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Genetic Diversity Studies of Bacteria Associated with *Brycinus longipinnis* from Eggua on Yewa River using Random Amplified Polymorphic DNA (RAPD) Markers

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Abstract: Twenty samples of *Brycinus longipinnis* were collected from Eggua landing site on Yewa river Ogun state, which lies on latitude 7°03'N and longitude 2°55'E. The water quality parameters (Temperature, Dissolve Oxygen and pH), weight and the morphometric characteristics (Total Length, Standard Length and Head Length) were taken. The bacteria were isolated from gills, gut and skin of *Brycinus longipinnis* and characterized. The genomic DNA of the bacterial isolates were extracted and subjected to Random Amplified Polymorphic DNA RAPD using five Oligonucleotide primers to determine the genetic relatedness. The bands obtained were scored and the data were analyzed using NTSYS software to draw the dendogram. Haemolysis was also done. The results of the water quality parameters (Temperature, Dissolved Oxygen and pH) values were 28.6°C, 5.51ppm and 8.30 respectively. The mean values of the Total Length, Standard Length and Head Length were 5.60±0.2, 4.60±0.2 and 1.00±0.1 respectively. Fifty seven polymorphic markers and 10 monomorphic markers were generated from the five RAPD markers used. The dendogram showed that the bacterial isolates were similar to 66% thereafter, they split into two major groups, and *Alcaligenes faecalis* is the only bacterial isolate that is genetically different from the others. *Proteus mirabilis* and *Proteus vulgaris* were the only bacterial isolates that were genetically similar. Haemolysis shows that 35% of the isolates had α-haemolysis (partial haemolysis or incomplete haemolysis), 20% had β-haemolysis (complete haemolysis) and 45% had no haemolysis.
Keywords: *Brycinus longipinnis*, Bacterial isolates, DNA extraction, RAPD, Haemolysis

INTRODUCTION

Fish is any member of a paraphyletic group of organisms that consist of all gill-bearing aquatic craniates animals that lack limbs with digits. Fish is an important source of food for people and it contributes about 60% of the world's supply of protein. It is man's most important source of high quality protein, providing approximately 16% of the animal protein consumed by the world's population[1]. 60% of the developing countries derive 30% of their annual protein from fish [2]. In Africa, fish is one of the cheapest sources of protein and it supplies 17% of protein [3]. In Nigeria, the increasing human population and the short supplies of animal protein together have raised the cost of animal protein to a level almost beyond the reach of the low income group [4]. The advantage of fish as food is that it is easily digestible and has high nutritional value. However, fish are vulnerable to a wide variety of bacterial pathogens, most of which are capable of causing diseases and are considered by some to be saprophytic in nature [5]. According to Cahill [6], the microbiological diversity of fresh fish muscle is dependent on the fishing grounds and environmental factors around it. Claucas and Ward [3] suggested that the type of micro-organisms that are found associated with a particular fish depends mostly on its habitat. Kvenberg [7] and Rodricks [8] classified the bacterial pathogens associated with fish as non-indigenous and indigenous. The non-indigenous contaminate the fish or the habitat one way or the other and examples include: *Staphylococcus aureus, Escherichia coli, Shigella dynteriae, Listeria monocytogens, Clostridium Botulinum and Salmonella*. The indigenous bacterial pathogens are found naturally living in the fish's habitat for example Aeromonas species and Vibrio species. The bacteria from fish only become pathogens when fish are nutritionally deficient, physiologically unbalanced, or there are other stressors, i.e., overstocking, poor water quality, which allow opportunistic bacterial infections to prevail. Pathogenic and potentially pathogenic bacteria associated with fish and shellfish include: *Vibrio spp., Aeromonas spp., Mycobacterium, Salmonella spp. Streptococcus spp*., and others [5].

RAPD consists in the random amplification of DNA fragments with a single primer with an arbitrary sequence, resulting in the final synthesis of several DNA fragments with different sizes. From this set of

fragments it is possible to determine polymorphism in the DNA sequence, which can be used as a genetic marker [9].

The RAPD analysis described by Williams *et al.;* [9] is a commonly used molecular marker in genetic diversity studies. This technique has been widely used in various studies in plants and animals because, in addition to its low cost, it allows polymorphism to be detected in a simple and fast manner[10, 11].

