



Isolation And Characterization Of Pathogenic Bacteria Associated With Rotten Tomatoes Sold At Tungan Goro Market, Minna. Niger State, Nigeria.

Erena N. B

Department of Integrated Science, School of Sciences, Niger State College of Education Minna. Niger State, Nigeria.

erenanuhu@gmail.com

Abstract: The identification of bacteria associated with rotten tomatoes from Tungan Goro Market, was conducted. Rotten tomatoes from Tungan-goro market were taken to the laboratory in a clean polythene bag. The tomatoes were subjected to microbiological test and Biochemical analysis were also conducted for isolation and characterization of bacterial strains. Five bacteria strains were isolated and characterized; *Bacillus subtilis*, *Bacillus anthracis*, *Bacillus cereus*, *Listeria monocytogenes* and *Pseudomonas aeruginosa*. Among the isolated organisms, four isolates, *Listeria monocytogenes*, *Bacillus cereus*, *Pseudomonas aeruginosa* and *Bacillus anthracis* are identified as pathogens. While *Bacillus subtilis* is the only non pathogenic organism isolated. The research recommended proper handling of harvested tomatoes fruits, proper screening of the harvested tomatoes fruits and provision of disease resistance varieties.

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Keywords: Rotten tomatoes, pathogenic bacteria, Tungan goro market.

Introduction:

Tomato (*Lycopersicon esculentum*). Is grown in many parts of Nigeria both as raining and dry season crop. Although most tomato production is at a small scale in backyards garden, though its production in the world in 2008 was about 130 million metric tone (Wokama, 2008, Pla *et al*; 2005). In northern Nigeria where dry season tomato is grown under furrow irrigation, foliar disease are less. Consequently, tomato crops grown in northern Nigeria have higher yields and better quality fruits.

Tomatoes are rich in 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 spoil 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). During most classes of spoilage, 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 (Trias *et al*; 2008).

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. Man hardly can do without consuming tomatoes whether as fruit or vegetable in his daily meal. Experts say tomatoes are the major dietary source of antioxidant, a substance that protects against cell damage. Tomatoes also contain lycopene, which helps reduce the risk of heart diseases and cancer (Spedro and Gullino, 2004).

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 (Awoh *et al*; 1983).

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 (Trias *et al*; 2008). The damaged tomatoes are

locally called 'Bage' by mostly Northern speaking languages, 'Esa' by the Yoruba-speaking people of Nigeria and the Eastern part of Nigeria that are predominantly Igbo speaking call it 'Omebigo' or 'Nmebi'. The rotten tomatoes are not thrown away but sold to consumers in large measures in order for the producer and seller to make maximum profit. The proliferation of microorganisms more especially in damaged tomatoes could be considered to be potentially harmful when such contaminated tomatoes are consumed in improperly cooked foods or eaten raw (Awoh *et al*; 1983).

According to Ghosh (2009), fungi were the source of spoilage of most of tomatoes samples accessed rather than bacteria. Among the fungi, it was found that *Aspergillus niger* and *Fusarium spp* were found in most of the spoiled samples with a few samples containing *penicillium spp*.

Methodology

Collection of Samples

Fresh and spoilt tomato samples were purchased from Tungan Goro market in Minna, Niger State of Nigeria. The samples were collected in separate sterile polythene bags and immediately transported to the laboratory for analysis.

Isolation of Bacteria

The media used for isolation of bacteria were nutrient agar, potato dextrose agar and plate count agar three grams of fresh healthy tomato and 3g of spoilt tomato samples were separately weight and differently transferred into sterile pestle containing 10ml of sterile distilled water and grinded with mortal to dislodge the microorganisms present in the tomato samples the mixture obtained water from the spoilt tomato sample was used to inoculation, inoculation was carried out at 37⁰c for 7 days for the PDA, while the other plates were incubated at 37⁰c for 24 hours, the plate were later examined for growth of bacterial strains which

were repeatedly sub-cultures to obtain pure cultures which were differently stored on agar slants in mac-canthney bottles.

Identification of Bacterial Isolates

After 24 hours the bacterial colonies showing different morphological characters were re-streaked several lines on pre-sterilized nutrient agar plate to obtain pure cultures of the isolates there after, the isolated bacterial strains from the tomato samples were subjected to different biochemical test for identification (Stilea and Holzpatel, 1997).

Gram Staining Reaction

The Gram-reaction was performed followed the procedure developed by thinly spread bacterial smear was prepared on a clean slide, dried in air and fixed by heating. The dried smear was flooded with crystal violet solution for one minute and washed in tap water for few seconds. It was again flooded with iodine solution for one minute and washed and blot-dried it was then decolorized with 95% ethyl alcohol by applying drop by drop until no more color flows from the smear and washed and blot dried finally slide were counter stained for about 10 second with safaranin red, washed and examined under microscope using oil immersion objective, isolate that appeared pink Gram-negative bacteria.

Biochemical Test

Biochemical screening were carried out to identify the bacterial strains, such as, catalase, oxidase, starch hydrolysis, Tween 80 Hydrolysis, coagulase, motility, sugar fermentation, methyl red, indole, urease, citrate utilization, mannitol salt ager tests etc.

