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Bacterial Flora Associated with Intestine of Tropical Estuarine Fish Species

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Abstract: The intestinal microflora of three fish species (*Tilapia guineensis*, *Sarotherodon melanotheron* and *Liza falcipinnis*) from three rivers (Bonny River, New Calabar River and Sombriero River) in the brackish water zone of the Nigerian Coast was investigated. Populations of aerobic heterotrophic bacteria present in the digestive tracts of fish species and in rivers were estimated using spread plate method. The total heterotrophic, proteolytic, amylolytic, hydrocarbon-degrading and total coliform bacteria in the digestive system of fish were abundant and ranged from 6.60 to 6.88logcfu/g, 5.38 to 5.53logcfu/g, 5.3 to 5.61logcfu/g, 5.68 to 5.95cfu/g and 5.20 to 5.70logcfu/g respectively. The counts varied among fish species and were higher than in water. The culturable bacterial genera in the digestive tract of fish were identified as *Aeromonas*, *Bacillus*, *Pseudomonas*, *Staphylococcus*, *Salmonella*, *Vibrio*, *Escherichia*, *Flavobacterium*, *Lactobacillus*, *Micrococcus* and *Enterobacter*. The results indicate that intestinal bacteria possess enzymatic activity that may be beneficial for the fish. The presence of opportunistic pathogens may predispose the fish to bacterial epizootics especially if populations are stressed by environmental contaminants.

Keywords: Gut microbiota, *Tilapia guineensis*, *Sarotherodon melanotheron* and *Liza falcipinnis*, brackish water.

INTRODUCTION

The normal microflora of aquatic organisms occupies vital niches on the surface of the body and in the digestive tract, thus preventing intrusion of pathogenic microorganisms^{2, 3, 4&5}. It was also proved that the microflora of the digestive tract of fish and shellfish plays an important role in the formation of resistance to infectious diseases, for it produces antibacterial materials preventing pathogenic bacteria from getting into an organism^{6, 7, 8&9}. The autochthonous microflora of the digestive tract of fish participates in the secretion of enzymes, vitamins, amino acids and other physiologically active materials and, therefore, is necessary for normal metabolism of an organism¹⁰. Gastrointestinal microorganisms feed on the food of the macroorganism which is digested by the enzymes produced by them and by the latter. As a result, chymous is formed the composition of which decides the abundance and qualitative composition of communities of gastrointestinal microorganisms¹¹.

It is well known that population level of bacteria associated with the digestive tract of hydrobiont is affected by nutritional and environmental factors. Therefore, estimation of the abundance and dynamics of autochthonous and petroleum hydrocarbon-degrading bacteria in the digestive tract of fish enables evaluation of participation of such bacteria in biodegradation of pollutants and in the process of self-purification of water¹⁰. This is because the microflora of the digestive tract of aquatic animals is proved to be the first to be affected by any pollutants appearing in water¹⁰. Petroleum and its products, apart from being toxic to the majority of bacteria in aquatic ecosystems, are sources of carbon and energy to some bacteria. The result of such an effect is a community of microorganisms with altered species diversity enriched in microorganisms degrading hydrocarbons¹². The authors stated that if the abundance of bacteria degrading hydrocarbons exceeds 10% of the total abundance of heterotrophs, the ecosystem is slightly and chronically polluted by petroleum products.

Extensive reviews have been published on various aspects of microbial flora associated with fish intestine and on relationships between intestinal microflora and the microflora of aquatic habitats^{10, 13, 14, 15&16}. However, these reviews are mainly on fishes from the temperate region of the world. Information on the microflora of the digestive tract of tropical fish species is scanty. The selected fish species (*Tilapia guineensis*, *Sarotherodon melanotheron* and *Liza falcipinnis*) are very common in the brackish water zone of Nigeria and most especially in the Niger Delta region. They are popular food fish species and source of income in commercial fishery business. They are also prominent in the ecology of tropical waters as well as in the resources of their aquatic systems. The brackish water zone of the Nigerian Coast which includes creeks, lagoons, rivers and mangrove swamps has tremendous potential for fish farming¹⁷. Knowledge about the fish intestinal microbiota would help to understand the disturbances, if there are any, brought about during disease outbreaks. Therefore, we examined the abundance of total heterotrophic, proteolytic, amylolytic, hydrocarbon-degrading and total coliform bacteria in the digestive tract of three tropical estuarine fish species (*Tilapia guineensis*, *Sarotherodon melanotheron* and *Liza falcipinnis*) from three rivers (Bonny River, New Calabar River and Sombriero River) in Rivers State of Nigeria. The culturable intestinal bacterial flora of *Tilapia guineensis* caught from the rivers was also established.

