

Production and Characterization of Monoclonal Antibodies Specific to Pangasius Catfish, Basa, and Tra

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ABSTRACT: Four IgG (subclass IgG1) class monoclonal antibodies (MAbs) strongly reactive to Asian farm-raised *Pangasius catfish*, tra (*Pangasius hypophthalmus*) and basa (*Pangasius bocourti*), have been developed. These MAbs were raised by immunizing an animal with thermal-stable crude sarcoplasmic protein extract of cooked tra. The MAbs were selected by screening hybridoma clones against more than 70 common fish and meat protein extracts. Two MAbs, T7E10 and T1G11, were found to be specific to the Asian *Pangasius catfish*, tra, and basa, with no cross-reactions with any of the common fish and meat species or with the food additive proteins (bovine serum albumin, soy proteins, milk proteins, egg proteins, and gelatin) tested. MAb T7E10 recognized 2 antigenic proteins (molecular weight approximately 36 and 75 kDa) in raw and cooked tra and basa extracts, while T1G11 bound to several proteins (molecular weight between 13 and 18 kDa) in tra and basa extracts. Two other MAbs, F7B8 and F1G11, recognized a common protein (36 kDa) and cross-reacted with all the fish extracts tested and with several mammalian species. These MAbs can be employed individually or in combination in various formats of immunoassays for rapid identification of *Pangasius catfish*, either raw or cooked. They can also be used to study the biological, biochemical, and physiological aspects of thermal-stable antigenic proteins. This is the first study identifying these thermal-stable antigenic proteins present in *Pangasius catfish* as species-specific biomarkers.

Keywords: basa, immunoassay, monoclonal antibody, pangasius catfish, tra

Introduction

Basa (*Pangasius bocourti*) and tra (*Pangasius hypophthalmus*) are members of the Pangasiidae family of catfish and have been the 2 most popular scaleless fish grown by Vietnamese and Cambodian fish farmers in cages along the Mekong Delta for decades. Basa is a white fish with a delicate texture. Tra grows comparatively faster and is cheaper to produce than basa, its eating quality is inferior to basa because of its thinner fillets and coarser texture. As most importers simply call tra “basa” or even just “catfish,” other importers have created a new brand name for basa, “Cajun Delight Catfish,” which implies that the basa is from the Mississippi, rather than the Mekong delta (SeaFood Business 2001). This labeling is no longer allowed in the United States following an FDA ruling that only species from the family Ictaluridae can be sold as true catfish in the United States (FDA 2002). However, there is still the problem that much of the “basa” currently sold is actually the cheaper substitute, tra and basa have become frequent substitutes for popular wild caught fish such as snapper or grouper in the restaurants. Although annual imports of these Asian *Pangasius catfish* into the United States had surpassed 31 million kilos by October 2008 (Nat'l. Marine Fisheries Service 2008), the terms “basa” and “tra” rarely appear on restaurant menus. According to numerous media investigative reports rampant species substitution in restaurants, where native catfish or high-valued other fish are replaced by imported *Pangasius*, is causing serious economic problems for the domestic seafood and restaurant industry. Species substitution

constitutes consumer fraud, as customers do not receive the premium product they have paid for. The Federal Food Drug and Cosmetic Act, Section 403(b) (FDA 1938) clearly states that a food shall be deemed to be misbranded if it is offered for sale under the name of another food. To discourage the widespread illegal practices of fish species substitution or mislabeling, an economic, reliable, and rapid test is therefore both necessary and urgently needed, especially for the identification of imported tra and basa *Pangasius catfish*.

Although DNA-based PCR assays and protein-based isoelectric focusing (IEF) techniques are currently available for fish species identification (Hsieh 2005), both require the use of major laboratory facilities, long assay times (hours to days), trained analysts to conduct the assays, and authentic fish standards for comparison. The PCR techniques suitable for cooked fish tissue are prone to contamination, expensive, require laborious extraction procedure of the DNA from samples, and suffer from a lack of refereed databases for accurate DNA sequence comparison, while the AOAC official IEF methods can be used only for raw fish identification. In addition, variable sample storage conditions and the presence of closely related species may complicate the data interpretation.

