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Comparism of DNA Restriction Patterns of *Aeromonas Hydrophila* Isolates from Humans by Agarose Gel Electrophoresis (AGE)

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ABSTRACT

Agarose Gel Electrophoresis (AGE) was used in the analysis of genomic DNA fragment of Aeromonas hydrophila (A.hydrophila) isolates that were digested with Bam H1 restriction enzymes from out patients attending Ahamadu Bello University Teaching Hospital, Zaria. All the 8 isolates had bands at the 900 bp DNA fragment level. Three isolates have a unique band at 1.0 kb molecular weight level. At the 950 pb, 4 prominent bands were also seen in the A.hydrophila strains. Two bands of 650 bp and 450 pb were also found in two isolates while one other isolate had a separate band at the 250 bp. One isolate had three faint bands at 1.5 kb, 650 and 450 pb. Restriction fragments were suggested as routine adjunct to the present method of characterization and identification of Aeromonas hydrophila. This could be useful in epidemiological diagnoses involving trace-back of true sources of infection in cases of aeromoniasis outbreaks in man and animals.

KEY WORDS: *Restriction digestion, Aeromonas hydrophila, Agarose Gel Electrophresis*

INTRODUCTION

Human health remains one of the primary targets of the public Health Veterinarian. The environment we live and the food we eat posse's serious health threat to man, since most of this surfaces are often contaminated by several microorganisms including *Aeromonas hydrophila* [8].

Aeromonas hydrophila is an emerging zoonotic disease caused by a gram negative coccobacillary bacterium of the family *Aeromonadaceae*. They are chemoorganotrophic bacteria that are native of both aquatic and terrestrial environments [7] and may share some similarities with coliforms. The organism is a straight, coccobacillary with round to coccoid ends, measuring 0.3 – 1.0 x 1.0 – 35 µm [9]. They occur in pairs, singly or in short chains and may have a single pollar flagellum, though peritrichous or lateral flagella may be formed in solid media by some strains. Hydrolytic enzymes produced by this organism includes: esterases, amylases, deoxy ribonucleases, peptidases, elastases, chitinases and lipases [3]. These virulent factors produced by this bacterium are responsible for intestinal symptoms in man characterized by diarrhea and extra intestinal symptoms characterized by meningitis and endocarditis.

The presence of *Aeromonas hydrophila* strains from a variety of retail foods such as fish and poultry products could be possible sources of human infection [2]. Due to the increasing bio-diversity and significance of this organism in diarrhoeic patients, morphological and biochemical methods are not sufficient enough to characterize this organism into distinct species and strains. Recently, DNA studies by Agarose Gel Electrophoresis (AGE) have shown that *Aeromonas hydrophila* have slight variations in genotypic traits [9].

The purpose of this study therefore is to use restriction fragment analysis of *Aeromonas* genomic DNA. This could provide necessary fragments which could be crucial in efficient surveillance of

Aeromonas infections in man and animals. This could be useful in tracing back the true sources the disease in outbreak areas in Nigeria.

MATERIALS AND METHODS

Aeromonas Isolates

Eight (8) *Aeromonas hydrophila* species were isolated from diarrhoiec stool of out patients attending Ahmadu Bello University teaching hospital (ABUTH), Zaria, using accepted laboratory methods for bacteriological examination of faecal samples as described by [4]. This involved pre-enrichment, enrichment, selective plating and detection of colonies, preliminary identification and complete biochemical identification.

Preparation of Aeromonas hydrophila cells

This was based on the methods of [6]. The pure cultures containing *A. hydrophila* isolates were grown on test-tubes containing Brain Heart Infusion (BHI) at 37^0 C for 24 hrs. After incubation at 37^0 C, it was then centrifuged at 10,000 g for 5 minute using Harous labofuge. Supernatants were decanted and residues (cells) were used for DNA extraction.

Preparation of *Aeromonas hydrophila* Genomic DNA

Total genomic DNA was extracted from harvested cultures using the Qiagen kit as adopted by (1). 20 µl of proteinase K was dispensed into a 1.5ml microcentrifuge tube, 200 µl of the separate organisms and 200 µl of the lyses buffer were added, vortexed and then incubated at 56^0 C. After 10 mins, 200 µl of absolute ethanol was added and then vortexed.

