



DIVERSITY AND MICROBIAL LOAD OF FUNGAL ISOLATES OF THE GASTRO-INTESTINAL TRACT OF CAPTURED AND CULTURED *Clarias gariepinus* IN ABEOKUTA, SOUTH WESTERN NIGERIA

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ABSTRACT

Fungi present in the different gut regions of the captured *Clarias gariepinus* were isolated, characterized and compared with that of cultured *Clarias gariepinus*. The fungi isolated from the captured fish were; *Penicillium chrysogenum*, *Fusarium* spp and *Rhizopus stolonifer* while the fungi isolates obtained from the cultured fish were; *Aspergillus flavus*, *Candida albicans* and *Aspergillus fumigatus*. The spore count of the fungi isolated from the fore gut, mid gut and hind gut of captured fish were 4.0×10^{-6} , 7×10^{-6} spore/g and 9.05×10^{-6} spore/g while the count obtained from the cultured fish were 3.0×10^{-6} , 7×10^{-6} and 9.05×10^{-6} spore/g. The results of this study revealed that; different species of fungi were observed to be present in different regions of both the captured and cultured *Clarias gariepinus*, the microbial load of fungi increased from the fore gut to the hind gut of both captured and cultured species of *Clarias gariepinus*.

Keywords: *Clarias gariepinus*, fungi, microbial load, gut.

INTRODUCTION

Studies on parasite and disease condition of fish under natural conditions have been documented because it serves as a basis for information on the potential risk of diseases expected under intensive culture. This is so because use of fingerlings, juveniles and sometimes parent broodstocks of most cultivated fish species in Africa are collected from the wild (Fagbenro *et al.*, 1993). Parasites and 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) and which, 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). *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). The hardiness of *C. gariepinus* due to the presence of arborescent air-breathing organ, and its ability to feed on variety of food coupled with its ability to withstand adverse environmental condition, its high fecundity and mass artificial seed technique

make it easier to culture them in the country (Haylor, 1992; Hecht *et al.*, 1996). The gut flora, in which fungi is a part, consists of the micro organisms that normally live in the digestive tract of animals. The term "gut flora" is interchangeable with intestinal microflora and intestinal microbiota. (O'Hara and Shanahan, 2006). 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). According to Wikipedia, fungi and protozoa also make up a part of the gut flora, but little is known about their activities. 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. The anaerobic gut flora of humans and other animals are known to be involved in varieties of functions including enzymatic digestion of food residues, synthesis of vitamins, suspension of reactions which result in the generation of carcinogenic metabolites and detoxification of potentially toxic substances (Guarner and Malagelada 2003 a and b, Sears, 2005). In the present study an attempt has been made to investigate diversity and microbial load of fungi in the foregut, mid gut and hind gut regions of captured and cultured *Clarias 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 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.

Isolation and characterization of microflora

Each organ was placed in sterile bottle containing 5ml sterile distilled water and vigorously shaken to allow the content dissociated in water. Then from each suspension 0.1ml was pour plated, using freshly prepared Sabouraud Dextrose Agar medium (SDA). The plates after being covered were gently swirled to evenly mix up and allowed to gel. The plates were allowed to stay in the inoculating chamber for 3–4 days. 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 culture. Microscopic examination of young, actively growing moulds was on the basis of structures bearing spores and on the spore themselves; presence or absence of septation, rhizoid or other tissues. The microbial isolates were identified by their micro-morphology as well as the colour and micro-morphology of their sporulating structures and conidia according to Onions *et al.*, (1981) **Microbial Count:** Colonies which developed after incubation were subjected to counting. The total fungal counts were expressed as spore/g. **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

Table 1: Spore count (spore/g) from different regions of the gut of both cultured and captured *C. gariepinus*

	Fore gut	Mid gut	Hind gut
Cultured <i>Clarias gariepinus</i>	$3.0 \pm 0.28 \times 10^{-6c}$	$7.0 \pm 0.56 \times 10^{-6b}$	$9.05 \pm 0.35 \times 10^{-6a}$
Captured <i>Clarias gariepinus</i>	$4.0 \pm 1.84 \times 10^{-6c}$	$7.0 \pm 2.12 \times 10^{-6b}$	$9.0 \pm 1.97 \times 10^{-6a}$

values with different superscripts in the same row are significantly different ($P < 0.05$) from each other

Table 2: Interaction between the environment of the fish and the spore count (spore/g) of the different regions of the gut of both cultured and captured *C. gariepinus*

	Fore gut	Mid gut	Hind gut
Cultured <i>Clarias gariepinus</i>	6.0×10^{-6a}	14.0×10^{-6a}	18.10×10^{-6a}
Captured <i>Clarias gariepinus</i>	8.0×10^{-6a}	14.0×10^{-6a}	18.0×10^{-6a}

Values in the table with the same superscript are not significantly different ($P > 0.05$)

