# MICROBIAL FLORA OF THE GASTRO-INTESTINAL TRACT OF NILE TILAPIA (*Oreochromis niloticus*) CAUGHT FROM RIVER DANDARU, IBADAN

*[1]JIMOH W.A., [2]OLADELE-BUKOLA M.O., [3]ADEBAYO M.D., [4]YUSUFF A.A., [3]AZEEZ A.F. & [4]O.O. SALAMI

1. Department of Fisheries Technology, Federal College of Animal Health and Production Technology, PMB 5029, Ibadan, Nigeria
2. Department of Livestock Improvement Programme, IAR&T, Ibadan, Nigeria
3. Department of Animal Health, Federal College of Animal Health and Production Technology, Moor Plantation, PMB 5029, Ibadan, Nigeria
4. Science Laboratory Department, Federal College of Animal Health and Production Technology, PMB 5029, Ibadan, Nigeria

*Correspondence: jawabus@gmail.com

**ABSTRACT**

*The study reports the microbial load and diversity in the gastro-intestinal tract of Nile Tilapia Oreochromis niloticus caught in River Dandaru, Ibadan. A set of adult samples of Nile Tilapia was caught from the river Dandaru, Ibadan. Microbial loads and characterization of micro organisms present in the gut region of the captured Clarias gariepinus were examined using standard microbiological procedures. The isolated fungi isolated were Geotricum candidum,, Aspergillus candidus, Penicillium expansum while the bacteria isolated were Proteus mirabilis, Bacillus licheniformis, Pseudomonas aeruginosa, Bacillus megaterium, Staphylococcus aureus, Flavobacterium aquatile, Pseudomonas cepacia, Lactobacillus brevis. The microbial count of Nile Tilapia caught from river Dandaru Ibadan were; the total plate count was 3.3 x $10^6$ CFUs/g, total coliform count was 2.4 x$10^4$ CFUs/g, total anaerobic count was 1.0 x $10^3$ CFUs/g, total faecal coliform count was 1.1 x $10^3$ CFU/ml, total fungi count was 7.5 x $10^3$ spore/g.*

**Keywords:** fungi, bacteria load, microbial diversity, tilapia

## INTRODUCTION

Studying the factors associated with the physiology and the development of the fish under natural conditions is important as it serves as a basis of evaluating the potential risk expected under intensive culture, most importantly in Africa where most of the fingerlings, juveniles and sometimes parent broodstocks of most cultivated fish species 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 un-curtailed, can result in mass mortalities of fish, or in some cases, infection of man and other vertebrates that consume them (Shawn, 1997). The term "gut flora" is interchangeable with intestinal microflora and intestinal microbiota and consists of the micro organisms that normally live in the digestive tract of animals (O'Hara and Shanahan, 2006). Shawn (1997) reported that bacteria are usually present in small numbers in most fish and in normal situation seldom cause any problem as the fish own quite adequate immune system which is more that capable of fending off any infection which may be chronic. There is scanty information on the microflora in the gastrointestinal tract of fish (Mondal *et al.*, 2008). Although some information is available on some microflora in fish digestive tract (Dixon, 2001, Jimoh *et al*, 2009 a and b). The anaerobic gut floral 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). The present study investigates the microflora of the gut of captured Nile tilapia.

## MATERIALS AND METHOD

### Collection of Specimen

A set of adult samples of Tilapia *Oreochromis niloticus* were collected from fishermen at river Dandaru, Ibadan, Oyo State. The samples collected from the wild were visually examined and confirmed to be apparently healthy.

### Dissection of the Specimens

Each of the specimens was dissected aseptically to remove the gut (the entire alimentary canal). The glass wares were sterilized in an oven at

160 $^0$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 moulds under study was on the basis of their micro-morphology as well as the colour and micro-morphology of their sporulating structures and conidia according to Onions *et al.,* (1981). For bacteria count and isolation, the gastro-intestinal tract (GIT) of each sample was placed in sterile bottle containing 5ml sterile distilled water and vigorously shaken to allow the content to dissociate in water. 1ml was taken and serially diluted to $10^{-6}$. Microbial load, isolation and characterization of microorganisms using serial dilution and pour plate method were carried out using nutrient agar. Representative colonies emerging from the plates after incubation at $37^0$C for 24hours were grouped according to their cultural characteristics, purified by repeated sub-culturing and maintained on appropriates agar slants as stock cultures. The bacterial isolates were characterized based on the methods of Claus (1992); Harrigan and McCance, (1976); Seeley and Van Demark (1972). The colonies were characterized and identified using the criteria of Holt (1994).

