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Bacteria Associated with Fresh Tilapia Fish (*Oreochromis niloticus*) Sold At Sokoto Central Market in Sokoto, Nigeria.

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ABSTRACT: A research was conducted on bacteria micro flora associated with fresh Tilapia fish (*Oreochromis niloticus*) sold at Sokoto central market, Sokoto. Nigeria. Sections of the skin, gills and intestine of ten randomly selected fishes were aseptically removed by means of a sterile scalpel and pair of sterile scissors. Four (4g) each of the sections were homogenized in 6 ml of sterile distilled water, which served as the original stock culture. A serial dilution up to 10^9 was carried out, and surface plated on nutrient agar. A total of nine (9) bacterial species were isolated and identified. Eight bacteria were identified to specie level and one to genus level. Six (6) were gram positive namely: *Bacillus megatanium, Listeria monocytogenes, Bacillus Pumilus, Bacillus alvei, Bacillus Licheniformis and Staphylococcus saprophyticus* and three gram negative bacteria namely: *Serratia mercescens, Providentia stuartii and Salmonella spp.* The frequency of occurrences of the isolated Bacteria indicated that *Bacillus pumilius* had the highest frequency of occurrence *(*19.35%), while *Salmonella spp.* had the least occurrence (3.2%). The mean viable Bacterial count from each section of the samples revealed 46.1 x10^7cfug^{-1} from the gills, 18.8 x 10^8cfug^{-1} from the intestine and 27.3 x 10^8cfug^{-1} from the skin. The isolates were found to be of medical importance.
Keywords: Bacteria, Tilapia fish and Sokoto central market

INTRODUCTION

Fish has been one of the main foods for humans for many centuries and still constitute an important part of the diet in many countries (Leisner *et al*., 1995). In Nigeria, the short supplies of animal protein together with the increasing human population have raised the cost of animal protein to a level almost beyond the reach of the low income group (Ezeri *et al*., 2001). As a result, there is a considerable increase in the demand for fish being the cheapest source of animal protein. (Ladipo *et al*., 1981).

The advantages of fish as a food are its easy digestibility and high nutritional value (Leisner *et al*., 1995). These important attributes makes the commodity readily susceptible to microbial attack particularly bacteria (Adams *et al*., 1999). Fish flesh naturally contains very low levels of carbohydrates and these are further depleted during the death struggle of the fish (Adams et al., 1999). This has two important consequences for spoilage. Firstly, it limits degree of post morten acidification of the tissue so that the ultimate pH of the muscles is 6.2-6.5 (Adams *et al.,* 1999).Disease breaks out in fish tank very quickly and you have to first identity the type of disease before you can take action.

The bacteria are transmitted by fish that have made contact with other diseased fish. Bacterial fish disease and infections are very common and are one of the most difficult health problems to Deal with (Douglas, 2007). Bacteria can enter the fish body through the gills or skin or it can stay on the surface of the body (Douglas, 2007).

There are four types of bacterial infections. Bacterial gill disease: The gills are the primary target,Systemic bacterial disease: bacteria inwades the fish's body and damages internal organs, bacterial body ulcears: Lesions on the fish body that can be shallow or deep and fin rot: Most likely resulting from environmental stress. (Douglass, 2007).

Secondly, the absence of carbohydrate means that bacteria present on the fish will immediately resort to using the soluble pool of readily assimilated nitrogenous material, producing off-odour. (Adasms *et al.,* 1999)

Shell fish such as Tilapia have a particular large pool of nitrogenous extractives and are even

more prone to raid spoilage, a factor which accounts for the common practice of keeping them alive until immediately prior to consumption (Adams *et al*., 1999). The speed with which a product spoils is also related to the initial microbial load on the product: the higher the count, the sooner spoilage occurs (Adams *et al*., 1999).

The fresh water or rivers and lakes have a complex flora of microorganisms which include genuinely aquatic species as well as component introduced from terrestrial, animal and plant sources. (Adams *et al*., 1999). The scale of human activities has had a detrimental effect on coastal waters. Many shell fishes used for food out particles from large volume of waters. If these waters have been contaminated with sewage, there is always the risk that enteric organisms from infected individuals may be present and will be concentrated by the filter feeding activities of shell fish (Adams *et al.,* 1999). Also during handling of the commodity, the natural flora of the environment may be contaminated with organisms associated with man such as members of the *enterobacteriaceae* and *Staphylococcus aureus* which can grow well at 30-37^{o}c (Miceal *et al.,* 2007).

