ISSN 1682-296X (Print)
ISSN 1682-2978 (Online)

Bio Technology

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Biotechnology 13 (6): 289-294, 2014
ISSN 1682-296X / DOI: 10.3923/biotech.2014.289.294

Evaluation of Protein Fractions of Indigenous Clariid Fish Species (*Clarias gariepinus* and *Heterobranchus bidorsalis*) and their Reciprocal Hybrids

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Abstract: Electrophoresis of myofibrillar and sarcoplasmic muscle proteins of African catfish: *Clarias gariepinus*, *Heterobranchus bidorsalis* and their reciprocal hybrids in South-West Nigeria was carried out to reveal the similarities and dissimilarities among species in order to aid the selection of suitable strains for aquaculture that could lead to production of new varieties of fishes to alleviate the problem of short supply of fast growing quality fish seeds. The study was aimed at analyzing the muscle protein profiles of *C. gariepinus, H. bidorsalis* and their reciprocal hybrids. Sixteen juveniles fish samples (comprising four samples from each mating combinations) artificially propagated and reared for sixteen weeks were analysed electrophoretically. Myofibrillar and sarcoplasmic fractions were prepared by homogenizing 150 mg of fish muscle in 1.5 mL of rigor buffer containing 10 mM Trismeleates, 60 mM KCl, 5 mM $MgCl_2$, 1 nM EDTA. Extracts were centrifuged in a tube at 10,000 g for 5 min at 4°C. The resultant pellets (myofibrilla) and supernatant (sarcoplasmic) were separated using 12.5% Sodium Dodecyl-Sulphate Polyacrylamide Gel Electrophoresis (1D SDS-PAGE). The relative concentration of individual protein bands were analysed using TotalLab™ 1D software. The individual protein bands in the electrophoregram were identified in relation to their molecular weights. A total of eleven and seven protein bands were resolved in myofibrilla and sarcoplasmic fractions, respectively. The 5th band with Molecular Weight (MW) of 52.23 kDa of the myofibrilla electrophoregram distinguished *C. gariepinus* from *H. bidorsalis* while the 3rd band with MW 119.04, 4th band with MWs 101.49 and 102.13; 8th band with MWs 29.24 and 29.39 kDa distinct the pure breeds from the hybrids. However, in sarcoplasmic fraction, the 3rd and 5th bands with MWs 92.11 kDa and 54.28 kDa, respectively distinguished the hybrids in the while the 7th band with MW 41.67 kDa distinct the pure breeds. Therefore, this research will serve as a bridge between the existing gaps of information available on the muscle protein profile of *C. gariepinus, H. bidorsalis* and their reciprocal hybrids and the study identifies the proteomic classification of clariid species with the aim of enlightening fish researchers and aquaculturist on the characterization of broodstock selection for successful breeding exercise.

Key words: Myofibrillar, sarcoplasmic, rigor buffer, *Clarias gariepinus*, *Heterobranchus bidorsalis*

INTRODUCTION

Clariids are widely considered as an important tropical catfish species for aquaculture. It is well known in both culture and artisanal environments in Nigeria where it serves both socio-cultural and research purposes in most regions. *Clarias gariepinus* and *Heterobranchus bidorsalis* are species of high aquaculture importance in South-Western Nigeria (Oyebola *et al*., 2013). They are widely cultured owing to their high market price, fast growth rate and ability to withstand adverse pond conditions especially low oxygen content (Idodo-Umeh, 2003). *Clarias gariepinus* and its hybrid which is reproductively viable, are cultured throughout Nigeria and most especially in the South Western zone. Hybridization between the two species is common and widely reported among farmers in order to improve quality of fish seed. Hybrids of the two species must be properly identified so as not to replace them with the pure of *Heterobranchus* spp. Intergeneric hybrids of *Heterobranchus* × *Clarias* have been observed to be fertile (Aluko and Shaba, 1999). It was stated that aquaculture practitioners in Nigeria have exploited the fertility of the F1 hybrids by using these hybrids as

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breeders for further propagation. This improper hatchery practice of indiscriminate use of F1 hybrids as breeders should be of immediate concern to conservationist and geneticists as the potential hazards of this process on the catfish gene pool could be the genetic loss of pure indigenous stock of *Heterobranchus* and *Clarias* species (Yisa and Olufeagba, 2005).