Haemolysis is the breakdown of red blood cell. A study of bacterial pathogen in sea foods shows that a number of bacterial illnesses may be caused from the consumption of seafood that has either been contaminated at source or which becomes contaminated during the processing and retail chain. Such illnesses may occur from infection with the bacteria themselves or by the ingestion of toxins formed in the foodstuff before consumption. This division is actually too simplistic: for example, the toxins of *Clostridium botulinum* and *Staphylococcus aureus* are preformed in food during bacterial growth, the toxin of *Clostridium perfringens* is usually only made when the bacteria sporulate in the intestinal tract while the toxin of *Vibrio cholerae* O1 is produced when the bacteria multiply in the intestinal tract [12].

Therefore, the objective of this research work is to know the genetic relatedness and the pathogenecity of the bacteria isolated from gills, gut and skin of *Brycinus longipinnis*.

MATERIALS AND METHODS

Samples collection

Twenty samples of *Brycinus longipinnis* were collected from Eggua landing site on Yewa river Ogun state, which lies on latitude $7^{o}03^{'}$N and longitude $2^{o}55^{'}$E. Yewa River is a perennial river.

Twenty samples of *Brycinus longipinnis* were collected and weighed using citizen sensitive balance after which swab sticks were used to swab the skin, the gut and the gill of each *Brycinus longipinnis*. The swab sticks were transported to the laboratory on ice packs for microbiological analysis.

WATER QUALITY PARAMETERS AND MORPHOMETRIC CHARACTERISTICS

The physio-chemical parameters such as Dissolve Oxygen (ppm) are using a D.O. meter, Temperature (^{o}C) and pH using Hanna multipurpose meter were taken and recorded.

The morphometric features of fish samples such as weight (g) using citizen sensitive balance (the fishes were batch-weighed), Standard Length (cm), Total Length (cm) and Head Length (cm) were measured and recorded.

ISOLATION OF BACTERIA AND EXTRACTION OF GENOMIC DNA

The medium used in the experiment was Nutrient agar which was prepared according to the manufacturer's specification (28.0g of Nutrient agar dissolved in 1 litre of distilled water). The medium was properly dissolved and then sterilized in an autoclave at 121^{o}C for 15 minutes at 106mmHg. The medium was allowed to cool before pouring 15.0ml into sterile petri dish for solidification.

Isolation was done by streaking the swab sticks on the agar plates. The plates were then incubated in an inverted position at 37^{o}C for 24 hours. Pure cultures of the bacterial isolates were obtained by sub-culturing on Nutrient Agar plates. Pure cultures were then maintained in nutrient agar slants and stored at 4^{o}C. The genomic DNA of the bacterial isolates was extracted using CTAB method Akinyemi and Oyelakin 2014.

PCR AMPLIFICATION OF DNA USING RAPD PRIMERS

The reaction mix was carried out in 20µl final volume containing 60ηg - 80ηg genomic DNA, 0.1 µM of the primers, 2mM $MgCl_2$, 125µM of each dNTP and 1 unit of Taq DNA polymerase. The thermocycler profiles has an initial denaturation temperature of 94^{0}C for 3minutes, followed by 45 cycles of denaturation temperature at 94^{0}C for 20seconds, annealing temperature of 37^{0}C for 40 seconds and primer extension temperature of 72^{0}C for 40seconds, followed by final extension temperature at 72^{0}C for 5 minutes was added.

PCR amplicon electrophoresis was carried out by size fractionation on 2.0% agarose gels. Agarose gels were prepared by dissolving and boiling 4.0g agarose in 200ml 0.5X TBE buffer solution. The gels were allowed to cool down to about 50^{0}C and 10µl of 5mg/ml ethidium bromide was added, mixed together before pouring it into an electrophoresis chamber set with the combs inserted. After the gel has solidified, the PCR amplicon was loaded in the well created. Electrophoresis was done at 100V for 2 hours. The integrity of the DNA was visualized and photographed on UV light source.

SENSITIVITY TEST

Nutrient agar was prepared by dissolving 2.8g of Nutrient agar in 100ml of distilled water and mixed thoroughly. The medium was boiled to dissolve completely and then sterilized in an autoclave at 121°C for 15 minutes. After sterilization, it was allowed to cool to a temperature less than 40°C. 5% of human blood was added and mixed to form blood agar. 20ml of the blood agar was poured into the petri dish and allowed to solidify. Air drying was done for 15 minutes using LTE Hot Air Oven. The blood agar plates were inoculated with the test bacterial isolates. The plates

were incubated at 37°C for 24 hours. After 24 hours, the plates were observed for haemolysis.

