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Commensal Bacterial Flora of *Synodontis nigrita* and *Clarias gariepinus* from River Osun, Southwest Nigeria, Nigeria

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Abstract: The microflora of the skin, gill and gut tissues of two freshwater fish species namely *Synodontis nigrita* and *Clarias gariepinus* from river Osun at Epe in southwestern Nigeria were investigated. Ten adult samples of each fish species were analyzed for the isolation of microflora using the biochemical test. Bacteria isolated were *Staphylococcus aureus*, *Baccillus* species, *Streptococcus* sp., *Baccillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa* from the skin, gill and gut of both fish species. The occurrence and isolation of potential pathogens from the skin, gill and gut of the fish samples obtained from river Osun is an indication that fishes could serve as vectors for the propagation of diseases among consumers of fishes from river Osun. The result of this study showed that fish can serve as bio-indicators of the microbial quality of water body from where they are caught. It also showed that River Osun stands the risk of a potential organic pollution especially with the presence of *Baccillus* species on the skin, gill and gut of *S. nigrita.*

Key words: Bacterial flora, *Synodontis nigrita*, *Clarias gariepinus* isolation, potential pathogens, consumers

INTRODUCTION

Fishes by reasons of their habitat are continually bathed in aqueous suspension of microbes; their external surface, therefore is in constant contact with these organisms. Some of these organisms may colonize the surface of the fish becoming part of the resident microflora. The presence of these microflora inhibits the arrival and subsequent colonization by other organisms that may be pathogenic to the fish. Bacteria inhabit other parts of the fish such as gill, mouth and gut (Cahill, 1990). A large stable population of bacteria inhabits the gut (Hamid *et al*., 1979). These populations are able to survive the harsh conditions of the gastrointestinal tract.

The microbial flora of freshly caught fish and other aquatic specimens is largely a reflection of the microbial quality of the waters from where they are harvested. Of particular significance is whether the water is sewage polluted in which case the fresh water food is potentially capable of transmitting various pathogenic micro organisms (Pelczar *et al*., 1998). The gastrointestinal tract of fish is a very harsh environment in which to live. In other to survive passage through the digestive tract and colonize it, bacteria must be able to resist low pH, digestive enzymes, the effects of lysozymes and immunoglobulins in gut mucus and possibly anaerobic conditions in some regions (Cahill, 1990).

Many studies have been carried out on the microflora of temperate fish species (Bergh *et al*., 1994; Campbell and Buswell, 1983; Camman *et al*., 1993; Gorbach, 1993; Conway *et al*., 1996). However, information on the bacterial microflora of tropical fish species is scanty. It seems likely that the gastro intestinal microflora has a role in the nutrition (Sugita *et al*., 1991, 1996), growth and disease susceptibility of the fish (Trust and Sparrow, 1974; Joborn *et al*., 1997) and it is thought that the intestinal microflora may even be essential in free living fish feeding on materials that are not immediately digested by the host or on materials lacking vitamins which the microflora can synthesize. The microflora may even represent a source of nutrients to the fish (Trust and Sparrow, 1974).

The microbial populations within the digestive tract of fish are rather large in size but they are considerably simpler in diversity than those found in endotherms (Ringo *et al*., 1995). The predominant species of bacteria found in the intestine of freshwater fish are *Aeromonas*, *Enterobacter*, *Flavobacterium*, *Pseudomonas* and *Acinetobacter* which are contrasting to the predominant species found in seawater fish which are *Vibrio*, *Pseudomonas*, *Achromobacter*, *Corynecbacterium*, *Flavobacterium* and *Micrococcus* in seawater fish (Ringo *et al*., 1995; Cahill, 1990).

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The gastrointestinal tract is a very harsh environment. In order to survive passage through the digestive tract and colonize, bacteria must be able to resist low pH, digestive enzymes, the effects of lysozymes and immunoglobulins in gut mucus and possibly anaerobic conditions in some regions (Cahill, 1990). Generally, there is a progressive increase in the number of bacteria from the foregut (stomach and interior portion of the intestine) to the hindgut (intestinal region) (Ringo *et al*., 1995). There is also a progressive increase in the size of the anaerobic populations along the digestive tract (MacDonald *et al*., 1986) whereas anaerobic bacteria are generally confined to the upper intestine and intestinal contents (Austin and Al-Zahrani, 1998).

The aim of this research is to provide information on the commensal microflora in the skin, gill and gut of two freshwater species; *Synodontis nigrita* and *Clarias gariepinus* from river Osun, Southwestern Nigeria.

MATERIALS AND METHODS

Freshly caught specimens of *Synodontis nigrita* and *Clarias gariepinus* were obtained from river Osun where it empties into Epe lagoon, Ogun State, Southwestern Nigeria and transported to the laboratory in polythene bags containing water from the River. All the glassware used were washed in detergent solution, rinsed in tap water and sterilized in hot air oven at 160°C for 1 h while the media were sterilized in the autoclave at 121°C and 1 kg cm^{-2} pressure for 15 min unless otherwise specified.