By monitoring the bacteria contents of fish organs, the quality of fish can be measured since these will affect the storage life and quality of the fishery products (Kaneko, 1971). In order to provide a predictive capability for possible disease outbreaks and provide an opportunity to design preventive management actions, detailed information of the bacterial load and types of bacteria associated with the organs of apparently healthy Tilapia fish is needed. An attempt is made in this paper to investigate the bacterial micro flora associated with fresh Tilapia fish, sold at the Sokoto central market, Sokoto, Nigeria.

MATERIALS AND METHODS

Sample collection: Ten different samples of Tilapia fish were collected from different locations in Sokoto market. Each fish sample was especially put into sterile polythene bag and taken to the microbiology laboratory of the Usmanu Danfodiyo University, Sokoto for analysis.

Preparation of stock cultures: Section of the gills, skin and intestine of ten (10) randomly selected fish were especially removed by means of a sterile scalpel and pair of scissors and kept in sterile Petri dishes. 4g each of these sections was pounded with mortal and pestle. Homogenization was carried out is to obtain uniform distribution of cells through stock culture.

Enumeration, isolation and identification of Bacteria: Six serial dilutions of the original stock culture from the gills, skin and intestine were prepared. Each dilution was plated on solidified freshly prepared nutrient agar and spread using a sterile glass rod and incubated at 37^{o}c for 24 hours after which the colonies that developed on the plates were counted. Those counts within 30-300 colony forming units (cfu) were reported as total viable count (TVC).

Distinct colonies from each plate were then picked by means of a sterile wire loop and sub cultured onto a freshly prepared nutrient agar medium contained in sterile plates. This was done with a view to obtaining pure culture of the growth. The plates were incubated at 37^{o}c for 24 hours.

Characterization of the pure isolates was performed and involved colonial chracteristics, cell micro morphology, motility test and bichemical test of gram reaction, catalase test, glucose, sucrose and lactose utilization, citrate test, motility test, indole test, urease test, hydrogen sulfide production, gas production, methyl red test, voges praskaure test, coagulase test and spore staining. These tests were done to identiy isolates to generic level as contained in Chessbrough (2000) and Cowan and Steel (1999).

RESULTS

From the results obtained table 1 shows the result of the total viable count of bacterial isolates from the gills, skin and intestine of ten sampled tilapia fish.

The mean total viable count revealed 46.1 x 10^{7} cug^{-1} from the gills 18.8 x 10^{8} (cfug^{-1}) from the intestines and 27.3 x 10^{8} (cfug^{-1}) from the skin. A range of total viable count from the three sites analyzed revealed 3.1 x 107- 8.1 x 10^{7} cfug-1 from the gills, 1.2 x 10^{8} – 2.9 x 10^{8} (cfug-1) from the intestine and 1.1 x 10^{7}- 1.8 x 10^{8} (cfug-

1) from the skin. The mean count computed for each fish part sampled show that gill had the least count of 6.0 x 10^7 cfug-^{1} and intestines had the highest count of 1.49 x 10^8 cfug-Table 2 indicated the biochemical characterization used to identify the bacterial species isolated.

Table 3 showed the frequency of occurrence of bacterial isolates as well as their percentage in the ten analyzed fishes. From the table, *Bacillus pumilus* has the highest occurance (occurance 6) with a percentage of 19.4%, while Salmonella spp. is the least (1) with a percentage occurance (3.2%).

DISCUSSION

The result from this research shows that the Bacterial load varies in the three segments of the fishes analyzed, the skin, gills and intestine. The bacterial load in all sample was high (Gills, 6 07, intestines 1.49 and may be attributed to the high ambient temperature in the river where it was caught which is close to optimum for many mesophilic bacteria. Bacterial load in fish might increase with the increase of water temperature (Fernandes *et al*, 1997, Hossain *et al*, 1999). Choudhury *et al*, (1989) reported intestinal bacterial load of Tilapia fish as 5.5 x 109 cfug. This count is comparable to the results in this research at similar temperature. The high bacterial count on the skin may be attributed to contamination by genuinely aquatic specie as well as those that contaminate the commodity during handling. The gills had the lowest bacterial population compared to the intestine and skin. According to Trust (1975), the number of bacteria associated with the gills are actively maintained at low level, there by implying that fish probably has mechanism which enables it to keep the bacteria number low, and therefore afford it some degree of protection against bacteria invasion by the gill microflora (Ezeri *et al.,* 2001).