MATERIALS AND METHODS

Sample Collection: Brackish water samples were collected in sterile plastic bottles from Bonny River, New Calabar River and Sombriero River in the Niger Delta of Nigeria. The fish species (*Tilapia guineensis*, *Sarotherodon melanotheron* and *Liza falcipinnis*), from the same rivers, were collected in sterile plastic bags.

Bacterial Isolation: Populations of aerobic heterotrophic bacteria, proteolytic and amylolytic bacteria, hydrocarbon degrading bacteria and total coliforms present in brackish water and in the digestive tract of marine fishes were separately estimated using spread plate method. The fishes were cleaned externally with ethanol and the intestines dissected under sterile conditions. One gram of intestinal content was aseptically transferred to 9ml sterile physiological saline in a test tube. This gave 10^{-1} dilution from where subsequent 10-fold serial dilutions were carried out up to 10^{-6} . The Brackish water samples were diluted in a range 1:10 to 1:1000. Subsamples of 0.1ml of three dilutions expected to give between 30 and 300 colony –forming units were plated on five media in triplicates. The media chosen were: Nutrient agar (for isolation of total heterotrophic bacteria), milk agar (for isolation of proteolytic bacteria), starch agar (for isolation of amylolytic bacteria), MacConkey agar (for isolation of total coliform bacteria) and mineral salt medium (for isolation of hydrocarbon degrading bacteria). Mineral salt medium was used to selectively isolate hydrocarbon utilizing bacteria using the vapour phase transfer method [16].

The modified crude medium comprises of 10g NaCl, 0.42g MgSO₄.7H₂O, 0.29g KCl, 0.83g KH₂PO₄, 1.25g Na₂HPO₄, 0.42g NaNO₃, 20g Agar-Agar and distilled water to 1L. After inoculation of the mineral salt agar plates, Whatman (9cm) No. 1 filter papers were soaked in crude oil and introduced on the cover of the Petri dishes. The thin layer crude oil spread on the filter paper served as a hydrocarbon source and the plate with filter paper without crude oil was used as control. All the plates were incubated at 37°C for 24-48 hours except for Mineral salt agar plates which were incubated at 30°C for 5-7days. The bacterial colonies appearing on each plate were counted and the colony forming units per gram (cfu/g) of the intestinal contents were calculated. Proteolytic bacteria were identified according to zone of protein (casein) hydrolysis on milk agar. Amylolytic bacteria were determined according to zone of starch hydrolysis on starch agar under the action of iodine solution.

Isolates with distinct colony morphology were picked and streaked repeatedly on nutrient agar plates until pure. The purified isolates were identified to generic level based on their morphological and physiological characteristics¹.

Statistical analysis: The differences in microbial counts were assessed using the one-way analysis of variance (ANOVA). In all cases treatments were considered significantly different if $p<0.05$.

RESULTS AND DISCUSSION

The viable counts of bacteria in the digestive tract of tropical estuarine fish species (*Tilapia guineensis*, *Sarotherodon melanotheron* and *Liza falcipinnis*) caught from Bonny River, New Calabar River and Sombriero River are shown in Figures 1, 2 and 3 respectively. The results reveal that dense bacterial populations occur in the digestive tract of fish. The bacterial counts in water were significantly lower in comparison with their numbers in the digestive tract. This indicates that the digestive tract provides favourable ecological niches for these organisms. These results are in accordance with those found for other fish species^{10, 15, 16, 18-22}.

The total heterotrophic, proteolytic, amylolytic, hydrocarbon-degrading and total coliform bacteria in the digestive system of fish were abundant and ranged from 6.60 to 6.88logcfu/g, 5.38 to 5.53logcfu/g, 5.3 to 5.61logcfu/g, 5.68 to 5.95cfu/g and 5.20 to 5.70logcfu/g respectively. The counts varied among fish species. Heterotrophic counts are representative of a small group of active bacteria that react immediately to changes in nutrient supply²³. The stability of the ecological balance of microflora of the digestive tract of aquatic organisms depends upon the trophism, productivity, toxicity and other chemical and physical parameters of water bodies¹³. This explains why the viable counts of different types of bacteria in the digestive tract varied with fish species.

