The Use of Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis in Fish Blood Serum Analysis

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Abstract
This study considered the use of Native-Polyacrylamide Gel Electrophoresis (N-PAGE) and Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) in analyzing fish blood serum protein as a tool in aquaculture management. Blood samples were obtained from African catfish (*Clarias gariepinus*) collected from Niger State, Nigeria. Serum protein obtained from the blood samples were analyzed using 5.5% of N-PAGE and 5.5, 7, 8, 10 and 12% SDS-PAGE. The resulting gels from 12% SDS-PAGE analysis showed better resolution of the protein bands compared to 10% SDS-PAGE, while 5.5%, 7% and 8% SDS PAGE did not show the presence of any protein band. In addition, 12% SDS-PAGE showed better resolution of the fish serum samples with more protein bands than N-PAGE. This observation could be useful in analyzing fish serum protein for generating dendrogram grouping in determining the relationship between fish from the same location or between hybrid and parent stocks.

Key words: Blood serum, N-PAGE, SDS PAGE, *Clarias gariepinus*

INTRODUCTION

Aquaculture management is the application of scientific knowledge in order to provide solutions to the problems facing optimum yield of commercial fish production. This could only be realized when the underlying biological principles of species employed in aquaculture practices are well understood. Identification of individual species of fish is very important in aquaculture production as this helps in maintaining purity between the wild and cultured fishes.

Identification of fish has been studied using different molecular techniques. These range from Native Polyacrylamide Gel Electrophoresis, N-PAGE (Cruz *et al.*, 1982; Avtalion, 1984), Allozyme (Galman *et al.*, 1988; Liu and Cordes, 2004), mtDNA (Usmani *et al.*, 2003), nuclear DNA (Nielsen *et al.*, 2004), Amplified Fragment Length

Polymorphism, AFLP (Liu *et al.*, 1998) and Sodium Dodecyl Sulfate Polyacrylamide gel electrophoresis, SDS-PAGE(Alarcón *et al.*, 2001). The use of electrophoretic technique has been widely applied for the direct study of fish identification. This has been employed in studying population structures of different fish species including salmon (Allendorf and Utter, 1979), clarias (Agnése *et al.*, 1997; Rognon *et al.*, 1998), tilapia (Avtalion and Wodjani, 1971; Moralee *et al.*, 2000). Visualization of protein band is possible by making polypeptide molecules migrate in polyacrylamide gels. However, the use of SDS-PAGE has been limited, most especially with fish serum analysis. Piæeiro *et al.* (1999) reported the use of SDS-PAGE in analyzing fish species in raw and heated processed muscle samples but reports on the use of blood sample are scarce. The advantage of analyzing fish using blood serum is significant as this helps in preserving the fish alive, i.e. without sacrificing it as compared to when tissue sample is used. In addition, the serum collected can easily be transferred to the laboratory without any negative effect on further analysis as occurs when tissue samples are involved. Moreover, SDS-PAGE analysis is cheaper and faster as this does not involve the long procedures of DNA isolation and it requires no specialized technical know-how and costly equipment.

The genus Clarias is the third most diversified catfish genus in Africa (Teugels, 1997). One of the six subgenera of the genus is Clarias (*Clarias*), which includes two species: *Clarias anguillaris* and *Clarias gariepinus* and these are native to most African countries. *C. gariepinus* is of great importance in aquaculture as a result of its huge potentials which include high resistance to extreme environmental conditions, faster attainment of edible size and their wide distributions. Hence, this study was on the use of N-PAGE and SDS-PAGE for protein analysis using fish blood serum obtained from *C. gariepinus*.

MATERIALS AND METHODS

Collection of samples

Fish samples were collected from National Institute for Fresh-water Fisheries Research (NIFFR), Niger State, Nigeria. Blood was taken from each of the fish sample with the aid of heparin-lined syringes (5 ml). To every X ml of blood, ⅔X ml of 0.85% saline solution was added in the syringe (Avtalion, 1984). This was transferred into serological tubes furnished with a wooden spill-around. Clot was allowed to form in about 1 h at room temperature.

The clot was removed and centrifugation was achieved at 2,500 rpm for 10 minutes. The supernatant was quickly withdrawn into Eppendorf tubes. These samples were stored at -20 °C for further analysis.

