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CHARACTERISTICS AND ANTIBIOTICS SUSCEPTIBILITY PROFILE OF BACILLUS SPP ISOLATED FROM SOME FROZEN FOODS IN RANDOMLY SELECTED AREAS IN IBADAN, OYO STATE, NIGERIA

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ABSTRACT

Aerobic spore formers present in food samples are important due to the formation of the spores by the bacterium which makes them resistant to heat, freezing, chemicals and other adverse environment that these samples pass through during handling, processing and preparation. It is important to understand the dynamics of microorganisms present in food samples and their rate of resistance. Samples of *Urophycis tenuis* (Panla fish), *Scromber scombru* (Titus fish) and *Meleagris gallopavo* (Turkey meat) from selected markets in Ibadan was evaluated for *Bacillus sp* using cultural, morphological and biochemical characterization. Turbidmetric method of measuring bacteria growth was used to determine their growth at different temperature. A total number of 46 bacteria isolates were obtained, from which 6 were aerobic spore formers of Bacillus species. The colony count ranged from 2.2×104 cfu/g to 160×104 cfu/g and all aerobic spore formers were able to survive at temperature of between -15 to 70°C. The aerobic spore formers isolated were *Bacillus cereus* (50%), *B. subtilis* (33%) and *B. megaterium* (17%). *B. cereus* and *B. subtilis* had the highest antibiotics resistance while *B. megaterium* had the least resistance to the tested antibiotics. Environmental sanitation and proper handling should reduce incidence of such spore formers in frozen foods and enhance the keep ability of their nutritional values.

Keywords: Aerobic, Antibiotic Resistance, Frozen Foods, Isolates And Spore Formers.

I. INTRODUCTION

Spoilage causing bacteria deteriorate the frozen food product and develop unpleasant odors, tastes, and textures. The spoilage of frozen food products by bacterial constitute a significant economic problem which give rise to an increase loss in the food industry, mostly caused by inappropriate freezing/refrigeration conditions.

Bacteria species of Staphylococcus, Klebsiella, Bacillus, Proteus causes food deterioration but are not responsible for serious illness. Although when consumed in large amount can result into gastrointestinal tract discomfort. Food contaminated by pathogens and some microbial toxins could lead to serious infections or diseases (Tewari and Abdullah, 2015; Van Cauteren et al., 2017)

Some Bacillus specie can cause spoilage of food or food-borne illness. The differentiation is dependent on motility, toxin crystal, hemolytic activity and rhizoid growth. Food borne illness or food spoilage has been a major concern to consumers. These organisms come in contact with frozen food samples through different ways which include but not limited to; improper handling by food handlers, materials used in handling the foods, some environmental conditions.

There is a great need to understand the survival and growth conditions of *Bacillus sp* in frozen food samples; therefore the research aim to understand the microbiological quality of some selected frozen food samples sold in common retail outlets in Ibadan.

II. METHODOLOGY

2.1 Sample Site and Sample Collection

A total of 18 frozen food samples were collected from different sales outlets of 3 different markets in Ibadan, Oyo State. These markets include; Aleshinloye, Bodija and main Iwo road. The different frozen foods samples purchased for this work were *Urophycis tenuis* (Panla fish), *Scromber scombru* (Titus fish) and *Meleagris gallopavo* (Turkey meat). All samples were collected into sterile ziploc bags and carefully transported to Laboratory for further work.

2.2 Preparation of Culture Media

Each of the media used was weighed and prepare according to manufacturers' instruction. The media used were Nutrient agar (HIMEDIA), Nutrient Broth (HIMEDIA, Tryptone soy Agar (LABM), Tryptone soy Broth (OXOID) and Bacteriological Agar (BIOLAB). Each was weighed, dissolved and sterilized using an autoclave at 121°C for 20 minutes and allowed to cool down to about 45°C before using.