At present, there is no rapid immunoassay, such as a strip test or enzyme-linked immunosorbent assay (ELISA) kit, capable of identifying the species of any fish, including tra and basa. All immunoassays use antibodies, either polyclonal or monoclonal. Monoclonal antibodies (MAbs) are homogeneous populations of antibodies produced by cell-fusion hybridoma technology. They have defined biological activities, consistent specificity, and unlimited production capability (Hsieh and Others 1999). Species-specific MAbs raised against thermal-stable flesh proteins can be used to construct inexpensive rapid (complete in minutes) field

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immunoassay test kits, enabling untrained inspectors or fisherman to verify the true species of a fish sample regardless of any heat treatment and whether it is raw, frozen, cooked, or canned. However, as yet no species-specific MABs are commercially available due to the difficulties associated with developing MABs with desired species specificity. There are a wide variety of finfish species on the domestic and international markets, and most MAB trials conducted so far have resulted in MABs that cross-react with many other nontarget fish species. Research on the development of MABs for finfish species identification is in its infancy. Huang and others (1995) reported 2 MABs, C1C1 and C2A2, for red snapper identification, but noted that against 36 fish and meat species both MABs cross-reacted with 4 other snapper species, making it necessary to use 2 MABs to differentiate red snapper from the other snappers. Asensio and others (2003a) reported the development of MAB 3D12 for the identification of raw grouper fillets, testing the MAB against 19 common fish species obtained from markets, although not against meat or other food proteins. The same group went on to develop another antigrouper MAB, 1A4, raised against heated extract of grouper (Asensio and others 2003b). Although this MAB may have the potential to identify grouper in cooked products, it cross-reacted with wreck fish. There are no reports of the development of any antibodies, polyclonal or monoclonal, specific to Asian *Pangasius* fish species. The objectives of this study, therefore, were (1) to develop MABs specific to both raw and cooked *Pangasius* fish and (2) to characterize these MABs for possible future use in a rapid sandwich enzyme immunoassay.

Materials and Methods

Materials

Tris-buffered saline, 0.5 M Tris-HCl buffer (pH 6.8), 1.5 M Tris-HCl (pH 8.8), TEMED (*N,N,N',N'*-tetra-methyl ethylenediamine), Precision Plus Protein Kaleidoscope Standards, 30% acrylamide/bis solution, Tris/glycine buffer, 10 × Tris/glycine/SDS buffer, supported nitrocellulose membrane (0.2 μ m), and thick blot paper were obtained from Bio-Rad Laboratories Inc. (Hercules, Calif., U.S.A.). Hydrogen peroxide, horseradish peroxidase conjugated goat antimouse IgG (Fc specific), ABTS (2,2'-azino-bis 3-ethylbenzthiazoline-6-sulfonic acid), and β -mercaptoethanol were purchased from Sigma-Aldrich Co. (St. Louis, Mo., U.S.A.). Brom phenol blue sodium salt was purchased from Allied Chemical Corp. (N.Y., U.S.A.). Sodium chloride (NaCl), sodium phosphate dibasic anhydrous (Na_2HPO_4), sodium phosphate monobasic anhydrous (NaH_2PO_4), bovine serum albumin (BSA), sodium bicarbonate (NaHCO_3), sodium carbonate (Na_2CO_3), citric acid monohydrate, sodium dodecyl sulfate, Tween 20, and all other chemicals, reagents, syringe filters (0.45 μ m Millipore, Millex®-HV), Whatman nr 1 paper (Whatman®), and 96-well polystyrene microplates (Costar 9018) were purchased from Fisher Scientific (Fair Lawn, N.J., U.S.A.). All solutions were prepared using distilled deionized pure water (DD water) from a NANOpure DIAMOND ultrapure water system (Barnstead Intl., Dubuque, Iowa, U.S.A.). All chemicals and reagents were analytical grade.

Fish samples

Thirty-one of the 54 species of common food fish samples used were authenticated fish obtained from the Florida Dept. of Agriculture and Consumer Services, Tallahassee, Fla., U.S.A. The remaining of the fish and shellfish samples, either fresh or frozen were purchased from reliable seafood markets in Apalachicola and Tallahassee, Fla., U.S.A., and referred to according to the label on each (Table 1). Scientific species names of unauthenticated fish ob-

tained from the marketplace are not provided in the table because their exact species names were uncertain or unavailable. Fresh beef loin, lamb shoulder, pork loin, frozen dressed rabbit, whole turkey and chicken, and frozen frog legs were purchased from local supermarkets. Horse meat was obtained from a private source. Rat muscles were obtained from the Biological Research Facilities, Florida State Univ. All samples were stored at -80°C until use.

