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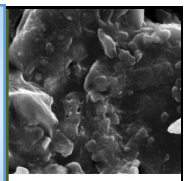

Enumeration of Most Common Microbes Responsible for Tomatoes Spoilage

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ABSTRACT

This study investigated the microorganisms associated with the spoilage of tomato *(Lycopersicum esculentum*) obtained from two markets in Jahun town, Jahun Local Government in Jigawa State, Nigeria. A total of five species of bacteria were isolated and identified viz: *Bacillus subtilis*, *B. aureus*, *Escherichia coli*, *Klebsiella aerogenes* and *Staphylococcus aureus*. The most prevalent bacterial isolate was *Bacillus subtilis* with 24% and was found in all samples from the two markets. *Klebsiella aerogenes* was the least prevalent isolate with 0.8% and was found in samples from Jahun Central Market only. The fungal isolates were *Penicilium notatum, Mucor mucido* and *Aspergillus niger*. Whereas, *Mucor mucido* was the most prevalent with 26.4% and was found in fruit samples from all the markets, *Penicillium notatum* had the least prevalence of 7.3% and occurred both in Jahun Central and Kabala Markets. The mean microbial count ranges were 10 to 40 × 10^4 CFU/g for Jahun Central Market and 5 to 30 × 10^4 CFU/g Kabala Market. The presence of toxin producing fungi *Aspergillus niger*, which are capable of causing food poisoning as well as some bacterial isolates raises concern over public health risks that may be associated with the consumption of spoiled tomato fruits.

INTRODUCTION

The current increase in world population has posed lot of problems spanning form food shortage and economic burdens on global scale. These issues have warranted several nations to embark on economic diversification, among which agriculture is included[1]. In particular, Nigeria has itemized agricultural business to be one of the frontiers for economic diversification. Along this line, one of the encouraged national agribusiness policies is that of tomato farming. Economically, tomato tops the list in value among edible vegetables in Nigeria[2].Tomato *(Lycopersicon esculentum)* been one of the world's most important vegetable crops with a current world wide fresh weight production of 80 million tons from a cropped area of about 3 million hectare[3]. It has its origin in Western South America and Central America. China is the largest producer followed by United States and Turkey[3]. The fruit is one of the most important vegetable in most regions of the world and constitute an important source of food as well as cash in Nigeria [4,5]. The fruit contributes to a healthy, well balanced diet. It is rich in vitamins, minerals, essential amino acids, sugars, and dietary fibers. Yellow varieties have high vitamin A content than red ones, but red tomato fruits contain lycopene, an anti-oxidant that may contribute to protection against carcinogenic substances [6].Although production figures are not available, production is seasonal resulting in a glut during the seasons and scarcity at off seasons. Tomato also does well with soil pH of between 5 and 7. Generally, harvested ripe fruits at room temperature (28±1˚C) can store for 5 days.

Despite the fact that some tomato fruits referred to as iron tomatoes that can stay for a maximum of 10 days if kept in a favorable environment [7], unfortunately, large percentage of tomato fruits produced in Nigeria are being lost to post-harvest deteriorations caused by microorganisms[4]. Among the microbes infecting tomato fruits, fungal plant pathogen can cause extensive loss of the fruits [8]. Bacterial contamination is more dangerous, this is because the presence of highly dangerous toxin and bacteria spores is often not detected until after an outbreak of food poisoning and when laboratory examination and

experiments uncovers the infective agent [9]. The biological structure of tomato when disrupted (during harvesting, transportation and storage or by insect) can serve as a route of entry for opportunistic pathogen [10]. Due to their soft textures the fruits, are easily bruised through harvesting and other postharvest handling operations such as packaging, transportation and storage[5]. Petriacq et al., [11] states that due to poor storage conditions, resistance of fruits and vegetables to natural disease usually decline, leading to infection by pathogens. For most microbial deteriorations to occur successfully, it usually requires cool weather temperatures with high relative humidity. The disease incidence increases with heavy rainfall [3].

The use of heat treatment at 380°C for 24 hours together with the application of *Pichia guilliermondii* is one of the most effective techniques in controlling post-harvest fungal spoilage in tomato fruit. The heat treatment inhibited hyphae growth and spore germination of *Rhizopus stolonifer, while P.guilliermondii* cells used on tomato after heat treatment multiplied rapidly in fruit wounds and had a strong capability of adhesion to the hyphae of *R.stolonifer* (Yan *et al*., 2010). The aim of this work therefore, is to outline the most common microbes responsible for tomatoes spoilage in two major markets in Jahun and Kabala markets.