In the past, the identification of fish species was carried out mainly by examining the external morphological characteristics. In the present day, electrophoresis of whole muscle protein, sarcoplasmic proteins, serum proteins, liver proteins, salivary proteins and a number of enzymes often have been used by some researchers as an aid in the species identification of fish (Pineiro *et al*., 2001; Yilmaz *et al*., 2007; Lamy *et al*., 2008). Although, a few serum electrophoresis research Diyaware *et al*. (2011, 2012), Akinwande *et al*. (2012), Majolagbe *et al*. (2012) and Oyebola *et al*. (2013) on *Clarias* and *Heterobrachus* sp. have recently surfaced but there appears dearth of information on the fish muscle protein of clariid species in Nigeria (Agbebi *et al*., 2013).

Muscle is the principal component of meat and contains water, proteins, fat, carbohydrates and inorganic constituents (Forrest *et al*., 1975). Water, as a major constituent, accounts for 65-80% of the total muscle weight. Proteins comprise from 16-22% of the total muscle mass and are the principal component of the solid matter. Muscle proteins are generally categorised as described by Huss (1995) as structural proteins (myofibrillar), sarcoplasmic and collagen. Myofibrillar proteins (actin, myosin, torpormyosin and actomyosin) constitute 70-80% of the total protein content, sarcoplasmic proteins (myoalbumin, globulin and enzymes) constitute 25-30% of the protein while connective tissue proteins (collagen), constitute approximately 3% of the protein in teleostei. Myofibrillar proteins are soluble in neutral salt solutions of fairly high ionic strength (0.5 M) while sarcoplasmic which are soluble in neutral salt solutions of low ionic strength (<0.15 M). Their solubility in the various concentrations of salt solutions make them independently separable in electrophoresis.

In 1D-electrophoresis, myofibrillar and sarcoplasmic proteins are separated on polyacrylarnide gel. Most of the electrophoretic studies carried out are related to either total proteins or myofibrillar protein or corneal proteins (Chakravarty *et al*., 2013). However, the proteins in the sarcoplasmic fraction are excellently suited to distinguishing fish species, as each species has a characteristic band pattern when separated by isoelectric methods (Huss, 1995). Therefore, this research will serve as a bridge between the existing gaps of information available on the muscle protein profile of *C. gariepinus*, *H. bidorsalis* and their reciprocal hybrids and also establish the similarities and dissimilarities between the species and their hybrids for the purpose of identification.

MATERIALS AND METHODS

Muscle sample collection: The experimental fish was produced through intergeneric hybridization between *C. gariepinus* and *H. bidorsalis* obtained from Hepa Fish Farm, Asero, Abeokuta, Nigeria. The following crosses were carried out for parental cross: *C. gariepinus* × *C. gariepinus* and *H. bidorsalis* × *H. bidorsalis* and hybrids *C. gariepinus* × *H. bidorsalis*-(Clariabranchus) and *H. bidorsalis* × *C. gariepinus* (Heteroclarias).

Sixteen juveniles fish samples (comprising four samples from each mating combinations) were transported live in well open plastic rubber bowls to the Biotechnology Laboratory Unit of the Animal Breeding and Genetics of the Federal University of Agriculture, Abeokuta. Muscle were isolated from the epiaxial muscle in the region below the cranial to the dorsal fin, above the lateral line of the experimental fish with the aid of a new surgical blade for each species (El-Serafy *et al*., 2006) after fish killing by medullar transaction (El-Serafy *et al*., 2006). Myofibrillar and sarcoplasmic extracts were prepared by homogenizing 150 mg of muscle with the aid of a mortar and pestle (on ice) in 1.5 mL of rigor buffer containing 10 mM Trismeleates, 60 mM KCl, 5 mM $MgCl_2$, 1 nM EDTA (Agbebi *et al*., 2013). The extracts were prepared according to Agbebi *et al*. (2013).

Sample preparation for SDS-PAGE analysis and gel staining: Protein fractions were centrifuged for 5 min at 10,000 × g at room temperature prior the electrophoretic analysis. Samples were loaded into 1 mm × 12 cm × 14 cm 12.5% polyacrylamide gel with 3% stacking gel. First, gel capillary chamber was loaded with a broad range molecular weight standard (BioRad Laboratories, Hercules, CA). Proteins were separated by applying constant voltage of 300 V for 2 h, 30 min until the dye front reached the bottom of the gel. After electrophoretic separation, gels were stained overnight with gentle agitation on an orbital shaker. Staining buffer contains (400 mL methanol, 50 mL glacial acetic acid and Coomassie Brilliant Blue G250) and subsequently destained with 10% acetic acid. Images were digitized and acquired employing a CCD camera (Samsung ES25-Samsung Opto, Electronics Co. Ltd). Raw images were subsequently imported into TotalLab 1D software (Nonlinear Dynamics, Newsacastle upon Tyne, UK) and analyzed (band detection and molecular weight calculations). User defined parameters employed are background subtraction-rolling ball,

radius = 300; band detection: Minimum slope = 30; noise reduction = 3; percent maximum peak = 1.0; Gaussian volumes fitted to peaks-no advanced fitting; R_f calibration aligned with no lane and use curve lines and snap R_f lines to bands were checked (Reddish *et al*., 2008).

