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

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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 allochthonous. 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 cultural 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 (opportunistic 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 km^2 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 Heterobratis were collected from a fish pond in Otuaba Community, Ogbia L.G.A of Bayelsa State. Each adult species of the fish was held in a separate glass containing unchlorinated water during the

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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 distill 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 ^{o}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**
Silver Catfish								
GIT	60	10	5	-	10	5	20	10
Gill	40	3	9	-	2	8	11	7
Tilapia								
GIT	150	40	25	10	20	6	30	19
Gill	80	15	5	-	17	21	22	-
Clarias								
GIT	80	5	-	-	10	20	25	20
Gill	70	-	-	23	18	14	15	-
Heterobratis								
GIT	100	10	30	24	20	-	16	-
Gill	90	6	10	-	-	18	31	25
	670	89 (13.28%)	84 (12.33%)	57 (8.51%)	97 (14.48%)	92 (13.73%)	70 (25.37%)	81 (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 Cled agar Blood agar	Smooth, glossy, translucent, rose pink colonies Smooth, circular, 1.5 mm diameter, yellow opaque colonies Colonies surrounded by zone of haemolysis	*Escherichia coli*
Blood agar	Large, round, golden-yellow colonies, with haemolysins	*Staphylococcus aureus*
Nutrient agar MacConkey agar Blood agar	Moist, translucent, round disks (1-2 mm in diameter) with a bluish tiny in transmitted light colonies Colonies became reddish on prolonged incubations The greenish zone initially appeared around the colonies and later became clear due to haemodigestion	*Vibrio* sp.
MacConkey agar	Mucoid red colonies with fishy smell	*Proteus* sp.
MacConkey agar	undulated white translucent, mucoid colonies	*Klebsiella* sp.
Cled agar	Heavily dull surface and irregular lines appeared with bluish green colour pigment	*Pseudomonas* sp.
MacConkey agar Blood Agar Salmonella – shigella	Non lactose fermenting, smooth, and pale colonies Non-hemolytic smooth white colonies Non fermenting colonies with black center	*Salmonella* 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^{14} 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 Staphylococci 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 culturable 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 heath 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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