Journal of Fisheries & Livestock Production
ISSN: 2332-2608
Adeosun et al., J Fisheries Livest Prod 2018, 6:2
DOI: 10.4172/2332-2608.1000273
Review Article
Open Access

Sensitivity of Bacteria Isolated from Smoked Cayenne Pepper (*Capsicum minimum*) Treated *Clarias gariepinus*

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Abstract

Microbiological examination of fish aims at evaluating hygienic quality of fish, including temperature abuse, and the possible presence of pathogenic microorganisms in the fish. Microbial activity is responsible for spoilage of most fresh and of several light preserved sea foods. This paper evaluates the sensitivity of bacteria isolated from smoked *Clarias gariepinus* to different concentrations of ground Cayenne pepper and commercial antibiotics. The bacteria isolated from the samples are *Bacillus subtilis, Staphylococcus saprophyticus, Escherichia coli and Micrococcus* species. *Bacillus subtilis* was the most abundant found in the fish samples throughout the four weeks of storage while *Micrococcus* species was the least occurring bacteria all through the four weeks of storage. Sensitivity results revealed that all bacteria isolates were resistant to all levels of cured smoked fish samples with pepper except *Escherichia coli* which was intermediate to 1% pepper. It was noticed that as the level of the pepper increases, the bacteria isolates showed resistance which infers that the less the pepper, the more effective it is to inhibit bacteria isolates. Results of effect of commercial antibiotics on the bacteria isolated from the smoked cured fish samples showed that all bacteria isolates were significantly susceptible to Ofloxacin and Pefloxacin while Amoxycillin was the least effective in all the commercial synthetic antibiotics used. The findings of this study revealed a lower microbial count. The bacteria load obtained from the smoked fish fell within range. The microorganisms isolated and identified from the fish samples can be said to be normal flora of the fish.

Keywords: Pefloxacin; *Bacillus subtilis*; Sulphides; Metabolism

Introduction

Fish and fisheries products are among the most perishable commodities worldwide mainly due to microbial spoilage in fish [1]. Microbial growth and metabolism is a major cause of fish spoilage which produce amines, biogenic amines, such as organic acids, sulphides, alcohols and ketones with unpleasant and unaccepted off flavours [2,3]. Composition of micro-floral on newly catch fish depends on the microbial content of the water in which the fish live. Fish micro-flora includes bacteria in fish such as *Pseudomonas, Alcaligens, Vibrio serrtia* and *Micrococcus* [3].

Materials and Method

12 kg table size *Clarias gariepinus* samples were selected for the experiment. The fish samples were killed, gutted, washed and allowed to drain. Subsequently, they were divided into two batches A and B. Fish samples in batch A were treated with salt and this serves as control. Fish in batch B were divided into six (6) groups of five (5) sample each and soaked in 1%, 2%, 3%, 4%, and 5% cayenne pepper solution for 10minutes respectively. The fish were drained and smoked using CORAF/WECARD smoking kiln for 10 hours at a temperature ranging from 75-85°C and after smoking fish were allowed to cool to room temperature and stored in different boxes. This was done to mimic commercial practices. Samples were drawn weekly from the control and treated sample for four weeks. Smoked fish samples were analysed for Total Bacterial count on Nutrient Agar, Isolation of microorganisms from the stock culture and Sensitivity of isolated bacteria to pepper and commercial antibiotics was determined using the method of Clinical and Laboratory Standards Institute (2012).

Results

The microbial load of bacteria isolated from the smoked cured fish samples at different levels (1-5%) during the storage periods are as presented in Table 1. The total bacteria count ranged from 0.0 to 0.8×10^6 CFU/g. The total bacterial count obtained in this study was lower than the recommended values by the International Commission on Microbiological Specifications for foods [4]. Table 2 revealed the occurrence of bacteria isolates in the fish samples with the control smoked cured samples harbouring all the bacteria isolates: *Bacillus subtilis, Staphylococcus saprophyticus, Escherichia coli and Micrococcus* species. *Bacillus subtilis* was the most abundant found in the fish samples throughout the four weeks of shelf life while *Micrococcus* species was the least occurring bacteria all through the four weeks of storage. Table 3 revealed that all bacteria isolates were resistant to all levels of cured smoked fish samples with pepper except *Escherichia*

Treatment	Week 0	Week 1	Week 2	Week 3	Week 4
	TBC × 10 ^{6} CFU/g				
Control	0.1	0.1	0.8	0.3	0.2
1%	0.3	0.3	0.3	0	0
2%	0.3	0.3	0.2	0	0
3%	0.1	0.2	0.1	0.1	0
4%	0.1	0.1	0	0	0
5%	ND	ND	0	0	0.1

TBC: Total Bacteria Count; ND: Not Determined

Table 1: Average bacteria count of fish samples from week one to week four of shelf life.

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Received May 20, 2018; **Accepted** June 02, 2018; **Published** June 05, 2018

Citation: Adeosun O, Olateju GB, Akinyemi AA (2018) Sensitivity of Bacteria Isolated from Smoked Cayenne Pepper (*Capsicum minimum*) Treated *Clarias gariepinus*. J Fisheries Livest Prod 6: 273. doi: 10.4172/2332-2608.1000273

Treatment	Week 0	Week 1	Week 2	Week 3	Week 4
Control	*Escherichia coli*	*Escherichia coli*	*Bacillus subtilus, Staphylococcus saprophyticus,*	*Bacillus subtilus,*	*Bacillus subtilus, Staphylococcus saprophyticus*
			Escherichia coli	*Staphylococcus saprophyticus*	
1%	*Bacillus subtilus*	*Bacillus subtilus, Staphylococcus saprophyticus*	*Bacillus subtilus*	Nil	Nil
	Staphylococcus saprophyticus				
2%	*Staphylococcus saprophyticus*	*Staphylococcus saprophyticus, Escherichia coli*	*Bacillus subtilus*	Nil	Nil
	Escherichia coli				
3%	*Bacillus subtilus, Micrococcus species*	*Bacillus subtilus, Micrococcus species*	*Bacillus subtilus*	*Bacillus subtilus*	Nil
4%	*Escherichia coli*	*Escherichia coli*		Nil	Nil
5%	ND	ND	*Micrococcus* species	Nil	*Staphylococcus saprophyticus*

Table 2: Occurrence of bacteria in smoked cured fish samples.

