ISOLATION AND IDENTIFICATION OF BACTERIA ASSOCIATED WITH SELECTED CANNED TOMATOES SOLD IN UTAKO MARKET, ABUJA.

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Abstract

The increase in microbial activities in canned food leading to spoilage and poisoning is on the rise and of health concern. Identification of bacteria associated with the increase level of bacterial activity in canned tomato was analyzed according to standard microbiological standard. The samples (De-Rica, Gino and Sonia) canned tomatoes were randomly purchased from Utako market, Abuja State but from different vendors at two (2) samples per product in the market area. The samples were carefully transported to Nile University's microbiology laboratory and stored at room temperature of about (25-27 ^{0}C) until it was time for analysis. The tomatoes samples were subjected to several microbiological and biochemical test analysis such as coagulase test, catalase test, motility test, methyl red test, gram staining as well as microscopy were also conducted for isolation and characterization of bacterial strains from the canned tomato. Some bacteria strains where isolated and characterized during this analysis such as *Escherichia coli* and *Streptococcus pyogens.* The electrophoregram of the various isolates indicates that they had a molecular band weight of 800kb. The research recommended proper handling of harvested tomatoes fruits, proper screening of the harvested tomatoes fruits and provision of disease resistance varieties as well as proper storage using canned processing.

Keywords: De-Rica, Gino and Sonia, Coagulase, Catalase, Motility, Methyl red, Gram staining, Electrophoregram, Tomato

Introduction

Tomato (*Lycopersicumesculentum*) is grown in many regions of Nigeria both as raining and dry season crop (Gorska, 2017). Research study has shown that tomatoes are rich in several materials such as carbohydrates and poor in proteins with pH value from slightly acidic to 7.0, and on the average, about 6.4 percent total solids, of which 3.5 percents is invert sugar, 0.5 percent citric acid, 0.6 percent ash, 0.9 percent protein, 0.55 percent crude fibre, and about 0.05 percent fat. When tomatoes undergo spoilage as a result of the life processes of bacteria, yeasts, and molds the sugar are rapidly used up, being changed principally into acetic acid, lactic acid, alcohol, and carbon dioxide, the amounts of these substance depending on the types of organisms which are most active in the particular sample in question (Ogbomo, 2011).

Tomato whether classified as fruit (according to botanist) or as a vegetable (according to nutritionists), indisputably serves immeasurable nutritional value to man no matter what purpose it is used for. Tomato fruits are highly perishable due to their high water content and hence they are prone to spoilage by microorganisms. The activities of these microorganisms bring about high levels of post-harvest losses especially after harvesting. Being perishable tomato is more susceptible to injury because of its shape and structure and its relative soft texture which is associated with high moisture content, and these lead to deterioration in transit and storage which is more rapid under conditions of high temperature and humidity, hence, heavy losses are encountered. Microbial spoilage of fruits and vegetable is known as rot, which manifests as loss of texture (soft rot) changes in colour (black or grey) and often off odor (Triase*t al;* 2008).

During this spoilage stage, the citric acid is also rapidly decomposed, so that its amount serves as a valuable index in detecting decomposition. It is very easy to detect spoilage in tomato pulp or canned tomatoes, as such products, when perfectly sound, contain no volatile acids and a considerable percentage of citric acid and invert sugar, and when spoiled quite large amounts of volatile acids are present with little or no invert sugar or citric acid (Triase*t al;* 2008; Yakubu, 2011).

The major key to a successful canning process can be attributed to understanding the acidity and spoilage factor of the canned food in question. There are two types of food that can undergo canning process which can be categorized as low acid (vegetables, meat, and seafood) and high acid (fruits and tomatoes). These foods categorized can successfully be canned using pressure. This method of using pressure is thus the best method for canning low acid food. As canning became a processing and preservation method, so did the invading and contamination by bacteria become a major issue (Oladejo, 2011).

