Assessment of Bacteria and Fungi Responsible for the Spoilage of Tomato Sold in Gada-Biu Market, Jos, Plateau State

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Abstract
This study assessed bacteria and fungi associated with spoilage of tomato fruit (Lycopersium esculentum). Tomatoes contain a large amount of water, which makes them more susceptible to spoilage by bacteria and fungi. This study was therefore carried out to isolate, characterize and identify bacteria and fungi associated with the spoilage of tomato fruit. A total of 20 tomatoes were obtained from 5 different retail stands in Gada-biu market in Jos. Bacteria isolated and identified were Bacillus subtilis, Klebsiella aerogenes, Aeromonas hydrophila, Pseudomonas aeruginosa, and Staphylococcus aureus. The most prevalent bacterial isolate was Bacillus subtilis with 41.93%. The fungal isolates were Aspergillus niger, Aspergillus flavus, Penicillum notatum, Fusarium oxysporum, and Saccharomyces cerevisiae. Aspergillus niger was the most prevalent with 37.12%, while Saccharomyces cerevisiae was the least prevalent with 4.55%. The presence of toxin-producing fungi such as Aspergillus niger and Fusarium oxysporum, which are capable of causing food poisoning as well as some bacteria isolates, raises concern over public health risks that may be associated with the consumption of wholesome tomatoes. Proper handling and adequate storage facilities must therefore be employed to prolong the shelf life of tomato fruits.

Keywords: *bacteria, fungi, spoilage, tomato fruit.*

Introduction

Lycopersicum esculentum, or tomato, is an annual plant with a weak woody stem covered in glistering reddish yellow glandular hairs. The tomato plant is widely cultivated in many parts of the world. The tomato fruit has a smooth skin. It is green when immature but becomes bright red or yellow as it ripens. The fruit varies greatly in size and shape (Agrios, 2005).

Tomato fruit is a common vegetable eaten raw as a salad or for garnishing various cooked foods in Nigeria as well as in many parts of the world. The fruit contains a high concentration of carbohydrates, fats, organic acid, water, minerals, vitamins, and pigments. It is estimated that ripe tomato fruits contain approximately 94% water, 4.3% carbohydrate, 1% protein, 1.0% fat, 0.6 fibre and vitamins. The nutrients support the growth of microorganisms, such as bacteria and fungi, which produce enzymes that degrade the nutrients (Sharma, 2016).

Tomato fruits contain a lot of water, which makes them more susceptible to spoilage by microorganisms. Also, the high water content makes storage and transportation of this vegetable difficult. These microorganisms reduce not only the nutritional value but also the market value of tomato fruits (Obunukwu et al., 2018).

In recent years, the incidence of diseases in tomato fruits has been a cause for global concern and intensive research has been undertaken to comprehend the measures which can be taken to effect some radical control (Beuchat, 2011). The parameters during quality control include various factors such as time of harvesting, temperature and moisture during storage, selection of agricultural products prior to processing, decontamination conditions, addition of chemicals, and final product storage (Bello et al., 2016). The work is aimed at assessing bacteria and fungi associated with the spoilage of tomato fruits sold in Gada-biu market, Jos, Plateau State.

Study Area

Four (4) samples each of spoilt tomatoes were purchased from five (5) different retail stands (20 samples in total), from Gada-biu market in Jos, Plateau State. The tomatoes were then transported separately in sterile polythene bags and brought to the laboratory.

Isolation of Microorganisms

Nutrient and Potato Dextrose Agar were prepared according to the manufacturer's instructions and then were used for the isolation of bacteria and fungi respectively.

The diseased portion of the tomato fruit was cut under aseptic conditions into small bits and placed in a sterile dish with the aid of a knife, which was then flamed over a Bunsen burner. A serial dilution of up to 10^{-4} of the homogenate was made in sterile test-tubes, 1ml of the serially diluted tomato sample was pipetted into 9ml of sterile distilled water in a test-tube. The test tube was shaken vigorously to homogenize. A 1ml portion of the third and fourth factors were aseptically transferred and plated in duplicate sets using sterile nutrient agar for bacterial isolates and potato dextrose agar for fungal isolates. The total microbial count was carried out on the spoilt tomato fruit samples using pour plate method. The plates were subsequently incubated at 37^{o}C for 24 hours for bacteria and 72hours for fungi. Discrete colonies that developed after incubation were counted and enumerated as colony forming units (CFU/g) after multiplying with the dilution factor (Mbajiuka & Emmanuel, 2014). Colonies from the primary plates were aseptically picked with sterile wire loop and transferred onto a freshly prepared sterile nutrient agar and potatoes dextrose agar, with a streaking technique such that discrete colonies appear at the ends of streaked lines after incubation. The sub-cultured plates were incubated as described by Mbajiuka and Emmanuel (2014).

