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Research Article

Microorganisms responsible for the spoilage of tomato fruits, *Lycopersicum esculentum*, sold in markets in Benin City, southern Nigeria.

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Abstract: This study investigated the microorganisms associated with the spoilage of fresh fruits of tomato, *Lycopersicum esculentum* obtained from four markets in Benin City, southern Nigeria. A total of nine species of bacteria isolated and identified were: *Bacillus subtilis*, *B. cereus*, *B. aureus*, *Escherichia coli*, *Klebsiella aerogenes*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Proteus mirabilis* and *Staphylococcus aureus*. The most prevalent bacterial isolate was *Bacillus subtilis* with 49.2% and was found in all samples from the four markets. *Proteus mirabilis* was the least prevalent isolate with 13.1% and was found in samples from Vegetable market only. The fungal isolates were *Penicilium* sp., *Mucor* sp., *Aspergillus niger*, *Fusarium* sp. and *Saccharomyces cerevisae*. Whereas *Mucor* sp. was the most prevalent with 57.7% and was found in fruit samples from all the markets, *Saccharomyces cerevisiae* had the least prevalence of 9.1% and occurred only in Vegetable and Santana markets. The mean microbial count ranges were: 2.0 x 10^4 – 35.0 x 10^4 for New Benin market; 1.0 x 10^4 – 25 x 10^4 for Vegetable market; 2.0 x 10^4 – 23.0 x 10^4 for Oba market and 1.1 x 10^4 – 9.3 x 10^4 for Santana market. The antibiotic susceptibility profile of bacterial isolates obtained from spoilt tomato fruit samples was determined using the disc-diffusion method. *Bacillus subtilis* was the most sensitive to all the antibiotics used while *Pseudomonas aeroginosa* and *Salmonella typhi* showed the highest resistance. The presence of toxin producing fungi *Aspergillus niger*, which are capable of causing food poisoning as well as some bacterial isolates with multiple antibiotics resistance, raises concern over public health risks that may be associated with the consumption of spoilt tomato fruits.
Keywords: microorganisms, prevalence, spoilage, tomato fruits, antibiotics, susceptibility, resistance

INTRODUCTION

Tomato, *Lycopersicum esculentum*, is an annual plant, having a weak woody stem covered with 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.

Tomato fruit is a common vegetable eaten raw as salad or for garnishing various cooked food in Nigeria as well as in many parts of the world. The fruit contains high amount of carbohydrates, fats, organic acids, water, minerals, vitamins and pigments. It is estimated that ripe tomato fruits contain approximately 94% water, 4.3% carbohydrates, 1% protein, 0.1% fat, 0.6% fibre and vitamins. The nutrients support the growth of microorganisms such as fungi and bacteria, which produce enzymes that degrade the nutrients [1]. 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. The microorganisms reduce not only the nutritional value but also the market value of tomato fruits.

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 [2]. The parameters during quality control include various factors such as time of harvesting, temperature and moisture during storage, selection of agricultural products prior processing, decontamination conditions, addition of chemicals and final product storage.

There are a few reports of studies on microorganisms associated with spoilage of tomato fruits in Nigeria [3, 4]. Similar research reports on tomato fruits in Benin City are not available. Nevertheless, it has been observed that the high cost of fresh ripened tomato fruits sold in local markets in Benin City has tended to lure the unwary public to patronize spoilt tomato fruits because they are relatively cheaper.

This study was undertaken to isolate and identify microorganisms that are associated or responsible for the spoilage of ripened tomato fruits sold in some markets within Benin City metropolis. In addition, the study investigated the toxin producing capacity and the susceptibility of the microorganisms to some antibiotics.

MATERIALS AND METHODS

Collection of Samples

All samples of tomato fruits were collected from four markets: Oba, New Benin, Santana and Vegetable, in Benin City. The ripened tomato fruits selected were fresh, undamaged, firm and healthy. The samples were taken to the laboratory, washed and drained of water. The fruit samples were kept free from dust and insects at room temperature for up to 14 days to undergo a natural process of spoilage before being used in this study.

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 was 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 37^0C 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 subcultured to obtain pure cultures which were stored at 4^0C and 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, colour and transparency. The isolates were further characterized and identified following biochemical procedures as described by [5]. These included catalase, coagulase, indole and sugar fermentation tests.

Antibiotic Sensitivity Testing

The standardized disc diffusion method as described by [6] and the zone size interpretation chart were used for the determination of the bacterial sensitivity to the various antibiotics selected.

The following commercially prepared paper discs impregnated with the various antibiotics were assessed against the isolates: gentamycin (10µg/ml), streptomycin (10µg/ml), septrin (30µg/ml), chloramphenicol (30µg/ml), ciproflaxacin (10µg/ml), amoxycilin (30µg/ml), augumentin (10µg/ml), ampiclox (30µg/ml), erythromycin (10µg/ml) and ampicilin (30µg/ml).