Protein extraction from fish and meat samples

Half-frozen fish fillets were cut into smaller pieces to obtain samples of the desired weight. Around 10 g of half frozen fish samples from each species were weighed into beakers. The beakers were covered with aluminum foil, sealed with adhesive tape, and heated in a boiling water bath for 15 min. After being cooled at room temperature, the cooked samples were mashed into fine particles using a glass rod. Extraction buffer (0.15 M NaCl) was then added to the mashed samples (5 mL/g) and the mixture homogenized for 1 min at 11000 rpm. The homogenized samples were allowed to stand at 4°C for 2 h and then centrifuged at $5000 \times g$ for 30 min at 4°C . The supernatants were filtered through Whatman nr 1 filter paper and stored at -20°C until use. The raw fish protein extracts were prepared by adding 35 mL of 0.15 M NaCl to 5 g of minced raw fish in a beaker, and the mixture homogenized for 2 min at 11000 rpm followed by standing at 4°C for 2 h. The mixture was then centrifuged and filtered as described previously and again stored at -20°C . The nonfish samples, included poultry and meat, were previously ground and then prepared in the same fashion as the fish samples.

Immunization

The crude protein extract of cooked tra prepared as described previously was dialyzed in 10 mM PBS for 24 h using a dialyzing tube with a molecular weight cut-off of 10 kDa. The dialyzed protein extract was filtered through a 0.45- μ m disposable filter into sterile tubes (1 mL/tubes). This crude protein extract was used to immunize animals for monoclonal antibody development. Three female BALB/c mice were immunized subcutaneously with 100 μ g/mouse of the dialyzed extract in phosphate buffered saline (PBS) (immunogen) emulsified with an equal volume of Freund's complete adjuvant. Three booster injections prepared in the same manner using Freund's incomplete adjuvant were applied to each mouse at 4-wk intervals. Test sera from the mice were collected 8 d after each boost by tail bleeding. The titer of the sera was determined by indirect ELISA. The mouse showing the highest titer was injected intraperitoneally with 100 μ g of the immunogen 4 d prior to fusion.

Hybridoma procedures

The spleen cells from the immunized mouse were fused with murine myeloma cells (P3 $\times$ 63.Ag8.653, ATCC CRL 1580) for hybridoma production. The general procedures as described by Köhler and Milstein (1975) were followed with modifications. Those hybridomas secreting antibodies that react with the target immunogen were selected, cloned twice by limiting dilution, and expanded. The initial screening against the immunogen (cooked tra thermal-stable proteins) was performed using indirect ELISA. For a secondary selection, the positive cells from the initial screening were expanded and the supernatants screened for reactivity with the native (raw) tra protein extract to ensure the MABs reacted to both native and heated antigenic proteins, protein extracts from the cooked samples from tra and other fish and seafood species, meats, and various food protein extracts (Table 1). Selection of desirable IgG MAB clones was achieved by using IgG γ -chain specific secondary antibody as a probe in the ELISA screening procedures. The subclass of each MAB was determined using a commercial kit

Table 1 – Immunoreactivity of MAbs T7E10, T1G11 F7B8, and F1G11 against cooked fish and nonfish samples determined by indirect ELISA.

Market name (Scientific name)	T7E10	T1G11	F1G11	F7B8
Basa (<i>Pangasius bocourti</i>)	++	++	+++	+++
Tra (<i>Pangasius hypothalamus</i>)	+++	+++	++	+++
Black Sea Bass (<i>Centropomus striata</i>)	–	+	++	+++
Striped bass (<i>Morone saxatilis</i>)	–	–	+++	+++
Blue catfish (<i>Ictalurus furcatus</i>)	–	–	+++	+++
Channel Catfish (<i>Ictalurus punctatus</i>)	–	–	+++	+++
Hybrid catfish	–	–	+++	+++
Trout cod (<i>Epinephelus maculatus</i>)	–	+	+++	+++
Atlantic Croaker (<i>Micropogonias undulatus</i>)	–	–	+++	+++
Southern flounder (<i>Paralichthys lethostigma</i>)	–	–	++	+++
Camouflage grouper (<i>Epinephelus polyhekadion</i>)	–	–	+++	+++
Dusky Tail Grouper (<i>Epinephelus bleekeri</i>)	–	–	+++	+++
Gag grouper (<i>Mycteroperca microlepis</i>)	–	–	+++	+++
Orange spotted grouper (<i>Epinephelus coioides</i>)	–	+/-	+++	+++
Red grouper (<i>Epinephelus morio</i>)	–	+/-	+++	+++
Redmouth grouper (<i>Aethaloperca rogaa</i>)	–	–	+++	+++
Squaretail grouper (<i>Plectropomus areolatus</i>)	–	–	+++	+++
Wavy lined grouper (<i>Epinephelus undulosus</i>)	–	–	++	++
Tomato Hind (<i>Cephalopholis Sonnerati</i>)	–	–	+++	+++
Dolphine Mahi Mahi (<i>Coryphaena hippurus</i>)	–	+/-	++	+++
Orange roughy (<i>Hoplostethus atlanticus</i>)	–	–	+++	+++
Sheephead (<i>Archosargus probatocephalus</i>)	–	–	+++	+++
Spotted seatrout (<i>Cynoscion nebulosus</i>)	–	+/-	++	+++
Caribbean red snapper (<i>Lutjanus purpureus</i>)	–	–	++	+++
Cubera snapper (<i>Lutjanus cyanopterus</i>)	–	–	++	+++
Gray snapper (<i>Lutjanus griseus</i>)	–	–	++	+++
Lane snapper (<i>Lutjanus synagris</i>)	–	+/-	+++	+++
Red snapper (<i>Lutjanus campechanus</i>)	–	–	+++	+++
Vermillion snapper (<i>Rhomboplites aurorubens</i>)	–	+/-	++	+++
Yellowtail snapper (<i>Ocyurus chrysurus</i>)	–	+	++	+++
Coral trout (<i>Plectropomus leopardus</i>)	–	–	+++	+++
Amberjack	–	–	+	++
Cobia	–	–	++	+++
Cod	–	–	++	++
Black grouper	–	–	+++	+++
Yellow edge grouper	–	–	+++	+++
Haddock	–	–	+++	+++
Alaskan halibut	–	–	+++	+++
Mullet	–	–	++	+++

