



## Gene Sequencing and Haemolysis of Bacteria in *Clarias gariepinus* From Ajilete Location on Yewa River

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### Authors' contributions

This work was carried out in collaboration between all authors. This work was initiated by authors AAA and O. O. Ololade. The laboratory work was carried out by authors ARO, YAB and O. O. Ololade while authors O. O. Oyelakin and AAI prepared the manuscript. All the authors read and approved the final manuscript.

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### ABSTRACT

Gene sequencing and haemolysis of bacteria in *Clarias gariepinus* post juveniles sampled from Ajilete location on Yewa River, Ogun state, Nigeria was carried out. Bacteria were isolated from the gut, gills and skin of the fish. Thirty bacteria isolates were selected and the DNAs were extracted using hexadecyltrimethylammonium bromide (CTAB) method and Polymerase Chain Reaction (PCR) amplification of the isolates was carried out using universal primer for bacteria. The purification of PCR product was done using ethanol precipitation and thereafter the DNAs were sequenced using automated DNA sequencer. The sequence data were analyzed on the GenBank database (NCBI) using Basic Local Alignment Search Tool (BLAST) to find the nearest related sequence, and to construct the phylogenetic relationship. The haemolysis of the selected isolates were also carried out. Data collected were subjected to descriptive statistics. Nine genera of

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bacteria isolated were *Escherichia*, *Staphylococcus*, *Enterobacter*, *Proteus*, *Pseudomonas*, *Klebsiella*, *Citrobacter*, *Streptococcus* and *Bacillus*. The 16S rRNA gene sequencing method identified the isolates as strains of *Brevibacillus agri*, *Bacillus cereus*, *Ignatzschineria indica*, *Comamonas kerstersii*, *Proteus vulgaris*, *Lysinibacillus sphaericus*, *Proteus hauseri*, *Clostridium bifermentans*, *Proteus mirabilis*, *Pseudomonas putida*, *Escherichia coli*, *Comamonas jiangduensis*, *Enterobacter cloacae*, *Serratia marcescens*, *Alcaligenes faecalis*, *Lysinibacillus fusiformis*, *Myroides odoratimimus*. Haemolysis showed that *M. odoratimimus*, *B. cereus*, *C. kerstersii*, *P. vulgaris*, *C. bifermentans*, *E. coli* and *E. cloacae* all had  $\beta$ -haemolysis. *A. faecalis* displayed  $\alpha$ -haemolysis while, *B. agri*, *I. indica*, *L. sphaericus*, *P. hauseri*, *L. fusiformis*, *S. marcescens* were non-haemolytic. This study confirms the reliability of the 16S rRNA gene sequencing method. This study also concludes that there are different pathogenic bacteria species that are associated with *C. gariepinus*.

**Keywords:** *Bacteria; Clarias gariepinus; DNA sequencing; haemolysis; cluster analysis.*

## 1. INTRODUCTION

Fish is a major source of nutrients for humans, providing a significant portion of the protein intake in the diets of a large proportion of people in developing countries where it represents one of the cheapest sources of animal protein. However, freshly harvested fish is highly perishable; and depending on harvesting techniques and handling, may deteriorate and spoil within six hours of landing [1]. Apart from the high perishability of fish, consumer safety is an issue to be considered because fish is a good medium for rapid bacteria multiplication particularly when exposed to unsanitary conditions. [2] observed that bacteria associated with freshly caught fish are principally a function of the environment where it is caught. Thus there is a need to investigate the bacteria associated with fish if only to safeguard public health. Moreover, contamination of water body by various activities of man has been frequently associated with transmission of diseases causing bacteria such as *Vibrio*, *Salmonella*, *Bacillus*, *Staphylococcus*, *E. coli* [3]. Diverse group of microorganisms exist in the aquatic environment where they live as saprophytic, pathogenic or heterotrophic organisms. Microorganisms share common environment with fish in their natural habitat and thus become resident fish flora, but they can cause epizootics under stressful conditions. These stressful conditions may be brought about by chemical, physical and or biotic factors [4]. [5] showed that there is an established connection between public health and fish consumption in terms of communicable diseases, malnutrition and non-communicable diseases. However, the presence of potential human pathogens suggests that fish improperly handled, consumed raw or undercooked may lead to disease to susceptible host.

