USE OF MULTI-LOCUS DNA FINGERPRINTING FOR STRAIN IDENTIFICATION IN CHANNEL CATFISH, *ICTALURUS PUNCTATUS*

Brian G. Bosworth
and
William R. Wolters
U.S. Department of Agriculture, Agriculture Research Service
Catfish Genetics Research Unit
P.O. Box 38
Stoneville, Mississippi 38776
Ph. (601)-686-5460 / FAX (601)-686-3044

Introduction

Channel catfish culture is the largest aquaculture industry in the United States. More than 430 million pounds of catfish were processed in 1994 with an average price paid to producers of $0.77 per pound (USDA 1996). Development and commercial use of improved germplasm (e.g. faster growing, disease resistant) have dramatically increased production efficiency in many livestock species and similar production increases should be possible through genetic improvement of catfish. Therefore, one of the mission objectives of the Catfish Genetics Research Unit (CGRU), USDA-ARS is to develop genetically improved catfish strains for release to the industry. As part of the CGRU germplasm development program, we are attempting to identify strain-specific molecular markers for strains scheduled for release. Strain-specific markers will be useful for maintaining strain integrity and providing proof of strain-type following release of fish to the industry.

Polymorphisms at isozyme loci can be used to distinguish among blue catfish (*I. furcatus*), channel catfish and their hybrids, but levels of polymorphism at these loci are too low within channel catfish to be useful for strain identification (Carmichael et al. 1992). Multi-locus DNA fingerprinting, a technique used to visualize restriction fragments at numerous, highly polymorphic loci, has been used to estimate relationships among populations of other fish species (Dahle 1994, Spruell et al. 1994, Naish et al. 1995) and may be useful for strain identification in catfish. The objectives of this research were to identify restriction enzyme/multi-locus probe combinations useful for DNA fingerprinting channel catfish and to identify strain-specific DNA fingerprint patterns in CGRU strains.

Methods

DNA isolation and fingerprint preparation were performed using techniques described for DNA fingerprinting of poultry (Dunnington et al. 1990). Genomic DNA was isolated from whole blood by phenol:chloroform extraction and ethanol precipitation, 10 ug samples of DNA were digested with 10-20 units of restriction enzyme, and digested DNA was electrophoresed in a 0.8% agarose gel in 1X TBE at 35 volts for 60-65 hr. After electrophoresis, DNA was transferred to a nylon membrane (MSI) by capillary action, the membrane was hybridized with alkaline phosphatase conjugated repeat probes (FMC BioProducts or Zeneca) and subjected to stringency washes. Membranes were exposed to X-ray film (Amersham) for 2-4 hours at 37 C. Autoradiograms were scanned (Sharp JX-330) and fingerprint patterns were analyzed with Advanced Quantifier® 1-D software (BioImage). Band sharing was calculated as: 2(Nab)/(Na + Nb), where Nab = number of bands shared between samples a and b, Na = total number of bands

in sample a, and Nb = total number of bands in sample b. Error tolerance (the amount a band can deviate in size and still be considered a match) was set at 1.5 %.

Five restriction enzymes: *Alu* I, *Dpn* II, *Hae* III, *Hinf* I, *Rsa* I, and 13 probes: CAn, GAn, ACGn, CACn, CGCn, CTCn, ACAGn, ATCCn, AGATn, Alu/Sli, MV1 middle repeat (FMC BioProducts), 33.15 and 33.6 (Zeneca) were tested to identify restriction enzyme/probe combinations useful for DNA fingerprinting channel catfish. Fingerprints were produced with DNA isolated from blood of individual fish and from blood pools (mixes of equal amounts of blood from 10 individuals). Three pooled samples (a total of thirty fish) from each of five CGRU catfish strains (albino, Mississippi normal, USDA-102, USDA-102 select, and USDA-103) were fingerprinted and band sharing within and among strains was calculated. Nineteen individuals from each of two strains being developed for release (USDA-102 select and USDA-103) were fingerprinted and within-strain band sharing was calculated. Fingerprints of individual and pooled samples were examined to identify banding patterns unique to USDA-102 select and to USDA-103 strains. Nine USDA-102 selects, 8 USDA-103s, and 18 fish from three commercial catfish farms were fingerprinted to determine if level of band sharing at diagnostic bands could be used to correctly identify strain-type.

Results

Useful fingerprints were produced by digesting DNA with *Alu* I, *Dpn* II, or *Hinf* I and hybridizing with CACn, CGCn, CTCn, ATCCn, and AGATn probes. Probe hybridization and wash conditions are listed in Table 1. The enzyme/probe combinations used typically produced 25-30 scoreable bands (size range 6-23 kb) per sample.

Table 1. Hybridization temperature and stringency wash conditions for oligonucleotide probes used to DNA fingerprint channel catfish.