Continued.

Table 1 – Continued.

Market name (Scientific name)	T7E10	T1G11	F1G11	F7B8
Ocean perch	–	+/-	++	++
Pollock	–	–	++	+++
Pompano	–	–	++	++
Farm salmon	–	–	++	+++
Wild salmon	–	–	+++	+++
Scamp	–	–	+++	++
Hog snapper	–	+/-	+++	+++
Mangrove snapper	–	–	+++	++
Sunfish	–	–	++	+++
Swordfish	–	–	–	+
Tilapia	–	–	+++	++
Idaho rainbow trout	–	–	+++	+++
Yellowfin tuna	–	–	++	+++
Wahoo	–	+/-	++	+++
Whiting	–	–	+	++
Shrimp	–	+/-	+++	+++
Crab	–	–	+	++
Scallop	–	–	+	+++
Chicken, breast	–	–	++	++
Chicken, thigh	–	–	+++	+++
Turkey, breast	–	–	–	–
Turkey, thigh	–	–	+++	+++
Pork	–	–	+/-	+
Beef	–	–	–	++
Lamb	–	–	–	+
Rabbit	–	–	–	+
Horse	–	–	–	+/-
Deer	–	–	–	–
Elk	–	–	–	+
Rat	–	–	+	+++
Frog, legs	–	–	+++	+++
Gelatin	–	–	–	–
Egg albumin	–	–	–	++
Soy concentrate	–	–	–	–
Nonfat dry milk	–	–	–	–
Bovine serum albumin	–	–	–	–

Absorbance readings at 410 nm: < 0.15: “–”; 0.15 to 0.199: “+/-”; 0.2 to 0.499: “+”; 0.5 to 0.999: “++”; > 1: “+++”.

mouse MAb isotyping kit (Sigma) according to the manufacturer's protocol.

Indirect ELISA

One hundred microliters (100 μ L) of each properly diluted sample protein extract containing 2 μ g of soluble protein in 0.06 M carbonate buffer (pH 9.6) was coated onto the wells of a 96-well polystyrene microplate (Costar 9018, Fisher) and incubated at 37 °C for 2 h. The plate was then washed 3 times with PBST [0.05% (v/v) Tween-20 in 10 mM PBS, pH 7.2] and incubated with 200 μ L/well blocking solution (3% NFDM in PBS) at 37 °C for 2 h, followed by another washing step. The undiluted or appropriately diluted MAb supernatants (100 μ L) in antibody buffer [1% (w/v) BSA in PBST] were added to each well and incubated at 37 °C for 2 h. After washing 3 times with PBST, diluted (1:3000 in antibody buffer) horseradish peroxidase-conjugated goat antimouse IgG-Fc specific solution was added. The plate was incubated at 37 °C for 2 h and then washed 5 times before the addition of the substrate solution (22 mg of ABTS and 15 μ L of 30% hydrogen peroxide in 100 mL of 0.1 M phosphate-citrate buffer, pH 4). The color was developed at 37 °C for 20 min. The enzyme reaction was stopped by adding 0.2 M citric acid solution, and the absorbance was then measured at 410 nm using a microplate reader (Model MQX200R, BioTek). This optimized indirect ELISA procedure was used for hybridoma screening as well as MAb characterization.

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE)

SDS–PAGE was performed to separate the soluble proteins in different sample extracts based on their molecular weights according to the method of Laemmli (1970) with modifications. Briefly, soluble proteins (3 μ g of protein in 10 μ L per lane) from the samples were loaded on the stacking gel (5%, pH 6.8) and then separated on the polyacrylamide separating gel (14%, pH 8.8). The gel was subjected to electrophoresis at 200 V for 45 min using a Mini-Protein 3 electrophoresis cell (Bio-Rad, 161-3301) connected to a power supply (Model 3000, Bio-Rad).

