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Rapid Isolation of DNA for Genetic Screening of Catfishes by Polymerase Chain Reaction

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Abstract.—We evaluated two methods, boiling and microwave irradiation, for the rapid isolation of DNA from barbel, adipose fin, and blood of channel catfish *Ictalurus punctatus*. Compared with routine DNA isolation methods, these procedures were fast (microwaving, 2–4 min; boiling, 10–18 min), simple, and inexpensive (about US$0.10/fish). Samples of DNA isolated from barbels of small (mean ± SD, 2.9 ± 1.0 g) and large (290 ± 43 g) fish were of high purity (ratios of absorbances at 260 and 280 nm, A_{260}/A_{280} = 1.72–1.90) and were between 0.29 and 0.67 μg/μL in concentration. Samples of DNA isolated from barbel, adipose fin, and blood of small fish, by either method, were used successfully for analysis by polymerase chain reaction (PCR). Samples isolated by boiling of barbel and blood from large fish also proved useful for PCR analysis. These DNA isolation procedures would be useful for rapid genetic screening of channel catfish. Removal of the barbel for tissue analysis would also enable direct marking of fish, and after analysis, individuals designated for further study could be identified.

Screening of large numbers of individuals for specific genetic markers is valuable for genetic improvement and study of fishes. Standard procedures involve killing small fish or collecting material from large fish to perform DNA hybridization analysis. The polymerase chain reaction (PCR) is a recently developed technique allowing the in vitro amplification of specific DNA sequences by use of primers designed specifically for a target gene (Taylor 1993). The polymerase chain reaction has become one of the most widely used techniques of molecular biology because it is rapid, inexpensive, and produces useful amounts of DNA from small quantities of source DNA. Standard procedures for isolation of DNA (e.g., Sambrook et al. 1989) are not practical for screening large numbers of fish by PCR. And if biopsy material is used for DNA isolation, live fish must be tagged so that certain individuals can be identified after analysis.

Channel catfish *Ictalurus punctatus* is a major aquacultural species, and an industry based on this species has been established in the southern United States. Interest in the genetic improvement of channel catfish has grown in recent years. Methods are needed to obtain tissues from live fish for rapid genetic analysis. Tissues in ictalurid catfishes that can be sampled for genetic research without causing death include eight barbels around the mouth, a fleshy adipose fin, and blood. Such tissues have enabled investigators to study live catfishes by protein electrophoresis (e.g., Carmichael et al. 1992). In this study we sought rapid methods of isolating DNA from these tissues of channel catfish for analysis by PCR. The objectives were to (1) develop procedures for isolation of DNA from barbel, adipose fin, and blood by boiling or microwave irradiation; (2) measure purity and concentration of the isolated DNA; and (3) evaluate the isolated DNA for use in analysis by PCR. In addition we describe a useful one-step method for tissue collection and identification of individual channel catfish.

Methods

Tissue collection.—Channel catfish were raised at the Aquaculture Research Facility, Louisiana State University (LSU), Baton Rouge. Small fish (mean ± SD, 2.9 ± 1.0 g) and large fish (290 ± 43 g) were studied. Blood was collected from large fish by syringe and from small fish by severing the tail, and was placed into acid–citrate–dextrose solution (Becton-Dickinson vacutainer 4606) and stored in 3-mL vacutainers at −20°C until use. Barbel and adipose fin were removed from fish and stored in 1.5-mL microcentrifuge tubes at −20°C. Sterile scalpels were used to eliminate the possibility of fish-to-fish contamination.

DNA isolation.—We evaluated various proce-

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TABLE 1.—Purity and concentration (means ± SDs) of DNA isolated from different tissues of small (mean ± SD, 2.9 ± 1.0 g) or large (290 ± 43 g) channel catfish by boiling or microwaving procedures. For each variable (purity or concentration), mean values for boiling and microwaving followed by the same letter were not significantly different ($P > 0.05$).