Therefore, the objective of this study is to identify bacteria flora from gills, guts and skin of *C. gariepinus* post juveniles using 16S rRNA gene sequence so as to know if there are different organism associated with these organs and to construct a cluster analysis to find evolutionary ties among the bacteria and also to evaluate the bacteria haemolysis.

## 2. MATERIALS AND METHODOLOGY

### 2.1 Samples Collection

The study was carried out at Ajilete station on Yewa River which has six landing site. It is situated at 6° 22' North and 2° 55' East. Bacteria samples were taken from the gill, skin and gut of *C. gariepinus* with the use of swab stick according to [6]. All swab sticks were streaked on both Nutrient agar and Mac Conkey agar by BioMark Laboratory, the samples were later incubated for 18 hours at 37°C.

### 2.2 Water Test and Morphometric of Fish Sample

The standard length, total length, the head length in centimeter (cm) were measured using a measuring ruler and recorded after weighing the fish sample in grams (g) using sensitive weighing balance. The water quality was tested on Temperature, Dissolved Oxygen and pH from difference point on the landing site.

### 2.3 Isolation of Bacteria and Morphological Characterization

Isolation of bacteria from the gills, guts and skin of the fish samples was carried by standard method. Each of the swab stick taken from the

gills, guts and skin was inoculated on Nutrient agar and MacConkey agar (Oxoid, UK) and incubated at 37°C for 24 hours. Bacteria colonies obtained were purified on Nutrient agar and incubated at 37°C for 24 hours for growth pure discrete colonies. Colonial and cellular morphology was performed by phenotypic examination and Gram staining respectively. Each bacteria isolates were biochemically characterized according to [7].

## 2.4 Identification of the Bacteria Isolates by 16S rRNA Gene Amplification

Bacteria isolate was grown overnight in Nutrient Broth and spun at 14,000 rpm for 2 mins, the DNA was extracted using CTAB method [8]. The DNA was later resuspended in 100 µl of sterile distilled water. DNA concentration of the samples was measured and the genomic purity was determined. PCR analysis was done using MJ Research Thermal Cycler (PTC-200 model). The primer used for PCR amplification was 16S universal primer for bacteria, the sequence for the forward primer was 5'AGAGTTTGATCCTGGCTCAG3' and reverse primer was 5'ACGGCTACCTTGTACGACTT3'. The PCR mix comprises of 1 µl of 10X buffer, 0.4 µl of 50 mM MgCl<sub>2</sub>, 0.5 µl of 2.5 mM dNTPs, 0.5 µl 5 mM Forward primer, 0.5 µl of 5 mM Reverse primer, 0.05 µl of 5 units/µl Taq with 2 µl of template DNA and 5.05 µl of distilled water. The PCR profile used has initial denaturation temperature of 94°C for 3 mins, followed by 30 cycles of 94°C for 60 seconds, 56°C for 60 seconds 72°C for 120 seconds and the final extension temperature of 72°C for 5 minutes and the 10°C hold forever.

The PCR product was further purified before the sequencing using 2M Sodium Acetate washing techniques. The pellet was resuspended in 5 µl sterile distilled water. The PCR mix used includes 0.5 µl of BigDye Terminator Mix, 1µl of 5X sequencing buffer, 1 µl of 16S Forward primer with 6.5 µl Distilled water and 1 µl of the PCR product making a total of 10 µl. The PCR profile for Sequencing is a Rapid profile, the initial Rapid thermal ramp to 96°C for 1 min followed by 25 cycles of Rapid thermal ramp to 96°C for 10 seconds Rapid thermal ramp to 50°C for 5 seconds and Rapid thermal ramp to 60°C for 4 minutes, then followed by Rapid thermal ramp to 4°C and hold forever. The PCR sequence product was purified before the sequencing using 2M Sodium Acetate washing techniques. The pellet was re-suspended in 5 µl

sterile distilled water [9]. The combination of 9µl of Hi di Formamide with 1 µl of Purified sequence making a total of 10 µl was prepared and loaded on Applied Biosystem (AB1 3130xl model).