STATISTICAL ANALYSIS

The bands obtained from the gel were manually scored '1' for the presence and '0' for the absence; and the binary data were subjected to analysis using NTSYS software to draw the dendogram for the bacterial isolates. Descriptive statistics was used to analyze data on morphometric and water parameter.

Results and discussions

Water quality parameter and morphometric characteristics

The water quality parameters of Eggua landing site on Yewa River are shown in Table 1. From the Table, temperature, dissolved oxygen and pH values were 28.6°C, 5.51ppm and 8.30 respectively. The average weight of the fish sample is 12.25g. Table 2 shows the mean values of the morphometric characteristics (total length, standard length and head length) of African Long-Finned Tetra (*Brycinus longipinnis*) from Eggua landing site on Yewa River.

RAPD PCR ANALYSIS

Table 3 shows that there were 57 polymorphic markers generated from the five RAPD markers. There were also 10 monomorphic markers from the primers. A total number of 67 markers were generated. Fourteen (14) alleles from the primer OPB-12, 16 alleles from the primer OPB-20 and 17 alleles from primer OPH-08. Table 4 shows the percentage polymorphism of the primers. Plates 2, shows the agarose gel electrophoresis of RAPD PCR amplicons for OPB-20 Figure 1 shows the dendogram of the bacteria isolated from *Brycinus longipinnis*. From the Figure, it was observed that *Alcaligenes faecalis* is the only bacterial isolate that is genetically different from the other isolates. *Proteus mirabilis* and *Proteus vulgaris* were the only bacterial isolates that are genetically similar. From the phylogenetic tree, the bacteria isolates were grouped into two main genotypes, 19 isolate were grouped together to form the first group while one isolate formed the second group. This shows that there is a strong relationship among the bacteria isolates obtained from *Brycinus longipinnis* Akanji *et al.;* [13].

HAEMOLYSIS

Table 4 shows the haemolytic activity of the bacteria associated with African Long-Finned Tetra (*Brycinus longipinnis*). From the Table, it was found that 35% of the isolates had α-haemolysis (partial haemolysis or incomplete haemolysis), 20% had β-haemolysis (complete haemolysis) and 45% had no haemolysis. Half of the bacterial isolates that had β-haemolysis (complete haemolysis) were gotten from the gill, 25% each from the gut and skin. Pathogenic and potentially pathogenic bacteria associated with *Brycinus longipinnis* include; *Pseudomonas putida, Enterobacter cloacae, Pseudomonas aeruginosa, Pseudoalteromonas sp., Escherichia coli, Comamonas jiangduensis, Proteus mirabilis, Alcaligenes faecalis, Pseudomonas fluorescens*, Serratia marcescens, *Proteus vulgaris, Enterobacter sp., Comamonas aquatica* [5].

Table 1: Water quality parameters of Eggua landing site on Yewa River

Water parameters	Values
Temperature (°C)	28.60
Dissolve Oxygen (ppm)	5.51
pH	8.30

Table 2: Morphometric characteristics values of *Brycinus longipinnis*

Morphometric features (cm)	Values (Mean±SE)
Total Length	5.60±0.2
Standard Length	4.60±0.2
Head Length	1.00±0.1

