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Comparison of Crude Protein Profiles of Isolates of *Aeromonas hydrophila* from *Tilapia zili* by SDS-Page

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Sodium dodecyl sulphate polycylamide gel electrophoresis (SDS-PAGE) was used in the analysis of protein profiles for the characterization of ten isolates of *Aeromonas hydrophila* from naturally infected fish captured for human consumption from ABU dam between November, 2005 to January, 2006. All the six isolates had a band at the 25 KDa protein level. five isolates had a band at the 60 KDa level. This indicates that all the isolates were related and could belong to the same species. Five of the isolates were more closely related to one another, than the others, by having three similar protein bands at the 70,65 and 60 KDa level. SDS-PAGE is suggested as a routine adjunct method to the present method of characterization, classification and molecular identification of *Aeromonas hydrophila.*

Key words: Protein profiles, Isolates, *A. hydrophila,* SDS- Page.

Aeromoniasis is a fatal infectious disease of fishes, reptiles amphibians and other aquatic, terrestrial, laboratory and zoo animals caused by a gram-negative, ubiquitous pathogen belonging to the genus *Aeromonas* (Carter *et al.,* 1995). It is one of the most prevalent infectious diseases of fish that is readily transmitted to man (Francis-Floyd, 2002).

Aeromonas hydrophila (*A. hydrophila*) is one of the most important member of the genus *Aeromonas*. The organism is a motile, mesophilic organism commonly associated with aquatic and terrestrial environment. Aeromoniasis is important in fishes in both tropical and temperate regions of the world (Figueras *et al.,*2000). The economic importance of the disease is estimated to cause losses of millions of US dollars, as result of loss of productivity in terms of reproductive disorders, low fish hatchability, skin lesions, eventual scarification and eventual death, leading to decreased in productivity, aesthetic and market value of the fish (Gavriel *et al.*,1998).

Biochemical studies indicated that, the isolates of *Aeromonas hydrophila* have been found to have slight variations to other *Aeromonas* species (Krieg and Holt, 1984). Due to the wide lost range, geographical distribution of the disease and the nature of the organism, it is evident that clinical, cultural, morphological and biochemical methods are not enough to characterize *Aeromonas hydrophila* into distinct strains or species therefore,

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more studies are needed to characterize these organisms.

The aim of this investigation is to use other techniques such as Sodium Dodecyl Sulphate poly acryl amide Gel Electrophoresis (SDS-PAGE) to analyze the protein patterns of isolates of *Aeromonas hydrophila* from fish for easy identification of the variations within the isolates to enhance possible characterization into distinct species

Methodology

Research design

The research was based on investigation of gut contents of apparently healthy fishes belonging to the specie *Tilapia zillii.* The fishes were bought alive from fishermen within ABU dam and collected in plastic buckets then transported to the laboratory for bacteriological examination. Gut contents were collected from the fishes based the pitting method as described by Kwaga *et al* (1988). Standard culture method of detection of *Aeromonas hydrophila* species was done as described by Cowan and steel (1978). This involves enrichment, selective plating, detection of colonies, preliminary identification and complete biochemical identification .

Preparation of whole cell protein

Cultures of the isolates on MacConkeys agar plates were harvested in ependorf tubes and washed three times in phosphate buffered saline, PH 7.2, by centrifugation at 10,000 g for 5 mins. The washed cells were suspended in sample treatment buffer (double working strength of sample buffer) containing 125 mm tris-Hcl, 4 % SDS, 2 % Mercaptoethanol, 20 % w/v glycerol and then boiled for 10 minutes.

The suspension was then centrifuged at 1400 g for five minutes and the supernatant transferred to fresh ependorf tubes. Sample buffer (Laemmli, 1970) containing 2 % SDS, 4 % mercaptoethanol, 10 % w/v glycerol, 0.1 % Bromophenol blue dissolved in 0.65mm tris Hcl PH 6.8 was added to the supernatant and used as *Aeromonas hydrophila* whole cell proteins.

SDS-PAGE

A discontinuous SDS-PAGE was performed with 4% sacking gel and a 12 % separating gel. The various extracts were solubilized by boiling for 5 minutes at 100^0 C in Laemmli solution (containing 2 % SDS, 4 % mercptoethanol, 10 % w/v glycerol, 0.1 % Bromophenol blue dissolved in 0.625 mm Tris Hcl PH 6.8).

The samples were loaded at 20 ml per lane and separated in 0.75 mm thick gel slabs in the mini protein 11 Dual slab gel (Biorad laboratories, Rockville, NY). Electrophoresis was carried out at a constant voltage of 200 volts for 45 minutes until the stacking dye was approximately I cm from the bottom of the gel.

Pre-stained molecular weight marker (Biorad laboratories, Rockville, NY) containing lysozyme 14400 Daltons, Soya bean, trypsin inhibitor 21,500 Daltons, carbonic anhydrase 31,000 Daltons, oval bumin 45,000 Daltons, Bovine serum albumin (BSA) 66,200 Daltons, Phosphorylase B92, 500 Daltons, B. Galactosidase 116, 200 Daltons, and myosin 2000,000 Daltons were included as reference proteins. Bands were visualized by fixing gels and staining for 1-2 hours in a solution of 0.2 % Coomasie blue R. 250 (biorad laboratories, Rockville, NY) in 50 % methanol and 10 % acetic acid.

Apparent molecular weights were calculated by comparism with known molecular weight standards. Apparent molecular weight was determined by plotting a graph of the RF (distance migrated by known protein/distance from origin to the end of the gel), against the logarithm of their corresponding molecular weights. The point on the graph which corresponded to the value for the protein of unknown molecular weight was located and the value which corresponds to this on the logarithmic scale was taken as the value of the estimated molecular weight of the protein.

