Isolation of Aerobic Bacteria Flora in the Gills and Gastrointestinal Tract of Culturable Freshwater Fish from Ogbia Bayelsa State

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Article info  
Received 26 August 2020  
Revised 9 March 2021  
Accepted 14 March 2021  
Published online 31 March 2021  
Regular article

Abstract  
Fish is in high demand as food, food additives, and supplements as they are a rich source of carbon, proteins, vitamins, and minerals. Fish has been established to possess bacterial populations on or in their skin, gills, digestive tract, etc. with their microbial diversity often reflecting the bacterial populations of the surrounding water which are either allochthonous or autochthonous. This study isolated and enumerated aerobic bacteria flora in the gastrointestinal tract and gills of four culturable freshwater fish (Silver catfish, Tilapia, Clarias, and Heterobranchus). These species of culturable freshwater fish were obtained and each adult specie held in a separate glass containing unchlorinated water and transferred to the laboratory. The quantitative and qualitative estimation of the bacteria flora present in the gill and Gastro-Intestinal Tract (GIT) of fish were investigated. The Mean total plate count on Nutrient Agar (NA), Blood Agar (BA), MacConkey Agar (MCA), Cysteine-Lactose-Electrolyte-Deficient Agar, (CLED Agar) and Salmonella – Shigella Agar (SSA) was found to be 60 and 40 CFU, 150 and 80 CFU, 100 and 90 CFU and 80 and 70 CFU respectively. Most of the isolates were of public significance. The results showed that fish contains a large number of microbiotas which may play a role in nutrition and health.

1. Introduction

Fish is in high demand as food, food additives and supplements as they are a rich source of carbon, proteins, vitamins, and minerals. Fish has been established to possess bacterial populations on or in their skin, gills, digestive tract, and light-emitting organs, internal organs (kidney, liver, and spleen) with their microbial diversity often reflecting the bacterial populations of the surrounding water (Austin, 2002). These microbiotas are either allochthonous bacteria (normal flora) or allochthonous (opportunist and transient) (Ringo et al., 1995). The composition of the allochthonous intestinal tract microbiota is highly variable and is affected by many environmental conditions as salinity, temperature, etc. (Liu et al., 2008; Pond et al., 2006; Ringo et al., 1995), but stable in fish kept in defined conditions (Pond et al. 2006). Food accessibility, composition and changes may affect the bacterial diversity in a fish intestine (Ringo & Strom, 1994; Ringo et al., 2006). The diversity of the microbiotas of the fish intestine has been shown to be largely dependent on the bacterial colonization during their early development (Ringo & Birkbeck 1999; Ringo et al., 1995) and often reflect those of the surrounding water (Austin, 2002). However, some studies have also reported a wider diversity of the gut microflora than previously believed (Ringo et al. 2006; Hovda et al. 2007; Ward et al. 2009), especially in the intestinal contents of freshwater fish (Cantas et al., 2012; Gonzalez et al., 1999; Spanggaard et al., 2000; Wu et al. 2010). This study is aimed at isolating and enumerating the aerobic bacteria flora from the gastrointestinal tract of culturable freshwater fish from a fish pond in Ogbia, Bayelsa State.

2. Material and methods

2.1 Sample Site

The samples were collected at Ogbia (4° 39’ 00" N 6°16’00" E), a Local Government Area of Bayelsa State in the Niger Delta region of Nigeria. It has an area of 695 km² and an estimated population of 179,926. It is headquartered to Oloibiri where crude oil was first discovered in Nigeria in 1956.

2.2 Sample Collection

The fish sample was collected with aquatic dip net into clean containers, appropriately labeled and taken to the laboratory for analysis.

2.3 Isolation of Microbes

Samples of silver cat fish, Tilapia, Clarias and Heterobranchus were collected from a fish pond in Otuaba Community, Ogbia L.G.A of Bayelsa State. Each adult specie of the fish was held in a separate glass containing unchlorinated water during the
transfer to the laboratories. They were sacrificed by pithing. The ventral surface of the fish was carefully scrubbed with 1 % iodine solution for surface decontamination (Trust & Sparno, 1974) and dissected under aseptic conditions. The gill portion and GIT portion were homogenized individually with distilled water and 1 ml of the sample plated in triplicate on nutrient agar for evaluation of the total plate count. Salmonella – shigella agar for total salmonella shigella counts, MacConkey agar for total coliform count and blood agar (as a selective media) for streptococcus and staphylococcus count. The Plates were incubated at 37 °C for 24 hours aerobically to count bacteria colonies. The distinct colonies (based on their different morphological, character (color, colony, size, surface, margin and opacity), were sub cultured on the respective media to obtain pure culture.

2.4 Identification and Characterization of Microbes

Phenotypic identification of microbes was performed according to standard methods (Barrow and Feltham, 2003). Expressed microbial morphological traits examined include the orientation, size, and pigmentation which were performed by visual inspection of microbial isolates on petri-plates, as well as cell wall characteristics which was performed by Gram staining of the isolates. Expressed biochemical traits examined include: the production of coagulase enzyme (coagulase test); the production of catalase enzyme (catalase test); the production of urease enzyme (urease test); biodegradation of tryptophan to produce indole (indole test); utilization of citrate as a sole carbon source (citrate test); production of stable acids from glucose fermentation (methyl red test); production of acetoin as the main end product with small quantities of mixed acids from glucose metabolism (Voges-Proskauer test); and motility.