## 2.5 Haemolysis Determination

Antibiotic susceptibilities of the isolated bacteria were analysed by using the Kirby-Bauer disc diffusion method. An overnight culture was standardized to a turbidity equivalent to 0.5 McFarland standards (1.5×10<sup>8</sup> cfu/ml) with sterile 1% peptone water. The standardized overnight broth culture was spread on Mueller-Hinton agar plates using sterile swabs. Antibiotic-impregnated discs (Abtek, U.K) were placed on seeded plates and were incubated at 37°C for 24 hours. Zones of inhibition were measured after 24 hours of incubation [10]. The strains were classified as 'resistant (R)', 'intermediate sensitive (I)' or 'sensitive (S)' using standard recommendations of Clinical and Laboratory Standards Institute.

## 2.6 Nucleotide Sequence and Statistical Analysis

Corrected sequences were aligned to 16S rRNA gene sequences in the Genbank DNA database and the homology of the sequences were analyzed using Basic Local Alignment Search Tool (BLAST) program of the National Centre for Biotechnology Information (NCBI) in order to determine the bacterial identities and the statistical analysis to determine the phylogenetic tree was done using Darwin software while descriptive statistics was used to analyze data on morphometric and water parameter.

## 3. RESULTS AND DISCUSSION

### 3.1 Water Parameters and Morphometric Characteristics of the Fish

There is a range of biological, physical and chemical components that affect the quality of water. These variables provide general indication of water pollution, whereas others enable a direct tracking of the sources of pollution [11]. The Physico-chemical parameters recorded at the study site are temperature, pH and Dissolved oxygen. The temperature value range between 28.7-29.4°C, this value recorded was similar to [12] who recorded temperature value range of 26.8°-27°C, this result is within WHO standard. The pH recorded was 6.6 which was within the

WHO standard, The Dissolved Oxygen value was 3.15 mg/l, this value is low and it is below the WHO standard limit of 5 mg/l for sustenance of aquatic life, any value below the standard will affect aquatic biological life adversely, a concentration below 2 mg/l may eventually lead to death of most fishes [12]. The sampling site was divided into three points (point A, B and C). The average total length, standard length, head length and average weight of the fish sampled at point A were 28.35±0.716 cm, 23.57±0.711 cm, 5.03±0.111 cm and 52.42±0.945 g respectively while that recorded at point B were 26.77±0.292 cm, 21.79±0.332 cm, 4.98±0.103 cm and 50.17±0.695 g respectively and point C had 26.787±0.398 cm, 22.15±0.318 cm, 4.07±0.110 cm and 48.13±0.313 g respectively.

**Table 1. Physico-chemical parameters of the river at the location**

| Parameter        | Values      | WHO (2006) |
|------------------|-------------|------------|
| Dissolved oxygen | 3.15 mg/L   | 5 mg/L     |
| Temperature      | 28.7-29.4°C | <40°C      |
| pH               | 6.6-6.9     | 6.5-9.5    |

Keys: WHO= World Health Organization, °C= Degree Celsius

Table 2 showed the result whereby the total length, standard length of fish sampled in point A was significantly different from the other two points. The head length of fish sampled at point C was significantly different from point A and B, while the average weight recorded from point A; point B and C are significantly different from one another.

### 3.2 Morphology of Bacteria

The morphology of the isolated bacteria is presented in Table 3. *Enterobacter spp* is a gram negative, round-shaped, motile organism, *Pseudomonas fluorescens* is a gram negative, round-shaped, motile organism while *Streptococcus spp* is a gram positive, round-shaped, non-motile organism. The total bacterial count in the guts, gills and skin of advanced *C. gariepinus* juveniles showed that gut has the

highest bacteria count ( $4.72 \times 10^4$ ), followed by the skin with  $3.5 \times 10^4$  while the gill recorded the least count of  $2.53 \times 10^4$ .

### 3.3 Molecular Characterization by Gene Sequencing

The size of the amplified band with 16S universal primer was 1600bp for the 26 samples, this is similar to the work of [9]. The DNA target for amplification using PCR technique was 16S ribosomal RNA, this is because 16S rRNA has its presence in all bacteria [13], often existing as a multi gene family, or operons and the function of the 16S rRNA gene over time has not changed, suggesting that random sequence changes are a more accurate measure of time (evolution); and the 16S rRNA gene (1,500bp) is large enough for informatics purposes.