2.3 Sample preparation

Different part of the samples (including gills of fishes, bones of turkey, intestine and flesh) were cut out using sterile knife and macerated. 10g of the sample was added to 90ml of sterile water, another 10 ml was taken from the 90ml to tube containing sterile water till the 4th dilution factor was reached. 0.1 ml of 3rd and 4th dillution was measured into 9ml of prepared soy broth in a lamina flow.

2.4 Isolation and Identification of Microorganism

0.1 ml of the 3rd dilution factor of the incubated broth was carefully measure and inoculated on Tryptone soy Agar and Nutrient Agar using pour plate method and incubated for 24hours at 37°C. Colonies on each plate were enumerated and recorded. Gram reaction and shape were microscopically studied using an 18 h culture from agar plate. Biochemical characterization involve test like; spore staining, motility, catalase, nitrate reduction, methyl red, voges proskaeur and carbohydrate fermentation using glucose, fructose, mannose, lactose and mannitol. Each of the tests was carried out according to procedure stated in Bergey's manual of systematic bacteriology.

2.5 Bacteria Growth and Survival at Different Temperature

Growth and survival rate of spore formers were determined by inoculating selected organism into sterile Nutrient broth prepared into macCartney bottles and incubated at different temperatures. Temperature range and incubation period were -10°C for 48hours, 4°c for 48 h, 45°c, 50, 55, 60, 70 and 75 for 24 h. After incubation, growth density and count was determined using a spectrophotometer at 480nm wavelength. Bacteria count in cfu/ml was determined using equation derived from standard curve obtained by using absorbance value from bacteria culture.

2.6 Antibiotic Sensitivity Testing

Antibiotics susceptibility tests of selected isolates were performed based on disc diffusion method (Bauer et al., 1996). Smaller wafers containing antibiotics cotrimoxazole, cloxacillin, erythromycin, gentamicin, augmentin, streptomycin, tetracycline and chloramphenicol were used. The degree of susceptibility of the test organism were determined and interpreted as either sensitive (S), partially sensitive (PS) or resistant (R) by measuring the diameter of zones of inhibition around the antibiotic disc (Akubuenyi, Otu & Nyong, 2018).

III. RESULTS AND DISCUSSION

3.1 Total Bacteria count

The total bacteria count of the samples plated on nutrient agar and tripticase soy agar ranged from 2.2×10^4 cfu/g to 16×10^{10} cfu/g. Total bacteria count of sample on different media are represented in Table 1a and 1b which exceeded the high range and moderate range of microbial contamination (HPA, 2004). This indicates that these frozen samples are potentially injurious to health. This microbial contamination might have originated from unhygienic handlers, transportation, poor means of storage, infected equipment used in food handling (Tallent, Kotewicz, Strain & Bennet, 2012). The total viable count of bacterial obtained from this study was much higher than findings reported by Adebayo, Odu, Esen & Okonko (2012); Chakra Borty Shuvho, Chowdhury, Khan, Monir & Islam (2015) and was somewhat related to findings reported by Moshood, Haziyamin, Tengku & Hamid (2012); Saranraj & Geetha (2012).

A total number of 46 bacterial isolates were evaluated from the frozen food samples consisting of 10 Gram positive rods, 31 Gram positive cocci, 4 Gram negative cocci and 1 Gram negative rod. From the 10 Gram positive rods, 6 were aerobic spore former which was our focus for the study. Bacterial isolation from frozen snack conducted by Chakra Borty Shuvho et al. (2015) also documented about 31% of Bacillus species a mist other microorganisms.

Table 1a: Total Bacterial Count from Samples of Frozen Foods on Nutrient Agar

Sample Codes	AL	BD	IR
UT1	20×10^4	5.4×10^5	12×10^5

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UT2	4.0×10^4	9×10^4	3×10^4
SS1	12×10^4	3.3×10^5	16×10^{10}
SS2	3.5×10^4	2.7×10^4	6×10^4
MG1	20×10^4	2.5×10^5	3.2×10^5
MG2	2.2×10^4	3×10^4	10×10^4

Table 1b: Total Bacterial Count from Samples of Frozen Foods on Trypticase Soy Agar