Native-PAGE Analysis
Gel preparation

The protocol used was according to Avtalion (1984). Native gel (5.5%) was prepared measuring 10 ml of 3.0 M Tris-HCl, 15.7 ml acrylamide/N'N' bis-methylene-acrylamide solution, 54.3 ml distilled water and 43.95 mg of ammonium persulfate. The solutions were added together in a 250ml flask, warmed at 37°C and deaerated in a vacuum for 1-2 minutes. Thereafter, 25µl of TEMED (N,N,N,N-tetramethylene acetylene diamine was added, the flask was vigorously shaken to allow proper mixing of the solutions and pour immediately and the comb was placed appropriately. This was allowed to polymerize before further analysis was carried out.

Preparation of sample for Native-PAGE Analysis

Fish blood serum (10µl) was withdrawn into 2ml eppendorf tube, 20µl of 40% sucrose and 2µl of 1% bromophenol solutions were added. Ten microlitres (10 µl) of each of the samples was loaded into the wells on the gel. Electrophoresis was carried out using 220/2.0 power supply chamber. Current was applied for the first 30 min at 150V and later increased to 180V to allow proper separation of the proteins. The analysis was carried out using BIORAD/ Mini protean II kit and the improvised kit by Avtalion (1984).

SDS-PAGE Analysis

The SDS-PAGE analysis was achieved using the Bio-Rad Mini Protean II Cell. Method employed was a discontinuous buffer system. The gel was cast using 12% resolving gel and 4% stacking gel. The procedure was according to the stipulated protocol in the BIO RAD user's guide supplied with the equipment.

Preparation of sample for SDS-PAGE analysis

Fish blood serum (10µl) was withdrawn into eppendorf tube and sample buffer containing 7.5% β-mecaptoethanol (Roth) was added in order to denature the protein. Both low and high molecular weight markers (Sigma) were employed in this study. These were solubilized according to the manufacturer's instructions. The blood samples and the markers were heated at 95°C with PCR

thermocycler machine for 5 min. After removal, they were allowed to cool for 2 minutes before loading them in the wells accordingly. Bio-Rad Electrophoresis Power Supply Model 200/2.0 and the Bio-Rad Mini Protean II Cell were used at 150 V for 45 min. Gels were removed and stained using Commassie Brilliant Blue (R250, Sigma).

Staining and destaining of gels

Staining solution was prepared with 40% Methanol, 10% glacial acetic acid and 0.1% commassie brilliant blue. Gels were stained for 30-45 min. Destaining solution was prepared using 40% Methanol and 10% glacial acetic and the gels were destained for 3 h. Both staining and destaining of the gel were carried out using an electric shaker (R100/TN Rotatest shaker, LUCK HAM).

RESULTS AND DISCUSSION

The gels obtained from Native-PAGE analysis of fish blood serum of *Clarias gariepinus* are presented in Figures 1 and 2 while those obtained from SDS-PAGE analysis are shown in Figure 3. Visual observation of the gels showed various protein bands ranging from 8-10 bands in the Native PAGE gels and 23-26 bands in the SDS PAGE gels. This observation can be attributed to the application of SDS detergent which denatured the polypeptides present in the blood sera Sonia (2001).

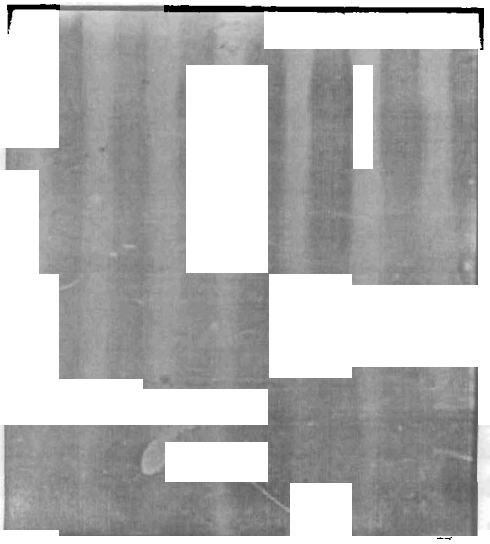

Figure 1: Representative of Native PAGE gels produced using improvised kit.