Tissue and fish size	N	Purity (A_{260}/A_{280})		Concentration ($\mu g/\mu L$)	
		Boiling	Microwaving	Boiling	Microwaving
Barbel					
Small	21–24	1.72 ± 0.13 zyx	1.89 ± 0.18 zy	0.29 ± 0.12 w	0.62 ± 0.34 x
Large	18–20	1.90 ± 0.09 z	1.86 ± 0.06 zy	0.67 ± 0.34 x	0.46 ± 0.19 xw
Adipose fin					
Small	21–24	1.54 ± 0.14 x	1.84 ± 0.11 zy	0.26 ± 0.12 w	0.43 ± 0.29 xw
Large	18–20	1.65 ± 0.15 yx	1.62 ± 0.25 x	0.61 ± 0.40 x	0.55 ± 0.32 xw
Blood					
Small	21–24	1.03 ± 0.01 u	1.34 ± 0.10 w	1.69 ± 0.24 z	1.16 ± 0.32 y
Large	18–20	1.14 ± 0.12 v	1.19 ± 0.14 v	1.24 ± 0.45 y	1.23 ± 0.56 y

dures in preliminary studies, including the grinding of frozen tissues. Of these, the most effective procedures were chosen for further development and are reported here. One drop (about 20 μL) of whole blood, or 5 mg of barbel or adipose fin tissue were placed into 1.5-mL microcentrifuge tubes, and 100 μL of 0.1 N NaOH was added. The solution was boiled at 110°C (Thermolyne Dri-Bath, model 17615, Dubuque, Iowa) for about 7 min (blood), 10 min (adipose fin), or 15 min (barbel). For the microwaving method, tubes were placed in a rack and heated in a standard microwave oven (model R310T, Amana Refrigeration Co., Inc., Amana, Iowa) on full power (700 W) for a total of 45 s (blood) or 60–90 s (adipose fin and barbel). Because overheating by microwaving can cause explosion of tubes, we divided the treatment time into 15-s intervals. A plastic clip was used to seal the tubes to minimize the chance of sample loss and contamination. After heating, tubes were centrifuged at 12,000 revolutions/min for 5 min, and the supernatant was used in PCR experiments. Purity and concentration of DNA was measured with a Beckman Du® series 60 spectrophotometer (Beckman Instrument, Inc., Fullerton, California). Purity was estimated by calculating the ratio of the absorbance measured at 260 nm (A_{260}, maximum absorbance of DNA) and the absorbance at 280 nm (A_{280}, maximum absorbance of protein). An A_{260}/A_{280} value of around 1.80 is considered optimal (Sambrook et al. 1989). Concentration of DNA [DNA] was calculated by the formula

$$[\text{DNA}],\ \mu g/\mu L = (A_{260} \times 50 \times 10)/1{,}000;$$

50 is a calculation constant for double-stranded DNA (in $\mu g/\mu L$), and 10 is the dilution factor.

Polymerase chain reaction.—Primers were designed by use of PC/Gene computer software (IntellGenetics, Inc., Mountainview, California) to target the Ch4 exon of the channel catfish gene encoding the constant region of the immunoglobulin M heavy chain, based on the published sequence of the gene (Wilson et al. 1990). Primers were synthesized by the LSU Gene Probes and Expression Systems Laboratory at Baton Rouge. The sequences of the bases (adenine, cytosine, guanine, and thymine) were TCCCCAAGGTTTACTTGCTCGCTCC and CGATGGATCTGGATATTGGCGCAC (5′ to 3′), and from these primers a DNA fragment of 303 base pairs was expected to be amplified by PCR. For analysis, 1 μL of DNA from blood, or 7 μL of DNA from adipose fin or barbel (Table 1), was added to a 100-μL reaction mixture containing 1 μL of each of the primer preparations (30–38 μM), 12.0 μL of 10× PCR buffer, and 8 μL of deoxyribonucleotide triphosphates (1.0 mM each of dATP, dTTP, dCTP, and dGTP). Two units of Vent DNA polymerase (New England Biolabs, Beverly, Massachusetts) were added per reaction, and 30 cycles of amplification were performed with a Perkin–Elmer P3090 thermal cycler. Following a 2-min denaturation step (95°C) in the first cycle, subsequent cycles consisted of denaturation at 95°C (30 s), annealing at 59°C (30 s), and DNA elongation at 72°C (30 s). Two control reactions were included in each analysis. One—channel catfish DNA isolated from blood by the phenol–chloroform method (Tiersch et al. 1992)—was used as a positive control. The other control—human DNA isolated from blood by the phenol–chloroform method—was used to identify the expected sizes of DNA fragments resulting from contamination by human cells.