Canned tomatoes that have been under processed will be unable to destroy moulds and heat-resistant bacteria during storage. One of the major micro-organism that aid spoilage of canned tomatoes is the *bacillus coagulans* which is heat-resistant and causes flat-sour spoilage in the canned tomatoes.

Images of Canned Tomatoes.Source: (Oladejo, 2011)

This study is hoped to provide important information on the various bacteria present in the tomatoes which may alter the preservation state and cause spoilage in these canned food (tomato)

Materials and Method

Sample collection

The samples were randomly purchased from Utako market in Abuja. The samples purchased were undamaged and not swollen or bloated. They were carefully transported to Nile university microbiology laboratory and stored at room temperature of about 27 ^{0}C until it was time for analysis.

Selected canned tomatoes

Three types of canned tomatoes were chosen for this project. The brands of canned tomatoes used for this project included Gino, Sonia and De-Rica.

CANNED TOMATO	NET WEIGHT	NAFDAC REG. NO	PRODUCTION DATE	EXPIRY DATE
GINO	400g	08-8925	21/02/2021	20/12/2023
SONIA	400g	A8-2062	04/11/2021	08/11/2021
DE-RICA	400g	01-5188	08/05/2021	08/11/2023

Sample And Media Preparation

Sterilization of Glasswares and Work Area

All glass wares were sterilized using the autoclave at 121^{o}C for 15 minutes. The work area and other equipment were swabbed with ethanol and cotton wool.

Media Preparation

The media used for this experiment was Nutrient Agar (NA) and it was prepared according to the manufacturer's recommendations and directions.

Serial Dilution

Using standard microbiological technique of serial dilution, the canned tomato samples were serially diluted into different concentrations of 10^{-1} to $10^{-4}$with the use of distilled water. The sample was mixed properly, and from 10^{-1}, 1ml was pipette into 10^{-2} and the process was repeated at each dilution factor using different pipette tips to avoid contamination. The steps were repeated for the remaining samples. (Koch *et al.*, 1883)

Sample Innoculation and Culure of Bacteria

Pour plate method was method of inoculation used. The glass tubes containing the diluted sample were flamed to avoid any contamination and then it was poured inside the petri- dishes. The lid of the Petri-dishes was opened aseptically and the nutrient agar was poured into the petri-dishes. The inoculated plates were then incubated at 37^{o}C for 24 hours (De souse-lima *et al.*, 2020).

Pure Culture

With the use of a flamed wire loop, discrete colonies were transferred from the petri dish and then streaked aseptically into freshly prepared nutrient agar to isolate pure colonies. The petri dishes were labeled accordingly to be able to identify each pure colony after growth. Plates were then incubated at 37^{o}C for 24 hours. At this step the pure culture of the microorganisms were obtained (De souse-lima *et al.*, 2020).

Gram Staining and Biochemical Identification of Bacterial Isolates

Gram Staining

A thin smear of the pure isolate colony was made on a glass sterile slide, dried in air and heat fixed. After heat fixing, the smear was covered with crystal violet for one minute before washed down with water, followed by iodine for thirty seconds before being rinsed. It was then decolorized with alcohol for twenty seconds, followed by an instant rinse. Safranin was added for thirty seconds as a counterstain before being washed with water. After air drying, the slide was examined using an oil immersion microscope with X100 objectives. Same procedure was repeated for all the isolates and the results were noted. Gram-positive bacteria showed up as purple, whereas Gram-negative bacteria showed up as pink or red (Deshmuck *et al.*, 2013).

Methyl Red Test

By using a sterile wire loop, the test organism was inoculated into the fresh, sterile prepared MR-VP medium and it was incubated at 37^{o}C for 48 hours. After incubation the broth was obtained from the incubator, 5 drops of 0.04% solution of alcoholic methyl red solution was added and mixed properly. Positive methyl red test was indicated by the development of red color after the addition of methyl red reagent and a negative methyl red test was indicated by no color change after the addition of methyl red reagent (Tankeshwar, 2013)

Catalase Test

A drop of 3% hydrogen peroxide was placed on the center of a slide and sterile glass rod was used to pick small portions of the suspected organism to be identified from nutrient agar into the slide for immediate gas bubble formation. Quick gas bubble or foaming in accordance to the method of Olutiola *et al.*, (1991) indicates positive result.

