Available online www.jsaer.com

***Journal of Scientific and Engineering Research*, 2019, 6(6):153-158**

Research Article

ISSN: 2394-2630
CODEN(USA): JSERBR

Identification and Enumeration of Microorganisms Associated with Post-Harvest Spoilage of Tomatoes *Lycopersicum esculentum* Sold in Gboko metropolis

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Abstract This study investigated the micro-organisms associated with the spoilage of fruits of tomatoes, *Lycopersicum esculentum* obtained from four markets in Gboko metropolis of Benue State, Nigeria. A total of 80 tomato fruits were sampled, out of which 40 were fresh and healthy while the other 40 were spoilt tomatoes. The microorganisms were isolated, characterized and identified using standard methods. The bacteria isolated and identified from the spoiled tomatoes were: *Bacillus spp, Salmonella spp, Proteus spp, Erwinia spp, Escherichia coli, and Pseudomonas spp.* The most prevalent bacteria isolate from the samples was *Bacillus spp* with 34.8% which was found in all samples from the four markets. *Salmonella spp* was the least with 6.50%. The fungal isolates were *Penicillium spp*, *Mucor*, *Aspergillus spp*, *Fusarium spp*, and *Saccharomyces cerevisiae*. Whereas *Mucor* was the most prevalent with 56.0% and was found in samples from all the markets, *Fusarium spp* had the least prevalence of 4.0%. The mean microbial count ranges were: 2.5 x 10^5 - 6.2 x 10^5 cfu/g for Ortese market, 2.1 x 10^5 - 7.1 x 10^5cfu/g for Ankyenge market, 2.4 x 10^5 - 7.1 x 10^5 cfu/g for Ubaganji market and 2.1 x 10^5 - 6.2 x 10^5 cfu/g for Tar-ukpe market. The study showed that the spoiled tomatoes were contaminated while the fresh tomatoes were free from these contaminants. The presence of these micro-organisms associated with tomatoes spoilage pose a great danger to humans and animals since they produce spores and toxins that cause food poisoning.

Keywords tomatoes, microorganisms, bacteria, fungal, spoilage

Introduction

Tomato (*Lycopersicum esculentum*) is a berry plant in the *solanaceae* family. It is a short lived perennial plant grown as an annual plant, typically growing about 3-5m approximately in height. The fruit is edible, brightly red colored berry [1]. Tomato is one of the widely consumed fresh fruit worldwide since it contributes to a healthy well-balanced diet which is rich in vitamins such as vitamin A, B, C and E. Carbohydrates such as fructose and glucose; Minerals which include phosphorous, sodium, potassium, calcium, magnesium and trace elements like iron, copper, Zinc and Dietary fibers [2]. The Deep red coloration of ripened tomato is due to the presence of lycopene, a form of β-carotenoid pigment and a powerful antioxidant that help to protect against prostate cancer, cardiovascular disease and diabetes [3], thus there is an appeal and demand for the fruits by consumers as a result of their knowledge that they are healthy, tasty, convenient and fresh [4]. Tomato, however, have serious challenges to their existence these include; climatic condition, pest, bacterial and fungal attack and over the years, there has been an increase in the need to identify and isolate the microorganisms associated with their spoilage. Spoilage refers to any change in the condition of food in which the food becomes less palatable, or even toxic. These changes may be accompanied by alteration in taste, smell, appearance or texture [5]. Numerous microbial defects (Signs and Symptoms) of tomatoes are characterized by the type of micro-organism responsible for the deterioration, in the process of infection in the case of fungal invasion follows the