The PCR products were separated by electro-

TABLE 2.—Success rate (percentage and ratio, number of samples with identifiable bands/total number of samples) of polymerase chain reaction with DNA isolated from different tissues of small (mean ± SD, 2.9 ± 1.0 g) or large (290 ± 43 g) channel catfish by boiling or microwaving procedures. No difference in success rate was observed among the tissues sampled ($\chi^2 = 0.37$; df = 2; $P > 0.05$).

Method	Small fish				Large fish				Overall, pooled by method[a]
	Barbel	Adipose fin	Blood	All samples	Barbel	Adipose fin	Blood	All samples	
Boiling	96% (23/24)	96% (23/24)	71% (17/24)	88% (63/72)	90% (18/20)	45% (9/20)	85% (17/20)	73% (44/60)	81 ± 20% (107/132)
Microwaving	90% (19/21)	95% (20/21)	90% (19/21)	92% (58/63)	44% (8/18)	30% (6/20)	56% (10/18)	43% (24/56)	68 ± 28% (82/119)
Overall, pooled by fish size[b]				90 ± 10% (121/135)				59 ± 24% (68/116)	

[a] Mean ± SD; success rates significantly different between methods ($\chi^2 = 4.34$; df = 1; $P < 0.05$).
[b] Mean ± SD; success rates significantly different between fish sizes ($\chi^2 = 30.61$; df = 1; $P < 0.001$).

phoresis on a 3.0% agarose gel (Amresco, Solon, Ohio) containing ethidium bromide (0.5 μg/mL) in 1× TAE buffer (0.1 M tris-HCl, 12.5 mM sodium acetate, 1 mM EDTA; pH 8.1). The banding of DNA fragments was visualized and photographed on an ultraviolet light transilluminator. The success rate of PCR was based on the presence or absence of a readily identifiable DNA fragment of the expected size for each sample within a treatment. The fragment sizes (numbers of base pairs, bp) were estimated graphically by use of a standard curve derived from data on the mobility of the DNA size markers.

Statistical analysis.—Purity and concentration of isolated DNA were analyzed by three-way analysis of variance (ANOVA); the three factors in this analysis were tissue source (barbel, adipose fin, or blood), isolation method (boiling or microwaving), and fish size (large or small). Duncan's multiple-means comparison was used to identify differences among treatments. The DNA purity data (A_{260}/A_{280}) were transformed to arcsine square root values before analysis. The chi-square test of homogeneity was used to analyze the success rates of PCR for DNA isolated from barbel, adipose fin, or blood; for DNA isolated by the boiling or microwaving methods; and for DNA isolated from small or large fish. Treatments were considered significantly different when $P \leq 0.05$.

Results

Purity and Concentration of DNA

The purity of isolated DNA was significantly different ($P = 0.0001$) among the three tissues (Table 1). The A_{260}/A_{280} values for DNA isolated from barbel (mean ± SD, 1.85 ± 0.15) and adipose fin (1.70 ± 0.18) were significantly higher than the value for DNA isolated from blood (1.20 ± 0.16). No difference was found between DNA isolated from barbel and adipose fin. The purity of DNA isolated by microwave irradiation (1.67 ± 0.30) was significantly greater than that of DNA isolated by boiling (1.52 ± 0.33; $P = 0.0001$). No difference was found between the purities of DNA isolated from small fish and DNA isolated from large fish ($P = 0.4165$).

The concentration of isolated DNA was significantly different ($P = 0.0001$) among the three tissues (Table 1). The concentration of DNA isolated from blood (1.31 ± 0.41 μg/μL) was significantly higher than that from barbel (0.54 ± 0.31 μg/μL) and adipose fin (0.45 ± 0.32 μg/μL). No significant difference in DNA concentration was found between barbel and adipose fin. The concentration of isolated DNA was not significantly different ($P = 0.4011$) between boiling and microwaving, nor was it different between small and large fish ($P = 0.3843$).