MATERIALS AND METHODS

Description of the study area

The study was carried out in two markets in Jahun town viz Jahun Central Market and Kabala Market of Jahun local government Jigawa state. Jahun is located at 61km from the state capital Dutse. It has an area 117km2 and population of 229094 at 2016 census. The inhabitants of the area predominantly farmers, fishing, civil service (NIPOST, 2009). The study site lies on GPS coordinates: 12.077183 N 12°4'37.85736"(Latitude) and 9.619854 E 9°37'11.47332" (Longitude). The major tribe in the study area is the Hausa and Fulani while the major religion is Islam.

Media preparation

Nutrients Agar (NA), Potatoes Dextrose Agar (PDA) was prepared according to the manufacturer's instruction.

Collection of sample

A total of twenty spoiled tomatoes sample were randomly collected from Jahun Central and Kabala Markets (10 from each) and taken under aseptic condition (placed in sterilized polythene bag) to the microbiology laboratory in Jigawa State polytechnic Dutse for analysis.

Isolation of Microorganisms

Aliquot (1g) of each spoiled tomatoes samples was aseptically measured. A homogenous of each sample was made by blended one gram in 9ml of sterile water and shaking them together. Serial dilutions of up to $10^4$of the homogenate was made in sterile test tubes. 1ml of the serially diluted tomato sample was pipetted into each serially marked petri dish. The tomato fruit samples were culture 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°C for 24 hours for bacteria and 72 hours for fungi. At the end of incubation, Colonies that developed after incubation were counted, enumerated in colony forming unit per gram (Cfu/g) samples.

Characterization and Identification of Isolates

Discrete colonies that developed after incubation, was sub cultured to obtain pure cultures which were stored at 4°C and used subsequently for microscopic characterization and biochemical analysis. The distinct colonies that developed in the pure culture plates was observed for the morphological and cultural characteristics including the nature of margin, elevation, shape, color and transparency.

Gram straining

Gram staining was carried out according to standard protocol. The stained bacterial slides were allowed to air dries and then examined with oil immersion objective [12]. The isolates were further characterized and identified following biochemical procedures as described by [13]. These included catalase, coagulase and indole.

Indole test

The test organism was inoculated into a bijou bottle containing 3ml of sterile triphone water.
It incubated at 35-37°C for 48hours.
0.5ml of Kovac reagent was added to tested Indole by shaken gently and examined for red colour in the surface layer within 10seconds.

Catalase test

About 5ml of 3% Hydrogen peroxide solution was poured into a sterile test tubes
Sterile glass rod was used to convey several colonies of the samples and immersed into hydrogen peroxide solution.
Immediate bubbling was observed.

Coagulase test

Coagulase is an enzyme produced by *S. aureus* that convert soluble fibrinogen in plasma to insoluble fibrin. Coagulase test is used to differentiate *S.aureus* which is coagulase positive from Coagulase negative *Staphylococcus. S. aureus* produces two forms of Coagulase bound and free. The Slide Coagulase test is driven to detect bound Coagulase or clumping factor. On the other hand, tube Coagulase test is done to detect free Coagulase.

Slide Coagulase test procedures

The slide coagulase test was observed according to [12]. Briefly:
Drop of distilled water was placed on each end of the slide.
A colony of the test organism was emulsified in each of the drops to make two thick suspensions. A loopful of blood plasma was added to one of the suspension to differentiate any granular appearance of the organism from true Coagulase clumping.

RESULTS AND DISCUSSION

The results for morphological and biochemical enumerations for various sample locations and types of microorganism are presented in **Tables 1** to **4**.

Table 1. Characterization and identification of bacterial isolates from tomato fruit samples.

Cultural characterization					
Margin	Smooth	Smooth	Entire	Smooth	Entire
Colour	White	White	Pink	Yellow	White
Shape	Small and irregular	Small	Small	Medium	Large
Morphology					
Cell type	Rod	Rod	Rod	Cocci	Rod
Cell arrangement	Single	Single	Single	Cluster	Single
Gram staining	+	+	-	+	-
Coagulase	-	-	-	+	-
Catalase	+	+	+	+	+
Indole	-	-	-	-	-
Probable microorganism	*Bacillus subtilis*	*B. Auerus*	*E. Coli*	*S. Aureus*	*Klebsiella aurogenes*

Key: + = Positive
- = Negative

Table 2: The mean bacterial counts of tomato fruit samples from Jahun central market and kabala market.