Sample Codes	AL	BD	IR
UT1	4×10^5	3.5×10^5	20×10^{11}
UT2	4.0×10^4	9×10^4	3×10^4
SS1	12×10^4	3.3×10^5	16×10^{10}
SS2	3.5×10^4	2.7×10^4	6×10^4
MG1	20×10^4	2.5×10^5	3.2×10^5
MG2	2.2×10^4	3×10^4	10×10^4

Key;

AL: Aleshinloye Market; BD: Bodija Market; IR: IwoRoad Market

UT: *Urophycis tenuis* ; SS: *Scromber scombru* ; MG: *Meleagris gallopavo*

1- First Sampling; 2- Second Sampling

3.2 *Bacillus spp* of Isolates

The 6 Bacillus sp isolated grew and survived at temperature range of between -15oC to 70oC as shown in table 2. The optical density at different temperature is represented in table 3. The log number of cells shows that the organisms were able to grow at different temperature indicating their survival at high and low temperature. This spores produce by this group of organism may survive cooking temperature exposing consumers to low numbers of Bacillus sp consumption.

Table 2: Log no of cells (cfu/ml) for *Bacillus spp* at Different Temperatures

Isolate code	-15°C	4°C	45°C	50°C	55°C	60°C	65°C	70°C
TA1	11.99	13.42	89.99	53.07	17.09	6.10	22.34	16.45
TA2	31.89	23.45	31.57	45.10	4.83	12.95	5.15	14.54
TiA1	10.08	11.35	138.24	57.36	1.35	4.19	26.64	18.04
TiA4	8.65	16.28	59.90	41.92	10.24	10.88	20.75	14.54
TB1	25.84	21.22	124.23	13.74	4.99	5.30	28.07	15.02
PI2	18.19	16.61	64.21	13.90	13.11	14.86	18.04	14.22

Table 3: Optical Density (480nm) of *Bacillus spp* at Different Temperature

Isolate code	-15°C	4°C	45°C	50°C	55°C	60°C	65°C	70°C
TA1	0.093± 0.001	0.102± 0.001	0.583± 0.002	0.351± 0.001	0.125± 0.001	0.056± 0.001	0.158± 0.001	0.121± 0.001
TA2	0.218± 0.002	0.165± 0.001	0.216± 0.001	0.301± 0.002	0.048± 0.001	0.099± 0.001	0.050± 0.001	0.109± 0.002
TiA1	0.081± 0.001	0.089± 0.001	0.886± 0.001	0.378± 0.001	0.026± 0.001	0.044± 0.001	0.185± 0.001	0.131± 0.001

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TiA4	0.072± 0.001	0.120± 0.001	0.394± 0.001	0.281± 0.001	0.082± 0.001	0.086± 0.001	0.148± 0.001	0.109± 0.001
TB1	0.180± 0.001	0.151± 0.001	0.798± 0.001	0.104± 0.001	0.049± 0.001	0.051± 0.001	0.194± 0.001	0.112± 0.001
PI2	0.132± 0.001	0.122± 0.001	0.421± 0.001	0.105± 0.001	0.100± 0.001	0.111± 0.001	0.131± 0.001	0.107± 0.001

NOTE: T: Turkey meat; Ti: Titus Fish; P: Panla Fish

3.3 Biochemical Characteristics of Bacillus sp

The 6 different isolates were characterized on the bases of biochemical characteristics 50% was identified as *B. Cereus* while *B. Megatarium* and *B. subtilis* were 17 and 33% respectively. *B. Cereus* isolates were catalase, motility, V.P, gelatine hydrolysis, hemolysis, glucose, and nitrate reduction positive, negative to methyl red, indole, manittol and sucrose. B. Subtilis isolates were positive to catalase, motility, gelatin hydrolysis, hemolysis, V.P test, nitrate reduction and negative to indole. *B. Megaterium* was positive to catalase, methyl red, gelatin hydrolysis, mannitol and negative to nitrate reduction, hemolysis, V.P test, motility, indole, sucrose and glucose fermentation (table4).

