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Isolation and Identification of Bacterial Species Associated with Spoilage of *Clarias gariepinus.*

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Research Article

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ABSTRACT

This study was carried to isolate and identify the bacteria species that are associated with fish spoilage using standard bacteriological techniques. The results of the bacteriological quality of the cat fish showed variation in the total bacterial and coliform counts to different anatomical parts (skins, gills and intestine). The highest total bacterial counts was recorded from gills (83 x 105cfu/ml) and lowest in skin (53 x 105cfu/ml) from cat fish. The total coliform counts of the cat fish ranges from 16 x 103cfu/ml, 36 x 103cfu/ml and 43 x 103cfu/ml in skin, intestine and gills respectively. A total of 288 colonies belonging to eleven genera where identified after comparing the morphological characteristics, gram staining reaction, biochemical tests and sugar utilization with those of known taxa. The identified genera were *Streptococcus* sp., *Staphylococcus* sp., *Salmonella* sp., *Shigella* sp., *Pseudomonas* sp., *E. coli, Klebsiella* sp., *Enterobacter* sp., *Enterococcus* sp., *Campylobacter* sp., and *Proteus* sp. The prevalence of these genera shows that *Pseudomonas* sp (96.95%) was the most prevalent on all the anatomical parts followed by *E. coli* (14.64%) *Enterococcus* sp (9.74%), *Klebsiella* sp., (6.90%), *Enterobacter* sp (6.72%). The presence of these bacteria genera could pose serious health problem if consumed, it is therefore advised that proper care must be taken to prevent spoilage of the fish through major preservation techniques.

INTRODUCTION

The African catfish is farmed in concrete basins in Africa. Farming started in early 1970´s as field trials did show that African catfish is a fast growing fish which is very robust. African catfish tolerates a large variety of feedstuffs and is very resistant to changing and suboptimal water conditions [20]. It is found to be able to being farmed in high densities reaching production levels of 6-16 MT/ha on an annual basis when raised in monocultures and fed high quality fish feed. Most of the African catfish is sold alive into the market. The African catfish are harvested at an age of 6 to 8 month having a weight of 200-300g.The meat of African catfish is mildly flavoured and has a tender texture If heavy and continuous overfeeding occurs phytoplankton growth will bloom which occasionally leads to a muddy flavour of the meat [10]. Spawning in catfish is initiated artificially by hormone injection or injection of grinded pituitary glands of catfish or Tilapia into the ripe female. Spawning takes place in the night following injection where the female is placed with a male in a special net [14]. The following day both the female and the male are removed. The eggs do hatch and after 3- 4 days the larvae are developed and transferred to the nursing pond were they are grown for 3-4 weeks until they have developed to fingerlings and transferred to the growing pond. In Africa, fish are widely consumed as a remarkable some of animal protein. Fish production from rivers is influenced by a number of factors; hydrological requires, fishing pressure and environmental degradation [21]. The flesh of fish is usually infected with a wide range of microbes present in the water body. These bacteria are often found in the scales, gills, gut and alimentary tract of the fish. The bacteria present on the body of internal organs of fish indicate the extent of pollution of the water environment hence the bacteria flora of the fish depicts the bacteria flora of the water environment [16]. Fresh fish spoilage can be rapid after it is caught, the spoilage process (Rigor Mortis) will start within 12hours of their catch in the high ambient temperatures of the tropics [4]. Rigor mortis is the process through which fish loses its flexibility due to stiffening of fish mussels after few hour of its death [1]. Most fish species degrade as a result of digestive enzymes and lipases, microbial spoilage from surface

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bacteria and oxidation [3]. During fish spoilage, there is a breakdown of various components and the formation of new compounds. Composition of the microflora on newly caught fish depends on the microbial contents of the water in which the fish live. Fish microflora includes bacterial species such as *Pseudomonas, Alcaligens, Vibrio, Serratia* and *Micrococcus* [11]. Microbial growth and metabolism is a major causes of fish spoilage which produce amines, biogenic amines such as putrescine, histramine and cadaverine, organic acids, sulphide, alcohols, aldehydes and ketones with unpleasant and unacceptable off-flavors [7,9]. This study was carried out to isolate and identify bacterial species associated with the spoilage of body of fresh fish preserved at ambient temperature.

MATERIALS AND METHOD

Collection of fish samples

The fishes use for this research study were bought from local market, Oja-Ikoko in Owo, Ondo State.