Table 1: Total viable counts of bacteria in cfu / g of tilapia fish sampled from Sokoto

	Count in cfu/g		
Fish parts sampled	Total counts	Range	Mean (SD)
Gills	46.1 $_x$ 10^7	3.1 - 8.1 $_x$ 10^8	6 $_x10^7$
Intestines	18.8 $_x$ 10^8	1.2 – 2.9 $_x$ 10^8	1.49 x10^8
Skin	27.3 $_x$ 10^8	1.1 – 1.8 $_x$ 10^8	1.4 $_x$ 10^8

Key: SD = Standard deviation

Table 2: Biochemical characterization used to identify the bacterial species isolated

Bacterial Isolates	Colonial characteristics	Cell micromophology
B. megatarium	Large milky colonies with rough edges	Appears as positive rods
B. pumillus	Colonies appear pale and large in size	Gram positive rods
B. alvei	Colonies present with pinkish colour and Small and large	Gram positive rods
B. licheniformis	Produces translucent colonies with rough Edges	Gram positive rods And appeared scattered
L. monocytogenes	Small drop like colonies showing blue green indeces	Cell appeared as coccobacillus and in cluster
S. saprophyticus	Colonies are white with streaks of yellow with Large edges	Cells are cocci With appearance Seen in cluster
S. mercescens	Produces discrete translucent colonies	Gram Negative rod
P. stuartii	Colonies appeared colourless with rough edges	Gram Negative rod
Salmonella spp.	Colonies are grayish, white, smooth translucent and convex	Gram negative rod

Table 3: Frequency of occurrence of bacterial isolates

Bacterial isolates	Number of occurrence	% occurrence
Bacillus moratorium	3	9.67
Listeria monocytogenes	3	9.67
Bacillus pumilus	6	19.35
Bacillus alvei	4	12.90
Bacillus licheniformis	3	9.67
Staphylococcus saprophyticus	4	12.90
Serratia mercescens	4	12.90
Providential stuertii	3	9.67
Salmonella spp	1	3.22
Total	31	100%

Base on the percentage frequency of occurance , *Salmonella* spp. Showed the least frequency of occurance of 3.22%. The presence of Salmonella spp. Indicates faecal contamination of water from which the fishes were harvested. The percentage frequency of occurrence of *Bacillus pumilus* 19.35%. The presence of the isolated organism was not surprising since according to Draser and Hill (1976), fish lives in water habitat full of microogrganism. Okpokwasili and Alapiki (1990) confirmed that bacteria flora associated with a Nigerian water culture include the genera, Bacillus, Lactobacillus, Staphylococcus, Escherichia, Micrococcus, Proteus and others.

Bacillus spp. are implicated in causing a wide range of infectious diseases including abscesses, bacterimia/septicimia, wound and food borne infections, ear infections, endocarditis, meningitis, ophthalmitis, osteomyelitis peritonitis and respiratory and urinary infections (Morales *et al.,* 2004). *Serratia mercescens*, has been reported to cause lower respiratory tract infections and urinary track infection. *Providential stuartii* is also implicated in causing travelers diarrhea. *Staphylococcus saprophyticus* has been demonstrated to cause urinary tract infections (UTIs) in women. Salmonella spp. Has been reported to cause enteritis and systematic disease. (Adams *et al*., 1999) has demonstrated that fish and fish products are only occasionally associated with Salmonella and that filter feeding shell fish harvested from polluted water have been identified as higher risk products. *Listeria monocytogenes* is widespread in the environment and humans can be exposed to the bacteria in various ways, though many persons remain symptomless (Cowan and Steel, 1999). Subpopulation who could develop the disease which sometimes can be life threatening include pregnant women, new born and infant and adults with a compromised immune system (Marth 1988). *Listeria monocytogenes* produces a series of toxins hemolytic, lipolytic, hemorrhagic and pyrogenic which are involved in the disease process (Schlech 1988). Five forms of Listeriosis can be caused by infections with *Listeria moncytogenes*; pregnancy infections, granulomatosis infantiseptica, sepsis meningoencephalitis and focal infections. The bacterium can also invade the eye and skin cause conjunctivitis and skin lesions (Bahk and Marth, 1990).

Conclusively, this research has brought to light those bacterial species associated with fresh Tilapia fish and has shown that they are potentially pathogenic to humans. Hence adequate measures should be taken in processing the fish before consumption.

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