The mixture is carefully loaded into a Q lamp spin column (in a 2 ml collection tube) and centrifuged. After 1 min the spin column was transferred into another collection tube and 500 µl buffer AWI is dispensed into it and centrifuged at 800rpm for I minute. The spin column was again transferred to a clean collection tube and then centrifuged at 1400 rpm for 3 minutes, after which the spin column was transferred into a 1.5 ml micro at 1400 rpm for 3 mins, after which the spin column was transferred into a 1.5 ml microfuge tube and 200 µl distilled water added, incubated for 1 min at room temperature and then centrifuged at 800 rpm for I minute to elute the DNA. DNA calibration curve was prepared by standard solution of salmon sperm DNA was prepared at 50,100,150,200 and 250 µl/ml. the absorbance of each concentration was read at 260 nm, and the readings were plotted against the concentration values to obtain a standard curve. Purity of the DNA was assessed by taking absorbance of extracted *Aeromonas hydrophila* DNA at 260 nm and 280 nm and the average A 260/ A 280 were calculated. *Aeromonas* DNA extracts were further quantified by taking absorbance readings at 260 nm which was used to extrapolate for its concentration from the calibration curve.

Restriction Digestion and AGE

The restriction digestion was done according to accepted methods. The restriction endonuclease digestion of the *Aeromonas hydrophila* genomic DNA was done using B*am* H1 (Biorad laboratories). The enzyme B*am* H1 was pretested on the 10 DNA samples to determine whether it is appropriate for use. 5 (µl) of genomic DNA were dispensed unto different 1.5 ml eppendorf tubes, followed by 39 µl of sterile distilled water, 5 µl B*am* H1 and then 5 units of B*am* H1 and calf intestinal phosphatase (CIP). Each mixture was mixed and incubated at 37^0 C for 2 hrs to stop digestion, 5 µl of trace blue dye was added. After digestion, DNA fragments were separated by electrophoresis in 0.8% agarose gel in IXTAE buffer using 30 v for 15 hrs. After 15 hours, electrophoresis was terminated and the gels were visualized under UV transluminator and photographed using an MP3 Polaroid camera.

RESULTS

All the samples, including the molecular weight standard showed DNA bands on the gel by Agarose gel electrophoresis that can be visualized through UV transluminator. This is evidence that the DNA in the samples (*A.hydrophila*) were separated by the electrophoresis. However, differences in the DNA fragments from one sample to another were found.

Figure 1 shows that photograph of eight *A.hydrophila* isolates ran on agarose gel. The isolates were designed A, B, C, D, E, F, G and H. The *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* isolate E in lane 5, *A.hydrophila* isolate F in lane 6, *A.hydrophila* isolate G in lane 7 and *A.hydrophila* isolate H in lane 8. DNA bands were labeled. (a) For 1.5 kb (b) for 1.2 kb (c) for 1.0 kb (d) for 950 bp (e) for 800bp (f) for 650 bp (g) for 450 bp and (h) for 250 bp (d) and (a) represented faint DNA bands. Three prominent bands of appropriate molecular weight 1.0 kb can be visualize from *A.hydrophila* isolate A, B and D in lanes 1,2 and 4 respectively. 4 prominent bands were also visualized where bands of 950 bp which were found in all the eight lanes (1, 2, 3, 4, 5, 6, 7 and 8). In isolate of 800 bp was found in isolate (E) band of 650 and 450 bp were also found in lanes 3 and 5 respectively. The least band was of 250 bp was found in lane 6. (a) Has three faint bands at of approximate molecular weight of 1.5 kb at lane 1, 2, and 5. (e) Has a faint band at lane 1. While E has two faint bands of molecular weight 650 bo and 450 bp respectively at both lanes 6 and 8.