RESULTS

Figure 1a and b are the raw image of myofibrilla and sarcoplasmic muscle proteins of *C. gariepinus*, *H. bidorsalis* and their reciprocal hybrids.

Figure 2a and b shows the result of 12.5% SDS electrophoregram analysis. The blue diamonds denotes bands derived from electrophoregram by the TOTALLAB software.

The myofibrillar muscle fraction resolved a total of eleven different protein bands across the gel. The average number of protein bands resolved among the pure breeds; *Clarias gariepinus* and *Heterobranchus bidorsalis* were ten and nine, respectively in all examined species. In the hybrids; *Clariobranchus* and *Heteroclarias* ten protein bands each were detected. The 1st, 2nd, 6th, 7th, 9th, 10th and 11th bands were commonly detected across all mating combinations. In *Clarias gariepinus* the 5th band was present but absent in *Heterobranchus bidorsalis*. The 4th and 8th bands were also present in the pure breeds but absent in the hybrids. The hybrids also had the 3rd band present but absent in the pure breeds. There were no distinguishing bands between the hybrids in myofibrilla fractions.

In the male pure breeds, eight protein bands were resolved for both *C. gariepinus* and *H. bidorsalis* while the male hybrids *Clariobranchus* species and *Heteroclarias* species both resolved six bands. In the female seven protein bands were resolved in both pure breeds; *C. gariepinus* and *H. bidorsalis* and hybrids; *Clariobranchus* species and *Heteroclarias* species resolved. Although, there were species specific bands among individual species there was no clear sexual dimorphic band across the male or female species of all cross combinations.

The sarcoplasmic muscle fraction resolved a total of nine different protein bands were resolved across the gel. The 1st and 9th bands were only present in BRS (Broad Range Standard) therefore, seven protein bands were detected across the mating combinations. The 2nd, 4th, 6th and 8th bands were commonly detected across all mating combinations. The average number of protein bands resolved among the pure breeds; *Clarias gariepinus* and *Heterobranchus bidorsalis* were six and five, respectively in all examined species. In *Clarias gariepinus* the 7th band was present but absent in both *Heterobranchus bidorsalis*. In the hybrids; *Clariobranchus* and *Heteroclarias*, an average number of six and four protein bands each were, respectively detected. The 3rd and 5th bands were present in *Clariobranchus* but absent in *Heteroclarias*.

In the male pure breeds, five protein bands were resolved for both *C. gariepinus* and *H. bidorsalis* while the male hybrids; *Clariobranchus* and *Heteroclarias* species resolved five and four protein bands, respectively. In the females, five protein bands were resolved in pure breeds; *C. gariepinus* and *H. bidorsalis* while three protein bands were resolved in hybrids *Clariobranchus* and *Heteroclarias* species.

The molecular weights of each myofibrillar (Table 1) and sarcoplasmic (Table 2) are presented below for artificially propagated *Clarias gariepinus*, *Heterobranchus bidorsalis* and their reciprocal hybrids in Kilo-Daltons (kDa). Myofibrillar protein bands were within 210-10 kDa while the sarcoplasmic protein bands were within 140-30 kDa.

In the myofibrillar electrophoregram, the 5th band that distinguishes the pure breeds; *C. gariepinus* and *H. bidorsalis* had a molecular weight of 52.23 kDa. The distinctions between the pure breeds and the hybrids marked by the 4th band had molecular weights 101.49 and

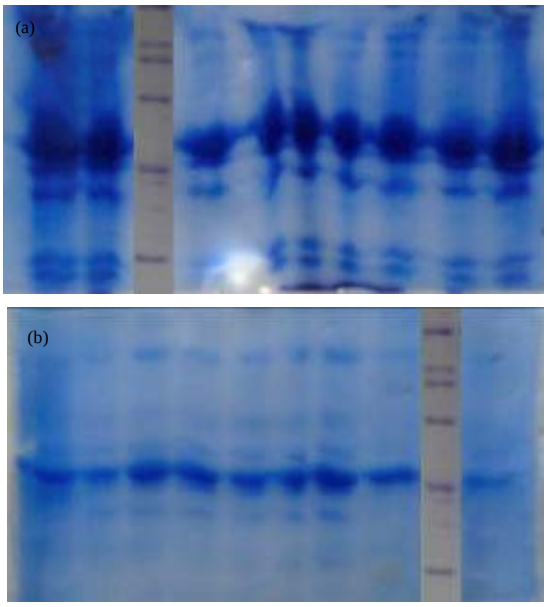

Fig. 1(a-b): Raw image of 12.5% SDS (a) Myofibrillar and (b) Sarcoplasmic muscle protein of artificially propagated *Clarias gariepinus*, *Heterobranchus bidorsalis* and their reciprocal hybrids