Some authors^{5&21} have also established that bacteria in the digestive tract of fish demonstrated proteolytic and amylolytic activities. The presence of these microorganisms in the digestive tract indicates a

significant role played by them during digestion of food. The presence of hydrocarbon-degrading bacteria in the gastrointestinal tract of fish is not surprising since microorganisms have the ability to utilize hydrocarbons¹⁰. The abundance of hydrocarbon-degrading bacteria in water and in fish digestive tract reflects the degree of contamination of the ecosystem with oil and its products. The ability of intestinal bacteria of aquatic organisms to use petroleum hydrocarbons as a source of carbon indicates that aquatic organisms participate in biodegradation of oil pollutants as well as self-purification of water¹⁰.

The abundance of total coliform bacteria in the digestive tract of the fish species indicates that the major pollution of the rivers originates from wastewaters. In the digestive tract of aquatic animals total coliform and hydrocarbon-degrading bacteria evoke undesirable changes in its bacterial flora²⁴. Total coliform bacteria are indirect indicators of fecal pollution. An increase in their abundance in the digestive tract of hydrobionts results in an imbalance in their immune system which directly affects a microorganism's physiological state¹³.

The qualitative composition of bacteria in the digestive tract of *Tilapia guineensis* caught from the rivers was the same. The culturable bacterial genera in the digestive tract of fish were identified as *Aeromonas*, *Bacillus*, *Pseudomonas*, *Staphylococcus*, *Salmonella*, *Vibrio*, *Escherichia*, *Flavobacterium*, *Lactobacillus*, *Micrococcus* and *Enterobacter*. Species of these genera have been isolated from various fishes^{18, 25, 26&27}. There are several possible sources for the establishment of intestinal gut flora and it is generally believed that the processes of bacterial colonization in fish are complex and depend upon the bacterial flora of live feed and water²⁸.

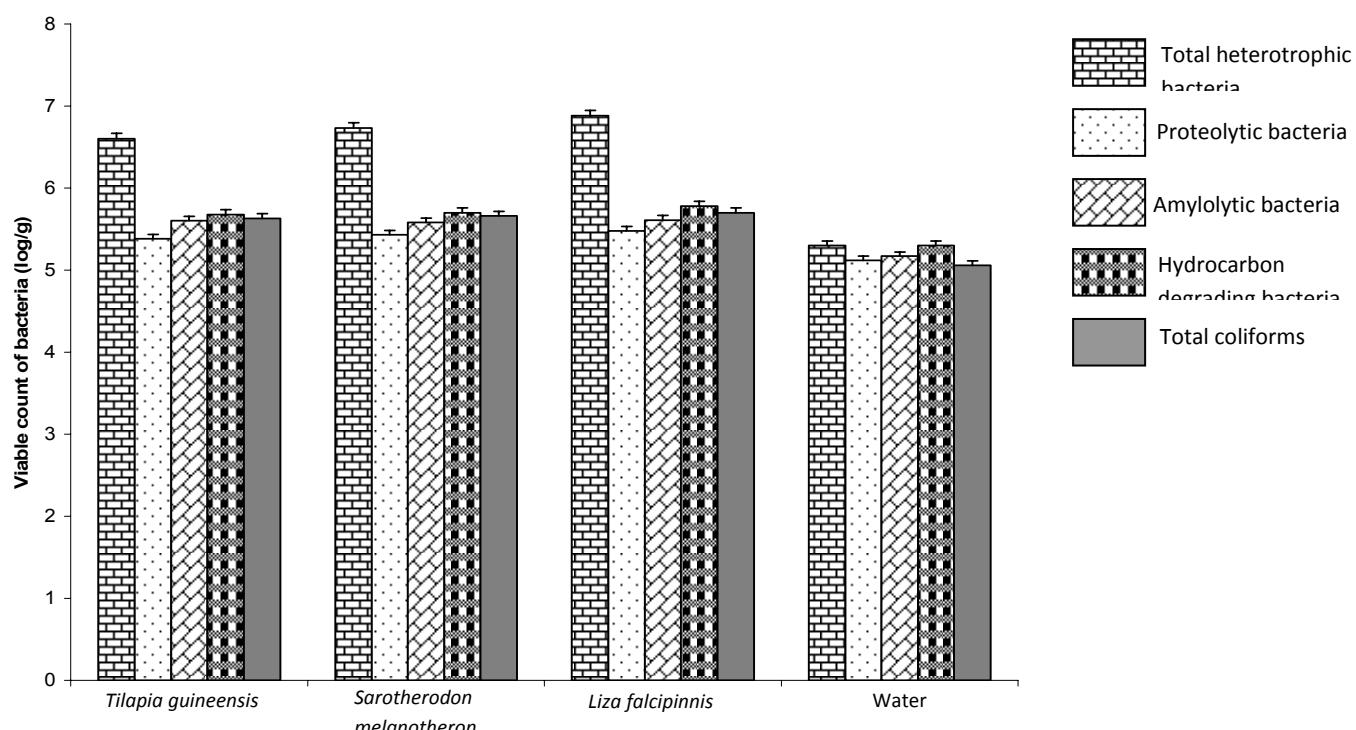


Figure 1: Viable count of bacteria (log/g) in the digestive tract of fish species and water from Bonny River