Indole Test

The test organism was inoculated in sterilized tube containing Tryptone broth using a sterile inoculating loop. While adopting aseptic techniques, the solution was incubated at 37^{o}C for 48 hours after which it was taken out and then 0.5mL (10 drops) of Kovac's reagent was added to

the inoculated tubes and was left to sit for 5 minutes. Observation for the presence or absence of ring took place and then the result was noted (Deshmuck *et al.,* 2013).

Coagulase Test

A clean dry sterile slide containing normal saline was obtained and a little amount of bacterial sample was applied. A drop of plasma was also placed on it and homogenized. The results were noted immediately. Colonies clumping together indicates a positive result. A coagulase test is used to distinguish between coagulase-positive and coagulase-negative Staphylococcus aureus (Deshmuck *et al.,* 2013).

Sugar Fermentation Test

The sugars tested for fermentation were glucose and lactose.This test is used to determine which sugars an organism can ferment and produce acid and gas from. The suspected organisms to be identified were aseptically inoculated into test tubes using a flamed inoculating loop. Durham tubes followed by 1-3 drops of phenol red were also added to each test tube. It was incubated at 37^{o}C for 48 hours and the results were read (Deshmuck*et al.,* 2013).

Citrate Test

Using a flamed sterile wire loop, the suspected organisms to be identified were inoculated and streaked into the simmons citrate agar and incubated at 37^{o}C degrees for 7 days. The results were recorded after (Deshmuck *et al.,* 2013). A positive citrate result is noted through change from green to blue which indicates the utilization of citrate.

Motility Test

This is a biochemical test which takes place to determine whether an organism is motile or not. The suspected organism to be identified was picked using a sterile wire loop and stabbed once to a distance of ½ inch in the middle of the tube keeping the needle in the same line it entered as it is removed from the medium. Incubation took place after at 37^{o}C and was examined daily for 7 days. A positive result will show diffusion, hazy growths that spread throughout the medium rendering it slightly opaque. While a negative result will show the growth that is confined to the stab-line, with sharply defined margins and leaving the surrounding medium clearly transparent (Deshmuck *et al.,* 2013).

Antibiotics Susceptibility

Antibiotic drug susceptibility was carriedout using 8 different against the different suspected bacteria isolates as described by Deshmuck *et al.,* 2016. They included ciprofloxacin, ofloxacillin, Amoxicillin + clavulanic acid, Nitrofurantoin, Ampicillin, ceftazimide, cefuroxime and gentamicin.

Molecular Tests

DNA was extracted from each bacterial isolate and the quality of DNA was checked by electrophoresis using agarose gel. These samples were sent to a DNA laboratory for sequencing in order to identify their individual species.

Statistical Analysis

The data was generated in triplicates and the mean value ± standard error of mean. Analysis of variance was also conducted by one way analysis of variance (ANOVA) using Graph pad prism 6, students T-test was used to consider the level of significance and values were considered to be significantly different at $p < 0.05$.

Results

Table.1 shows the colony count of bacteria isolates on nutrient agar medium. The tables show that De-rica tomato have the highest number of colonies (303) at a dilution factor of 10-3.

Table.2 shows the morphological characteristics of the isolated bacterial organism grown on nutrient medium. The bacterial organisms were characterized based on their colony morphology (color, shape, texture) from selected canned tomatoes gotten from utako market.

Table.3 shows the gram staining reaction of the suspected bacterial isolates from canned tomatoes after culturing on nutrient agar. The table shows that the probable bacteria were both gram positive and gram negative. It also points out the shapes of the bacteria when viewed under the microscope.

