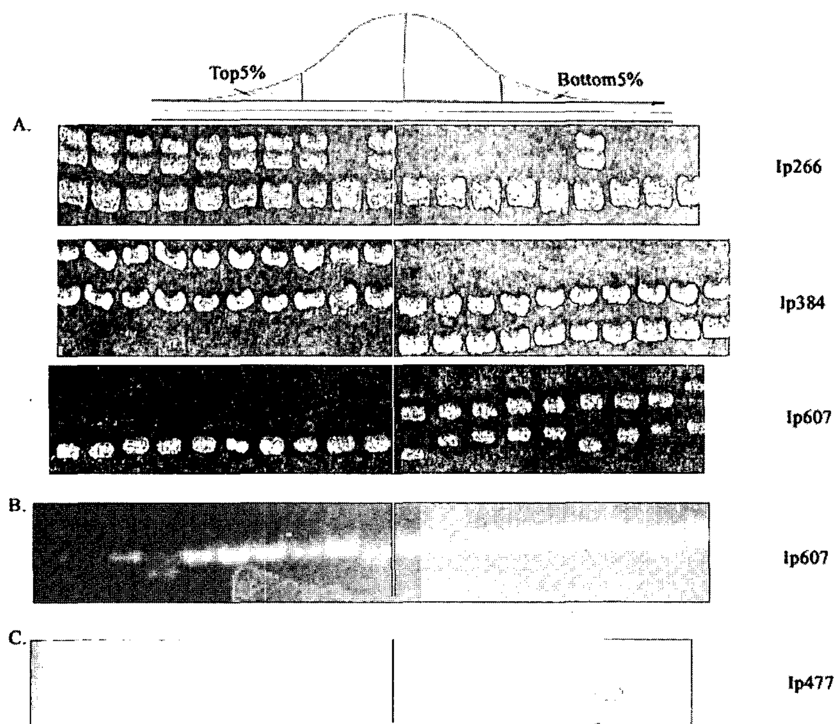

Fig. 1. Genotyping the best group and the worst group for feed conversion efficiency (A), growth rate (B) and disease resistance to enteric septicemia of catfish (C). The vertical middle line indicates the border line between the best group and the worst group. Products were separated on 2% agarose gel and stained with ethidium bromide.

conversion efficiency, consistent with the notion that growth rate and feed conversion efficiency are correlated traits in catfish. This also demonstrates, for the first time at the molecular level, that the QTL for growth and the QTL for feed conversion efficiency may either be the same or located in the same chromosomal region in the catfish genome. Not all the genotypes correlate with the phenotype groups. Theoretically, there are two possible reasons for this: recombination fraction and errors in phenotypic evaluation. The more distant the linked marker is to the QTL controlling the phenotype, the greater the recombination fraction should be. On phenotypic evaluation, certain traits such as growth rate can be accurately evaluated by body weight evaluation, while other traits such as disease resistance can be quite complex. The 4 linked DNA markers will be highly useful for MAS programs, and for directing further efforts of genomic mapping for important quantitative traits.

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