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ORIGINAL ARTICLE

RELATIONSHIP BETWEEN BACTERIA ASSOCIATED WITH FISH POND SEDIMENT, WATER AND THE FISH

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ABSTRACT: Among the bacterial strains isolated from fish pond water, sediment and fish using the enrichment method, the predominant ones were; in water sample *Aeromonas, Streptococcus, Flavobacterium* and *Bacillus* species while in sediment were *Vibrio* and *Flavobacterium* species, whereas that of the fish body surface (*Tilapia zilli*) include *Aeromonas* and *Pseudomonas*. Isolates from the body organs include *Pseudomonas, Vibrio* species, and *Aeromonas* were the most frequently occurring bacterial genera while *Pseudomonas* and *Streptococcus* species were the most frequently occurring bacteria genera in the gills.
KEYWORDS: Relationship, bacteria, Fish, Pond, Water, Sediment.

INTRODUCTION

Due to the high population increase, the hazards involved in cultural fishing methods and the rising need for protein food, man had to look for a better and more economical way of providing himself with protein foods. This attempt resulted in aquaculture. Surprisingly, Akwa Ibom, Cross River and other part of Nigeria and indeed some parts of West Africa, there is no indigenous tradition of aquaculture. Nevertheless, a few have been started in the past 50 years including state owned, private and community owned farms as documented by Ofuka and Marriott (1980). Although the richness of African cichlid fauna was unsuspected a mere century ago, detailed observation was made almost 5000 year ago on *Tilapia nilotica* which was frequently and exquisitely portrayed by Egyptian artists of early dynasties because it was regarded as sacred and went by the name 'Inet' symbolizing the hope of rebirth after death (Chitmits, 1957) but this doesn't prohibit its use as food. Bacteria are known to be active in breakdown of a wide variety of compounds thus helping in digestion of food components to fishes and also fertilize the pond water; apart from mineralization of organic matter, their biomass serves as food for micrograzers. Also quantitative nutrient enrichment of water can be determined by evaluation of bacterial flora since this is indicated by their biomass (Sieburth, 1965; Bezdek and Carlucci, 1972). In studying the roles of bacteria in aquatic ecosystem, Sorokin (1981) saw difficulty with small fractions used for cultural procedures but in the gastro intestinal tract (G.I.T) of fishes, Sugita *et al.,* (1987) stated that, 'there has been a lack of evidence because of the difficulty of successive sampling of intestinal contents while maintaining the host fish alive.

Snieszko, (1974) explained that various kinds of environmental changes are stressful and will also lower the resistance of both hatchery and aquarium-fishes to infection and other diseases. Environmental conditions like overcrowding, temperature fluctuations, inadequate dissolves oxygen, poor maintenance, toxins by other microbes and toxic wastes. Haller and Parker (1981) emphasized the advantage of tank culture over pond culture such that there is economic use of space as many are raised in small areas, it also permits closer control of environment and more precise management than possible in pond culture. Deming *et al.,* (1981) also present a great deal of useful practical information on the treatment of fish diseases. Bacterial flora of G.I.T. have been reported to contribute to the digestive activities within the intestine (MacDonald *et al.,* 1986) reported that chitin hydrolytic bacteria were abundant in the intestine of both fresh water

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and seawaters fishes in contrast with water samples. From this report, intestinal microflora of fresh water fishes were F*lavobacterium, Aeromonas* and *Vibrio* while that of sea water were *Pseudomonas* and *Aeromonas* species. Bacteria species such as *Proteus* and *Staphylococcus* may be exist as secondary invaders of damaged tissues, contaminants from water or intestines. Others like *Aeromonas salmonicida* and *Vibrio anguillarium* causes *Frunculosis* and *Vibriosis* respectively. The pathogenic bacteria present within the host or its environment (water and sediment) will only infect host under stressed conditions or when immune systems are weakened (Seki *et al.,* 1972). Yoshimizu *et al.,* (1980) regarded the fish intestinal microflora as being a reflection of that present in water and food, while Sakata *et al.,* (1980) did not detect any similarity between the bacterial groups isolated from water, intestine or fish diet. Nieto *et al.,* (1984) compared intestinal microflora of two different hatcheries and found that *Enterobacteria* and *Aeromonas* comprised 50% of the total microflora. The purpose of this study was to study the sediment/ water interface especially the microbes inhabiting them.