3. Results

| Table 1. The Prevalence of Aerobic Bacteria in GIT and Gills of Culturable Fresh Water Fish (CFU) |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| No of isolates                  | Escherichia coli| Staphylococcus aureus | Proteus sp | Pseudomonas sp | Salmonella sp | Vibrio sp | Klebsiella sp |
| GIT & Gills                     |                 |                   |               |                 |           |           |               |
| Silver Catfish                  | 60              | 10                | 5             | -               | 10            | 5           | 20             | 10 |
| Gill                            | 40              | 3                 | 9             | -               | 2             | 8           | 11             | 7  |
| Tilapia                         | 150             | 40                | 25            | 10              | 20            | 6           | 30             | 19 |
| Gill                            | 80              | 15                | 5             | -               | 17            | 21          | 22             | -  |
| Claris                          | 80              | 5                 | -             | -               | 10            | 20          | 25             | 20 |
| Gill                            | 70              | -                 | 23            | 18              | 14            | 15          | -              |    |
| Heterobratis                    | 100             | 10                | 30            | 24              | 20            | -           | 16             | -  |
| Gill                            | 90              | 6                 | 10            | -               | -             | 19          | 31             | 25 |
| 670                             | (13.28%)        | (12.33%)          | (8.51%)       | (14.48%)        | (13.73%)      | (25.37%)    | (12.80%)       |

Table 1 above showed that *E. coli*, *staphylococcus aureus*, *proteus sp*, *pseudomonas sp*, *salmonella sp*, *vibrio sp*, *klebsiella sp* were the bacteria isolated. *Vibrio* had the highest occurrence in the GIT and gill of the fish samples (28.6%) while *Proteus* (8.92%) had the least occurrence of bacteria.

| Table 2. Gram Negative and Positive Organisms Present |
|-----------------------------------------------------|
| Test                                               | Probable Organism |
|                                                    | *E. coli* | *S. aureus* | Proteus sp. | *Pseudomonas sp.* | *Salmonella sp.* | *Vibrio sp.* | *Klebsiella sp.* |
| Oxidase test                                       | -        | -           | +           | -                | +               | -           |
| Catalase test                                      | +        | +           | +           | +                | +               | -           |
| Coagulase test                                     | -        | +           | -           | -                | -               | +           |
| Indole                                            | +        | -           | -           | -                | +               | -           |
| Methyl red test                                    | +        | +           | +           | +                | -               | -           |
| Voges-Proskauer reaction                           | -        | +           | -           | -                | -               | +           |
| Urease                                            | -        | +           | -           | -                | -               | +           |
| Citrate utilization                                | -        | +           | +           | +                | -               | +           |
| Motility                                           | +        | -           | +           | +                | +               | -           |
| Gram staining                                      | -        | -           | -           | -                | -               | -           |
Table 3. Appearance of the Isolated Organisms on a Cultured Plate

| Media          | Appearance                                                                 | Probable Organism        |
|----------------|---------------------------------------------------------------------------|--------------------------|
| MacConkey agar | Smooth, glossy, translucent, rose pink colonies                            | Escherichia coli         |
| Blood agar     | Smooth, circular, 1.5 mm diameter, yellow opaque colonies                  | Staphylococcus aureus    |
| Blood agar     | Colonies surrounded by zone of haemolysis                                 | Vibrio sp.               |
| Nutrientagar   | Moist, translucent, round disks (1-2 mm in diameter) with a bluish tiny in transmitted light colonies | Proteus sp.              |
| MacConkey agar | Colonies became reddish on prolonged incubations                           | Klebsiella sp.           |
| Blood agar     | The greenish zone initially appeared around the colonies and later became clear due to haemodigestion | Pseudomonas sp.          |
| MacConkey agar | Mucoid red colonies with fishy smell                                       | Proteus sp.              |
| Blood agar     | undulated white translucent, mucoid colonies                              | Klebsiella sp.           |
| Salmonella shigella | Non lactose fermenting, smooth, and pale colonies                          | Salmonella sp.           |
| Cled agar      | Heavily dull surface and irregular lines appeared with bluish green colour pigment | Salmo sp.                |

4. Discussion

Fish living in a natural environment are known to harbor some pathogenic Enterobacteriaceae (Pillay, 1990). In this study seven bacteria viz. Escherichia coli, Staphylococcus aureus, Proteus sp., Pseudomonas sp., Salmonella sp., Vibrio sp. and Klebsiella sp. were isolated. According to Guzman et al. (2004), the invasion of fish muscles due to breakage of immunological barrier of fish by pathogens is likely to occur when the fish are raised in pond with faecal coliforms such as vibrio cholera, E. coli, S. aureus etc. with greater than 10^4 – 10^12 per 100 ml in pond water respectively. These bacteria isolated are all of public health significance and thus require close attention. Two of the isolates (Staphylococcus and Salmonella) are amongst the four most common types of food poisoning bacteria. The other two being clostridium and campylobacter. However, the other isolates apart from Pseudomonas have been frequently associated to food borne infections (CDC, 2019; Wang et al., 2010). The interesting thing about some of these organisms like Staphylococcus produce heat stable toxin that is not destroyed by cooking. The ingestion of contaminated fish or fish products that is not properly handled or cooked contributes significantly to cases of food borne illnesses. There is therefore a need to develop or adopt safe management practices for the production of fish or its product for human consumption (Teophilo et al., 2002).

5. Conclusion

Fish is in high demand as either food, food additives or supplements. This study aimed at isolating and enumerating the aerobic bacteria flora from the gastrointestinal tract of cultivable freshwater fish has demonstrated that the gills and guts of fresh water fish are a potential source of microorganisms of public health importance. If not properly prepared, consuming fresh fish form contaminated water can cause food borne diseases (poisoning and intoxication). Since there is a strong correlation between environmental contamination and the diversity of microbiome isolated from fish, it is vital that the proper environmental and public health attention and commitment be given to the fish habitats. It is also pertinent that there is an increased awareness of proper preparation of these fishes before consumption.

Declaration of interest

The authors report no conflicts of interest.

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