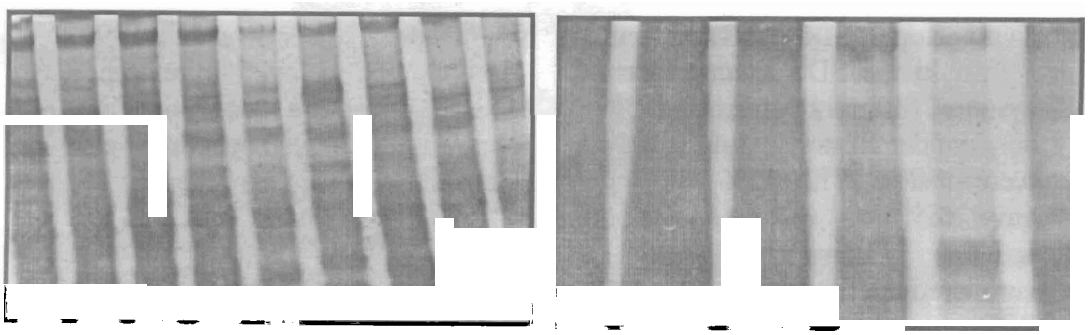

Figure 2: Representatives of Native PAGE gels produced using BIORAD protean II kit

Evaluation of the effect of heat treatment and denaturing agent

The electrophoretic protein bands obtained for the SDS-PAGE showed better and clearer band patterns with more number of bands compared to those obtained in the Native-PAGE. The effect of heat treatment (95°C) on serum protein with addition of Mercapthoethanol (a denaturing agent) in SDS-PAGE analysis disrupted the disulfide bonds that stabilized the higher order structure of proteins which resulted into breaking down of large proteins in the fish serum samples to smaller bands as shown on the gels (Figure 3). However, addition of Mercapthoethanol through already prepared sample buffer proved not to be adequate. This may be attributed to the possibility of Mercapthoethanol losing its denaturing potency with time. Therefore, the Mercapthoethanol was added to sample buffer at every electrophoretic run and better resolution was observed thereafter.

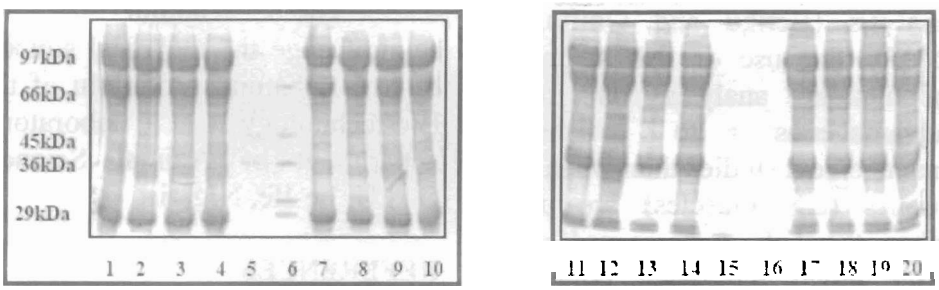

Figure 3: Representatives of Coomassie-stained SDS-PAGE profile of fish sera produced using BIORAD protean II kit (Lanes 5, 6, 15 & 16 are molecular markers).

Evaluation of SDS-PAGE systems

In the SDS Electrophoretic systems, different concentrations of acrylamide: Bisacrylamide solutions were tested. The 12% SDS gel gave the best resolution of the protein pattern (Figure 3) compared to 10%, 7.5% and 5.5% SDS gels (data not shown). Also, different sample to sample buffer ratios were investigated and the 1:1 ratio of sample to sample buffer proved a better combination than 1:2, 1:3 and 1:4 ratios.

Benefits accrue to the use of SDS PAGE system

From the above results, SDS system may be employed in analyzing fish serum samples. This is because with this method, the phylogenetic relationship of different species and populations of fishes can be obtained with the help of statistical software (e.g. after the protein band patterns have been obtained from SDS PAGE analysis (Betiku and Omitogun, 2007). The use of fish serum in population analysis is more advantageous to fisheries management studies than when fish tissues (e.g. muscles) are used, since the fish is preserved alive after blood has been collected. Hence, population with unique and important characteristics can be preserved for future breeding purposes. Similar report was produced by Piæeiro, *et al.* (1999) when they developed a reference method for the analysis and identification of fish species in raw and heat-processed samples.

CONCLUSION

The use of electrophoretic SDS-PAGE system gave better results than the Native PAGE system. The 12% concentration of acrylamide: Bisacrylamide gave the best resolution. The addition of Mercapthoethanol to sample buffer at every electrophoretic run together with the addition of heat (95°C) allowed better resolution and more protein bands. This observation could be useful in analyzing fish serum protein for generating dendrogram grouping in determining the relationship between fish from the same location or between hybrid and parent stocks.

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