Evaluation of Microbial Spoilage of Some Aquacultured Fresh Fish in Benin City Nigeria

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Abstract

A microbiological study of organisms associated with spoilage of fresh fish samples collected from two ponds located in Benin City, Nigeria was carried out. Samples from the fish skin, gills and flesh were cultured in three media; nutrient agar, Maconkay agar and potato dextrose agar and on examination the presence of five bacteria species namely; Staphylococcus aureus, Klebsiella sp., Salmonella sp., Escherichia coli, Pseudomonas sp., and four fungi species namely; Aspergillis niger, Geotrichum sp., Rhizopus sp. and Pennicillium sp. was confirmed. The highest colony count was obtained from the skin samples in all the media. Antibiotic sensitivity pattern showed that all isolates were resistant to Gentamicin and Amoxicillin and the presence of the above pathogens in the fresh fish samples could pose a potential public health threat especially to consumers. It is recommended that better handling and processing methods should be adopted to reduce or eliminate health risk to fresh fish consumers.

Introduction

This paper presents a microbiological evaluation to determine micro organisms associated with fresh fish spoilage in aquacultured fresh fish in Benin City, Edo State Nigeria. In recent times there has been renewed effort in fish production especially in artificial (concrete and plastic) ponds as a way of augmenting the protein requirement of the increasing human population in urban areas with the prohibitive price of beef and other animal protein sources. Fish along with shrimps, crabs, lobsters, shellfish etc. are generally categorized as seafood (Gram and Huss, 2001). Seafoods may harbor a number of biohazards as well as chemical contaminations such as biogenic amines, biotoxins, pathogenic bacteria and viruses (Gram *et al*., 2000 and Ashie *et al*., 2001).

The last two decades have seen appreciable increase in global fish trade and the need to enforce safety standards and regulations on imported consignment especially from developing nations fraught with unacceptable levels of microbiological contamination (EU, 1998 and ICMSF, 2005). Contamination concern has been on high loads of unsuspected spoilage by microorganisms like *Salmonella* sp., *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* (Bramsnacs, 1999 and Gram *et al.*, 2000). Spoilage patterns of fish have been well documented (Botta, 1995 and Doyle 2007) and usually varied according to species, feeding habits, seasonality, age of fish and also geographical location (Howgate 1982).

Fresh fish spoilage and high perishability are primarily due to large amount of non-protein nitrogen (like free amino acids), volatile nitrogen bases (ammonia, creatine, taurine, uric acid, carnosine and histamine) which support post mortem bacteria growth (Connell and Shewan, 1990; May and Ward, 1997 and Jay *et al*., 2005). Fish also possess a neutral to slightly acidic pH and high moisture content which favor growth of a wide range of microbes coupled with their poikilothermic nature (Herbert *et al.*, 1997). Fish spoilage essentially can be attributed to three main factors namely; microbial, enzymatic or autolytic and chemical spoilage (oxidative rancidity) of which microbiological contamination has been noted as the main cause of fish deterioration. Initial microflora on the surface of the fish is directly related to the surrounding aquatic environment while the bacterial flora in the gastrointestinal tract corresponds to the type of condition of the fish (Liston, 1980). According to Herbert *et al*., (1997), intrinsic and extrinsic factors determine the initial bacterial contamination.

In Nigeria fish is the preferred source of much desired animal protein compared to poultry, beef, mutton, pork and veal. It is comparatively cheaper and highly acceptable, with little or no religious bias, which gives it an advantage over pork or beef (Johnson et al., 1994 and Feldhusen, 2000). Currently there is scarcity of information on microbial spoilage fresh fish especially in Nigeria. This study therefore aims to identify the microorganisms associated with spoilage of aquacultured fish, determine the factors affecting fish spoilage and also obtain information on the incidence and distribution of microbial spoilage organisms of fresh fish samples.

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Methodology

Three species of commonly cultured fresh fish samples (*Clarias gariepinus, C. heterobranchus* and *Tilapia* sp.) were collected from two locations in Benin City; Benson Idahosa University fish farm and the Graduate Farmers Farm at Aduwawa. The fresh fish species were killed and macerated in a mortar and one gram of fish tissue was dissolved in test tube containing 9ml of sterilized distilled water to obtain a solution. Serial dilution up to 10^{-5} was carried out on extracts from the skin, gill and flesh. I ml of each of the samples from the 10^{-5} was transferred into petri dishes in replicates of two after which nutrient agar was added. This procedure was repeated for Maconkay and potato dextrose agar culture media respectively. The dishes were rotated by hand in a swirling motion so that the inoculum was uniformly dispersed in the medium. The agar was allowed to solidify and incubated at 37°C for 24 hours. Microbial colonies counts were taken using a digital colony counter (LABTECH) after incubation for the identified bacteria and fungi species. Colonies of each suspected bacteria species were subcultured in fresh nutrient agar plates.