**Microbial Count**

Bacteria Colonies which developed after incubation were subjected to counting and were expressed in Colony Forming Unit (CFU)/g and CFU/ml for faecal coliform count. The total fungal counts were expressed as spore/g.

**RESULTS**

Table 1 shows the identification of fungi isolates from the gastrointestinal tract of Nile tilapia caught from river Dandaru, Ibadan. The isolated fungi isolated were *Geotricum candidum, Aspergillus candidus, Penicillium expansum.* Table 2 shows the results of morphological and biochemical tests used in identification of bacteria isolates from the gastro-intestinal tract of Nile tilapia caught from river Dandaru, Ibadan.the bacteria isolated were *Proteus mirabilis, Bacillus licheniformis, Pseudomonas aeruginosa, Bacillus megaterium, Staphylococcus aureus, Flavobacterium aquatile, Pseudomonas cepacia, Lactobacillus brevis.* The microbial count of Nile Tilapia caught from river Dandaru Ibadan is as presented in table 3. The total plate count was 3.3 x $10^6$ CFUs/g, total coliform count was 2.4 x$10^4$ CFUs/g, total anaerobic count was 1.0 x $10^3$ CFUs/g, total faecal coliform count was 1.1 x $10^3$ CFU/ml, total fungi count was 7.5 x $10^3$ spore/g.

**Table 1: IDENTIFICATION OF FUNGI ISOLATES FORM THE GASTRO-INTESTINAL TRACT OF TILAPIA FISH CAUGHT FROM RIVER DANDARU, IBADAN**

| S/N | CULTURAL CHARACTERISTICS | SPORE CONIDIA UNDER MICROSCOPE | PROBABLE FUNGI ISOLATED |
|---|---|---|---|
| 1. | A fast growing colonies, white butyrous or membranous, odour somehow fruity. Advancing hypae dichotomously branched. | Conidial chains mostly aerial, conidia mostly nucleate, it has no dichotomously branched advancing hyphae. | *Geotricum candidum* |
| 2. | Pigmentation is absent and conidia are roughed colonies. Relatively slow-growing, colonies appear white initially and becoming cream to yellowish cream. | Conidiophores have large globose vesicles bearing inflated, club-shaped metulae and narrow phialides. Conidia are globose and smooth-walled | *Aspergillus candidus* |
| 3. | Colonies fast growing conidiophores in fresh isolates typically loosely symematous, giving the colony a zonate appearance. colonies are light green, reverse colorless yellow-brown conidiophores are smooth-walled. | penicillin 2-3 staged branched with numerous usually oppressed metulae, conidia sub globose to ellipsoidal, smooth-walled with aromatic odour, fruity and suggesting apple | *Penicillium expansum* |

**Table 2: Identification of bacteria isolates from the gastro intestinal tract of tilapia caught from River Dandaru, Ibadan**