Bacterial Isolates	Jahun central market CFU/g 10^4	Kabala market CFU/g 10^4
Bacillus subtilis	$40x10^4$	$13x10^4$
B. aureu	Nil	$5x10^4$
Escherichia coli	$10x10^4$	$30x10^4$
Staphylococcus aureus	$20x10^4$	$7x10^4$
Klebsiella aerogenes	$30x10^4$	Nil

Table 3. Morphological and cultural characteristics of fungal isolate.

Fungal Isolates	Macroscopy	Microscopy
Aspergillus niger	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 vesicle-like chains.
Mucor mucido	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.
Penicillium notatum	The colonies of *Penicillium notatum*. are rapid growing, flat, filamentous and velvety, Woolly, or cottony in texture.	Chains of single-celled conidia (ameroconidia) are produced in basipetal succession from a specialized conidiogenous Cell called a phialide.

Table 4: The mean fungal counts of tomato fruit samples obtained from Jahun central and kabala Markets.

Fungal Isolates	Markets CFU/g 10^4	
	Jahun Market 10^4	Kabala Market 10^4
Mucor mucido	22 x 10^4	50 x 10^4
Aspergillus niger	26 x 10^4	10 x 10^4
Penicillium notatum	14 x 10^4	8 x 10^4

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 [14]. The microorganisms present in samples of spoil 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 total five species of bacteria were isolated and identified viz: *Bacillus subtilis, B.aureus, Escherichia coli, Klebsiella aerogenes and Staphylococcus aureus.* The two species of *Bacillus* identified in this study differed from those reported by[15] who found, *Bacillus coagulans and B. Stearo thermophilus* from spoiled ripe tomato fruits. Besides, [16] isolated *Bacillus megaterium* and *B. laterosporus* from tomato fruit samples. However, the presence of *Escherichia coli*, *Staphylococcus aureus* in this study not confirmed findings reported earlier by [17].

The mean values of bacterial counts of fruit samples from the two markets in Jahun town, during the study period are presented in **Table 2**. The result showed that tomato fruit samples from Jahun Central Market recorded the highest bacterial count of 40 x 10^4 while the sample from kabala Market recorded the lowest mean bacterial count 5 x 10^4 CFU/mL. The bacterial count recorded indicated a high level of contamination of the tomato fruit samples from Jahun Central Market. The isolation of soil bacteria *Bacillus substilis,* from the fruit samples, was an evidence of opportunistic contamination from human activity. Also,the presence of *Staphylococcus aureus*, which are known to be associated with faecal matter, showed that the fruit samples were contaminated through poor human handling processes. However the mean bacterial counts in the spoiled tomato fruit samples investigated were similar to the counts reported by [18].

The cultural and morphological characteristics of fungal isolates are shown in **Table 3**. The colonization of fungi is a critical phase in the microbial spoilage of post harvested fruits. In this study, the fungal isolates from spoiled tomato fruit samples were: *Mucor mucido, Aspergillus niger, and Penicillium notatum*. Similar findings were reported by [18] who also asserted that *Aspergillus niger, Fusarium* sp. *and Penicillium* sp. 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. [19] Reported that *Fusarium oxysporum, Rhizopus stolonifer and Mucor sp.* were the fungi species responsible for the spoilage of tomato, fruits from three selected markets in Maiduguri, north eastern Nigeria. Samuel et al., [20] reported that the main tomato fruit spoilage fungi was *Aspergillus phoenicis.* They concluded that fungal *polygalacturonases* and *xylanases* were the main enzymes responsible for the spoilage of tomato fruits.

In this study, *Mucor mucido* was the most prevalent fungal isolate with 26.4% while *Penicillium notatum* was the least prevalent with 7.3%.The finding in this study of *Mucor* sp. and *Aspergillus* sp. as the most prevalent tomato fruit spoilage fungi is similar to an earlier report by [21].

The mean fungal counts of the tomato fruit samples are shown in **Table 4**. *Mucor mucido* had the highest mean fungal count of 50 x 10^4 while *Penicillium notatum* recorded the least count of 8.0 x 10^4. 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.

CONCLUSIONS

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 bacterial and fungal diseases.

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