Isolation of bacterial species from the fish

About 2g of the fleshy part of the head part, the trunk and tail region of the spoilt fishes were cut and mixed in 10mls of sterile distilled water and mixed gently several times. 1ml of the stock solution was used in serial dilution to make a dilution up to 10^3. Exactly 0.5ml of the fish part samples were introduced to the surface of already prepared nutrient agar (for total bacteria count), MacConkey (for total coliform count) and EMB (for total *E-coli* count). The plates were incubated at 37^0C for 24 hours.

Characterization of bacterial isolates

After 24 hours of incubation, the bacterial populations were counted; the morphological characteristics of the isolates were examined. Pure culture of the bacteria species were obtained on bjoe bottles before been subjected to gram staining reaction and biochemical tests such as oxidase, catalase, coagulase, citrate, sugar utilization as described by Cheesebrough [6]. The dichotomous key results were compared with the standard characterized bacteria in the Berger's Manual of Systemic Bacteriology.

RESULTS

Table 1showed the total bacterial and coliform count of different parts of cat fish. It showed that on the skin, the total bacterial count (TBC) was 53 x 10^5cful/ml, and total coliform count (TCC) was 16 x 10^3cful/ml. The TBC was 62 x 10^3 and TCC was 36 x 10^3 on the interface and on the gills, TBC was 83 x 105 while TCC was 43 x 10^3. Table 2 showed the gram staining and biochemical test of 260 pure isolates of the bacterial species. It showed that only 11 general specie were obtained from the whole total count. The probable bacteria were streptococcus sp., *Staphylococcus* sp., *Salmonella* sp., *E. coli*, *Enterobacter* sp., *Enterococcus* sp., *Campylobacter* sp., *Proteus* sp., *Pseudomonas* sp., *Klebsiella* sp. Table 3 showed the number of occurrence and percentage occurrence of these bacterial isolates. In the different part of the cat fish, on the skin, the dominant bacterial was *Staphylococcus* sp., (16), (27.59%) followed by *Shigella* sp. (12) (20.69%) while *Salmonella* sp. was the least (04) (6.90%). On the intestine, *Pseudomonas* sp. was the highest (08) (19.59%) followed by E. coli (15) (16.30%) while *Campylobacter* sp. was the least occurred bacterial species (07) (7.60). on the gills, *Pseudomonas* sp and *E. coli* were also the high occurring species with 23 (10.91%) and (19) (17.27%) colonies respectively. *Campylobacter* sp (09), (8.19%) and *Salmonella* sp. (06) (5.45%) were the least occurring bacteria. The bacteriological study of bacterial sp. Associated with different anatomical parts of cat fish showed that these parts contained at least eleven genera of bacteria. The total bacterial counts and total coliform counts obtained in the study don't concur with the results of Yagoub [19]. The occurrence of some gram negative bacteria (*Salmonella* sp, *Shigella* sp, *E coli, Enterobacter* sp, *Enterococcus* sp, *Proteus* sp, *Pseudomonas* sp, *klebsiella* sp and gram positive bacteria (*Streptococcus* sp, *Staphylococcus* sp, *Enterococcus* sp) is in concurrence with Turker and Usta [17]. In this study isolation of pathogenic enterobacteriacaea such as *Salmonella* sp, *Shigella* sp and the pathogenic *E. coli* from the collected samples indicated public health hazards and concern. The presence of *Salmonella* sp in the fishes sampled was in conformity with the result obtained by Hatha and Lakshmanaperumaisamy [12]. The isolation of *klebsiella* sp and *Proteus* sp, *Shigella* sp from this fish indicated feacal and environment pollution and this support the findings of Yagoub and Ahmed [18] and Najiah *et al.,* [15]. The occurrence of this pathogenic bacteria especially *E. coli* in food (fish and fish products) may influence human health by inducing diseases/infections and cause abdominal pain, acute gastroenteritis, bloody/aumucoid diarrhea nausea vomiting and fever [2].