Probe	Hybridization Temperature[a]	Stringency Wash Conditions[a]
CAC	47°C	Wash 1, 2x for 10 minutes at 47°C
		Wash 2, 2x for 10 minutes at 47°C
CGC	47°C	Wash 1, 2x for 10 minutes at 47°C
		Wash 2, 2x for 10 minutes at 47°C
CTC	45°C	Wash 1, 2x for 10 minutes at 45°C
ATCC	42°C	Wash 1, 2x for 10 minutes at 42°C
GATA	42°C	Wash 1, 2x for 10 minutes at 42°C

[a] Hybridization buffer and wash solutions are proprietary products provided by the probe supplier, FMC BioProducts.

Alu I produced fingerprints with resolvable bands, but the fingerprint patterns were too polymorphic to be useful for strain identification. The remaining restriction enzymes and probes were not useful for fingerprinting catfish due to presence of too many bands and/or intense background signal (Hae III, CAn, GAn, 33.15, and 33.6) or few to no bands (Rsa I, ACAGn, ACGn, Alu/Sli and MV1).

Band sharing within strains was generally 30-40% higher than band sharing among strains (Table 2). Band sharing within strains was lowest for abinos (mean for all enzyme/probe combinations = 60%) and highest for USDA-102 selects (86%). The USDA-102 select strain had high band sharing (78%) with its founder population, USDA-102. Within-strain band sharing values for the various enzyme/probe combinations used to fingerprint individual fish ranged from 37-53% for USDA-102 selects and from 33-44% for USDA-103s. No strain-

specific bands were found in USDA-102 selects or USDA-103s, but several enzyme/probe combinations revealed 2-3 bands present in more than 75% of individuals from each of these strains and absent or at a low frequency in other CGRU strains.

Table 2. Mean band sharing (%) within and among 5 channel catfish strains fingerprinted with 10 restriction/enzyme probe combinations.

Strain		Albino		USDA-103		Mississippi Select		USDA-102		USDA-102 select	
	Probe	*Dpn* II	*Hinf* I	*Dpn* II	*Hinf* I	*Dpn* II	*Hinf* I	*Dpn* II	*Hinf* I	*Dpn* II	*Hinf* I
	CACn	68	61	39	41	62	47	36	41	47	35
	CGCn	74	71	41	45	50	55	41	41	46	49
Albino	CTCn	53	58	43	39	52	44	42	30	44	34
	ATCCn	46	44	40	28	45	30	40	32	36	27
	ATAGn	58	69	46	52	51	59	46	48	41	50
	CACn			86	74	53	39	44	40	41	30
	CGCn			83	78	51	41	48	32	45	38
USDA-103	CTCn			87	76	52	44	34	41	31	43
	ATCCn			73	81	41	29	50	36	43	42
	ATAGn			84	71	49	38	56	36	51	42
	CACn					84	72	58	42	49	47
	CGCn					79	72	54	31	39	39
Mississippi	CTCn					76	66	45	41	44	39
Select	ATCCn					68	80	58	38	55	42
	ATAGn					84	77	59	51	45	43
	CACn							96	77	72	71
	CGCn							81	82	82	74
USDA-102	CTCn							92	87	88	79
	ATCCn							94	79	86	68
	ATAGn							98	80	81	75
	CACn									86	78
	CGCn									88	83
USDA-102	CTCn									91	86
Select	ATCCn									89	75
	ATAGn									96	84

Level of band sharing at diagnostic bands visualized with Hinf I/ATAGn and Hinf I/ATCCn resulted in correct strain assignment for all 9 USDA-102 selects tested (based on $\geq$50% band sharing at 6 diagnostic bands) and correct assignment of 7 of 8 USDA-103s ($\geq$40% sharing at 5 diagnostic bands). One of the 18 fish from the commercial farms was incorrectly assigned to the USDA-103 strain.

Discussion

DNA fingerprints were produced in channel catfish using two restriction enzymes (*Hinf I* and *Dpn II*) and five probes (CACn, CGCn, CTCn, ATCCn and ATAGn). Within-strain band sharing of individual USDA-102 select (37-53%) and USDA-103 catfish (33-44%) were similar to band sharing values reported in other fishes (Harris et al. 1991, Bosworth et al. 1994, Spruell et al. 1994). The high levels of band sharing among DNA pools within strains and between USDA-102 selects and their founder strain, USDA-102, indicate that band sharing among groups was reflective of their genetic similarity. Multi-locus fingerprinting also has been used to accurately estimate genetic similarities among tilapia strains (Naish et al. 1995), inbred poultry lines (Plotsky et al. 1995), and cattle (Mannen et al. 1993). The relatively low within-strain band sharing in alinos was surprising because it was thought that this strain was founded by a limited number of fish from a single strain. The apparent low genetic similarity in the alino strain may be the result of the founder population being a mix of two or more strains.