Results

The spoiled tomatoes were analysed and five strains of bacteria isolates were identified. The bacteria isolate includes, *Bacillus subtilis*, *Bacillus cereus*, *Bacillus anthracis* *Listeria monocytogenes* and *Pseudomonas aeruginosa*.

No of Plate	Morphological Appearance	Cell Shade	Gram Reaction	Catalase	Oxidase	Coagulase	Citrate	Urease	Methyl Red	Motility	Spore Formation	Lactose	Maltose	Citucose	Sucrose	Manitol	Organism
1.	Translucent, milky, raised, irregular translucent	Rod	+	+	-	-	+	+	+	+	+	AG	AG	AG	Ah	Ah	<i>Bacillus subtilis</i>
2.	Milky, that irregular translucent	Rods	+	+	-	-	+	+	+	+	-	Ah	Ah	Ah	Ah	Ah	<i>Listeria monocytogenes</i>
3.	Rectangular with square end	Rod	+	+	-	-	-	-	+	+	+	00	Ah	Ah	Ah	Ah	<i>Bacillus cereus</i>
4.	Rectangular with square end	Rod	+	+	-	-	+	-	+	-	+	Ah	Ah	Ah	Ah	Ah	<i>Bacillus anthracis</i>
5.	Water soluble, green-blue pigment	Rod	-	+	+	-	+	-	+	+	-	-	00	Ah	Ah	Ah	<i>Pseudomonas aeruginosa</i>

Key: (+) positive reaction, 00 – No reaction, (-) Negative reaction, (AG) Acid gas reaction, (Ah) Acid without gas.

Discussion

The research was able to isolate and identify five bacteria, *Bacillus subtilis*, *Bacillus anthracis*, *Bacillus cereus*, *Listeria monocytogenes* and *Pseudomonas aeruginosa*. As been found by a research conducted by

Ibrahim *et al*; (2011) where Some *Bacillus* species which include *Bacillus magatarium*, *Bacillus morgani* and *Listeria monocytogenes* were also isolated and among the isolates *Listeria monocytogenes* was the

only pathogenic bacteria isolate from spoil tomatoes in Sokoto state.

Similar research was reported by Gosh (2009). He stated that, *Bacillus* species are the major micro organism responsible for the spoilage of tomatoes, which he claimed is due to the water content of the tomato fruits which support the growth of *Bacillus* species. This observation might be due to the ubiquitous nature of micro organism and the ability to produce the required extra cellular enzymes to solubilize the tomato fruit into simpler components and utilize them for growth and metabolic activities, thereby causing soft rot tomatoes disease.

The appearance of *Pseudomonas aeruginosa* among the bacteria that spoiled tomato is no longer a surprise because a related studies reveals the presence of *Pseudomonas aeruginosa* as among the bacteria identified with post harvest rotting of tomatoes caused by microorganism. The research shows that, the pathogen could be the initial cause of tomato rot. When inoculation was made on unrounded tomato fruits, which indicate that the microorganism fail to penetrate directly through the waxy skin of tomato. But however small rounds created during the post harvest handling enable them to infect fruit tissue. The organism is known to be an opportunistic pathogen that causes several acute and chronic infections in human, and it has become an important cause of nosocomial infections and its antibiotic resistance ability (Feiroz *et al.*, 2017).

In also another studies by Taylor (1987) shows the presence of *Listeria monocytogenes* and *Bacillus cereus* among others to be association with deterioration of tomato fruit, the organisms were isolated four days of deterioration process and name them among the most dangerous food poisoning microorganism isolated. Wiessinger, *et al.*, (2000) also reported that the isolation of *Bacillus cereus* from tomato fruits was an evidence of contamination from human activity. The organisms are known to cause some array of infections. *Bacillus cereus* is commonly associated with reheated or inadequately cooked foods. In addition to being a rare cause of several acute infections such as pneumonia and septicemia (Pitt, 2015). While *Listeria monocytogenes* invades macrophages and most tissue cells of infected host (Rosquette and Berche, 1996).

Bacillus anthracis cause s Anthrax disease of animals and can be transmitted to human because it form spores that can be aerosolized and spread with the intent to kill, and can be use as biological warfare and bioterrorism (Missiakas and Schneewind, 2005).

The research discovered that few organism isolated are food borne pathogens, that can cause food poisoning in humans when consume. It is also revealed that the isolated bacteria that causes spoilage

of tomato gain access into the tomato fruits during the process of cultivating, harvesting and packing and also from environmental contaminant which have been involved in food poisoning.

Recommendation

Farmer's should handle the harvested tomatoes fruit with cautions. They should use proper packaging of the fruit when transporting to market. They should screen those tomato that has skin injuries with healthy ones. The government should provide the farmer's which disease resistance varieties. The government should also give sensitization to farmers on proper way of handling tomato fruit.

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Corresponding Author:

Erena Nuhu Bako
Department of Integrated Science,
School of Sciences
Niger State College of Education
PMB 39 Minna Niger State Nigeria.

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