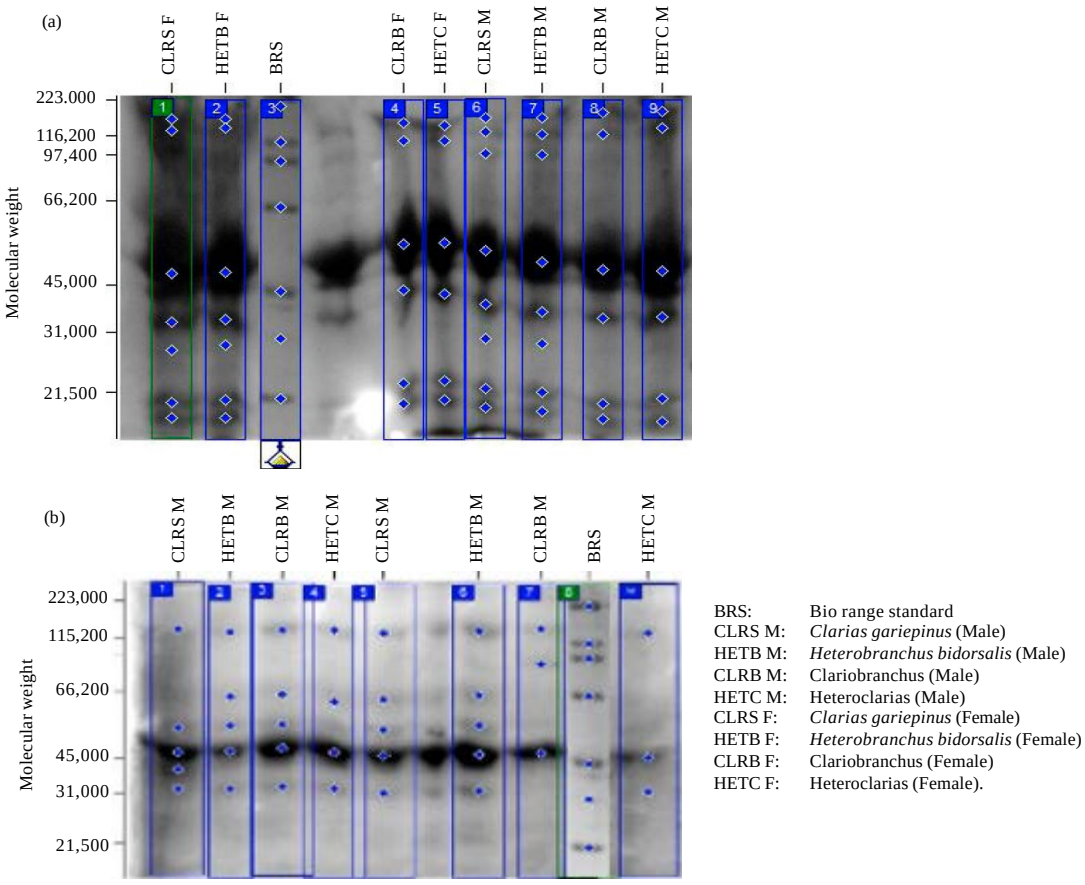

Fig. 2(a-b): Calibrated image of 12.5% SDS (a) Myofibrillar and (b) Sarcoplasmic muscle protein of artificially propagated *Clarias gariepinus*, *Heterobranchus bidorsalis* and their reciprocal hybrids

Table 1: Molecular weights of bands detected for calibrated image of 12.5% SDS myofibrillar muscle protein of artificially propagated *Clarias gariepinus*, *Heterobranchus bidorsalis* and their reciprocal hybrids

Band No.	BRS	CLRS	HETB	CLRB	HETC
1	223.00	175.25	179.35	201.17	205.50
2		137.63	141.60	148.99	154.03
3	116.20	-	-	119.04	119.04
4	97.40	101.49	102.13	-	-
5	66.20	52.23	-	53.78	54.12
6	45.00	48.43	49.72	47.28	46.83
7		38.09	37.71	37.25	37.65
8	31.00	29.24	29.39	-	-
9		22.39	22.18	23.10	23.48
10	21.50	20.35	20.38	20.80	21.37
11		18.38	18.38	18.25	17.93

102.13 kDa while the 8th band had molecular weights 29.24 and 29.39 kDa for *C. gariepinus* and *H. bidorsalis*, respectively. The 3rd band had the molecular weights 119.04 in both *Clariabranchus* and *Heteroclarias*.