Temperature and pH are limiting factors for the survival of bacteria in fish products, these facts are used during the process of pasteurization and heat treatment, particularly of offal [8]. In the technology of marine animal processing by cooking, the following critical aspects of marine animals are significant the duration of cooking, temperature of steam, water and other mediaracy of thermometer and other monitoring and timing devices. For unpreserved fish, spoilage is a result of gram-negative, fermentative bacteria (such as Vibrionaceae), whereas psychrotolerant gram-negative bacteria (such as *Pseudomonas* spp and *Shewanella* spp.) tend to spoil chilled fish

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[11]. It is therefore important to distinguish non spoilage microflora from spoilage bacteria as many of the bacteria present do not actually contribute to spoilage [21]. Bacteriological criteria for fishery products, sea shellfish and molluscs have been elaborated both on international (Codex Committee on Food Hygiene) and European levels (Competent European Institution). Microbiological criteria, including samples plans and methods of analysis, are laid down when there is a need to protect public health, microbiological criteria for fish and fishery products include quantification of the counts of *E. coli*, thermo-tolerant coliform, mesophilic aerobic bacteria and pathogenic *V. parahaemolyticus* is performed during the production. At the finished product stage, the measure monitored is the quantification of the count of *S. aureus* and detection of bacteria of salmonella genus as their presence indicates recontamination of a finished product (Council Directive 91/493/EEC). Enterobactereacea (*E. coli*, *Shigella* sp, *Salmonella* sp, *Enterobacter* sp, *Klebsiella* sp) was isolated from skin, intestine, guts of cat fish. A different species of enterobactereacea was earlier isolated from channel catfish in the united states [5,17] and cultured fish from other parts of the world [8,9]. Results of biochemical tests were similar to those reported by other investigators including [5,13].

Table 1: Total bacteria and coliform count of different parts of cat fish

Parts	TBC cfu/ml	TCC cfu/ml
Skins	53×10^5	16×10^3
Intestines	62×10^5	36×10^3
Gills	83×10^5	43×10^3

TBC = Total bacteria count
TCC = Total coliform count

Table 2: Gran staining reaction and biochemical test of pure culture isolate of bacteria from different parts of cat fish

Parameters	A	B	C	D	E	F	G	H	I	J	K
Grain reaction	+cocci	+cocci	-rod	-rod	-rod	-rod	-rod	-rod	+cocci	-rod	-rod
Catalase test	-ve	+ve	-ve	-ve	+ve	-ve	+ve	-ve	-ve	-ve	-ve
Citrate test	-ve	-ve	-ve	-ve	+ve	-ve	+ve	-ve	-ve	-ve	-ve
Oxidase test	-ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve
Coagulase test	-ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Ladole test	-ve	-ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve	+ve
Urease test	-ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve
Glucose test	+ve	+ve	-ve	-ve	+ve	-ve	-ve	-ve	+ve	-ve	+ve
Lactose test	-ve	-ve	-ve	-ve	-ve	+ve	+ve	+ve	-ve	-ve	-ve
Sucrose test	-ve	-ve	-ve	-ve	-ve	+ve	+ve	-ve	-ve	-ve	+ve
Manitol test	+ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve
Motality +ve	+ve	+ve	-ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	

Probable bacteria : A:Streptococcus, B:Staphylococcus, C:Salmonella, D:Shigella, E:Pseudomonas , F:E. coli, G:klebsiella, H: Enterobacter,I: Enterococcus, J: Campylobacter, K:Proteus

Table 3: Incidence of bacterial species on the different parts of cat fish

Parts/Bacterial isolated SKIN	No of occurrence	Percentage of occurrence (%)
Staphylococcus aureus	16	27.59
Pseudomonas sp.	06	10.34
Enterococcus sp.	06	10.34
Shigella sp.	12	20.69
Salmonella sp.	04	6.90
E. coli	06	10.34
Streptococcus sp.	08	13.79
TOTAL	58	100.00

CONCLUSION

From the results obtained, it could be conclude that fish spoilage organisms are mostly caused by the bacteria's although, some of the bacterial species are associated with the water environment of the fish.These bacterial species are capable of causing infections in man.

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Table 4: Incidence of bacterial species on the different parts of cat fish

Parts/bacteria isolated Intestine	No of occurrence	Percentage of occurrence (%)
Enterobacter sp.	07	7.61
Klebsiella sp.	09	9.78
Enterobacter sp.	06	6.52
Salmonella sp.	08	8.70
Shigella sp.	05	5.43
Enterococcus sp.	09	9.78
E. Coli	15	16.30
Campylobacter sp.	07	7.60
Proteus sp.	08	8.70
Pseudomonas sp	18	19.59
Total	92	100.00

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