RESULTS

All the samples, including the molecular weight standard should contain protein bands on the sodium dodecyl sulphate polyacryclamide electrophoresis gel that can be visualized. This is evidence that the proteins in the samples (*A. hydrophila*) were separated by electrophoresis. However, differences in the protein bands from one samples to another were found.

Figure 1 shows the photograph of 6 *A. hydrophila* isolates ran on a gel. The isolates were designated *A. hydrophila* A B C D E and F . The

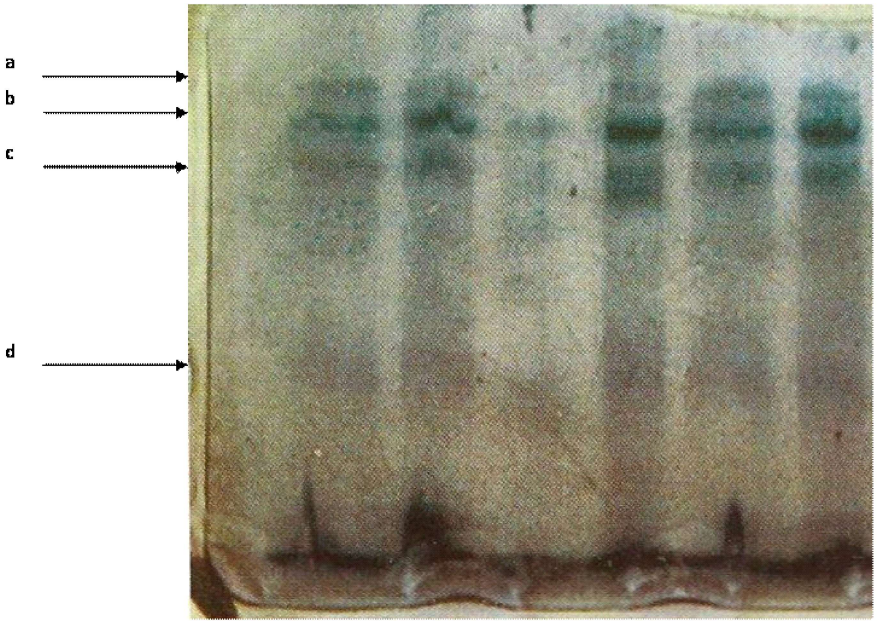

Fig. 1

A. hydrophila isolates were loaded thus; *A hydrophila* isolate A in lane 1, *A .hydrophila* isolate B in lane 2, *A hydrophila* isolate C in lane 3, *A. hydrophila* isolate D in lane 4, *A hydrophila* isolates E in lane 5, and *A..hydrophila* isolate F in lane 6. Three prominent bands of approximate molecular weight of 70, 65 and 60 KDa were visualized. The protein bands were labeled (a) for 70 KDa, (b) for 65 KDa, (c) for 60 KDa and (d) for the faint protein band. Three prominent band bands of approximate molecular weight of 70 KDa, 65 KDa and 60 KDa can be visualized from *A.hydrophila* isolates A, B,C,D, E, and F on lanes 1, 2,4, 5, and 6 respectively and one faint band at the lower level corresponding to approximately 25 KDa. Isolate C lane in 3 did not show prominent bands. Isolate C has three bands at the upper level with the highest corresponding to approximately 65 KDa. It also has one faint band at the 25 KDa level.

DISCUSSION

Our research conducted at the mary Hallaway Teaching Laboratory, Ahmadu Bello University Zaria, using Sodium Dodecyle Sulphate Polycrylamide Gel Electrophoresis (SDS PAGE) of whole cell protein pattern profiles of microorganisms may be utilized as an adjunct for their characterization and molecular identification. All the six *A.hydrophila* isolates used in this study showed distinct protein profiles.

All the isolates have a common band of approximately 65 KDa molecular weight. All the isolates also shared a faint band at the 25 KDa level. This is an indication of the genetic relatedness of the isolates as it has been shown that, proteins are genetically-directed and their patterns tend to express genetic identity of a particular organism as well as its relatedness to other microorganisms. Five of the isolates have an extra band of about 70 KDa, which could indicate their closer relation as members of the same species. Two isolates share 65 KDa, and may be grouped together. The banding patterns observed showed that, 5 of the 6 *A.hydrophila* isolates appear to belong to one strain while the remaining one could be grouped as a separate strain.

The heaviness and faintness of protein bands visualized could be due to proteins exhibited at the time of harvest of the organisms, or the quantity of the particular peptide (site specific hybrids) extracted during protein preparation, or better still the quantity of protein loaded during

electrophoresis (Makinde and Gyles,2000).Three protein bands of 65 KDa, 60 KDa and the faint 25 KDa were common to all the six *A. hydrophila* isolates, and these could be used as standard bands for the identification of *A. hydrophila* isolates. These observations agree with that of Shaibu and Adetosoye, (2008).

Strain differentiation is possible by comparison of protein profiles as the authors used similar technique to identify species of the family Enterobacteriaceae. Similarly, polyacrylamide gel electrophoresis of numerous microorganisms has also been used to enhance their characterization of their protein pattern in bacterial taxonomy. (Strom *et al.* (1976) suggested that if polyacrylamide gel electrophoresis is to be used as an adjunct for identification, it would be necessary to use freeze dried extracts of known standards or controls in each electrophoretic run.

CONCLUSION

SDS-PAGE could be a useful method for identification and molecular classification of *Aeromonas hydrophila*. Further studies could be directed through investigating the roles of these proteins in virulence and their use in vaccine development.

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