| CODE | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|---|
| **Gram Reaction** | | + | + | - | + | + | + | - | + |
| **Cell Morphology** | | R | R | R | C | C | R | R | R |
| **Catalase** | | + | + | + | + | + | + | + | - |
| **Oxidase** | | + | + | + | - | - | - | + | - |
| **Casein Hydrolysis** | | + | + | - | - | + | - | - | - |
| **Gelatin Hydrolysis** | | + | + | d | (+) | - | + | - | - |
| **Methyl Red** | | + | - | + | + | + | - | + | - |
| **VogesProskaer** | | + | - | - | **d** | + | + | - | - |
| **Nitrate Reduction** | | - | + | + | + | + | - | + | + |
| **Growth at temperature** | $60^oC$ | - | - | - | - | - | + | - | - |
| | $30^0C$ | - | - | - | - | - | - | + | - |
| **Coagulase** | | - | - | + | **d** | + | - | + | - |
| **Urease** | | - | - | + | - | + | - | + | - |
| **Growth at pH** | 3.9 | (+) | (+) | - | - | - | + | - | + |
| | 9.2 | (+) | (+) | + | + | + | + | + | + |
| **Starch Hydrolysis** | | + | + | - | - | - | - | - | + |
| **Growth NaCl** | | + | + | + | + | + | - | + | (+) |
| **Citrate Utilisation** | | + | - | + | - | + | - | + | + |
| **Motility** | | + | + | + | - | - | + | + | - |
| **IndoleTest** | | - | - | - | - | - | - | - | - |
| **Glucose** | | +G | +G | + | + | + | + | + | + |
| **Fructose** | | + | - | - | + | + | + | - | + |
| **Maltose** | | - | + | (+) | - | + | + | + | + |
| **Lactose** | | - | (+) | + | d | + | (+) | + | d |
| **Sucrose** | | (+) | (+) | + | + | d | + | + | d |
| **Galactose** | | + | + | + | + | - | - | + | + |
| **Xylose** | | + | + | - | - | (+) | - | - | - |
| **Arabinose** | | - | + | - | - | + | (+) | - | + |
| **Raffinose** | | - | - | - | + | + | - | - | w |
| **Rhamnose** | | - | + | - | - | + | (+) | - | - |
| **Dulcitol** | | - | - | - | - | - | + | + | - |
| **Mannitol** | | - | - | (+) | - | + | - | (+) | + |
| **Probable bacteria isolate** | | *Proteus mirabilis* | *Bacillus licheniformis* | *Pseudomonas aeruginosa* | *Bacillus megaterium* | *Staphylococcus aureus* | *Flavobacterium aquatile* | *Pseudomonas cepacia* | *Lactobacillus brevis* |

**w = Weak reaction +G = Positive with gas production S+ = Strongly Positive; W+ = Weakly Positive d = delayed R = Rod C = Coccus**

**Table 3: Microbial count in the gastro intestinal tract of tilapia caught from River Dandaru, Ibadan**

| | Parameters | Catfish |
|---|---|---|
| **Bacteria Count** | The total plate count | $3.3 \times 10^6$ |
| **(CFUs/g)** | Total coliform count | $2.4 \times 10^4$ |
| | Total anaerobic count | $1.0 \times 10^3$ |
| **(CFU/ml)** | Total faecal coliform count | $1.1 \times 10^3$ |
| **Fungi Count (Spore/g)** | Total fungi count | $7.5 \times 10^3$ |

## DISCUSSION

From the result of this study, it is evident that the gastro-intestinal tract of fish harbours diverse microflora though little is known about their activities (Guanar and Malagelada, 2003a). Sears (2005) reported that gut microflora play symbiotic role; helping in the proper development of the gut. However, in certain conditions, some species are capable of causing infection to the host (Guanar and Malagelada 2003b; Beaugerie and Petit 2004). Gut microflora plays an important role in the digestive process, growth and disease susceptibility of marine deposit feeders (Fenchel and Kofoes 1976; Yingst 1976). Idowu et al (2006 and 2008) indicated that the microflora carried by an organism is an indication of the flora in such environment. Bacteria are abundant in the environment in which fish live and it is therefore, to a certain extent impracticable to avoid them being a constituent of their diet (Strom and Olafsen 1990, Hansen et al., 1992). The bacteria entering along with the diet of fish during ingestion may adjust themselves in the gastrointestinal tract and form a symbiotic association (Clements 1991, Luczkovich and Stellwag 1993, Ringo and Strom 1994, Clements and Choat 1995). Within the digestive tract of fish large numbers of microbes are present which is much higher than in the surrounding water indicating that the digestive tracts of fish provide favorable ecological niches for these organisms (Austin and Al- Zahrani, 1988 and Sakata, 1990). The major bacterial genera/species isolated from most fish guts have been aerobes or facultative anaerobes (Sakata 1990, Bairagi et al., 2002, Ghosh et al., 2002). Total bacterial population recorded in this study is in agreement with earlier work by Mondal et al., 2008. These microflora according to Shawn, 1997 in normal situation seldom cause any problem as the fish own quite adequate immune system which is more that capable of fending off any infection which may be chronic.

## CONCLUSION

It can therefore be concluded that varied number of bacteria and fungi exist in the GIT of fish. The presence of these microflora in the gastro intestinal tract of fish portends a host of functions such as efficient utilization of feed to direct the host to store fats.

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