Isolation of bacterial flora was carried out from the skin, gills and stomach of the fish specimens by rubbing a sterile swab on the skin. The swab was immediately cultured on nutrient agar which had been prepared aforetime. The swab spot on the medium was then streaked out using a sterile inoculating loop. The plate was kept for incubation at room temperature in an inverted position for 24 h.

Isolation from gills: The fish was disabled using a pointed scalpel to puncture its brain and the operculum raised up and a sterile swab was used to rub the gills. This swab was immediately cultured on nutrient agar and streaked out using a sterile inoculating loop. The plates were incubated at room temperature for 24 h.

Isolation from stomach: Fish specimens were opened up with the aid of a sterile blade, the stomach cut into two and two pairs of sterile forceps were used tot turn the stomach inside out. A sterile swab was used to rub the exposed inner surface of the stomach and cultured on nutrient agar after which streaking was carried out using a sterile inoculating loop. The plates were then incubated at room temperature for 24 h.

Various bacterial isolates were subjected to morphological and biochemical tests for their identification according to the methods of Buchanan and Gibbson (1994). The results were analyzed by crosss reference to Bergey's Manual of Systematic Bacteriology (Buchanan and Gibbson, 1994).

RESULTS AND DISCUSSION

The microflora of the gill, gut and skin of *S. nigrita* consisted of *Staphylococcus aureus*, *Bacillus lichenformis*, *Streptococcus* sp. and *Escherichia coli* (Table 1) while *S. aureus*, *E. coli* and *Pseudomonas aeruginosa* were found associated with the skin, gill and gut tissues of *C. gariepinus*. Table 2 shows the isolates and the sites where they occurred in *S. nigrita* while Table 3 shows the isolates and the sites in which they occurred in *C. gariepinus* (Table 4).

Despite the large variations in numbers between individual fish, the composition of the bacterial flora was similar within each fish species.

Table 1: Bacterial isolates found associated with the gill, gut and skin of *Synodontis nigrita* from river Osun, Southwestern Nigeria

	Site of occurrence		
Isolates	Gill	Gut	Skin
Staphylococcus aureus	+	+	
Bacillus lichenformis	+		+
Bacillus subtilis		+	+
Streptococcus sp.	+		
Escherichia coli		+	

Table 2: Rate of bacterial occurrence on sampled *Synodontis nigrita* from river Osun, Southwestern Nigeria

	Site/Rate of occurrence			
Isolates	Gill	Gut	Skin	Total number
Staphylococcus aureus	12	25	-	37
Bacillus lichenformis	7		3	10
Bacillus subtilis		35	7	42
Streptococcus sp.	10			10
Escherichia coli		32		32
Total number	39	92	10	131

Table 3: Bacterial isolates found associated with the gill, gut and skin of *Clarias gariepinus* from river Osun, Southwestern Nigeria

	Site of occurrence		
Isolates	Gill	Gut	Skin
Staphylococcus aureus	+	+	+
Pseudomonas aeruginosa			+
Escherichia coli	+		+

Table 4: Rate of bacterial occurrence on sampled *Clarias gariepinus* from river Osun, Southwestern Nigeria

	Site of occurrence			
Isolates	Gill	Gut	Skin	Total number
Staphylococcus aureus	12	34	8	54
Pseudomonas aeruginosa	-	-	6	6
Escherichia coli	15	-	7	22
Total number	27	34	27	88

The variation in bacterial counts between individual fish have been observed previously (Trust and Sparrow, 1974; Yoshimizu and Kimura, 1976; Spanggaard *et al*., 2000) and were confirmed by the results. The dominating bacteria in *S. nigrita*: *S.aureus, Bacillus lichenformis* and *B. subtilis* belonged to a few phylogenetic groups (*Citrobacter*, *Aeromonas*, *Carnobacterium*) while in *C. gariepinus*, *Staphylococcus aureus* and *E. coli* were the dominant bacteria. The overall dominant flora consisting of fermentative gram-negative bacteria of Proteobacteria belonging to the genera *Citrobacter* and *Aeromonas* agrees with the previous studies (Trust and Sparrow, 1974; Nieto *et al*., 1984; Spanggaard *et al*., 2000).

It is generally contended that the fish intestine does not have a stable microflora although, the gastrointestinal tract provides an ecosystem distinctly different from the surrounding water. However, other investigators (Sakata *et al*., 1980) have not detected any similarity between bacterial groups isolated from water intestine or fish diet. Austin and Al-Zahrani (1998) distinguished between the flora of the gut and the associated gut wall flora in rainbow trout and noted that scanning electron microscopy showed only sparse colonization of the wall.