Table.4 shows the results of the biochemical test carried out on the isolates from the canned tomatoes (Gino, De-Rica and Sonia) to help in narrowing down the suspected organisms. The bacteria isolates are suspected to be*Escherichia coli* and *Streptococcus pyogenes.*

Table.5 shows the effect of the antibiotic disk on isolates from the different canned tomatoes used. Areas with a clear zone of inhibition were those with no bacterial growth around them and areas with some bacterial growth have no clear zone of inhibition. CPR was the most effective with clear zones of inhibition in all the plates.

FIG.1 shows the colony count of bacteria isolates on nutrient agar medium.

FIG.2 shows the zone of inhibition of the antibiotic susceptibility test of 8 different antibiotics against the bacteria isolate from De-rica (DA) tomato paste where OFL had the most effect and CRX had the least effect.

FIG.3 shows the zone of inhibition of the antibiotic susceptibility test of 8 different antibiotics against the bacteria isolate from sonia tomato (SA) paste where OFL had the most effect and GEN had the least effect.

FIG.4 shows the zone of inhibition of the antibiotic susceptibility test of 8 different antibiotics against the bacteria isolate from Gino tomato (GO) paste where GEN had the most effect and CAZ had the least effect.

Table.1 Colony count results

	NO. OF COLONIES	DILUTION FACTOR
GA	113	10^3
SA	262	10^2
DA	303	10^3

KEY: GA stands for Gino tomatoes, SA stands for Sonia tomatoes and DA stands for De-rica tomatoes.

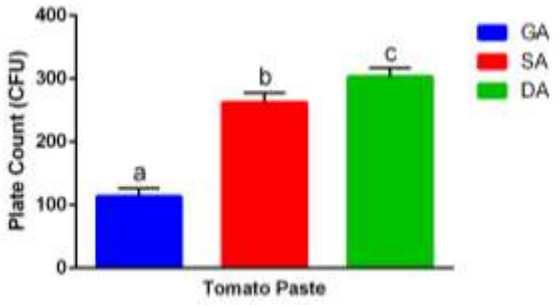


FIG.1 shows the colony count of bacteria isolates on nutrient agar medium.

Table.2 Morphological characteristics of isolated bacterial organisms

	NUTRIENT MEDIUM	COLOR	TEXTURE	SHAPE
GA	Nutrient Agar	Greyish white	Smooth	Round
SA	Nutrient Agar	Creamy	Smooth	Round
DA	Nutrient Agar	Greyish white	Smooth	Round

KEY: GA stands for Gino tomatoes, SA stands for Sonia tomatoes and DA stands for Derica tomatoes.

Table.3 Gram staining reaction and microscopic description of isolated bacterial organisms

	GRAM REACTION	MICROSCOPIC DESCRIPTION
GA	Gram Negative	Rod shaped
SA	Gram positive	Cocci
DA	Gram Negative	Rod shaped

KEY: GA stands for Gino tomatoes, SA stands for Sonia tomatoes and DA stands for Derica tomatoes

Table.4 Biochemical characterization of isolated bacterial organisms

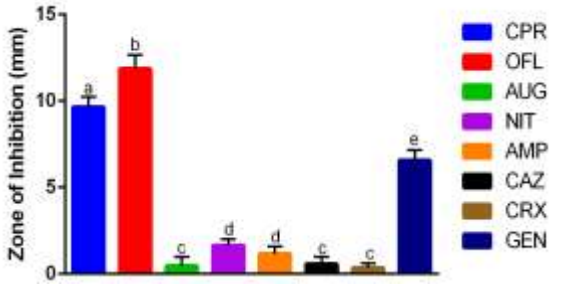
ISOLATES	GA	SA	DA
CATALESE	+	_	+
COAGULASE	_	_	_
METHYL RED	+	+	+
INDOLE	+	+	+
CITRATE	+	+	+
SF - GLUCOSE	+	+	+
SF - LACTOSE	+	+	+
MOTILITY	Motile	Non-Motile	Motile
PROBABLE BACTERIA	*Escherichia Coli*	*Streptococcus pyogens*	*Escherichia Coli*