MATERIALS AND METHODS

2.1. Sample Collection

The water and sediment samples were collected from the Institute of Oceanography, University of Calabar experimental pond using sterile MaCartney bottles, and in the absence of an icebex, the bottles were put in sterile aluminium foil containing ice blocks (to keep the temperature at approximately 4^0C) and covered with a black opaque polythene material. This was transported to the laboratory and processed immediately on arrival. The fish samples were obtained from the same pond before the morning feeding. The fish used in this study was Tilapia species. Three of them have average length (17cm, 18cm and 16.5cm) and were placed in plastic containers filled with water and transported to the laboratory for analysis.

2.2. Microbial Analysis

Sterilized test tubes and water were used to make serial dilutions of the water and sediment samples. Dilutions of 10^{-3} and 10^{-4} was selected to prepare pour plates by pouring a reasonably sufficiently quantity of both media (Zobell agar and Tryptone glucose extract agar) into different plates respectively, and 1ml of the dilution added and rotated to mix well with the medium and left to set on a clean sterile laboratory. The plates were labeled and incubated at 25^0C for 2-3 days. After this time, the different colonies were selected, picked and subcultured into different sterile plates by streaking using sterile wire loop. This was also incubated at 25^0C for 48 hours. This process continues till pure colonies were obtained. Colonies of the pure culture examined macroscopically for shape, size, edge and pigmentation. From fish samples, swabs from the skin surface after killing them by tapping gently on the head using sterile pestle. The swab was plated directly onto the media. Swab from the gills was also plated after washing the fish surface with 70% alcohol and the operculum cut open. The intestine was also asceptically removed as the ventral parts were dissected using sterile dissecting knife and scissors. The upper parts of the intestine was removed using sterile forceps, this section of it was cut to small pieces and homonized with sterile mortar. This was transferred into sterile test tubes of 10ml dilution blank and tenfold dilution were prepared and 1ml of 10^{-3} and 10^{-4} dilutions were poured on sterile petri-plates with agar and mixed. All experiment was done in triplicates. All the plates were incubated at 25^0C for 48 hours and the sediment sample was incubated in anaerobic jar. The viable plate count was done after this time and the number of bacteria was recorded as colony forming unit (cfu/ml). On the whole 50 different colonies were randomly picked up from the plates and subsequently purified by streaking on agar plates. Isolates were characterized to genus level according to the scheme of Shewan *et al.,* (1960) and Hendrie *et al.,* (1964).

2.3. Salt Requirement

Salt requirement for the samples was determined by culturing a 1ml of water and sediment sample and a 1ml of the serial dilution (10^{-3} and 10^{-4}) of the gut content in peptone water with different salt (NaCl) concentrations incorporated into each sample ranging from 0% to 5% concentration. Optimal salt requirement was determined turbidometrically by measuring the absorbance of light by the liquid cultures at 540nm using a colorimeter. This was done after incubation of culture at 25^0C for 48 hours.

RESULTS

3.1. Microbial Population

The aerobic heterotrophic and microaerophilic bacteria were isolated with higher counts obtained

from the Zobell agar (ZA) which had $3.03x10^5$cfu/ml as highest count in the water sample and $1.04x10^6$ cfu/ml in the sediment sample. On the other hand, tryptone glucose extract (TGA) agar had as its highest count $1.74x10^5$ cfu/g in sediment sample and $7.6x10^4$ cfu/ml in water. The highest count for gill swab obtained from the Zobell agar was $3.84x10^2$ cfu/ml, while the count obtained for body swab using TGA was $2.39x10^2$ cfu/ml. The count obtained for intestinal sample using TGA was $1.07x10^5$. However, the Zobell agar favoured the growth of most bacterial colonies than TGA. The result also indicates that the pond (water, sediment and fish samples) were as follows: the sediment sample was predominated by *Vibrio* followed by *Flavobacterium, Micrococcus* and *Enterobacterium*. The water sample was predominated by *Aeromonas, Streptococcus, Bacillus* and *Flavobacterium*. The intestinal sample was predominated by *Pseudomonas, Aeromonas, Vibrio* and *Enterobacterium*. The body swab was dominated by *Aeromonas*, while the gill by *Streptococcus* and *Pseudomonas* species.