E. coli was isolated from both fish species at different sites in the course of the investigation. *E. coli* is one of the micro-organisms designated as coliforms i.e., they are normal inhabitants of the large intestines of humans and other animals and consequently present in feaces (Pelczar *et al*., 1998). Thus, the presence of *E. coli* in water is an evidence of feacal pollution of human or animal origin. If *E. coli* is present in water, the way is also open for human intestinal pathogens to gain entrance into the water since they also occur in feaces (Pelczar *et al*., 1998). This water body is subjected to potentially dangerous pollution. Hence, the consumers of the water and fish from river Osun may be at risk of developing intestinal tract infection due to the presence of *E. coli* on both fish species as a normal microflora. *Staphylococcus aureus* is known to cause intoxication because they produce toxinswhich cause gastroenteritis in their human consumers. Pathogenic effect of bacteria can be directly related to the toxins they produce (Stewart and Amerine, 1992). Some genera including *Bacillus* and *Pseudomonas* sp. have been identified with fish spoilage. The spoilage causing bacteria in the fish are part of the natural flora of the external slime and intestinal content. When fish dies, bacteria on the skin multiply and rapidly invade the fish flesh. This is possible because the fish has lost its natural defense mechanisms. The bacteria (*Bacillus* and *Pseudomonas* sp.) feed on the fish fleshwhich they break down with the aid of their enzymes (Noguchi *et al*., 1987). Thus the abundance of food leads to an exponential growth in bacteria resulting in the presence of heavy slime on the skin and gill surface. The fish flesh softens and produces an offensive and an unpleasant odour. Hence, fish spoil will be more rapid than the normal rate and the fishes have to be preserved thoroughly and if cooked or fried, it should be done thoroughly to prevent and epidemic situation as a result of food poisoning or infection from spoilage. Also, the consumers of water from river Osun are prone to intestinal tract infection. The present of *Bacillus* sp. and the gill, skin and gut of *S. nigrita* may be responsible for the early deterioration of the species offish as soon as they are taken out of water. This has made the preservation of *S. nigrita* more difficult than *C. gariepinus* which has *Pseudomonas* sp. and then can be relatively easier to preserve. The presence of *Staphylococcus* sp. in all the target organs investigated except in *S. nigrita* is an indication of possible food poisoning as it may cause enterogastroenteritis in the unwary human consumers (Austin and Al-Zahrani, 1998). Although, *Bacillus* sp. are harmless saprophytes, many form exocellular enzymes that hydrolyze proteins leading to food spoilage. Moreover, because of the heat resistance of the endospore *Bacillus* sp. may survive inadequate heat treatment during cooking or smoking of *S. nigrita*. This is due to the fact that the endospore formed by *Bacillus* are extremely resistant to desiccation, staining, disinfections, chemicals, radiation and heat (Pelczar *et al*., 1998).

These microflora can also be advantageous as seen in the digestive processes of fish such as microbial breakdown of chitin, collagen, cellulose and the flora may also supply fatty acids and other vitamins to the host (Ringo *et al*., 1995). Also, these microflora prevent colonization of the fish by other microbes that might otherwise be pathogenic.

The reported microflora presented in this research may only serve as an indication of the microfloral composition based on the culturable bacteria. However, a striking characteristic of the indigenous bacteria in many environments such as water, soil and activated sludge is the lack of culturability of the majority of the living bacteria (Amman *et al*., 1983; Spanggaard *et al*., 2000). It has been reported that only a minor percentage of the

bacteria observed via direct microscopy is generally capable of growth on common laboratory media (Van Elsas and van Overbeck, 1998). The fish skin has similarly been found to contain <0.01 culturable bacteria (Bernadsky and Rosenberg, 1992). Although, microbiology of fish intestine is well studied (Trust and Sparrow, 1974; Horsely, 1977; Austin and Al-Zahrani, 1998; Munro *et al*., 1994), the investigations of the fish gut have all relied on the culturable part of the flora. It is not known how large a proportion of the fish microflora is actually characterized by traditional culture and isolation procedures (Spanggaard *et al*., 2000).

CONCLUSION

The microbial flora of *S. nigrita* and *C. gariepinus* from River Osun were studied using traditional culture and isolation procedures and the results presented. The dominant microflora in the gill, gut and skin of *S. nigrita* and *C. gariepinus* were culturable and effectively identified the classical method. This will provide information on the commensal microflora in two highly relished fish species of aquacultural importance.

The results of this study showed that *S. nigrita* and *C. gariepinus* from River Osun cannot be safely consumed half cooked. Hence, salting and smoking are the effective forms of preservation in the *C. gariepinus* and *S. nigrita* while freezing is also employed in the preservation of *C. gariepinus* and salting serves as anti-septic.

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