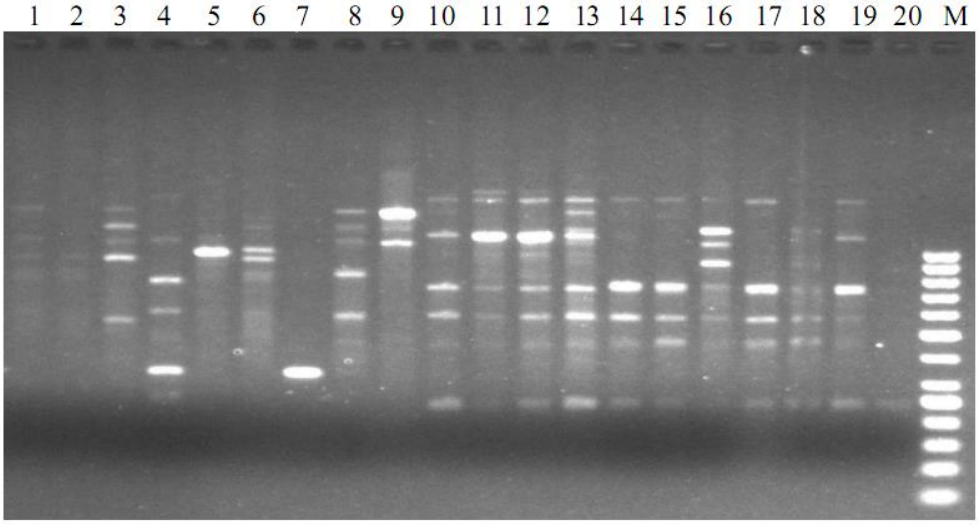

Plate 2: Electrophoresis gel for RAPD primer OPB 20

Table 3: Primers and percentage polymorphism

S/N	Primer name	No of Monomorphic markers	No of Polymorphic markers	Total no of markers	Percentage Polymorphism
1	OPB 12	Nil	14	14	100
2	OPB 20	01	15	16	94
3	OPH 08	01	16	17	94
4	OPH 12	03	05	08	63
5	OPH 19	05	07	12	58
	Total	10	57	67	

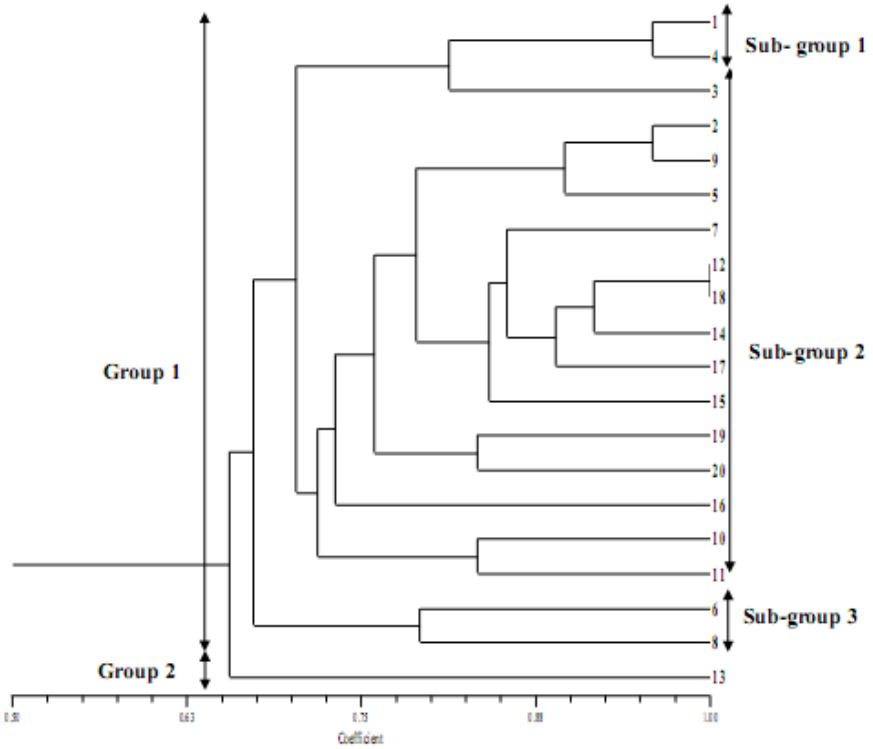

Fig 1: Dendogram for the 20 Bacterial Isolates

Key

1: Pseudomonas putida 2: Enterobacter cloacae 3: Pseudomonas putida 4: Pseudomonas putida 5: Pseudomonas aeruginosa 6: Pseudoalteromonas sp. 7: Escherichia coli 8: Pseudoalteromonas sp 9: Enterobacter cloacae 10: Comamonas jiangduensis 11: Escherichia coli 12: Proteus mirabilis 13: Alcaligenes faecalis 14: Proteus vulgaris 15: Pseudomonas fluorescens 16: Serratia marcescens 17: Proteus penneri 18: Proteus vulgaris 19: Enterobacter sp. 20: Comamonas aquatic

Table 4: Haemolytic activity of bacteria associated with African Long-Finned Tetra

Bacterial isolates	Tissues	Type of haemolysis
Pseudomonas putida	Gut	α-haemolysis
Enterobacter cloacae	Gill	β-haemolysis
Pseudomonas aeruginosa	Gut	β-haemolysis
Pseudoalteromonas sp.	Skin	No haemolysis
Escherichia coli	Gut	No haemolysis
Comamonas jiangduensis	Gill	No haemolysis
Escherichia coli	Skin	No haemolysis
Proteus mirabilis	Gut	No haemolysis
Alcaligenes faecalis	Gut	α-haemolysis
Pseudomonas fluorescens	Gill	β-haemolysis
Serratia marcescens	Gill	α-haemolysis
Proteus vulgaris	Gut	α-haemolysis
Enterobacter sp.	Gut	α-haemolysis
Comamonas aquatic	Skin	β-haemolysis

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