development of fungal penetrating structure. The colonization process involves the ability of the micro-organism to establish itself within the produce [6]. Susceptibility of tomato to microbial colonization is due to its differential chemical composition such as high level of sugar, low pH and its high water activity which favors the growth of micro-organism in tomato is recognized as a source of potential health hazard to man and animals. This is due to their production of toxins which are capable of causing disease like respiratory tract infection, meningitis, gastroenteritis, diarrhea in man following ingestion [7]. The contamination of tomato by micro-organism could be as a result of poor handling practices in the tomato production chain, storage condition, distribution, marketing practices and transportation [8]. Tomatoes were chosen for this study because they are referred to as ready-to-eat food since they are minimally processed and many people take tomatoes raw directly or via meals of salad usually served cold. Microbial spoilage and contaminating pathogens on this product poses a serious problem in food safety [9]. The centre of disease control and prevention (CDC) estimates that there are 76 million cases of food borne illness every year. Outbreaks with identified etiology are predominantly of microbial origins [10], so that studying about the microbial ecosystem of fresh raw and spoilt tomato is necessary. As consumers, we need to recognize that food safety is important for fresh fruits and vegetables. Also individuals of the population especially those in developing countries who usually use spoilt and slightly decaying tomatoes as a result of their cheaper prices should be educated that these spoilage are often not due to mechanical damages but microbial colonization and physiological decays [11], they should be made to know that these organisms produces toxins and spores which are relatively heat resistant and can cause severe food poisoning resulting in fatal outcome.

The aim of this work therefore is to isolate and enumerate the microorganisms responsible in tomato spoilage with a view of alerting the general public of the dangers of consumption of cheaper spoiled tomatoes in the study area.

Materials and Methods

Sample Collection

Collection of samples both fresh and spoilt tomatoes were carried out in 4 markets within Gboko metropolis, they include Ortese, Ankyenge, Ubangaji and Tar-ukpe markets. A total of 80 tomatoes were sampled, of which 10 samples of both fresh healthy intact tomatoes and spoilt ones were collected aseptically using hand gloves into sterile polythene bags from each of the markets and was taken to microbiology laboratory of the Department of Science Laboratory Technology, Fidei Polytechnic, Gboko, Benue state immediately for analysis.

Isolation of microorganisms

The fruit samples were ground using a sterile mortar and pestle. A homogenate of each sample was made by blending one gram in 9ml of sterile water and shaking them together. Serial dilutions of up to 10^4 of the homogenate were made in sterile test tubes. 1ml of the serially diluted tomato sample was pipetted into each serially marked petri dish. The total microbial count was carried out on the spoiled tomato fruit samples using the pour plate method. Nutrient agar and potato dextrose agar were used for bacteria and fungi respectively. The plates were subsequently incubated at 37 ºC for 24 hours for bacteria and 72 hours for fungi. At the end of incubation, developed colonies were counted and colonies forming units per unit gram of tomato fruit sample were calculated and recorded.

Characterization and Identification of Isolates

Discrete colonies that developed after incubation were sub-cultured to obtain pure cultures which were used subsequently for microscopic characterization and biochemical analyses. The distinct colonies that developed in the pure culture plates were observed for the morphological and cultural characteristics including the nature of margin, elevation, shape, color and transparency. The isolates were further characterized and identified following biochemical procedures as described by Holt [12]. These included catalase, coagulase, and sugar fermentation tests.

Results and Discussion

Fresh fruits have a natural protective barrier (skin) that acts effectively against most plant spoilage and pathogenic microorganisms. However, this protection may be eliminated and fruits may become contaminated during their growing in fields or during harvesting, post harvest handling and distribution [9].

The microorganisms present in samples of spoilt tomato fruits were identified based on their cultural, morphological and biochemical characteristics. The characterization and identification of the bacterial isolates are shown in Table 1.

The bacteria isolates were: *Bacillus spp, Salmonella spp, Proteus spp, Erwinia spp, Escherichia coli, and Pseudomonas spp.* The presence of *Escherichia coli*, and *Pseudomonas spp* in this study confirmed findings reported earlier by Jushi and Patel [13]. The occurrence of the bacteria isolates from tomato fruit samples obtained from the different markets is shown in Table 2.

From all the tomato fruit samples obtained from the four markets, *Bacillus spp* were the most prevalent with 34.8% followed by *Escherichia coli* 28.2% while *Pseudomonas spp* and *Erwinia spp* recorded 10.9%, *Proteus spp* 8.70%, *Salmonella spp* with the least prevalent 6.50% (Table 2).

The mean values of bacteria counts of tomato fruit samples from the four markets in Gboko, during the study period are presented in Table 3.