The blasting of the gene sequence for the isolates shows that there were 17 different bacteria strains present. The isolates identified were *Brevibacillus agri* strain, *Bacillus cereus* strain, *Ignatzschineria indica* strain, *Comamonas kerstersii* strain, *Proteus vulgaris* strain, *Lysinibacillus sphaericus* strain, *Proteus hauseri* strain, *Clostridium bifermentans* strain, *Proteus mirabilis* strain, *Pseudomonas putida* strain, *Escherichia coli* strain, *Comamonas jiangduensis* strain, *Enterobacter cloacae* strain, *Serratia marcescens* strain, *Alcaligenes faecalis* strain, *Lysinibacillus fusiformis* strain, *Myroides odoratimimus* strain (Tables 4, 5 and 6).

### 3.4 Cluster Analysis of the Bacteria

Fig. 1 showed the cluster analysis for the 26 bacteria strains. *Proteus spp* and *Comamonas spp* were grouped together, *Bacillus spp*, *Lysinibacillus spp*, *Pseudomonas putida* and *Escherichia coli* were also grouped together while *Serratia marcescens* is in the middle of the two groups. The cluster analysis showed that *Bacillus cereus* strain and *Lysinibacillus spp* are more related when compared with *Proteus vulgaris* strain and *Comamonas spp* on the other end.

**Table 2. Morphometric characteristics of the fish sampled (P<0.05)**

|                 | Point A     | Point B     | Point C      |
|-----------------|-------------|-------------|--------------|
| Total length    | 28.35±0.716 | 26.77±0.292 | 26.787±0.398 |
| Standard length | 23.57±0.711 | 21.79±0.332 | 22.15±0.318  |
| Head length     | 5.03±0.111  | 4.98±0.103  | 4.07±0.110   |
| Average weight  | 52.42±0.945 | 50.17±0.695 | 48.13±0.313  |

Length in centimeter, weight in grams

**Table 3. Morphology of isolated bacteria**

| Bacteria organism                   | Motility | Opacity     | Elevation | Edges  | Consistency | Color         | Shape     |
|-------------------------------------|----------|-------------|-----------|--------|-------------|---------------|-----------|
| <i>Enterobacter spp</i>             | +        | Opaque      | Flatten   | Smooth | Wet         | White         | Round     |
| <i>Pseudomonas fluorescens</i>      | +        | Opaque      | Flat      | Smooth | Wet         | Grey          | Round     |
| <i>Klebsiella spp</i>               | -        | Opaque      | Round     | Smooth | Wet         | White         | Round     |
| <i>Proteus spp</i>                  | +        | Opaque      | Round     | Smooth | Wet         | White         | Round     |
| <i>E. coli</i>                      | +        | Opaque      | Flatten   | Smooth | Wet         | White         | Round     |
| <i>Staphylococcus saprophyticus</i> | -        | Opaque      | Flatten   | Smooth | Wet         | Golden-yellow | Round     |
| <i>Bacillus subtilis</i>            | +        | Opaque      | Flatten   | Rough  | Dry         | Yellow        | Irregular |
| <i>Streptococcus spp</i>            | -        | Translucent | Flat      | Smooth | Wet         | Creamy white  | Round     |

**Table 4. Nearest relative of 12 selected isolates of bacteria from the gut samples**

| Isolates | Nearest relatives                       | Accession numbers | Homology (%) |
|----------|---|-------------------|--------------|
| AJU017   | <i>Ignatzschineria indica strain</i>    | KM272388.1        | 90           |
| AJU013   | <i>Proteus hauseri strain</i>           | JN092597.1        | 96           |
| AJU038   | <i>Lysinibacillus sphaericus strain</i> | JN700164.1        | 79           |
| AJU014   | <i>Pseudomonas putida strain</i>        | JQ782512.1        | 95           |
| AJU018   | <i>Comamonas jiangduensis strain</i>    | KJ806505.1        | 85           |
| AJU037   | <i>Proteus mirabilis strain</i>         | JX974560.1        | 94           |
| AJU007   | <i>Proteus vulgaris strain</i>          | JN384144.1        | 93           |
| AJU021   | <i>Lysinibacillus fusiformis strain</i> | JX485804.1        | 93           |

