

Bacterial isolates in the different gut regions of captured and cultured *Clarias gariepinus* in Abeokuta North Local Government

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ABSTRACT

The diversity and microbial load of the bacteria inhabiting the gut sections of captured and the cultured *Clarias gariepinus* were investigated in Abeokuta North Local Government, South Western Nigeria. The gut contents of the fish were isolated and characterized using standard methods. The bacteria isolated from the captured fish were *Escherichia coli*, *Proteus vulgaris*, *Klebsiella mobilis*, *Enterobacter spp*, *Shigella spp*, and *Micrococcus varians* while *Citrobacter spp*, *Proteus vulgaris*, *Enterobacter spp*, *Escherichia coli*, *Serratia marcescens* and *Shigella spp* were isolated from the cultured fish. The bacteria count obtained from the fore gut, mid gut and hind gut of the captured fish were; 1.4×10^4 cfu/g, 1.7×10^4 cfu/g and 2.7×10^4 cfu/g while that of cultured fish had 2.05×10^4 cfu/g, 2.65×10^4 cfu/g and 2.85×10^4 cfu/g respectively. The hind gut had the highest number of species and microbial load of bacterial in both captured and cultured fish than the other regions. There was significant difference ($P < 0.05$) in the microbial load of bacteria in the captured fish and the cultured fish. The results therefore suggested that there is diversity in microbial composition and microbial load in different sections of the gut of the cultured and captured fish which may plausibly be the reflection of the environment in which they were raised.

Keywords: *Clarias gariepinus*, bacterial, fish gut, microbial load.

INTRODUCTION

Clarias gariepinus is the most cultured fish in Nigeria and indeed Africa, and third in the world (Garibaldi, 1996). Other catfishes that are cultured include *C. anguillaris*, *Heterobranchius bidorsalis*, *H. longifilis*, *C. isheriensis*, *C. submarginatus*, *Chrysichthys nigrodigitatus*, *Bagrus sp.*, *Synodontis sp.*, but *C. gariepinus* is undoubtedly the fish of choice of farmers. It can then be inferred that Nigeria is the highest producer of this Clariid catfish in the world (Williams *et al.*, 2007). Its hardiness due to the presence of arborescent air-breathing organ, omnivore's ability to withstand adverse environmental

condition, high fecundity and mass artificial seed technique (Haylor, 1992; Hecht *et al.*, 1996) also ease its culture.

It is important to study the parasite and disease condition of fish under natural conditions because it serves as a basis for information on the potential risk of diseases expected under intensive culture all the more because fingerlings, juveniles and sometimes broodstocks of most cultivated fish species in Africa are collected from the wild (Fagbenro *et al.*, 1993). Diseases, which are caused by the presence of pathogenic microbial fauna or flora, reduce fish production by affecting the normal physiology of fish (Kabata, 1985). These, if left uncurtailed, can result in mass mortalities of fish, or in some cases, infection of man and other vertebrates that

consume them (Shawn, 1997).

Shawn (1997), reported that bacteria including the non pathogenic and pathogenic are usually present in small numbers in most fish. He further stated that in normal situations fish possess quite adequate immune system which is capable of fighting against bacterial infection which may be chronic. The information on gut microflora in fish is scanty and there is paucity of knowledge of microflora in the gastrointestinal tract of fish (Mondal *et al.*, 2008). Although some information is available on some microflora in fish digestive tract, almost nothing is known about their distribution in different regions of the gut. Bacteria make up most of the flora in the colon and 60% of the dry mass of feces. Somewhere between 300 (Guarner and Malagelada, 2003a) and 1000 different species live in the gut (Sears, 2005) with most estimates at about 500. However, it is probable that 99% of the bacteria come from about 30 or 40 species (Beaugerie and Petit, 2004). Bacteria producing antibacterial substances were recently isolated from marine fish intestines (Westerdahl *et al.*, 1991; Sugita *et al.*, 1997; Sugita *et al.*, 1998; Sugita *et al.*, 2002). In the present study an attempt has been made to investigate the distribution of microflora in the foregut, mid gut and hind gut regions of captured and cultured *C. Gariepinus*.