The result showed that tomato fruit samples from Ankyenge and Ubangaji markets recorded the highest bacteria counts of 7.1 x 10^5 cfu/g while the samples from Tar-ukpe market recorded the lowest mean bacteria count of 2.1 x 10^5 cfu/g. The bacteria counts recorded indicated a high level of contamination of the tomato fruit samples from Ankyenge and Ubangaji markets. The isolation of soil bacteria *Bacillus spp* from the fruit samples was an evidence of opportunistic contamination from human activity. Also, the presence of *Escherichia coli*, which are known to be associated with faecal matter, showed that the tomato fruit samples were contaminated through poor human handling processes. However the mean bacteria counts in the spoiled tomato fruit samples investigated were similar to the counts reported by Ghosh, [14].

The cultural and morphological characteristics of fungal isolates are shown in Table 4.

The colonization of fungi is a critical phase in the microbial spoilage of post harvested fruits. In this study, the fungal isolates from spoilt ripe tomato fruit samples were: *Penicillium spp, Mucor, Aspergillus spp, Fusarium spp*, and *Saccharomyces cerevisiae*. Similar findings were reported by Ghosh, [14] who also asserted that *Aspergillus niger, Fusarium spp* and *Penicillium spp* were the major microorganisms that are responsible for the spoilage of tomato fruits. Furthermore, the author maintained that fungi were the source of spoilage of most tomato fruit samples assessed rather than bacteria.

Akinmusire [15], reported that *Fusarium oxysporum, Rhizopus stolonifer* and *Mucor* were the fungi species responsible for the post harvest spoilage of tomato, *Lycopersicum esculentum*, fruits from three selected markets in Maiduguri, north eastern Nigeria. Al-Hindi [16], reported that the main tomato fruit spoilage caused by fungi was *Aspergillus phoenicis*. They concluded that fungal polygalacturonases and xylanases were the main enzymes responsible for the spoilage of tomato fruits. The occurrence of fungal isolates is shown in Table 5.

In this study, *Mucor* was the most prevalent fungal isolate with 56.0% while *Fusarium spp* was the least prevalent with 4.0% (Table 5). The finding in this study of *Mucor* and *Aspergillus spp* as the most prevalent tomato fruit spoilage agents is similar to an earlier report of Dennis and Davis [17]. The mean fungal count of the tomato fruit samples in this study is shown in Table 6.

Mucor had the highest mean fungal count of 7.1 x 10^5 cfu/g while *Fusarium spp* recorded the least count of 4.1 x 10^5 cfu/g (Table 6).

Susceptibility of tomato fruits could be largely due to differential chemical composition such as pH (near neutrality) and moisture content which are associated with their greater predisposition to fungal spoilage. The contamination of tomato fruits by fungi could also be as a result of poor handling, storage conditions, distribution, marketing practices and transportation. The occurrence of fungal spoilage of tomato fruits is a source of potential health hazard to man. This is due to their production of mycotoxins (naturally occurring toxic chemicals often of aromatic structure) compounds which are capable of inducing mycotoxicoses in man following ingestion. They however, differ in their degree and manner of toxicity.

Table 1: Characterization and identification of bacteria isolates from tomato fruit samples

Characteristics	Description of isolates					
Cultural						
Margin	Smooth	Smooth	Entire	Entire	Entire	Entire
Color	Creamy	White	White	Creamy	Pink	
Shape	Medium	Small and irregular	Large	Large	Small	Small
Morphological						
Cell type	Rod	Rod	Rod	Rod	Rod	Rod
Cell arrangement	Single	Single	Single	Single	Single	Chain
Gram reaction	-	+	-	-	-	-
Motility test	-	+	+	+	-	+
Sugar fermentation test						
Glucose	A	A	A	A	AG	AG
Lactose	-	-	-	-	+	-
Biochemical test						
Coagulase	-	-	-	-	-	-
Catalase	-	+	+	+	+	-
Oxidase	-	-	-	+	-	-
Probable microorganisms	*Salmonella spp.*	*Bacillus spp*	*Proteus spp*	*Pseudomonas spp*	*Escherichia coli*	*Erwinia spp*