In the sarcoplasmic electrophoregram, the 7th band that distinguishes the pure breeds; *C. gariepinus* and *H. bidorsalis* had the molecular weight 41.67 kDa. The 3rd and 5th bands that distinct the hybrids; *Clariabranchus* and *Heteroclarias* had the molecular weight 92.11 and 54.28 kDa, respectively.

Table 2: Molecular weights of bands detected for calibrated image of 12.5% SDS sarcoplasmic muscle protein of artificially propagated *Clarias gariepinus*, *Heterobranchus bidorsalis* and their reciprocal hybrids

Band No.	BRS	CLRS	HETB	CLRB	HETC
1	223.00	-	-	-	-
2	116.20	141.53	143.03	146.93	140.92
3	97.40	-	-	92.11	-
4	66.20	64.27	66.71	66.20	63.53
5	-	53.41	54.62	54.28	-
6	45.00	47.39	47.99	48.40	47.47
7	-	41.67	-	-	-
8	31.00	33.81	34.77	35.39	34.43
9	21.50	-	-	-	-

DISCUSSION

Electrophoretic separation by SDS-PAGE muscle myofibrilla and sarcoplasmic proteins reveal similarity and

dissimilarity among *C. gariepinus*, *H. bidorsalis* and their reciprocal hybrids in south-west Nigeria based on presence or absence and molecular weights of proteins bands of individual species and their hybrids. A total of eleven and seven protein bands were resolved in myofibrilla and sarcoplasmic fractions, respectively. This is not farfetched from results obtained by Machado and Sgarbieri (1991) in the partial characterization and nutritive value of proteins from Pacu. Dissimilarities among species are important for identification of the species within the genus. The 5th band of the myofibrilla electrophoregram distuinguished *C. gariepinus* from *H. bidorsalis* while the 3rd, 4th and 8th bands distinct the pure breeds from the hybrids. The bands of the hybrids were similar, hence no distinction could made for the the myofibrilla fraction. However, the 3rd and 5th bands distinguished the hybrids in the sarcoplasmic fraction, while the 7th bands distincts the pure breeds. Band similarity was high in both fractions; seven out of eleven in the myofibrilla fraction and four out of seven in sarcoplasmic fraction. Myofibrillar and sarcoplasmic protein bands were found within molecular weight ranges of 10-210 kDa of 30-140 kDa. It is therefore observed that both fractions: Sarcoplasmic and myofibrilla effectivdly characterized the pure breeds but sarcoplasmic identifies hybrids species effectively than myofibrilla fractions. This is in support with Yarmohammadi *et al.* (2012) using molecular technique such as Amplified Fragment Length Polymorphism (AFLP) applied to species identification of bester hybrids. Hybrids identification was performed by comparison of electrophoresis profiles with parental species.

Agbebi *et al.* (2013) identified the individual protein bands in each fraction of yellow perch (*Perca flavescens*) whole muscle, sarcoplasmic and myofibrilla in relation to their molecular weights and identified the sarcoplasmic protein in the whole muscle from myofibrilla fraction. In Chakravarty *et al.* (2013) muscle albumin proteins of three species of pomfrets-*Pampus argenteus*, *P. chinensis* and *Apolectis niger* observed difference between the sexes of three species using protein fraction, relative mobility and molecular weights. The finding does not corobonated with this result for no distinct classification is derived from the sexes of the two fractions. Even though sexually dimorphic band are not satisfactorily reported in this study but male and female fish species are easily distinguished from one another under field examination after they are about 10 cm long (Yisa and Olufeagba, 2005).

CONCLUSION

The challenges of morphological variations in *C. gariepinus*, *H. bidorsalis* and their reciprocal hybrids necessitated the use of molecular tool in establishing their biochemical relationship. This research will enable aquaculturists to recognize or identify the right species to use in breeding and genetic studies for accurate results and profitability, consequently solving the problem of poor growth performance and loss of pure indigenous stock of *Heterobranchus* and *Clarias* species.

ACKNOWLEDGMENT

The authors wish to express their profound gratitude to Mrs. Fadiaro of the Biotechnology Laboratory Unit of the Animal Breeding and Genetics of the Federal University of Agriculture, Abeokuta for her technical support.

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