**Table 5. Nearest relative of 9 selected isolates of bacteria from the gill samples**

| Isolates | Nearest relatives                       | Accession number | Homology (%) |
|----------|---|------------------|--------------|
| AJG023   | <i>Brevibacillus agri strain</i>        | KF600772.1       | 90           |
| AJG017   | <i>Comamonas kerstersii strain</i>      | NR025530.1       | 81           |
| AJG009   | <i>Proteus vulgaris strain</i>          | JN384161.1       | 83           |
| AJG013   | <i>Clostridium bifermentans strain</i>  | JX267051.1       | 91           |
| AJG038   | <i>Lysinibacillus sphaericus strain</i> | HM631831.1       | 94           |
| AJG014   | <i>Escherichia coli strain</i>          | JN578646.1       | 91           |
| AJG018   | <i>Enterobacter cloacae strain</i>      | JX514409.1       | 97           |
| AJG037   | <i>Serratia marcescens strain</i>       | AB244453.1       | 93           |
| AJG010   | <i>Serratia marcescens strain</i>       | AB244453.1       | 93           |

**Table 6. Nearest relatives of 9 selected isolates of bacteria from the skin samples**

| Isolates | Nearest relatives                       | Accession number | Homology |
|----------|---|------------------|----------|
| AJS023   | <i>Bacillus cereus strain</i>           | KJ865589.1       | 88       |
| AJS017   | <i>Bacillus cereus strain</i>           | FJ493043.1       | 97       |
| AJS009   | <i>Lysinibacillus sphaericus strain</i> | KM581344.1       | 87       |
| AJS013   | <i>Comamonas kerstersii strain</i>      | NR025530.1       | 81       |
| AJS038   | <i>Proteus mirabilis strain</i>         | KC456548.1       | 85       |
| AJS037   | <i>Alcaligenes faecalis strain</i>      | DQ857898.1       | 90       |
| AJS007   | <i>Bacillus cereus strain</i>           | KJ453111.1       | 81       |
| AJS040   | <i>Proteus hauseri strain</i>           | KM406467.1       | 85       |
| AJS061   | <i>Myroides odoratimimus strain</i>     | GQ359964.1       | 80       |