Western blot

The Western blot analysis was then carried out according to the method of Towbin and others (1979) with modifications to determine the molecular weights of the immunogenic components that reacted with each MAb. After separation of the proteins on the polyacrylamide gel by means of SDS–PAGE, protein bands were transferred electrophoretically (1 h at 100 V) from the gel to nitrocellulose membranes using a MiniTrans-Blot unit (Bio-Rad). Upon completion of the transfer, the membrane was washed with TBST (20 mM Tris, 500 mM NaCl, 0.05% Tween-20, pH 7.5), blocked with 1% BSA in TBS, and then incubated with the MAb supernatant diluted 1:1 in antibody buffer for 2 h at room temperature. The excess antibody reagent was removed by washing with TBST and the membrane incubated with goat antimouse IgG-alkaline phosphatase conjugate diluted 1:3000 in antibody buffer for 1 h at room temperature. After being washed, the membrane was incubated with 5-bromo-4-chloro-3-indolyl phosphate/*p*-nitroblue tetrazolium chloride (BCIP/NBT) in 0.1 M Tris buffer at pH 9.5 to develop the color. The color reaction was stopped by washing the membrane with distilled water. The appearance of a dark purplish band indicated an antibody binding site. Prestained broad range protein standards (Precision Plus Protein Kaleidoscope Standards, Bio-Rad, 161-0375) were used as molecular weight markers for both the SDS–PAGE and Western blot.

Results and Discussion

MAb development

Four IgG class MAbs, 7E10.D8.E6 (T7E10), 1G11.D3.D12 (F1G11), 1G11.D3.E2 (T1G11), and 7B8.G11.F1 (F7B8) were selected for this study and cloned twice to ensure their stability. All 4 belong to subclass IgG1. MAbs T7E10 and T1G11 are *Pangasius*-specific, while MAbs F7B8 and F1G11 are cross-reactive. The cross-reactive MAbs were selected for their potential utility when paired with a specific MAb in the future development of a sandwich type of immunoassay. Our success in developing these MAbs was due to our adoption of the following strategic approaches. The animals were immunized with dialyzed crude soluble protein/peptide mixtures extracted from cooked tra with 10 mM PBS. The heat treatment of the fish tissue ensured that the proteins that remained soluble in the solution after heating were heat-stable. This is vital, because most sarcoplasmic fish proteins become denatured and insoluble after cooking. The dialysis of the crude immunogen eliminated impurities and small molecules in the protein extract, which ensured that the retained thermal-stable proteins were all immunogenic. An advantage of monoclonal antibody development is that impure antigen can be used for animal immunization, as the ability of such a hybridoma clone to secrete MAbs of interest is primarily dictated by the screening and selection procedures used. We used crude immunogen for the development of the MAbs here as we were endeavoring to recognize a particular type of tissue (for example, tra or

basa fish tissue) rather than an individual protein in the tissue. This is because there is no information regarding a suitable biomarker (antigen) for the tissue currently available. Also, by using this partially purified protein extract for immunization all the individual thermal-stable proteins in the extract became potential antigens to elicit antibody production. Later characterization of these antibodies by Western blot would thus identify these thermal-stable antigens.

Species specificity and cross-reactivity

Species specificity of MAbs was determined by testing each MAb supernatant with both raw and cooked protein extracts from 57 common fish and shellfish species, 13 meat extracts, and 5 extracts from other proteins using indirect ELISA procedures. Four IgG class MAbs showed strong positive immunoreactivity to both raw and cooked extracts of *Pangasius catfish*, tra, and basa. Among these four, MAb T7E10 was specific only to basa/tra and had no cross-reaction with any of the other seafood, meat, and food proteins tested (Table 1). T1G11 was also specific to basa and tra, but had a feeble cross-reaction with a few other fish species. Both *Pangasius* fish specific MAbs showed a stronger reactivity with tra and a weaker reactivity with basa. These 2 *Pangasius*-specific MAbs would therefore be useful to differentiate *Pangasius catfish* from other non-*Pangasius* fish species, including domestic *Ictalurus catfish* such as channel catfish and blue catfish. The remaining 2 MAbs, F7B8, and F1G11, were cross-reactive to all fish and shellfish species as well as some of the mammalian species tested. Table 1 summarizes the species-specificity and cross-reactivity of these MAbs to proteins extracted from cooked (100 °C for 15 min) samples of different seafood and meat species, and several common food proteins (bovine serum albumin, egg albumin, gelatin, and nonfat dry milk proteins) using indirect ELISA. The indirect ELISA signals for raw samples were similar but weaker than the cooked samples because the targeted thermal-stable antigen was diluted several hundred-fold due to the presence of substantial amounts of heat-labile soluble proteins in the solution (data not shown). To improve the signal for this indirect ELISA, the raw sample extracts can be heated for few minutes to remove heat-labile proteins before coating the extract on the microtiter plate.