3.2. Percentage Occurrence of Bacterial Genera

Pond water Microflora

The percentage occurrence of the bacteria genera from the fish pond water were as followed: *Aeromonas* (18.0), *Streptococcus* (15.8), *Bacillus* (14.5), *Flavobacterium* (16.6), *Corynebacterium* (10.7), *Micrococcus* (5.7), *Enterobacterium* (6.0) and *Vibrio* (12.7) (Fig 2).

3.3. Pond Sediment Microflora

The sediment of the pond ecosystem had *Vibrio* (34.6), *Flavobacterium* (22.0), *Micrococcus* (11.0), *Enterobacterium* (10.3), *Streptococcus* (8.8), *Bacillus* (7.9) and *Corynebacterium* (5.9) (Fig 2).

3.4. Fish Microflora

The percentage occurrence of bacterial genera of the fish microflora consist of all the samples from gills, body and intestines which are as follows: The intestinal microflora had the *Pseudomonas* (25.0), *Aeromonas* (19.6), *Vibrio* (16.3), *Enterobacterium* (11.9), *Bacillus* (10.9), *Corynebacterium* (10.9) and *Streptococcus* (5.4). Gills Microflora had *Streptococcus* (27.8), *Pseudomonas* (31.4), *Bacillus* (7.4), *Corynebacterium* (9.3), *Flavobacterium* (9.3) and *Micrococcus* (14.8). Skin/Scale microflora had *Aeromonas* (40.0), *Vibrio* (10.0), *Pseudomonas* (30.0) and *Bacillus* (20.0) (Fig 1).

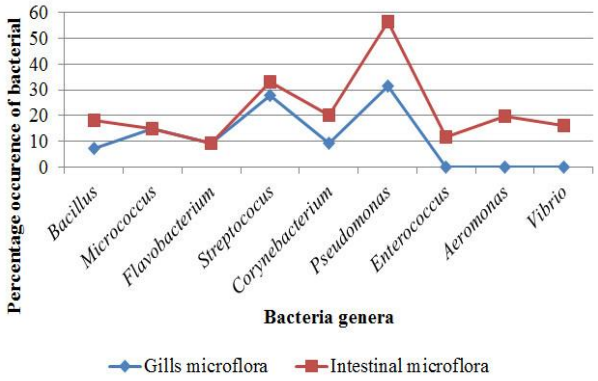

Figure 1: Percentage occurrence of bacteria genera in the Gills and Intestinal microflora

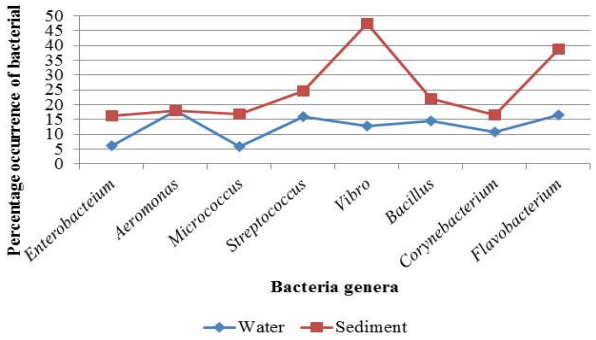

Figure 2: Percentage occurrence of bacteria genera in the water and sediment microflora