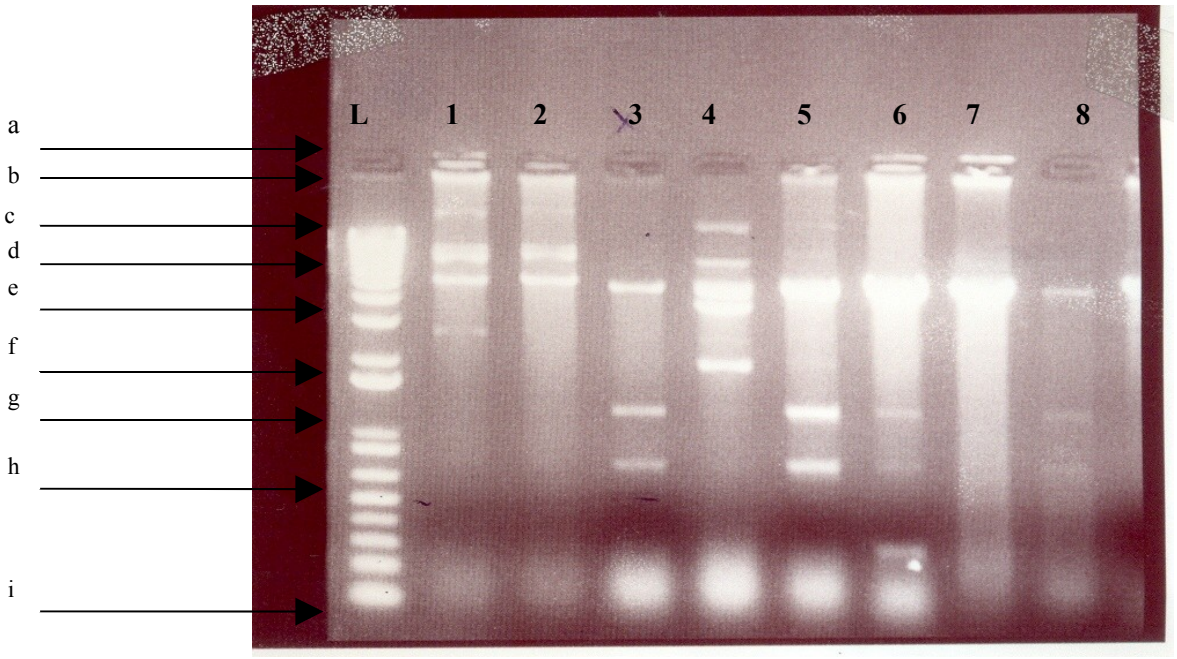

Fig. 1 Shows *A .hydrophila* isolates designated a-i. Lane 1: Isolate a, Lane 2: Isolate b, Lane 3: Isolate c, Lane 4: Isolate d, Lane 5: Isolate e, Lane 6: Isolate f, Lane 7: Isolate g, Lane 8: isolate h. Prominent protein bands are labeled b, c, e, g, h and i

DISCUSSION

High purity of the DNA isolates indicates the efficiency of the Qiagen Kit in extraction of *Aeromonas* genomic DNA [5]. The genomic DNA of *Aeromonas hydrophila* isolates were subjected to restriction digestion using B*am* H1 restriction enzyme showed dissipation of several DNA molecules generated in the B*am* H1 digestion of chromosomal DNA and was separated according to size in agarose gel. There could be possibility of total dissociation of the gene from the plasmid, leading to the multiple bands seen in various wells giving rise to varied molecular weight ranging from 250 bp to 1.5 kb.

All the 8 isolates have a common fragment of appropriately 800 bp. Isolate 3 and 5 share common bands as appropriate 650 and 450 bp. This is an indication of the genetic relatedness of the isolates as it has been shown that DNA fragments are genetically directed and their patterns tend to express genetic identity of a particular organism as well as its relatedness to other microorganisms. One isolate had an extra band at the 250 bp which could indicate their closer

relation with other microorganisms. Those isolates with same fragments could be grouped together while the one with different extra fragment could be grouped as a separate strain. The assay of the different fragments generated by B*am* H1 restriction enzyme shows the potential of molecular characterization of *Aeromonas hydrophila*. This could specially be suitable at determining evolutionary diversions wherever mutation occurs.

The heaviness or faintness of restriction fragments could be the fragments of DNA exhibited at time of harvest of the microorganisms or low quantity of DNA during DNA preparation of better still low quantity of DNA loaded during gel electrophoresis [9]. Two DNA faint bands were common in isolates 6 and 8 at 650 and 450 bp were common and the dense band at 950 bp could be used as standard band for the genetic identification of *Aeromonas hydrophila* isolates. These observations concur with that of [1]. This principle is applicable in classification, identification and epidemiological studies involving multiple gene arrays, trace-back analysis of true sources of infection, in paternity cases on whom is the true father of a child or a crime scene involving a suspect.

CONCLUSION AND RECOMMENDATION

The genomic DNA of *Aeromonas hydrophila* were extracted, purified and quantified various restriction fragments were produced. It is therefore recommended that cloning, sequencing and general assembly of the genome should be conducted to enhance functional surveillance programme for monitoring and control of aeromoniasis in Nigeria.

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