This is the 1st report of the development of species-specific MAbs that have no detectable cross-reactivity with other common fish and nonfish animal species. Although it is clearly impractical to collect and test samples of all the more than 5000 food fish species regularly consumed worldwide, based on the unambiguous testing results of the present study, which covered 57 of the most commonly consumed fish and shellfish species and included closely related species, it seems unlikely that these MAbs will recognize non-*Pangasius* fish. However, our efforts to test more fish species will continue.

Thermal-stable antigenic components

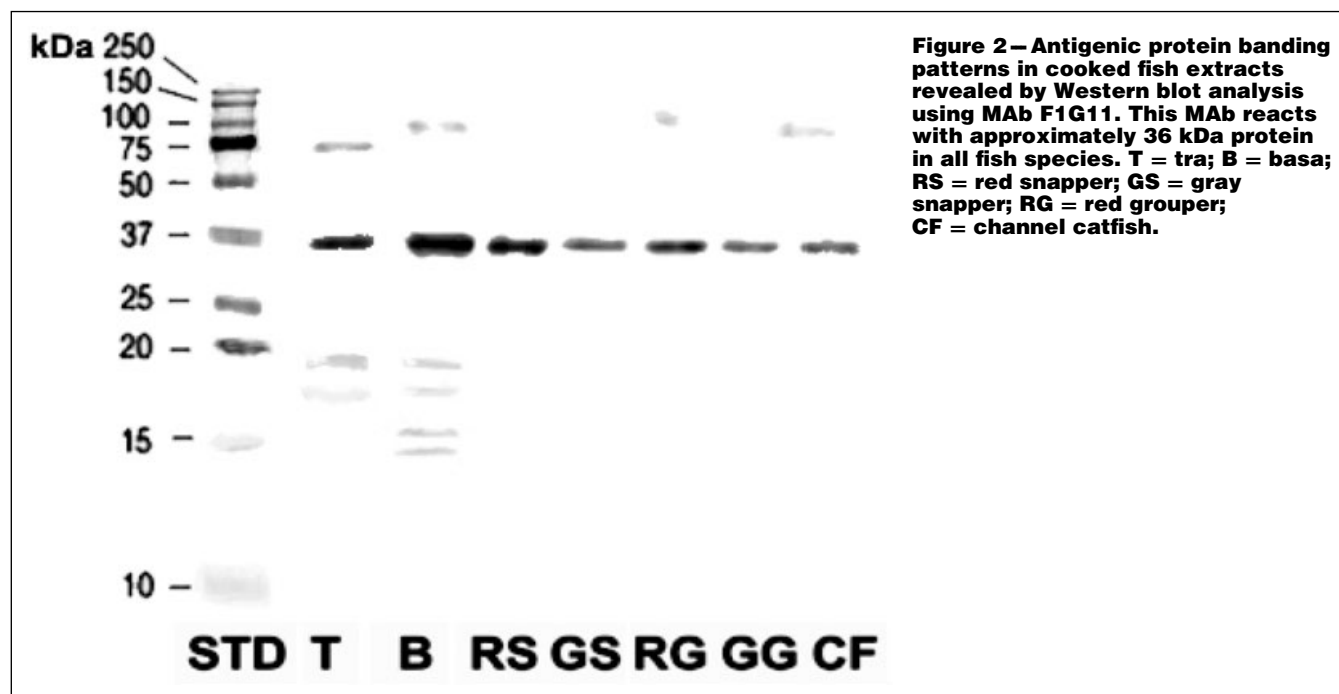
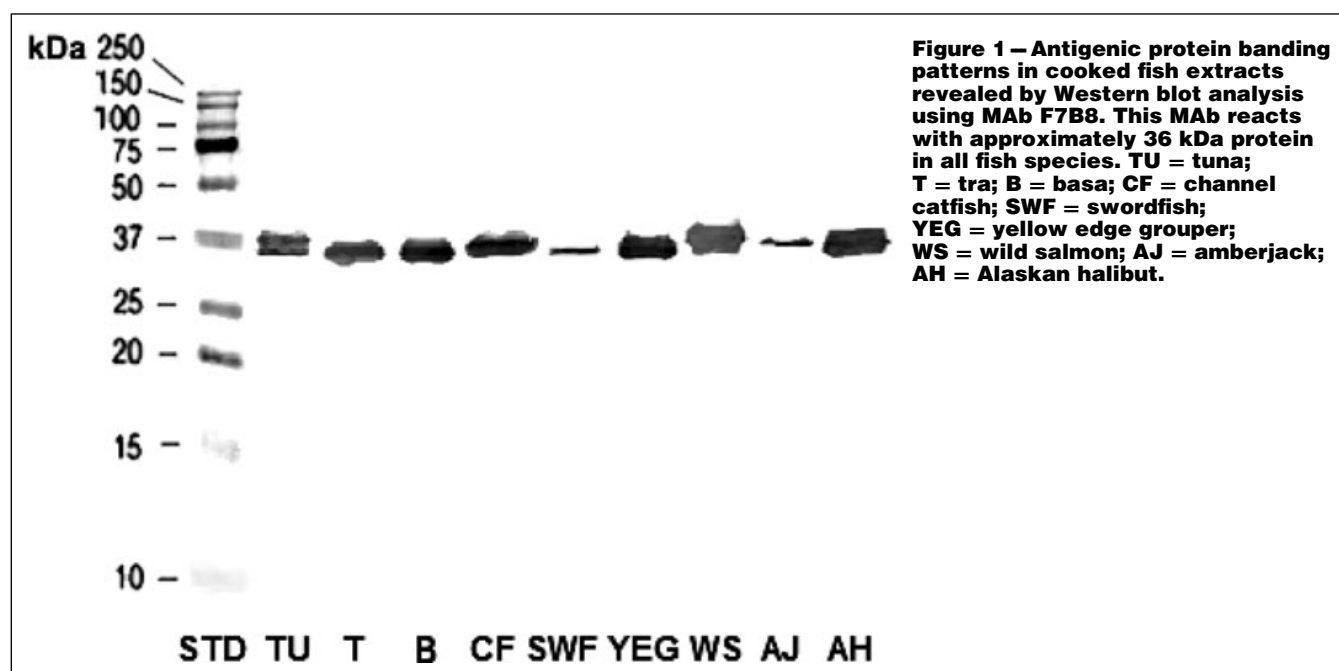
The antigenic proteins, the antigen or analyte in the fish tissue responsible for the binding of the MAbs, were determined by Western blot. While stained SDS–PAGE protein banding patterns of all cooked fish species were generally the same, primarily consisting of 2 major groups of protein bands at 37 and 12 kDa (Gajewski and Hsieh 2009), Western blot results revealed characteristic antigenic bands in different species blotted with different MAbs. Both MAbs F7B8 and F1G11 reacted with a major band around 36 to 37 kDa in the extracts of all the fish samples tested (Figure 1 to 2). Although the 2 *Pangasius*-specific MAbs, T7E10, and T1G11, exhibited similar specificity and ELISA reactivity pattern, they had distinctive binding characteristics, with MAb T7E10 binding to 2 major