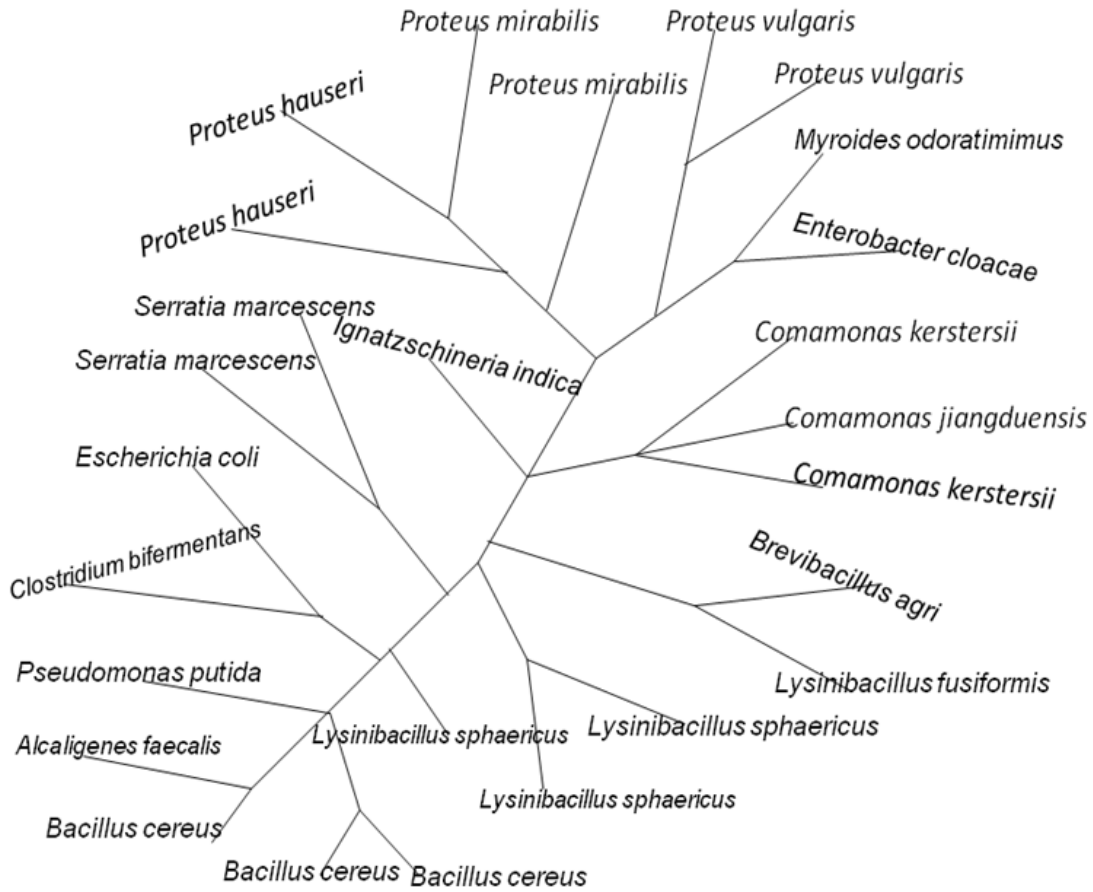


Fig. 1. Cluster analysis of the 30 selected isolates

Table 7. Haemolytic pattern of bacteria isolated

| Isolated organism                       | Haemolysis |          |           |
|---|------------|----------|-----------|
|   | α (alpha)  | β (beta) | γ (gamma) |
| <i>Brevibacillus agri</i> strain        | -          | -        | +         |
| <i>Bacillus cereus</i> strain           | -          | +        | -         |
| <i>Ignatzschineria indica</i> strain    | -          | -        | +         |
| <i>Comamonas kerstersii</i> strain      | -          | +        | -         |
| <i>Proteus vulgaris</i> strain          | -          | +        | -         |
| <i>Lysinibacillus sphaericus</i> strain | -          | -        | +         |
| <i>Proteus hauseri</i> strain           | -          | -        | +         |
| <i>Clostridium bifermentans</i> strain  | -          | +        | -         |
| <i>Escherichia coli</i> strain          | -          | +        | -         |
| <i>Alcaligenes faecalis</i> strain      | +          | -        | -         |
| <i>Serratia marcescens</i> strain       | -          | -        | +         |
| <i>Lysinibacillus fusiformis</i> strain | -          | -        | +         |
| <i>Myroides odoratimimus</i> strain     | -          | +        | -         |
| <i>Enterobacter cloacae</i> strain      | -          | +        | -         |
| <i>Comamonas jiangduensis</i> strain    | -          | +        | -         |
| <i>Proteus mirabilis</i> strain         | -          | +        | -         |
| <i>Pseudomonas putida</i> strain        | -          | +        | -         |

Keys: α= Complete haemolysis, β= Partial haemolysis, γ= Gamma haemolysis. += Positive reaction, -= Negative reaction

### 3.5 Antibiotics Susceptibility Pattern of the Bacteria Isolates Strained from *C. gariepinus*

Table 7 showed the haemolytic pattern of fish bacteria isolated. Only *A. faecalis* strain displayed complete haemolysis ( $\alpha$  alpha), *B. cereus* strain, *C. kerstersii* strain, *P. vulgaris* strain, *C. bifermentans* strain, *E. coli* strain, *C. jiangduensis* strain, *E. cloacae* strain, *M. odoratimimus* strain, *P. mirabilis* strain and *P. putida* strain displayed partial haemolysis ( $\beta$  beta), while *B. agri* strain, *I. indica* strain, *L. sphaericus* strain, *P. hauseri* strain, *S. marcescens* strain, *L. fusiformis* strain displayed no haemolysis ( $\gamma$  gamma).  $\beta$ -haemolysin is a very important bacterial virulence factors, Haemolysin and related proteins containing cystathionine  $\beta$  synthase (CBS) domains are bacterial toxins that tend to function by assembling identical subunits in a membrane-spanning pore [14]. Out of the 17 bacteria strains identified, 10 (58.82%) exhibited  $\beta$ -haemolysis; only *Alcaligenes faecalis* strain displayed partial haemolysis while 6 (35.29%) were non-haemolytic in blood agar. In addition, the concentration of the blood in the agar influence the observed haemolysis; high concentrations may cause  $\beta$ -haemolytic organisms appear as non-haemolytic organisms while low concentrations may make  $\alpha$ - or  $\beta$ -haemolysis difficult to determine.