Biochemical tests like catalase, coagulase, oxidase , indole, urease, citrate and methyl red were carried out as well as morphological characteristics like Gram staining and motility test were used to properly confirm identification of microbial isolates.

Results

Microbial Colony Count

The result of the total microbial colony counts expressed in cfu x 10^5 obtained from three separated media (Nutrient agar, Maconkay agar and potato dextrose agar) and cultured fresh fish samples obtained from the skin, gill and flesh is shown in Table 1. Higher values were recorded in nutrient agar medium in all parts of the fish cultured. Mean microbial counts ranged from a minimum count of 112 cfux10^5 recorded in the flesh sample to maximum value of 215 cfux10^5 recorded in the skin sample. For Maconkay agar the values ranged from 52.5 to 111.5 cfux10^5 and 6 to 25 cfux10^5 in potato dextrose agar. The trend of variation in colony count of the various parts of the fish sampled showed highest microbial load on the fish skin followed by gill and then the flesh. Generally, microbial load in the culture media varied in the order of Nutrient agar > Maconkay agar >Potato dextrose agar and in this order the highest mean value in each succeeding medium was less than the lowest mean value of the medium preceding it.

Morphological and Biochemical Characteristics of Isolates

Table 2 showed the result of morphological and biochemical criteria used in characterization and identification of the bacteria species isolated. Microscopic observation revealed details of cultural characteristics of suspected bacteria and result of biochemical test confirmed the presence of *Staphylococcus aureus, Klebsiella* sp., *Salmonella* sp., *Escherichia coli* and *Pseudomonas* sp.

Cultural and Morphological Characteristics of Fungal Isolates

Based on the pigmentation of the spores, nature of the mycelia and spore formers the following fungal species were observed from the fish samples; *Aspergillus niger, Geotrichum* sp. *Rhizopus* sp. *Penicillium* sp (Table 3).

Discussion

The results from this study revealed the presence of four fungal species namely; *Aspergillis niger, Geotrichum* sp., *Rhizopus* sp. and *Pennicillium* sp. and five bacterial species namely; *Staphylococcus aureus, klebsiella* sp. *Salmonella* sp., *Escherichia coli* and *Pseudomonas* sp. and these are the major pathogens associated with post-harvest fish spoilage. This finding is in agreement with previous findings by Gram and Huss (2001), who reported that these organisms were the major causes of microbial spoilage of fresh fish after capture and the microbial count on the different media suggests contamination. Higher microbial counts in the skin samples comparative to the gill and flesh may be attributable to handling during harvest and processing and high *Escherichia coli* in all the samples may be due to its ubiquitous nature as it could be found in almost all environment including human skin, water and air during processing. This result corroborated that of Majeed and MacRae (1991) who observed that most of the bacteria flora associated with spoilage of fish were Gram negative rod bacilli such as *E. coli, Salmonella* sp. and *Pseudomonas* sp. The total bacterial count on fish rarely indicate the quality of the fish but it gives an indication of the risk of spoilage induced since each of these organisms had different ways of affecting health conditions of

consumers of such contaminated fish (Gram *et al.*, 2000). It is generally accepted that fish with microbial load of $>10^6$cfu/ml is likely to be at the stage being unacceptable from the microbiological point of view and unfit for human consumption (Cheesbroughl, 2000).

Conversely, Miller *et al*., (1973) observed that not all bacteria present on fresh fish are spoilers but there are certain active spoilers which are the major pathogens on fish spoilage. These bacteria have the ability to reduce trimethylamine and produce hydrogen sulphide from sodium thiosulphate as asecondary metabolite that constitute fresh fish spoilage. The presence of *Klebsiella* and *Salmonella* spp. in the fresh fish samples is an indication that the water used for processing was faecally contaminated. *Pseudomonas* sp. was the fourth most common bacteria isolated and the observation in the present study was low compared to previous reports by Gram *et al*., (2000) owing to the fact that during storage at 0^oC they had fewer isolates. Therefore *Pseudomonas* sp. is the major pathogen associated with fresh fish spoilage during refrigeration (Gram, 1993). The presence of *Staphylococcus aureus* a normal flora of skin and mucous membrane of humans can be attributed to human contact during handling and processing (Dalgaard *et al*., 2006). *Staphylococcus aureus* produces a variety of extra cellular enzymes and toxins that have been found to be responsible for food poisoning and can rapidly develop resistance to many antimicrobial agents and pose therapeutic problems (Thrower, 2000).