Characterization and Identification of Fungal Isolate

Identification and characterization of the fungal isolates was based on macroscopic and microscopic examination. It was done by comparing the result of their morphological characteristics with existing stock cultures and references were also made to fungi atlas. Identification of fungi was based on growth, patterns, color of mycelia and microscopic examination of vegetative and reproductive structures (Obunukwu *et al*., 2018).

Characterization and Identification of Bacterial Isolates

The characterization and identification of bacterial isolates was based on gram's staining and some selected biochemical tests which include; Catalase test, Oxidase test, Methylred (MR) test, Citrate test, Coagulase test, Motility test and Indole test as described by Cheesbrough (2007).

Results and Discussion

Results

Table 1: Microbial Load Of Spoilt Fresh Tomatoes Stored At Ambient Temperature (Cfu/G)

SAMPLES	TOTALBACTERIAL COUNT	TOTALFUNGAL COUNT
S1	$7.5X10^2$	$12x10^2$
S2	$10.8x10^2$	$8.3x10^2$
S3	$8.7x10^2$	$12.0x10^2$
S4	$11.3x10^2$	$8.4x10^2$
S5	$11.6x10^2$	$8.6x10^2$

Key
1-S 5, Sample 1-Sample 5.

Table 2: Morphological and Biochemical Characterization of Bacteria Isolates

Sample	Shape	Elevation	Surface	Opacity	Color	Texture	Gram-staining	Catalase Test	Oxidase Test	Methyl-Red	Citrate Test	Coagulase Test	Motility Test	Indole Test	Probable
S1	Rd	Um	Sth	Trans	White	Mucoid	-rod	+	-	-	+	-	-	-	*Klebsiella aerogenes*
	Ir	Um	Rgh	Opq	White	Dry	+rod	+	-	-	-	-	+	-	*Bacillus subtilis*
	Rd	Ra	Sth	Opq	D/gn	Mucoid	+rod	+	+	+	+	+	+	+	*Aeromonas hydrophila*
S2	Rd	Ra	Sth	Opq	D/gn	Mucoid	-rod	+	+	+	+	+	+	+	*Aeromonas hydrophila*
	Rd	Um	Sth	Trans	White	Mucoid	-rod	+	-	-	+	-	-	-	*Bacillus subtilis*
	Ir	Um	Rgh	Opq	White	Dry	+rod	+	+	-	-	-	+	-	
S3	Rd	Ra	Sth	Opq	D/gn	Mucoid	-rod	+	+	+	+	+	+	+	*Aeromonas hydrophila*
	Ir	Um	Rgh	Opq	White	Dry	+rod	+	+	-	-	-	+	-	*Bacillus subtilis*
	Oval	Um	Sth	Trans	Green	Mucoid	-rod	+	+	-	+	-	+	-	*Pseudomonas aeruginosa*
	Rd	Um		Opq	White	Mucoid	+cocci	+	-	+	-	+	-	-	*Staphylococcus aureus*
S4	Rd	Ra	Sth	Opq	D/gn	Mucoid	-rod	+	+	+	+	+	+	+	*Aeromonas hydrophila*
	Ir	Um	Rgh	Opq	White	Dry	+rod	+	+	-	-	-	+	-	*Bacillus subtilis*
S5	Ir	Um	Rgh	Opq	White	Dry	+rod	+	+	-	-	-	+	-	*Bacillus subtilis*
	Oval	Um	Sth	Trans	Green	Mucoid	-rod	+	+	-	+	-	+	-	*Pseudomonas aeruginosa*
	Rd	Ra	Sth	Opq	D/gn	Mucoid	-rod	+	+	+	+	+	+	+	*Aeromonas hydrophila*

Key: Rd – Round, Ir-Irregular, Um-Umbonate, Ra-Raised, Sth-Smooth, Rgh-Rough, Opq-Opaque, Trans-Translucent, D/gn-Dark-green

Table 3: Bacterial Isolates from Tomatoes Sample

SAMPLES	BACTERIAL ISOLATES
SAMPLE 1	*Klebsiella aerogenes, Bacillus subtilis, Aeromonas hydrophila*
SAMPLE 2	*Aeromonas hydrophila, Klebsiella aerogenes, Bacillus subtilis*
SAMPLE 3	*Aeromonas hydrophila, Bacillus subtilis, Pseudomas aeruginosa, Staphylococcus*
SAMPLE 4	*Aeromonas hydrophilia, Bacillus subtilis*
SAMPLE 5	*Bacillus subtilis, Pseudomonus aeruginosa, Aeromonas hydrophila*