Polymerase Chain Reaction

The calculated size of the single fragment amplified from channel catfish DNA was 303 bp, which was the size expected. The banding intensity of PCR products obtained from human DNA was weak, and the sizes of the two fragments (338 and 598 bp) were each different from the size of the DNA fragment obtained from channel catfish DNA.

No significant difference was found in success rate of PCR for DNA isolated from the three different tissues. The success rate was higher when DNA isolated by boiling was used than when DNA isolated by microwaving was used (Table 2). The difference between the two methods was largely

due to the lower success of the microwaving technique in isolating DNA from tissues of larger fish. The DNA isolated from small fish had a significantly higher success rate for PCR than did DNA isolated from larger fish (Table 2).

Discussion

Our study allows for an evaluation of two approaches for the rapid genetic analysis of channel catfish. Both of the DNA isolation procedures were simple and inexpensive, and both minimized the potential for contamination. The entire microwaving protocol required only 2–4 min, and the boiling protocol required 10–18 min. Barbel and adipose fin were easy to collect from large and small fish. Other procedures, such as freezing and grinding of tissues, present a greater risk of contamination because of increased handling and processing of samples. The equipment and chemicals used in this study were readily available, and the greatest expenses were the microcentrifuge tubes and sodium hydroxide. The cost per fish for DNA isolation was about US$0.10.

Techniques for rapid isolation of DNA have been developed for a variety of taxa. The use of microwave irradiation in a procedure different from ours was reported for DNA isolation from prokaryotes, fungi, plants, and reptiles (Goodwin and Lee 1993). Isolation of DNA by boiling frozen and ground tissues in distilled water has been shown to be suitable for PCR amplification of human tissue (Jackson et al. 1993). We found freezing and grinding prior to boiling to be ineffective for processing DNA of channel catfish (data not shown). The DNA isolated by either method from barbel had a purity comparable to DNA isolated by standard methods such as phenol–chloroform extraction (Sambrook et al. 1989; Ausubel et al. 1993). With further purification, this DNA could be suitable for experiments requiring DNA of high purity.

Quality and quantity of DNA are important for successful use in PCR. Generally 2.0–4.0 μg of DNA isolated from barbel or adipose fin, or 1.0–1.8 μg of DNA isolated from whole blood were suitable for PCR analysis. Although no difference was found between small and large fish in purity and concentration of isolated DNA, the DNA from small fish had a significantly higher rate of success in PCR. The reason for this difference is unclear, but tissue samples from the larger fish appeared to possess a greater amount of lipid than did tissues from smaller fish. Lipids or other molecules present in the tissues of the larger fish could have inhibited the action of the DNA polymerase.

Contamination by human cells did not cause false positive reactions when channel catfish Ch4 primers were used. Two DNA fragments of sizes different from that of the target DNA of channel catfish were amplified from the human genome with the reaction-cycle settings we used. Immunoglobulin Ch4 primers would therefore provide a positive control for channel catfish DNA by which the quality of template DNA could be evaluated when screening for the presence of a transgene. This offers utility in gene transfer research, which is becoming widely used to study mechanisms of gene manipulation, and has been applied to genetic study of a variety of plants and animals, including some fishes (e.g., Maclean and Penman 1990).

An important consideration in genetic research is the marking of fish during sampling. One of our sample sources (barbel) allowed simultaneous tissue sampling and marking of individuals. Various methods have been used to mark fish for communal stocking (e.g., Wydoski and Emery 1989). Most effective are internal tags, but they are expensive and cannot be used in small fish. Clipping of adipose fin is used often in the hatchery because it is easily performed, and the adipose fin does not regenerate after removal. Barbel provides a valuable source of tissue for DNA isolation and enables easy identification of individual fish. Channel catfish have eight barbels in a discrete arrangement: two large, lateral barbels; two dorsal, central barbels; and four ventral barbels. Clipping different combinations of barbels allows individual marking of up to 256 fish. In combination with removal of the adipose fin or a portion of caudal fin, hundreds of fish could be marked individually while providing tissue for genetic screening.

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