DISCUSSION

In this study, about 74% of the total isolates were motile and only 26% were non-motile. This result is similar to those observed by Oliver and Edwardsmith (1982) who reported 76% motile form while Quigley and Colwell (1968) reported 72% motile bacteria, despite the fact that they were working with marine bacteria. The dominant bacterial group isolated from the pond water during the sampling period are *Aeromonas, Flavobacterium, Bacillus* and *Streptococcus*. This result is in agreement with the report of Sakata *et al.*, 1980; Newman *et al.*, 1972 and Nieto *et al.*, 1984 who studied the taxonomy of bacteria in freshwater bodies. In contrast to this however, *Pseudomonas* reported by Nieto *et al.*, (1984) was not recovered as a member of the pond water. Since, they stated that this organism has a higher fluctuation in the water sample; it is regarded as being influenced by some factors due to the seasonal changes. Other members that are greatly influenced by the water quality are *Enterobacteria*, *Micrococcus, Vibrio* and *Corynebacteria*.

Comparison between predominant bacteria in fish and water samples revealed that *Aeromonas* and

Streptococcus occur in parallel in fish and water. Whereas *Pseudomonas* and *Aeromonas* were prevalent in the organs examined. From the sediment sample, *Vibrio* and *Flavobacterium* which are dominant could also be seen among the dominant ones in the intestines. *Aeromonas*, *Streptococcus* and *Bacillus* which are dominant in the water sample could be observed to dominate the skin and gills of fish. Therefore, on close examination, it could be observed that sources (sediment and water) may act as reservoir of bacteria which are transferred to fish samples and into some organs. Apparently, these organs may also serve as transient environments to the bacteria which may come in contact with them in various ways for example while feeding, the fish may swallow the feed with sediment and water bacteria which then pass down into its intestines where some that resist the digestive enzyme action remain in antagonism while others are passed out via the faeces. While breathing, the fish takes a large volume of water which passes out through the operculum. Here, the gills due to its filter nature will trap most bacteria cells from the water and those that can survive in highly aerated environment like the *Pseudomonas, Aeromonas* and *Streptococcus* will proliferate in such area, as could be observed in this result.

Those bacteria that can resist the antimicrobial action of the mucus produced on the body of the fish exists there in constant antagonism as transient population of such type *Aeromonas* was the most dominant one. The bacterial population of the water sediment and fish are in a dynamic equilibrium or constant transition. Commercial fish feeds have been found to harbor a large microbial load and therefore have to be routinely examined for the presence of the pathogenic organisms. Such bacteria affect the quality of the feed by production of extracellular enzymes, they also play a role in infection of fish and man such as *Fluorescent, Pseudomonas* and *Salmonella sp* respectively (Bullock 1964). Fish and its products have been implicated in clostridia intoxication of man (Frazier, 1967). However, Virulence test carried out by Nieto *et al.*, 1984 revealed that *Aeromonas, Vibrio* and *Pseudomonas* displayed some degree of virulence with *Pseudomonas* and *Vibrio* displaying a high degree of virulence for rainbow traut. Two species of *Pseudomonas* have been referred to as potential fish pathogens these are *P. fluorescens* and *P. anguilliseptic* (Ahne *et al.*, 1982; Nakai and Muroga, 1982) that causes septicemia. *Vibrio* strains of *V. anguillanum* species cause several epizootics in fresh water. Others are *Yersinia ruckeric* and *Edwardsiella tarda* of the class *Enterobactericeae, Staphylococcacea* and *Streptococcacea*. VanDuijn (1967) has reviewed the diseases of fish and also presents a great deal of useful practical information on the treatment of fish diseases.

From this study it could be deduced that there is no adapted resident flora in the fish specimen since all the bacterial group were present in either the water or sediment samples. The only exception being Pseudomonas which was absent in both water and sediment samples, but however, this could not be regarded as being apparently unique only to the fish intestine since it was it was also recovered from the gills. Therefore these organs only supply them with favourable environmental conditions and medium for growth. Most of the bacteria that cause diseases to the fishes can be antagonized thus: uneaten feed should be removed; or rather sufficiently weighed quantity of the feed should be given to the fish so that the ration is consumed by them so as to prevent bacterial bloom. Faecal and other excretory products should be taken care of since it goes a long way to lower oxygen tension, increase ammonia level of water, and lower resistance of the fish.

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