KEY: GA stands for Gino tomatoes, SA stands for Sonia tomatoes and DA stands for Derica tomatoes. '+' stands for positive and '-'stands for negative

Table.5 Antibiotics susceptibility test for bacterial isolates ANTIBIOTICS

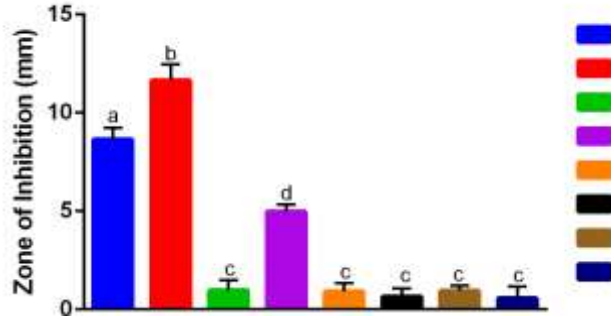
	CPR	OFL	AUG	NIT	AMP	CAZ	CRX	GEN
GA	**susceptible**	**susceptible**	**Not susceptible**	**Not susceptible**	**Not susceptible**	**Not susceptible**	**Not susceptible**	**susceptible**
SA	**susceptible**	**susceptible**	**Not susceptible**	**susceptible**	**Not susceptible**	**Not susceptible**	**Not susceptible**	**Not susceptible**
DA	**susceptible**	**susceptible**	**Not susceptible**	**Not susceptible**	**Not susceptible**	**Not susceptible**	**Not susceptible**	**susceptible**

KEY: GA stands for Gino tomatoes, SA stands for Sonia tomatoes and DA stands for Derica tomatoes.



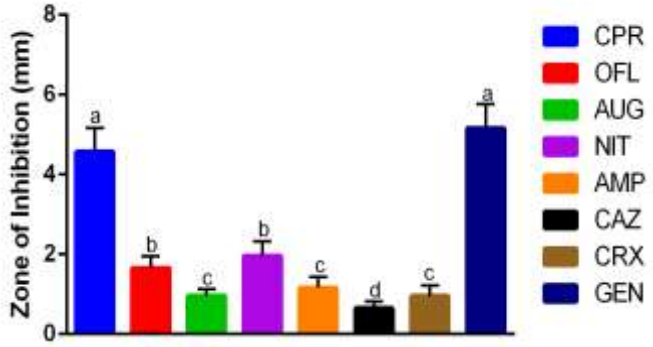
Isolate from DA

FIG.2 shows the zone of inhibition of the antibiotic susceptibility test of 8 different antibiotics against the bacteria isolate from De-rica(DA) tomato paste where OFL had the most effect and CRX had the least effect.



Isolate from SA

FIG.3 shows the zone of inhibition of the antibiotic susceptibility test of 8 different antibiotics against the bacteria isolate from sonia tomato (SA) paste where OFL had the most effect and GEN had the least effect.



Isolate From GA

FIG.4 shows the zone of inhibition of the antibiotic susceptibility test of 8 different antibiotics against the bacteria isolate from Gino tomato (GA) paste where GEN had the most effect and CAZ had the least effect.

Discussion

Tomato has long been known as one of the perishable fruits that are classified under berries. Because of the juicy nature of it, it is capable of harboring an array of microbes (bacteria); especially during deterioration (Prescott *et al*., 2018). This research was able to isolate and identify certain probable bacteria including *Escherichia coli* and *Streptococcus pyogens*.