Table 4 shows antibiotic sensitivity pattern of the bacteria isolates from the fresh fish samples using the agar disc diffusion method with Augmentin, Tetracyclin, Gentamicin, Nitrofuranton, Cotrioflaxacin, Amoxicillin and Chloroamphenicol. Gentamicin was found to be most sensitive to all bacteria isolates. Maximum resistance of the bacteria isolates was found in Amoxicillin. However, infective dose was dependent on individual susceptibility and the onset and severity of disease may depend on quantity of toxin in the fish and quantity of fish ingested.

Conclusion

The presence of these bacterial and fungal isolates in fresh fish spoilage is indicative of public health risk in contacting diseases associated with these organisms. Compliance with standard microbiological measures to prevent contamination by these organisms becomes very necessary and should be ensured. Lack of proper storage facility after capture and insanitary conditions during processing are the major sources of contamination identified in this study. In view of the findings of this research work it is therefore recommended that good hygienic conditions and use of clean water during processing should be strictly adhered to. After harvest, fresh fish should be properly stored at low temperatures so as to inhibit survival of mesophilic bacteria

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Table 1 Total microbial count from skin, gill and flesh of the fresh fish samples

SAMPLES	COLONY COUNT ($X10^5$ $Cfuml^{-1}$)								
	Nutrient Agar		Mean	Maconkay Agar		Mean	Potato Dextrose Agar		Mean
Skin	216	214	215	121	102	111.5	26	24	25
Gill	189	179	184	87	62	74.5	11	4	7.5
Flesh	137	87	112	75	30	52.5	9	3	6

Table 2 Morphological and Biochemical Characteristics of bacterial isolates

Test	Isolates				
CULTURAL CHARACTERISTICS	**1**	**2**	**3**	**4**	**5**
Cell form	Circular	Circular	Circular	Circular	Circular
Colony margin	Entire	Flat	Entire	Entire	Undulate
Elevation	Convex	Convex	Convex	Flat	Flat
Texture	Smooth	Mucoid	Mucoid	Smooth	Rough
Pigmentation	Cream	Pinkish	Light cream	White	Cream
Optical Characteristics	Opaque	Opaque	Opaque	Opaque	Opaque
Morphology					
Gram staining	+ve	-ve	-ve	-ve	-ve
Cell shape	Cocci	Rod	Rod	Rod	Rod
BIOCHEMICAL					
Catalase	+	+	+	+	-
Coagulase	+	-	-	-	-
Oxidase	-	-	-	-	+
Indole	-	-	+	+	-
Urease	-	-	-	-	-
Citrate	-	+	+	-	+
Methyl red	+	+	+	+	+
FERMENTATION					
Glucose	A/G	A	A/G	A/G	-
Lactose	-	+	-	A/G	-
Sucrose	A/G	A	G	A	G
Suspected organism	*Staphylococcus aureus*	*Klebsiella* sp.	*Salmonella* sp.	*Escherichia coli*	*Pseudomonas* sp.

+ = positive, - = negative, A = acid production, A/G = acid and gas production, G = gas production

Table 3 Cultural and Morphological Characteristics of Fungal Isolates

Fungal species	**Spore color**	**Stolon**	**Surface**	**Hyphae**	**Spore-former**
Aspergillus niger	Brownish	Absent	Fluffy	Septate	Conidiophore
Geotrichum sp.	White	Absent	Colon-like	Non-septate	Chlamydophore
Rhizopus sp.	Black	Absent	Fluffy	Non-septate	Sporangiophore
Penicillium sp.	Green	Absent	Colon-like	Septate	Conidia

Table 4 Antibiotics Susceptibility Test

Antibiotics	**Test Isolates**				
	Escherichia coli	***Staphylacoccus aureus***	***Salmonella* sp.**	***Pseudomonas* sp.**	***Klebsiella* sp**
Augmentin (mm)	25 (R)	10 (S)	8 (S)	-	6 (S)
Tetremycin (mm)	4 (S)	25 (R)	22 (R)	30 (R)	19 (R)
Gentamicin (mm)	28 (R)	38 (R)	18 (R)	27 (R)	16 (R)
Nitrofuranton (mm)	15 (R)	19 (R)	2 (S)	5 (S)	7 (S)
Cotrioflaxacin (mm)	20 (R)	4 (S)	9 (S)	25	2 (S)
Amoxicillin (mm)	10 (S)	4 (S)	2 (S)	10 (S)	7 (S)
Chloroamphenicol (mm)	3 (S)	7 (S)	5 (S)	10 (S)	24 (R)

(R) = Resistant (above 12mm), S = Susceptible (below 12mm)