### 4. CONCLUSION

This study showed that the isolated bacteria from the gills, gut and skin of *Clarias gariepinus* post juveniles from Ajilete location on Yewa River included genera strains of *Ignatzschineria*, *Proteus*, *Lysinibacillus*, *Pseudomonas*, and *Comamonas* with the genera *Proteus* dominating the guts. The bacteria genera identified in the gills included strains of *Brevibacillus*, *Comamonas*, *Proteus*, *Clostridium*, *Lysinibacillus*, *Escherichia*, *Enterobacter*, and *Serratia* with the genera *Serratia* dominating the gills. While the strains of *Bacillus*, *Lysinibacillus*, *Comamonas*, *Proteus*, *Alcaligenes*, and *Myroides* were identified on the skin with the genera *Bacillus* dominating the skin.

### COMPETING INTERESTS

Authors have declared that no competing interests exist.

### REFERENCES

- Narain N, Nunes ML. Marine animal and plant products. In: Handbook of meat, poultry and seafood quality, Nollet LML, Boylston T, eds. Blackwell Publishing, 2007;247.
- Austin B. The bacterial microflora of fish. The Scientific World Journal. 2002;2:558-572. DOI: 10.1100/tsw.2002.137
- Fobes BA, Sahm DF, Weisfeld A. Baily and Scott's diagnostic microbiology, Mosby, St. Louis, Mo, USA, 11<sup>th</sup> edition; 2002.
- Ekundayo FO, Diyaolu DO, Fasakin EA. Composition, distribution and antimicrobial sensitivity of bacteria associated with cultured *Clarias gariepinus*. Malaysian Journal of Microbiology. 2014;10(2):72-79.
- Tiamiyu AM, Emikpe BO, Adedeji OB. Isolation and identification of aerobic bacteria flora of the skin and stomach of wild and cultured *Clarias gariepinus* and *Oreochromis niloticus* from Ibadan, Southwest Nigeria. Journal of Applied Sciences Research. 2011;7(7):1047-1051.
- Akinyemi AA, Oyelakin OO. Molecular characterization of bacteria isolates from farm-raised catfish *Clarias gariepinus*. British Microbiology Research Journal. 2014;4(12):1345-1352.
- Cowan ST, Steel KJ. Enterobacteriaceae, in Barrow GI, Feltham RKA, (Eds). Manual for the identification of medical bacteria (3<sup>rd</sup> edition). Cambridge University press, United Kingdom. 1993;213-218.
- Thottappilly G, Mignouna HD, Onasanya A, Oyelakin O, Singh NK. Identification and differentiation of isolates of *Colletotrichum gloeosporioides* from yam by random amplified polymorphic DNA markers. African Crop Science Journal. 1999;7:197-207.
- Oyelakin OO, Akinyemi AA, Oloyede AR, Agboola AK, Oloye IO, Akinduti PA. Molecular characterization and antibiotic resistance profile of bacteria associated with *Brycinus longipinnis* from Eggua station on Yewa River. British Journal of Applied Science & Technology. 2016; 15(2):1-7.
- Ekelemu JK, Akinyemi AA, Oyelakin OO, Oloyede AR, Agbasi VO. Molecular characterization and antibiotic resistance profile of bacteria associated with *Clarias gariepinus* (Burchell, 1822) from

- Ebute-Ibora location on Yewa River. American Journal of Experimental Agriculture. 2016;11(5):1-8.
11. Olatunji MK, Kolawole TA, Albert BO, Anthony IO. Assessment of water quality in Asa River (Nigeria) and its indigenous *Clarias gariepinus* fish. Int. J. Environ. Res. Public Health. 2011;8:4332-4352.
  12. Taiwo AG, Adewunmi AR, Ajayi JO, Oseni OA, Lanre-Iyanda YA. Physico-Chemical and microbial analysis of the impact of Abatoir effluents on Ogun River course. International Journal of ChemTech Research CODEN (USA): IJCRGG. 2014; 6(5):3083-3090.
  13. Janda JM, Abbott SL. 16S rRNA gene sequencing for bacterial identification in the diagnostic laboratory: Pluses, perils, and pitfalls. Journal Clinical Microbiology. 2007;45(9):2761-2764.
  14. Oladipo IC, Bankole SO. Nutritional and microbial quality of fresh and dried *Clarias gariepinus* and *Oreochromis niloticus*; International Journal of Applied Microbiology and Biotechnology Research. 2013;1-6.

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