It can be argued that the contamination of these canned foods could be a reflection of many factors including the quality of raw materials, under processing, pre-processing contamination and the level of stringency in their production which agrees with a study by Ogundipe *et al*., (2014) which describes that the bacteria often isolated from tomatoes could be due to the fact that most of these bacteria access tomatoes either from the soil or from the water used to wash the tomatoes after the harvest. Oftentimes, these contaminations are further increased by cross-contamination with contaminated surfaces, vessels and hands (Adebanwo *et al*., 2002). *E. coli* contamination may also arise from fecal contamination from farmyards and manure.

Furthermore, Antimicrobial drug susceptibility test was carried out by testing eight commonly used antibiotics against the suspected bacterial isolates. These antibiotics included ciprofloxacin, ofloxacillin, Amoxicillin + clavulanic acid, Nitrofurantoin, Ampicillin, ceftazimide, cefuroxime and gentamicin. Most of the isolates were susceptible to gentamicin and ofloxacillin, but none of the isolates expressed resistance to ciprofloxacin. However, high resistance was observed for Ampicillin, ceftazimide and cefuroxime. The varying antibiotic prevalence has been previously reported by Wogu and Ofuase (2014) in a previous study on tomatoes in Benin City, Nigeria. The difference in resistance may be associated with varying functional groups of antibiotics and bacterial species. The presence of bacteria with antibiotic resistance associated with tomatoes sampled in this study shows the potential risk of tomatoes to consumers and since they are resistant to

some of these antibiotics, they should not be used in treating infections or diseases caused by them.

Finally, DNA was extracted from each bacterial isolate and the quality of DNA was checked by electrophoresis using agarose gel. The results from this showed that the isolates had a band width of 800kb which agrees with a study by Howard Ochman *et al.*, (1995) that states that some strains of *Escherichia coli* ranges from 5 to 1,800 kb.

Conclusion

This study was able to establish that most on-the-shelf canned tomato products can possibly harbor organisms of public health importance. The probable pathogenic bacteria identified were *Escherichia coli* from GA (Gino) and DA (De-rica) and *Streptococcus pyogenes* from SA (Sonia). The presence of these bacteria could also be explained by the fact that shelf stable canned foods packed in hermetically sealed containers are not absolutely sterile and may contain injured and suppressed micro-organisms that could proliferate, if storage conditions and integrity of the container is compromised.

Due to the fact that processed tomato products are usually widely distributed, most produce related outbreaks can result in widespread epidemics across communities and among consumers

Thus, to prevent an outbreak of disease, the government should ensure that companies and factories producing canned tomatoes maintain microbiological standard and proper hygiene, wastes generated are properly disposed and the producers encourage packaging their tomatoes in sterile containers.

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APPENDIX

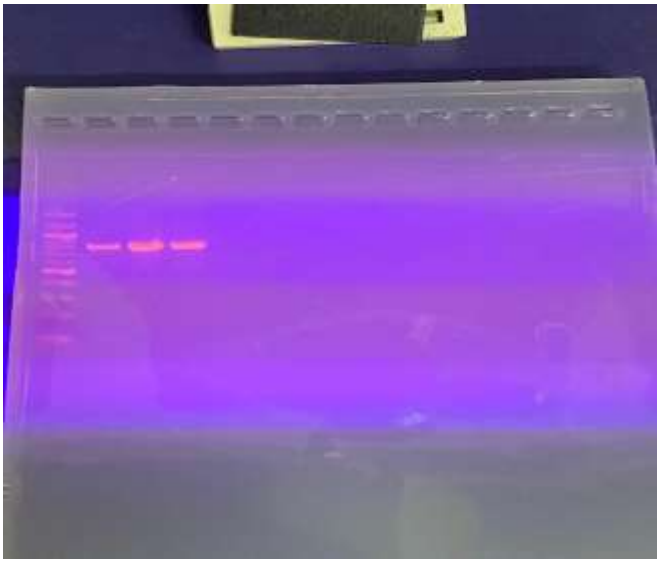

PLATE 1: Results from gel electrophoresis