Table 4: Macroscopic and Microscopic Identification of Fungal Isolates

Samples	Macroscopic Examination	Microscopic Examination	Probable Organism
Sample 1	Blackish colony, with blackish spores, rapidly spreading, cream color on reverse.	Septate branching hyphae, with conidiophores that are non septate.	*Aspergillus niger*
	Rapid colonies with a dull green and white margin, colorless to white on reverse	Conidiophore is simple and smooth with a septate hyphae. Conidia is borne in loose columns.	*Penicillium notatum*
Sample 2	Blackish colony, with blackish spores, rapidly spreading, cream color on reverse.	Septate branching hyphae, with conidiophores that are non-septate.	*Aspergillus niger*
	A clear green color with a smoother velvety appearance, cream color on reverse	Conidiophores are present with a short columnar conidial heads. Mycelium is slightly visible.	*Aspergillus flavus*
Sample 3	Rapid colonies with a dull green and white margin, colorless to white on reverse.	Conidiophore is simple and smooth with a septate hyphae. Conidia is borne in loose columns.	*Penicillium notatum*
	Blackish colony, with blackish spores, rapidly spreading, cream color on reverse	Septate branching hyphae, with conidiophore that are non-septate.	*Aspergillus niger*
Sample 4	Initially white and cottony but later develop pink center with a lighter periphery.	Septate hyphae with canoe-shaped macroconidia,	*Fusarium oxysporum*
	Rapid colonies with a dull green and white margin, colorless to white on reverse.	Conidiophore is simple and smooth with a septate hyphae. Conidia is borne in loose columns.	*Penicillium notatum*

Sample 5	A clear green color with a smoother velvety appearance, cream color on reverse.	Conidiophores are present with a short columnar conidial heads. Mycelium is slightly visible.	*Aspergillus flavus*
	Blackish colony, with blackish spores, rapidly spreading, cream color on reverse.	Septate branching hyphae, with conidiophores that are non-septate.	*Aspergillus niger*
	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 present, *Saccharomyces spp.* Produces ascospores.	*Saccharomyces Cerevisiae*

Table 5: Fungal Isolates from Tomatoes Samples

Samples	**Fungal Isolates**
Sample 1	*Aspergillus niger, Penicillium notatum*
Sample 2	*Aspergillus niger, Aspergillus flavus*
Sample 3	*Penicillium notatum, Aspergillus niger*
Sample 4	*Fusarium oxysporum, Penicillium notatum*
Sample 5	*Aspergillus flavus, Aspergillus niger, Saccharomyces cerevisiae*

Table 6: Frequency of Occurrence of Bacteria Isolates Associated with Tomatoes Samples from Gada-Biu Market

Bacteria Isolates	**Sample 1**	**Sample 2**	**Sample 3**	**Sample 4**	**Sample 5**	**Frequency**	**Percentage**
Klebsiella aerogenes	10	20	-	-	-	30	24.19
Bacillus subtilis	25	3	4	8	12	52	41.93
Aeromonas hydrophila	5	5	4	10	6	30	24.19
Pseudomonas aeruginosa	-	-	5	-	2	7	5.64
Staphylococcus aureus	-	-	5	-	-	5	4.03
Total	**40**	**28**	**18**	**18**	**20**	**124**	**100**

Table 7: Frequency of Occurrence of Fungi Isolates Associated with Tomatoes Samples from Gada-Biu Market

Fungi Isolates	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Frequency	Percentage
Aspergillus flavus	2	20	-	-	11	33	25
Aspergillus niger	15	10	15	-	9	49	37.12
Penicillum notatum	8	-	7	9	-	24	18.18
Fusarium oxysporum	-	-	-	20	-	20	15.15
Saccharomyces cerevisiae	-	-	-	-	6	6	4.55
Total	**25**	**30**	**22**	**29**	**26**	**132**	**100**

Discussion

The results on the assessment of bacteria and fungi responsible for the spoilage of tomatoes are described as follows. The microbial load of spoilt fresh tomatoes stored at ambient temperature is shown in Table 1. The result shows that tomato fruit samples from sample 5 show the highest bacteria count of $11.6x10^2$ and sample 1 shows the lowest bacteria count of $7.5x10^2$ cfu/ml, while the samples from sample 1 recorded the highest fungi count of $12.5x10^2$ cfu/ml and sample 2 recoded the lowest fungi count of $8.3x10^2$ cfu/ml. The result agreed with the work of Akinmusire (2011), who reported and concluded that fungus may be the major organism responsible for the spoilage of tomato fruits due to poor sanitation, overcrowding, poor storage, and unhygienic practises by the fruit handlers.

Table 2 shows the morphological and biochemical characterization of bacteria isolates.

The bacteria isolates identified from fresh spoilt tomatoes were: *Bacillus subtilis, Klebsiella aerogenes, Pseudomonas aeruginosa, Aeromonas hydrophila,* and *Staphylococcus aureu*s. These isolated organisms agree with the work of Wogu and Ofuase (2014). The presence of these organisms in spoilt tomatoes is an indication that tomato fruits were exposed to faecal contaminated water or organic manure.