MATERIALS AND METHODS

Collection of Specimens

Three adults of *C. gariepinus* captured from Oyan dam, via Abeokuta were obtained from fishermen at Olomore market, Abeokuta and three cultured samples were obtained from Teaching & Research Aqua Farm of Crescent University, Abeokuta. The average standard length of captured and cultured samples was 30.46 ± 3.81 cm. The samples collected from the wild and pond were visually examined and confirmed to be healthy.

Dissection of the Specimens

Each of the three specimens was dissected aseptically to remove the gut (the entire alimentary canal). The average length of gastro intestinal tract of both captured and cultured sample was 9 ± 1 cm. The glass wares were sterilized in an oven at 160°C for 90 minutes. Absolute alcohol was used to sterilize the surface of the working table. The gastro-intestinal tract (GIT) of each sample was cut into fore gut, hind gut and mid gut and prepared for isolation and characterization of microflora.

Isolation and characterization of microflora

Each organ was placed in sterile bottle containing 5ml sterile distilled water and vigorously shaken to allow the content dissociate in water and serially diluted to 10^{-6} . Then from each suspension 0.1ml was pour plated using freshly prepared McConkey agar. The plates after being covered were gently swirled to evenly mix up and allowed to gel. The McConkey agar treated plates were inverted and incubated at 37°C for 24 hours. Representative colonies emerging from the plates were grouped according to their cultural characteristics, purified by repeated sub-culturing and maintained on appropriate agar slants as stock cultures. The bacterial isolates were tested for Gram reaction (Claus 1992), morphology, motility, catalase and oxidase reactions, citrate utilization, coagulase production, starch hydrolysis and sugar fermentation (Harrigan and McCance, 1976; Seeley and Van Demark, 1972).

Microbial Count

Colonies which developed after incubation were subjected to counting and were expressed in Colony Forming Units/g (CFU).

Statistical analysis

Data were analyzed using 2x3 Factorial Experiment in Completely Randomized Design. Fishers' Least Significant Difference was used to separate means where significant difference ($P < 0.05$) occurred.

RESULTS

The change in microbial load of the fore gut, mid gut and hind gut region of both cultured and captured *Clarias gariepinus* in cfu/g are represented as Table 1.

Table 1. The mean bacteria count from different regions of the gut of cultured and captured *Clarias gariepinus* in cfu/g

	Cultured <i>Clarias gariepinus</i>	Captured <i>Clarias gariepinus</i>
Fore-gut	$2.05 \pm 0.29 \times 10^{-6}a$	$1.40 \pm 0.14 \times 10^{-6}b$
Mid-gut	$2.65 \pm 0.21 \times 10^{-6}ab$	$1.70 \pm 0.28 \times 10^{-6}b$
Hind-gut	$2.85 \pm 0.63 \times 10^{-6}a$	$2.70 \pm 0.28 \times 10^{-6}a$

The mean bacteria counts obtained from the fore-gut of the cultured *Clarias gariepinus* was $2.85 \pm 0.29 \times 10^{-6}$, while the mean count for the mid gut was $2.05 \pm 0.21 \times 10^{-6}$ and the hind gut recorded average microbial count of $2.65 \pm 0.63 \times 10^{-6}$ cfu/g. The mean bacteria count obtained from the fore gut, mid gut, and the

hind gut of captured *Clarias gariepinus* were $1.40 \pm 0.14 \times 10^{-6}$, $1.70 \pm 0.28 \times 10^{-6}$ and $2.70 \pm 0.28 \times 10^{-6}$ respectively. The result showed that there was significant difference ($P < 0.05$) in the bacteria count in the different gut regions of *Clarias gariepinus*. It is also evident that there was significant interaction ($P < 0.05$) between the environment in which the fish was raised and the microbial load of the gut bacteria of the fish. The hind gut had the highest bacteria load of $2.65 \pm 0.63 \times 10^{-6}$ and $2.70 \pm 0.28 \times 10^{-6}$ for both captured and cultured *Clarias gariepinus* respectively followed by mid gut then fore gut for both captured and cultured *Clarias gariepinus*.