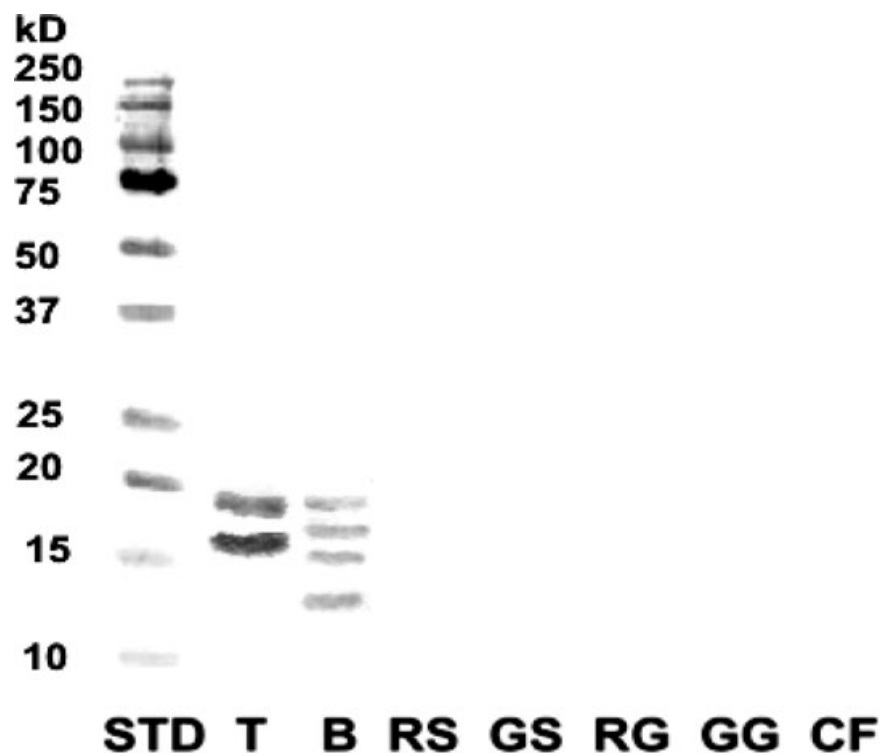
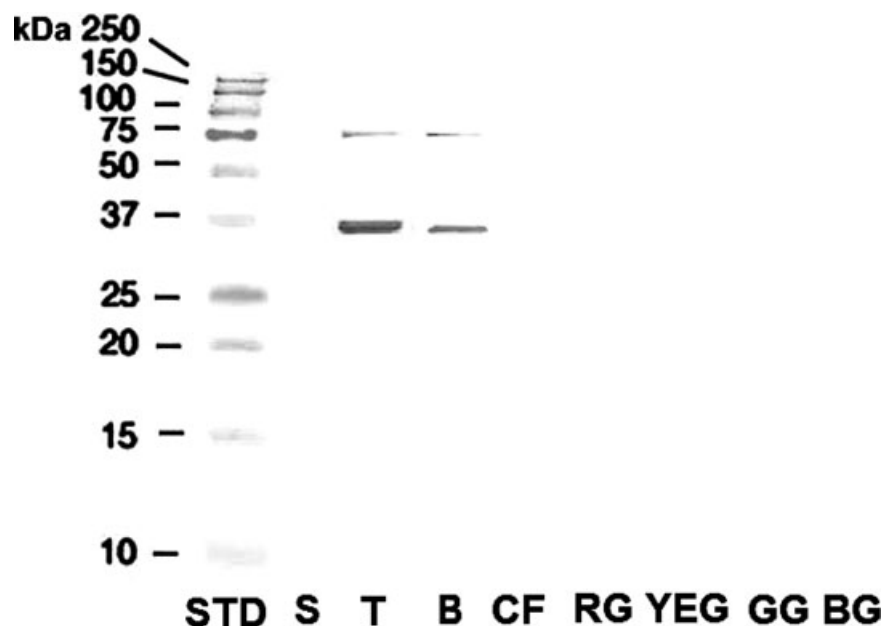
proteins (36 and 75 kDa) in cooked tra and basa extracts (Figure 3), while MAbT1G11 recognized several smaller proteins between 13 and 18 kDa in the basa and tra extracts (Figure 4). Thus, Western blot techniques can be used to differentiate between basa and tra, which is not possible using ELISA. No antigenic protein bands were observed in any of the other samples tested, including snappers, groupers, and *Ictalurus* catfish species (Figure 3 to 4). It is important to stress that these 2 MAb's recognized *Pangasius* catfish but did not react with the domestic catfish species in the *Ictaluridae* family (Channel catfish, Blue catfish, and Hybrid catfish). The 75 kDa band blotted with T7E10 in the cooked tra extract is probably a dimer of the 36 kDa peptide. This band did not appear in the raw sample extract where the quantity of the thermal-stable antigenic proteins is hundreds of times lower in raw fish extract