Table 3 shows the bacterial isolate of each sample.

The results on the macroscopic and microscopic identification of fungi isolates are shown in Table 4. The fungi isolated were *Aspergillus niger, Pencillium notatum, Aspergillus flavus, Fusarium oxysporum,* and *Saccharomyces cerevisiae*. Table 5 shows the fungal isolates of each tomato sample.

The frequency of occurrence of bacteria isolates associated with tomato samples is present in Table 6, which indicates that from all the tomato fruit samples obtained, 124 bacteria were isolated, of which *Bacillus subtillis* was the most prevalent with 41.93%, followed by *Klebsiella aerogenes* (24.19%), *Aeromonas hydrophila* (24.19), Pseudomonas aeruginosa (5.64%) and *Staphylococcus aureus* (4.03%). The isolation of *Bacillus subtilis* from the fruit samples suggests evidence of opportunistic contamination from human activities.

In Table 7, shows the frequency of occurrence of fungi isolates associated with tomato samples; in the characterization of fungi, a total of 132 organisms were isolated, of which *Aspergillus niger* had the highest of 37.12%, *Aspergillus flavus* (25%), *Pencillium notatum* (18.18%), *Fusarium oxysporum* (15.15%), and Saccharomyces cerevisiae (4.55%). *Aspergillus niger* had the highest percentage occurrence of 37.12% in the spoilt tomato fruits examined, while *Saccharomyces cerevisiae* had the lowest percentage occurrence of 4.55% in the fruit examined.

The result is similar to the work of Akinmusire (2011) but with variation in the frequency of occurrence. They reported that *Aspergillus niger* had the highest rate of occurrence of 47.27% in the tomato fruits, while in this present study, *Aspergillus niger* had the highest rate of occurrence of 37.12%. This may be due to the variation in the market, poor practises or unhygienic practises by the fruit handlers. Wogu and Ofuase (2014) isolated *Aspergillus niger* (47.27%), *Aspergillus flavus* (30.1%), *Penicillium notatum* (15.3%), *Fusarium oxysporum* (12.73%) and *Saccharomyces cerevisiae* (3.64%),) from spoilt tomato fruits, which is also similar to the organisms isolated from these present study, whereas, there is variation in the frequency of occurrence. These isolated fungi could probably be due to fungal spores usually found in the environment, their spores can be carried on air, and thus can infect exposed tomato fruits, as well as farm tools. The implication of microbial contamination and growth on tomato produce causes spoilage, decreased sensory appeal and decreased shelf life, leading to loss and wastage of product that have significant economic consequences. The prevalence frequency of occurrence of fungi was higher than that of bacteria as shown in table 6 and 7, which demands the appropriate control measures against infection should be employed. Adequate microbiological knowledge and handling practices of these produce would help minimise wastes due to deterioration.

It is therefore, important that both the farmers who harvest the fruits into bags for transportation, the marketers, and consumers take necessary precautions in prevention contamination and eating contaminated fruits. This will however, enhance reduction in the risk of microbial toxins that are deleterious to human health which are produced from these microorganism that have been isolated.

Conclusion and recommendation

Conclusion

This study showed that bacteria and fungi are associated with spoilage of' tomato in Gada-biu market. . The result showed that tomato fruit samples from sample 5 had the highest bacteria count of $11.6x10^2$ and sample 1 showed the lowest bacteria count of $7.5x10^2$ cfu/ml, while the samples from sample 1 recorded the highest fungi count of $12.5x10^2$ cfu/ml and sample 2 recoded the lowest fungi count of $8.3x10^2$ cfu/ml.

It was also revealed that mechanical injuries such as bruises or cuts that occur during harvesting, post-harvesting and packaging could provide infections sites for spoilage pathogens. These infections sites can therefore be greatly reduced and brought to a minimal by proper storage and handling of the vegetable. The major organisms associated with the spoilage of tomato fruits may be due to poor sanitation, poor packaging and storage, and unhygienic practises by the fruit handlers.

Recommendation

1. Tomatoes must be thoroughly washed with clean water and properly cooked before consumption.
2. Proper cleaning and sanitation of ware houses and disinfection of packaging containers.
3. Proper handling of the vegetable during harvest should be done to prevent bruises and scars or other mechanical injuries.
4. The environment in which the tomatoes are sold should also be kept clean since most of the bacteria isolated are associated with dirty environment.
5. Tomatoes farmers as well as other vegetable farmers should be advised to avoid the use of contaminated waste water for irrigation during cultivation as well as, using organic manure that contains animal or human fertilizer which are potential habitants for *Klebsiella aerogenes* and *Pseudomonas aeruginosa.*

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