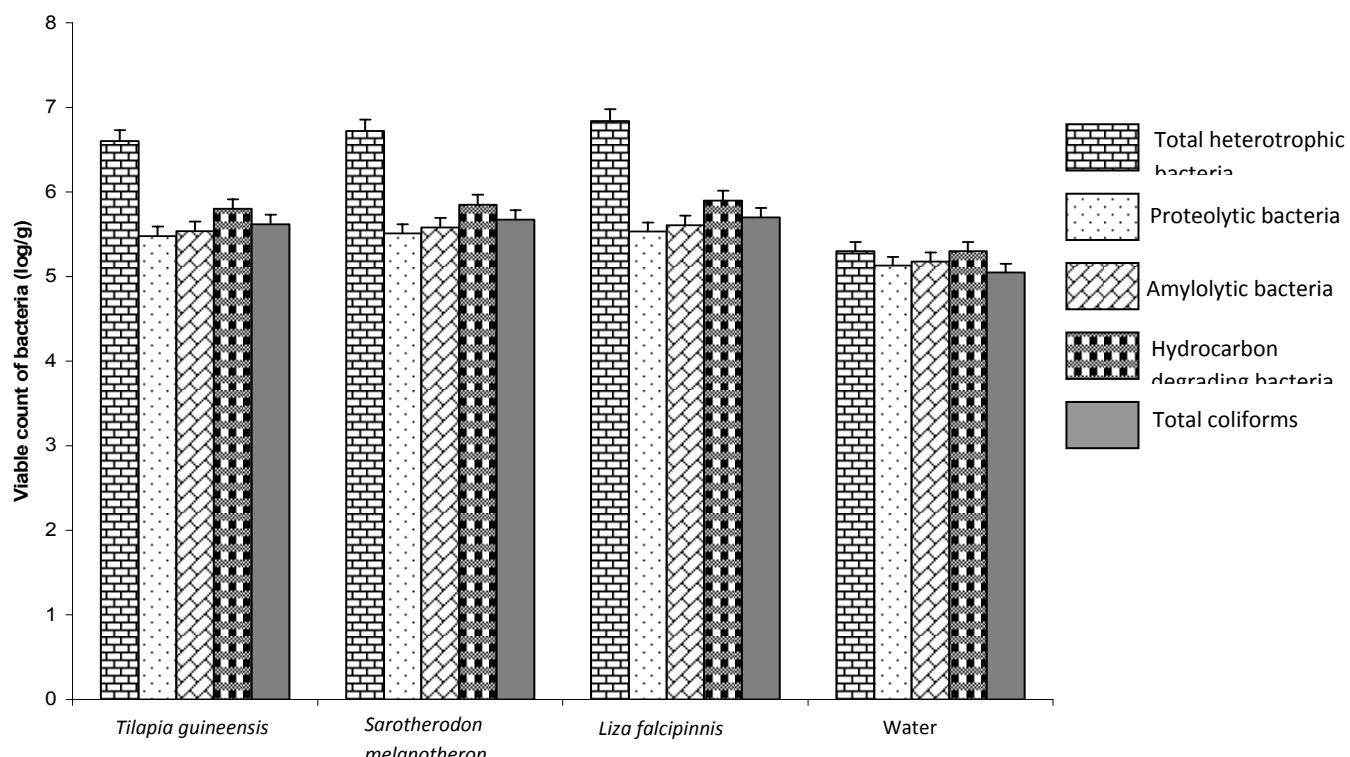


Figure 2: Viable count of bacteria (log/g) in the digestive tract of fish species and water from New Calabar River