B. Cereus is commonly found in frozen foods samples (Moshood et al., 2012; Saranraj & Geetha, 2012). *B. Cereus* are found naturally in soil and water, they can easily gain entry into food samples causing food spoilage or deterioration. The characteristic of *B. Cereus* found in this study agrees with previous study of Gordon, Haynes & Pang (1973) and Bergey's manual of Determinative Bacteriology by Buchanan. *B. Subtilis* are mostly found in soil and gastrointestinal tract of ruminants and humans. A study compared the density of *B. Subtilis* spores found in soil to that found in human fecal sample which was about 10^6 spores/gram and 10^4 spores/gram respectively. It was therefore concluded that spores evaluated in human fecal samples were too high to be attributed to consumption of contaminated food (Hong et al., 2009).

B. megaterium is widely diverse in habitat and has been documented to grow at temperature range from 3°C to 45°C. Saranraj & Geetha (2012) documented that *Bacillus sp.* is mostly responsible for the spoilage of different bakery products while Singh, Kaushal, Tyagi & Sharma (2011) observed that *Bacillus sp.* are responsible for spoilage of milk and milk product. This present study shows the presence in frozen food samples which may be as a result of contact with soil, surfaces and not been properly rinsed or during the cooling processes where their spores germinate.

Table 4: Biochemical Characteristics of Isolates

S/N	Isolate Code	Cellular Characteristics	Gram Reaction	Motility	Spore Staining	Catalase	Methyl Red	V.P	Gelatin Hydrolysis	Indole	Hemolytic Activity	Glucose fermentation	Mannitol Fermentation	Sucrose Fermentation	Nitrate Reduction	Probable Organism
1	TA1	Rod	+	+	+	+	-	+	+	-	+	+	-	-	+	*B. cereus*
2	TA2	Rod	+	-	+	+	+	-	+	-	-	-	+	-	-	*B. megatarium*
3	TiA1	Rod	+	+	+	+	-	+	+	-	-	-	+	+	+	*B. subtilis*
4	TiA4	Rod	+	+	+	+	-	+	+	-	+	+	-	-	+	*B. cereus*
5	TB1	Rod	+	+	+	+	-	+	+	-	-	-	+	+	+	*B. subtilis*
6	PI2	Rod	+	+	+	+	-	+	+	-	+	+	-	-	+	*B. cereus*

3.4 Antibiotics Susceptibility testing of Bacillus sp

The antibiotic susceptibility testing carried out on aerobic spore former's showed that most of the organism were resistant to the antibiotics. The antibiotics employed are mostly used for treatment of gram positive bacteria food borne illness and most of the antibiotics are not successful in treating the infection.

Table 5: Antibiotics Susceptibility Profile of the Bacillus species

Isolate code	GEN	AUG	STR	TET	CHL	COT	CXC	ERY
TA1	R	R	R	R	R	R	R	R
TA2	S	R	S	PS	PS	R	R	PS
TiA1	S	R	R	PS	PS	PS	R	S
TiA4	R	R	R	R	R	R	R	R
TB1	R	R	R	R	R	R	R	R
PI2	R	R	R	R	R	R	R	R

Key;

GEN: Gentamicin ; AUG: Augmentin ; STR: Streptomycin ; TET: Tetracycline ;

CHL: Chloramphenicol ; COT: Cotrimoxazole ; CXC: Cloxacillin ; ERY: Erythromycin

S: Susceptible; R: Resistant; PS: Partially susceptible

IV. CONCLUSION

The research shows that frozen foods obtained from these area has high microbial load and the aerobic spore formers of focus were able to survive quite a large range of temperature which indicates possibility of surviving cooking temperature. Therefore, Health and Environmental officers need to educate food handlers and consumers on hygienic food handling methods. The general public should be sensitized on high risk of handling food improperly which might lead to food born infection and illnesses. This study also recommends the use of molecular analysis to further understand the organisms isolated in this study which can help researchers in development of new antibiotics that can be used to treat infections caused by these multi resistant isolates.

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