Organisms	1%	2%	3%	4%	5%	CPX
Staphylococcus saprophyticus	4	0	0	0	0	36
Bacillus subtilis	5	0	0	0	0	37
Escherichia coli	13	11	4	10	0	34
Micrococcus species	12	2	5	4	0	36

Ciprofloxacin (10UGml-1) {Positive control}

Resistance: 0-12; Intermediate: 13-18; Susceptible: 19-Above (CLSI, 2012).

Table 3: Sensitivity of bacteria isolates to different treatments of pepper.

coli which was intermediate to 1% pepper. It was noticed that as the level of the pepper increases, the bacteria isolates showed resistance which infers that the less the pepper, the more effective it is to inhibit bacteria isolates. Table 4 showed the effect of commercial antibiotics on the bacteria isolated from the smoked cured fish samples. All bacteria isolates were significantly susceptible to Ofloxacin and Pefloxacin while Amoxycillin was the least effective in all the commercial synthetic antibiotics used.

Discussion

This study showed that pathogenic bacteria are present in the smoked cured *Clarias gariepinus*. According to International Commission on Microbiological Specification for Food [4], the maximum recommended bacteria count for good quality product is 5.0×10^5 (5.7Log cfu/g). The findings of this study revealed a lower microbial count, compared to that obtained for the skin, intestine and gills of fish samples in an investigation [5,6] - the bacterial load ranged from 10×10^{12}-14×10^{12}cfu/g. Higher microbial level was reported by Al-Harbi and Uddin (2008) ranging from 4.3×10^6 to 1.6×10^7 cfu/g in gill filaments and 8.7×10^9 to 5.4×10^{10}cfu/g in intestines of common carp, *Cyprinus carpio* cultured in ponds in Saudi Arabia. Similar trend of bacterial growth has been reported by several workers. In a study of microbial load of fresh fish samples in Benin City, a range of 2.8×10^6 – 4.4×10^6 for bacterial count was recorded [7].

The bacteria load obtained from the smoked fish fell within range. The microorganisms isolated and identified from the fish samples can be said to be normal flora of the fish e.g. Bacillus sp. [8]. The normal microbial flora of the fish are not initially harmful, as they even help in preventing the invasion of the fish flesh by other microorganisms but they become pathogenic when there is an enabling environment that promotes their growth. Bacteria present in the fish samples include, *Bacillus subtilis*, *Staphylococcus saprophyticus, Eschericia coli* and *Micrococcus species.* The occurrence of *Staphylococcus saprophyticus* and *Eschericia coli* in the smoked fish samples had been reported by Martin [9] when he stated that these organisms were the commonest micro-organisms associated with smoked fish. The presence of

Antibiotics	***Staphylococcus saprophyticus***	***Escherichial coli***	***Bacillus subtilis***	***Micrococus specie***
Pefloxacin	33	30	27	29
Cotrimoxazole	12	20	26	22
Ciprofloxacin	30	34	33	25
Erythromycin	31	22	28	22
Amoxicillin	12	2	11	20
Ofloxacin	36	37	32	30
Streptomycin	4	14	25	26
Chloramphenicol	14	21	20	20
Cefuroxime	20	21	35	23
Gentamicin	25	15	22	22

Resistance: 0-12 (resistance to antibiotics); Intermediate: 13-18

Susceptible: 19- above (not resistance to antibiotics) (CLSI, 2012).

Table 4: Sensitivity of bacteria isolates to commercial antibiotics.

Staphylococcus saprophyticus in fish samples according to Okonko et al. [10] might have been through contamination by handling.

The bacteria group *Staphylococcus* according to Herman et al. [11] reported that it was one of the most common causes of human disease and they constitute the normal flora of the human skin and mucous membrane without resulting to a disease condition. This bacteria class may also cause superficial and systemic infections such as boils, impetigo and folliculitis while more serious and more common infections could be pneumonia, bacteremia and other infections of the bones and wounds. Also, *Escherichia coli* usually cause diarrhea and kidney damage as well as uncomplicated community acquired urinary tract infections. The presence of the organisms could be as a result of handling during smoking and also cross contamination during storage, after smoking and handling during sales of smoked fish.

According to Thampuran et al. [2], *E. coli* is commonly associated with sea food contamination in the tropics, where it is encountered in high numbers. Thampuran et al. [2] isolated *E. coli* in finfish samples acquired at the retail market, and although typical *E. coli* or labile toxin-producing *E. coli* was not detected, the isolation of strains with the ability to produce hemolysis in human blood was a fact worth

mentioning. Marin et al. [12] detected *E. coli* when researching the bacteriological quality of *Cynoscions quamipinnis* and *Lutjanus gutattus* fish samples marketed in Costa Rica. Koo et al. [13] reported having isolated pathogenic strain from rockfish sold in South Korea, and alerting to the presence of *E. coli* pathogen in seafood.

All bacteria isolates were significantly susceptible to Ofloxacin and Pefloxacin. It was revealed that the microorganisms are resistant to different treatments of pepper. The use of antimicrobial agents in aquaculture and then possibility of antibiotics resistance among bacteria flora from fish have been identified [14,15].

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