Key: + = Positive, - = Negative, A = acid production only, AG = acid and gas production

Table 2: The occurrence of bacteria isolates in samples from all the markets

Bacteria isolates	Number of occurrence	% of occurrence
Salmonella spp	3	6.5
Bacillus spp	16	34.8
Proteus spp	4	8.7
Pseudomonas spp	5	10.9
Escherichia coli	13	28.2
Erwinia spp	5	10.9
Total	**46**	**100.00**

Table 3: The mean bacteria counts of tomato fruit samples from the different markets

Bacteria Isolates	Markets			
	Ortese (cfu/g)	**Ankyenge (cfu/g)**	**Ubangaji (cfu/g)**	**Tarukpe (cfu/g)**
Salmonella spp	2.5×10^5	2.2×10^5	3.2×10^5	5.3×10^5
Bacillus spp	5.4×10^5	4.1×10^5	5.0×10^5	6.2×10^5
Proteus spp	3.8×10^5	4.1×10^5	2.7×10^5	2.1×10^5
Pseudomonas spp	4.8×10^5	2.1×10^5	2.4×10^5	2.9×10^5
Escherichia coli	6.2×10^5	7.1×10^5	4.9×10^5	3.2×10^5
Erwinia spp	5.2×10^5	6.2×10^5	7.1×10^5	3.7×10^5

Table 4: Morphological and Cultural characteristics of Fungal Isolates

Fungal Isolates	Macroscopy	Microscopy
Aspergillus spp	Greenish, filamentous with profuse proliferation of black velvety spores.	Septate hyphae, branched condiophore with secondary branches. The condiophore is enlarged at the tip forming rounding vesiclelike chains.
Mucor	Grows quickly and cover agar surface with white fluff that later turns grey, reverse side is white.	Hyphae practically non-septate, sporangiophores are long, often branched and bear terminal spore filled sporangia.

Fusarium spp	Initially white and cottony but later develop pink centre with a lighter periphery.	Septate hyphae with canoe-shaped macroconidia, condiophores bear conidia singly or cluster.
Penicillium spp	The colonies of *Penicillium* sp. are rapid growing, flat, filamentous and velvety, woolly, or cottony in texture.	Chains of single-celled conidia are produced in basipetal succession from a specialized conidiogenous cell called a phialide.
Saccharomyces cerevisiae	Colonies of *Saccharomyces spp* grow rapidly. They are flat, smooth, moist glistening or dull, and cream to tannish cream in color.	Multilateral budding is typical Pseudohyphae, if present are rudimentary. Hyphae are absent. *Saccharomyces* sp. produces ascospores.

Table 5: The occurrence of fungal isolates from the different markets

Fungi genera	Number of Occurrence	% of Occurrence
Mucor	28	56.0
Aspergillus	9	18.0
Penicillium	7	14.0
Fusarium	2	4.0
Saccharomyces	4	8.0
Total	**50**	**100.00**

Table 6: The mean fungal counts of tomato fruit samples obtained from different markets

Fungal Isolates	Markets			
	Ortese (cfu/g)	Ankyenge (cfu/g)	Ubangaji (cfu/g)	Tarukpe (cfu/g)
Mucor	1.1×10^5	4.0×10^5	7.1×10^5	1.4×10^5
Aspergillus spp	5.0×10^5	7.0×10^5	6.0×10^5	2.0×10^5
Penicillium spp	4.2×10^5	5.1×10^5	4.3×10^5	4.7×10^5
Fusarium spp	5.0×10^5	5.0×10^5	4.1×10^5	7.0×10^5
Saccharomyces cerevisiae	6.1×10^5	5.1×10^5	4.3×10^5	4.4×10^5

Conclusion

Several genera of bacteria and fungi have been identified in this study as being associated with the spoilage of tomato fruits. Therefore concerted efforts should be made by the relevant health workers to discourage or stop the display and sale of spoilt tomato fruits in local markets. The general public should also be enlightened about the health risks that may be associated with the consumption of relatively cheaper but spoilt ripe tomato fruits, as these could be agents in food borne bacteria and fungal diseases

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