Our preliminary data indicate that multi-locus fingerprinting can be useful for catfish strain identification. Although the sample size was small (35 fish), the ability to correctly identify strain-type in 16 of 17 fish based on level of band sharing at 'diagnostic' bands was encouraging. However, additional work is needed to clearly define levels of similarity at diagnostic bands needed for accurate determination of strain classification. In particular, a large number of fish from different commercial farms should be screened to ensure that bands identified as diagnostic for a strain are not present at high frequencies in existing commercial stocks.

Multi-locus DNA fingerprinting with non-radioactive probes can be conducted in labs with minimal equipment and allows simultaneous screening of numerous polymorphic loci. However, disadvantages of multi-locus fingerprinting include the time needed to produce results (5-6 days), amount of DNA required (5-10 ug per sample), and variation in banding patterns due to slight differences in transfer efficiency or hybridization and wash conditions (O'Reilly and Wright 1995). Therefore, we are also examining the ability to identify catfish strains based on polymorphisms at microsatellite loci. Although initial costs for developing primers for a large number of microsatellite loci are high, PCR amplification of microsatellites requires small amounts of DNA and will allow rapid and consistent genotyping of large numbers of fish.

Regardless of the techniques used to visualize polymorphisms, the use of highly variable DNA markers appears to have potential for strain identification in catfish. Use of strain-specific markers will allow producers who propagate and distribute released strains, to maintain genetic integrity of their stocks and provide proof of strain integrity to individuals interested in purchasing a particular strain.

Acknowledgments

The authors thank Dr. Geoffrey Waldbieser for manuscript review and we also thank the Mississippi Agriculture and Forestry Experiment Station, Lando Fratesi and Leigh Holland for providing fish used in this research. Mention of a trade name, proprietary product, or specific equipment does not constitute a guarantee or warranty by the U.S. Department of Agriculture and does not imply approval to exclusion of other products that may be suitable.

References

Bosworth, B.G., E.A. Dunnington, G.S. Libey, and L.C. Stallard (1994). Restriction enzyme/multi-locus probe combinations useful for DNA fingerprinting of the striped bass, white bass and their F1 hybrid. Aquaculture 123:205-215.

Carmichael, G.J., M.E. Shcmidt and D.C. Morizot (1992). Electrophoretic identification of genetic markers in channel catfish and blue catfish by use of low-risk tissues. Transactions of the American Fisheries Society 121:26-35.

Dahle, G. (1994). Minisatellite DNA fingerprints of Atlantic cod (*Gadus morhua*). Journal of Fish Biology 44:1089-1092.

Dunnington, E.A., O. Gal, Y. Plotsky, A. Haberfeld, T. Kirk, A. Golberg, U. Lavi, A. Cahaner, P.B. Siegel, and J. Hillel (1990). DNA fingerprints of chickens selected for high and low body weight for 31 generations. Animal Genetics 21:247-257.

Harris, A.S., S. Bieger, R.W. Doyle, and J.M. Wright (1991). DNA fingerprinting of tilapia, *Oreochromis niloticus,* and its application to aquaculture genetics. Aquaculture 92: 157-163.

Mannen, H., S. Tsuji, F. Mukai, N. Goto, and S. Ohtagaki (1993). Genetic similarity using DNA fingerprinting in cattle to determine relationship coefficient. Journal of Heredity 84:166-169.

Naish, K.A., M. Warren, F. Bardakci, D.O.F. Skibinski, G.R. Carvalho and G.C. Mair (1995). Multilocus DNA fingerprinting and RAPD reveal similar genetic relationships between strains of *Oreochromis niloticus* (Pisces: Cichlidae). Molecular Ecology 4:271-274.

O'Reilly, P. and J.M. Wright (1995). The evolving technology of DNA fingerprinting and its application to fisheries and aquaculture. Journal of Fish Biology 47:29-55.

Plotsky, Y., M.G. Kaiser, and S.J. Lamont (1995). Genetic characterization of highly inbred chicken lines by two DNA methods: DNA fingerprinting and polymerase chain reaction using arbitrary primers. Animal Genetics 26:163-170.

Spruell, P, S.A. Cummings, Y. Kim and G.H. Thorgaard (1994). Comparison of three anadromous rainbow trout (*Oncorhynchus mykiss*) populations using DNA fingerprinting and mixed DNA samples. Canadian Journal of Fisheries and Aquatic Sciences 51: 252-257.

USDA (U.S. Department of Agriculture) (1996). Farm Raised Catfish Processor Report. National Agricultural Statistics Service, Agricultural Statistic Board, USDA, AQ-1, January 1996.