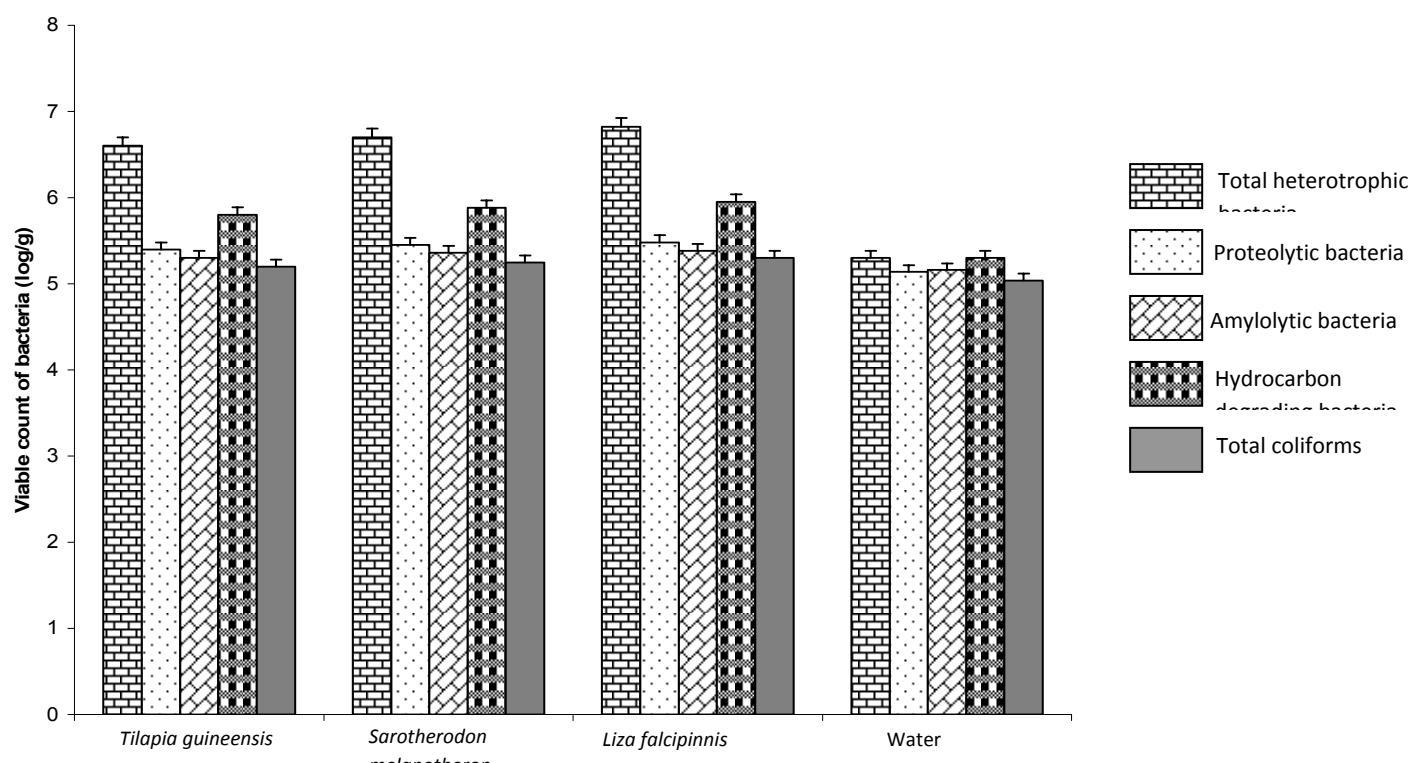


Figure 3: Viable count of bacteria (log/g) in the digestive tract of fish species and water from Sombriero River

Members of the genus *Enterobacter* are common in the digestive tract of freshwater and marine fishes. For example, they have been found in the intestine of rainbow trout²⁵ and Yellow grouper (*Epinephelus awoara*)²⁹. The importance of *Vibrio* in aquaculture has been discussed^{30&31}. Some certain strains of these bacteria isolated in this study possess the virulence factors necessary to induce disease. It has been proposed that fish digestive tract is a reservoir for many pathogens²⁵. The presence of opportunistic pathogens in the intestine of investigated fish indicates the potential for epizootics if the fish species are stressed by environmental contaminants

CONCLUSION

The abundance of total heterotrophic bacteria fluctuated among fish species. The differences in results may be due to the differences in feeding efficiency of fish. The results also showed that bacteria in the digestive tract of fish demonstrated proteolytic and amylolytic activities. Efficient food utilization by the fish is probably promoted by these activities. The abundance of total coliform and hydrocarbon-degrading bacteria in the digestive tract of the investigated fish species reflects the degree of contamination of the ecosystem with wastewater and petroleum hydrocarbon respectively. Most of the bacteria found in the intestinal content are potential pathogens indicating that fish digestive tract is a reservoir of many opportunistic pathogens which may predispose the fish to bacterial epizootics especially if populations are stressed by environmental contaminants.

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