than in the cooked extract for the same concentration of total soluble proteins separated in the gel. The results for the immunoreactivity obtained from Western blot generally agreed with those obtained using ELISA. Three of the 4 MAb's recognized a common antigen with a molecular weight of approximately 36 kDa; characterization of this antigenic protein is currently underway in our laboratory.

Conclusions

Four MAb's raised against crude protein extract from cooked tra tissue exhibiting specific reaction patterns were developed in this study. Two MAb's (T7E10 and T1G11) strongly reacted with *Pangasius* catfish, basa and tra, but not other fish and nonfish proteins. These *Pangasius*-specific MAb's recognize different antigenic





peptides in fish extracts and can therefore be used not only to differentiate between *Pangasius catfish* and other non-*Pangasius catfish*, but also to further differentiate between basa and tra catfish based on their different antigenic components. The other 2 MAbs (F7B8 and F1G11) raised for this study are cross-reactive with all the fish species tested and are potentially useful partners for the *Pangasius*-specific MAbs for future development of a sandwich immunoassay. The MAbs developed in this study can be employed in various formats of ELISA, including indirect noncompetitive ELISA, competitive ELISA, and sandwich ELISA, as well as in various immunoassay systems, such as 96-well microtiter plates,

lateral flow strip tests, immunosticks, immunoblots, immunodots, and immunosensors, to rapidly differentiate between Asian catfish, tra, and basa, and other fish species. In addition, these MAbs offer valuable information for those studying the chemical, biological, and physiological properties of these species-specific, thermal-stable proteins that are reported in this study.

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