Table 2 and 3 reveal the results of morphological and biochemical test used in characterization of the bacteria isolates from different gut regions of *Clarias gariepinus*.

Table 2. Identification of the Bacteria isolates in the different gut regions of cultured *Clarias gariepinus*

TESTS	FORE GUT		MID GUT		HIND GUT		
Morphological Test							
Colour	Cream	Off-white	Creamy	Creamy	Grey	Pinkish	Creamy
Surface	Rough	Smooth	Rough	Rough	Smooth	Smooth	Smooth
Shape	Irregular	Round	Irregular	Irregular	Round	Irregular	Irregular
Elevation	A little raised	Undulate	Raised	A little raised	Low convex	Undulate	Raised
Edge	Entire	Flat	Entire	Entire	Entire	Entire	Entire
Cell characteristics	Rod	Short	Small rod	Rod	Rod	Rod	Rod
Biochemical Test							
Gram reaction	-	-	+	+	+	+	-
Motility	+	+	-	-	-	-	-
Spore	-	-	+	+	+	+	+
Catalase	+	+	-	-	-	-	-
Coagulase	-	+	-	-	-	+	-
Citrate	+	-	-	+	-	-	-
Sugar Fermentation							
Glucose	AG	AG	AG	AG	AG	AG	A
Fructose	AG	AG	AG	AG	AG	AG	A
Manitol	AG	-	AG	AG	AG	AG	A
Lactose	AG	-	AG	-	AG	-	-
Maltose	AG	AG	AG	AG	AG	AG	A
Probable Bacterium	<i>Citrobacter Spp</i>	<i>Proteus vulgaris</i>	<i>Enterobacter spp</i>	<i>Citrobacter spp</i>	<i>Escherichia Coli</i>	<i>Serratia Marcescens</i>	<i>Shigella Spp</i>

KEY: +: positive -: negative AG: Acid and Gas production A: Acid

Table 3: Identification Of Bacteria Isolates In The Different Gut Regions Of Captured *Clarias gariepinus*.

Tests	Fore gut		Mid gut		Hind gut		
Morphological Test							
Colour	Grey	Off white	Creamy	Creamy	Off white	Creamy	yellow
Surface	Smooth	Smooth	Glittering	Rough	Smooth	Smooth	Smooth
Shape	Round	Round	Round	Irregular	Round	Irregular	Cocci
Elevation	Low convex	Undulate	Raised	Raised	Undulate	Raised	Raised
Edge	Entire	Flat	Entire	Entire	Flat	Entire	Entire
Cell characteristics	Rod	Short rod	Small rod	Small rod	Short rod	rod	Cocci
Biochemical Test							
Gram reaction	-	-	-	-	-	-	+
Motility	+	+	+	+	+	-	-
Spore	-	-	-	-	-	-	-
Catalase	+	+	-	+	+	-	-
Coagulase	-	+	+	-	+	-	-
Citrate	-	-	-	-	-	-	-
Sugar Fermentation							
Glucose	AG	AG	AG	AG	AG	A	AG
Fructose	AG	AG	AG	AG	AG	A	AG
Manitol	AG	-	AG	AG	-	A	AG
Lactose	AG	-	AG	AG	-	-	AG
Maltose	AG	AG	-	AG	AG	A	AG
Probable Bacteria	<i>Escherichia coli</i>	<i>Proteus vulgaris</i>	<i>Klebsiella spp</i>	<i>Enterobacter spp</i>	<i>Proteus vulgaris</i>	<i>Shigella spp</i>	<i>Micrococcus varians</i>

KEY: +: positive -: negative AG: Acid and Gas production A: Acid

Table 4 shows distribution of isolated bacteria in the gut sections of *Clarias gariepinus*. The bacteria species isolated from the fore gut of cultured *Clarias gariepinus* were *Citrobacter spp* and *Proteus vulgaris*, that of mid gut were *Enterobacter spp* and *Citrobacter spp* while *Escherichia coli*, *Serratia marcesens* and *Shigella spp* were isolated from the hind gut.