Table 1.0 shows the spore count obtained from different regions of the gut of both cultured and captured *Clarias gariepinus* in spore/g. The spore counts obtained from the fore gut of the cultured *Clarias gariepinus* was $3.0 \pm 0.28 \times 10^{-6}$, while the count for the mid gut was $7.0 \pm 0.56 \times 10^{-6}$ and the hind-gut recorded microbial count of $9.05 \pm 0.35 \times 10^{-6}$ spore/g. The various spore count obtained from the fore-gut, mid-gut, and the hind-gut of captured *Clarias gariepinus* were $4.0 \pm 1.84 \times 10^{-6}$, $7.0 \pm 2.12 \times 10^{-6}$ and $9.0 \pm 1.97 \times 10^{-6}$ respectively. There existed a significant difference in fungal count ($P < 0.05$) in the gut regions of *Clarias gariepinus*. Table 2 is a contingency table showing the interaction

between the environment of the fish and the spore count of the different regions of the gut of both cultured and captured *Clarias gariepinus* in spore/g. There was no significant difference ($P > 0.05$) in the interaction between fungi count in the gut region of the fish and the environment in which it was raised revealing that there was no significant effect ($P > 0.05$) of environment on the fungal count in the gut of cultured and captured *Clarias gariepinus*. The hind gut had the highest fungi load followed by mid gut then fore gut for both captured and cultured *Clarias gariepinus*

Table 3 and 4 revealed the results of morphological characterization of the fungi isolates from different gut regions of *Clarias gariepinus*.

Table 3: Identification of fungi isolates from the different gut regions of captured *Clarias gariepinus*.

	Fore guts	Midguts	Hindguts
Cultural Characteristics	Green mould growth	White cotton-like mycelium with black base	White cotton-like mycelium
Spore conidia under microscope	Septate mycelium bearing single conidiophores which are branched near the apex	Spore have well developed collumela spore are of different shapes	Non- Septate Hyphae and coenocytes. Sporangiphore have well developed collumela spore are of various
Fungi Isolates	<i>Penicillium chrysogenum</i>	<i>Fusarium</i> spp	<i>Rhizopus stolonifer</i>

Table 4: Identification of fungi isolates from the different gut regions of cultured *C. gariepinus*.

	fore guts	midguts	hindguts
Cultural characteristics	White base with black colour in appearance.	Greenish spore	White base with brown conidiophore which make it appear brown in colour.
Spore conidia under microscope	Conidia head radiate surface containing many flask shaped phialides and chains of conidia. Non septate and no collumela.	Mycelium are not extensive conidia, forming short chains by budding which are produced on mycelium epically or laterally.	An upright conidiophores which are one celled and vesicle glubose. Non- septate.
Fungi Isolates	<i>Aspergillus flavus</i>	<i>Candida albicans</i>	<i>Aspergillus fumigatus</i>

Table 5 revealed the distribution of isolated Fungi in the gut sections of *Clarias gariepinus*. The fungi isolates in the foregut, mid gut and hind gut of the captured *Clarias gariepinus* were *Penicillium chrysogenum*, *Fusarium spp* and *Rhizopus stolonifer* respectively. While the

fungi isolates in the fore gut, mid gut and hind gut regions of cultured *Clarias gariepinus* were *Aspergillus flavus*, *Candida albicans* and *Aspergillus fumigatus*

Table 5: Distribution of fungi isolates in the gut sections of *C. gariepinus*

Isolates	Culture environment	Fore gut	Mid gut	Hind gut
<i>Penicillium chrysogenum</i>	W	+	-	-
	P	-	-	-
<i>Fusarium spp</i>	W	-	+	-
	P	-	-	-
<i>Rhizopus stolonifer</i>	W	-	-	+
	P	-	-	-
<i>Aspergillus flavus</i>	W	-	-	-
	P	+	-	-
<i>Candida albicans</i>	W	-	-	-
	P	-	+	-
<i>Aspergillus fumigates</i>	W	-	-	-
	P	-	-	+

Legend: P- Pond , W - Wild

DISCUSSION AND CONCLUSION

The results of the present study indicate that a diverse fungi flora exist in the gastrointestinal tracts of both captured and cultured. According to Wikipedia, fungi also make up part of the gut flora but little is known about their activities. The micro organisms present in the gut perform a host of functions such as fermenting unused energy substrate, training the immune system, preventing growth of harmful species (Guaner and Malageladar, 2003a; Sears 2005) regulating the development of the gut, producing vitamins for the host and producing hormones to direct the host to store fats. However, in certain

conditions some species are thought to be capable of causing disease by causing infection to the host. (Guaner and Malageladar, 2003b; Beaugerie and Petit, 2004).

The results of the study also reveal that the fungi flora existing in the gastrointestinal tracts of both captured and cultured *Clarias gariepinus* are highly colonized in the hindgut rather than in the foregut region. Guamer and Malagelada, 2003a 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 caecum and ascending colon which has a low pH and slow in the descending colon, with an almost neutral pH.

The role of the predominant fungi in the gut of captured and cultured fish could not be established in this study but the majority of these species may be of beneficial advantage to the fish. The microflora present in gut perform a host of functions such as efficient utilization of feed; fermenting unused energy substrate, training the immune system, preventing growth of harmful species, regulating the development of the gut, producing vitamins for the host and producing hormones to direct the host to store fats (Sears, 2005). These microflora according to Shawn,

(1997) in normal situation seldom cause any problem as the fish has an adequate immune system which is more than capable of fending off any infection which may be chronic. The different fungi observed in the gut content of fish are a reflection of the environment in which they are raised and it is an indicator that they are the predominant organisms in the fish culture medium. It has been reported that the microflora carried by animals in an environment is an indication of the flora in such environment (Idowu *et al.*, 2006; Idowu *et al.*, 2008) so also Osungbemi (2005) observed that quantitatively the microbial flora in fish appears to be a function of their environment. It is therefore rather impossible to avoid them being a component of the fish diets (Strom and Olafsen 1990; Hansen *et al* 1992).

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