The bacteria isolates identified from the fore-

gut of captured *Clarias gariepinus* were *Escherichia coli*, *Proteus vulgaris*. Those of the mid gut were *Klebsiella mobilis* and *Enterobacter spp* while the hind gut was dominated by *Proteus vulgaris*, *Shigella spp* and *Micrococcus varians*.

Table 4. Distribution of isolated bacteria in the gut sections of *Clarias gariepinus*

Isolates	Culture Environment	Fore gut	Mid gut	Hind gut
<i>Citrobacter spp</i>	W	-	-	-
	P	+	+	-
<i>Proteus vulgaris</i>	W	+	-	+
	P	+	-	-
<i>Enterobacter spp</i>	W	-	+	-
	P	-	+	-
<i>Escherichia coli</i>	W	+	-	-
	P	-	-	+
<i>Serratia marcescens</i>	W	-	-	+
	P	-	-	-
<i>Shigella spp</i>	W	-	-	+
	P	-	-	+
<i>Enterobacter spp</i>	W	-	+	-
	P	-	-	-
<i>Micrococcus varians</i>	W	-	-	+
	P	-	-	-
<i>Klebsiella mobilis</i>	W	-	+	-
	P	-	-	-

P- Pond, W - Wild; + Present , - Not Present

DISCUSSION

Generally, bacteria are abundant in the environment in which fish live and it is therefore, rather impossible to avoid them being a component of their diet (Strom and Olafsen, 1990; Hansen *et al.*, 1992). Osungbemi (2005) observed that quantitatively the microbial flora in fish appears to be a function of the environment. The bacteria entering along with the diet of fish during ingestion may adapt themselves in the gastrointestinal tract and form a symbiotic association. Within the digestive tract of fish large numbers of microbes are present (Trust *et al.*, 1979; Rimmer and Wiebe, 1987; Clements, 1991; Luczkovich and Stellwag

1993; Ringo and Strom, 1994; Clements and Choat, 1995), which is much higher than in the surrounding water indicating that the digestive tracts of fish provide favorable ecological niches for these organisms (Trust and Sparrow, 1974; Horsley, 1977; Austin and Al-Zahrani, 1988; Sakata 1990). However, the gastrointestinal microflora of fish appears to be simpler than those of endotherms. While the digestive tracts of endotherms are colonized mainly by obligate anaerobes (Finegold *et al.*, 1983), the predominant bacterial genera/species isolated from most fish guts have been aerobes or facultative anaerobes (Trust and Sparrow, 1974; Horsley, 1977; Sakata, 1990; Bairagi *et al.*,

2002; Ghosh *et al.*, 2002). In the present study, attention has been focused on the microflora in the gut of cultured and captured *Clarias gariepinus*. Total bacterial population was recorded highest in the hindgut region of the cultured and captured *Clarias gariepinus*. This report is in agreement with earlier work by Mondal *et al.*, (2008). Buras *et al.*, (1987) reported that fish in general usually contain high numbers of bacteria (including pathogenic micro-organisms: *Aeromonas*, *Pseudomonas*, *Salmonella* etc.) in the digestive tract, mucus, gills, internal organs and flesh. This is in agreement with what was observed in this study. Chandrasekaran (1985) reported that animals in the aquatic environment carry bacterial flora, which is a reflection of the flora in the environment. Gut microflora plays an important role in the digestive process, growth and disease susceptibility of marine deposit feeders (Fenchel and Kofoes, 1976; Yingst, 1976).

CONCLUSION

In conclusion, the results of the present study indicate that a diverse bacteria flora exist in the gastrointestinal tracts of both captured and cultured *Clarias gariepinus*, which are highly colonized in the hindgut rather than in the foregut region. Guarner and Malagelada, (2003) explained that the acid in the stomach, as well as bile and pancreatic secretions, might hinder colonization of most microflora in the stomach and proximal small intestine. The first part of the colon is mostly responsible for fermenting carbohydrate, while the latter part mostly breaks down proteins and amino acids. Bacterial growth is rapid in the cecum and ascending colon which has a low pH and slow in the descending colon, which has an almost neutral pH.

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