Each inoculum of the bacterial isolates was grown in separate tubes at 37^0C in Mueller-Hilton broth (agar plates) for 18 hours, with shaking and subsequently diluted to an optical density of 0.1 (0.5 McFarland standard) and stored at 4^0C. The paper discs were gently but firmly placed on the inoculated plates using sterile forceps. The plates were incubated at 37^0C for 24hours after which zones of inhibition were measured and interpreted according to [7]. Results obtained were classified as resistant or sensitive.

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 [8].

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 bacterial isolates were: *Bacillus subtilis*, *B. cereus*, *B. aureus*, *Escherichia coli*, *Klebsiella aerogenes*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Proteus mirabilis* and *Staphylococcus aureus*.

The three species of *Bacillus* identified in this study differed from those reported by [9] who found, *Bacillus coagulans* and *B. stearothermophilus* from spoiled ripe tomato fruits. Besides, [10] isolated *Bacillus megaterium* and *B. laterosporus* from tomato fruit samples. However, the presence of *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas* sp. in this study confirmed findings reported earlier by [11].

The occurrence of the bacterial isolates from fruit samples obtained from the different markets is shown in Table-2.

From all the tomato fruit samples obtained from four markets, *Bacillus subtilis* was the most prevalent with 49.2% while *Klebsiella aerogenes* and *Proteus* mirabilis were the least prevalent recording 1.6% (Table 2).

The mean values of bacterial counts of fruit samples from the four markets in Benin City, during the study period are presented in Table 3.

The result showed that tomato fruit samples from New Benin market recorded the highest bacterial count of 54.0 x 10^4 while the samples from Santana market recorded the lowest mean bacterial count of 2.3 x 10^4. The bacterial counts recorded indicated a high level of contamination of the tomato fruit samples from New Benin 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 [12].

The sensitivity patterns of the bacterial isolates to different antibiotics are shown in Table 4.

Bacillus subtilis recorded the highest sensitivity to all the antibiotics and had no resistance to any of the antibiotics. *Pseudomonas aeruginosa* and *Salmonella typhi* had the highest resistance to all the antibiotics used.

With the exception of *Bacillus* subtilis, the other eight bacterial isolates exhibited varied levels of sensitivity and resistance to antibiotics. The presence of bacterial isolates with multiple antibiotic resistance in the spoiled tomato fruit samples, highlights the potential risk to effective treatment against infectious diseases in consumers of such fruits.
The cultural and morphological characteristics of fungal isolates are shown in Table 5.

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* sp., *Mucor* sp., *Aspergillus niger*, *Fusarium* sp., and *Saccharomyces cerevisiae*. Similar findings were reported by [12] 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.

[13] reported that *Fusarium oxysporum*, *Rhizopus stolonifer* and *Mucor* sp. were the fungi species responsible for the spoilage of tomato, *Lycopersicum esculentum*, fruits from three selected markets in Maiduguri, north eastern Nigeria. [14] 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. The occurrence of fungal isolates is shown in Table 6.

In this study, *Mucor* sp. was the most prevalent fungal isolate with 52.7% while *Fusarium* sp. was the least prevalent with 5.5% (Table 6). 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 of [15]. The mean fungal counts of the tomato fruit samples are shown in Table 7.

Mucor sp. had the highest mean fungal count of 70.1 x 10^4 while *Fusarium* sp. recorded the least count of 4.3 x 10^4 (Table 7).

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 bacterial isolates from tomato fruit samples

CHARACTERISTICS	DESCRIPTION OF ISOLATES								
CULTURAL									
Margin	Smooth	Smoot h	Smooth	Entire	Smooth	Entire	Entire	Smooth	Entire
Colour	White	White	White	Pink	Yellow	White	Creamy	Creamy	White
Shape	Small and irregular	Small	Small	Small	Medium	Large	Large	Medium	Large
MORPHOLOGICAL									
Cell type	Rod	rod	rod	Rod	cocci	rod	rod	rod	rod
Cell arrangement	Single	single	single	single	cluster	single	single	single	single
GRAM REACTION	+	+	+	-	+	-	-	-	-
MOTILITY TEST	+	+	+	-	-	+	+	-	+
SUGAR FERMENTATION TEST									
Glucose	A	A	A	AG	A	AG	A	A	A
Lactose	-	-	-	+	+	+	-	-	-
BIOCHEMICAL TEST									
Coagulase	-	-	-	-	+	-	-	-	-
Catalase	+	+	+	+	+	+	+	-	+
Oxidase	-	-	-	-	-	-	+	-	-
Indole	-	-	-	-	-	-	-	-	-
Probable Microorganisms	*Bacillus Subtilis*	*B. cereus*	*B. aureus*	*Escherichi a coli*	*Staphylococc us aureus*	*Klebsiella aurogenes*	*Pseudomonas aeruginosa*	*Salmonell a typhi*	*Proteus mirabilis*

KEY: + = Positive - = Negative

A = acid production only. AG = acid and gas production

Table 2: The occurrence of bacterial isolates in samples from various markets.

Bacterial Isolates	**Number of occurrence**	**Percentage of occurrence**
Bacillus subtilis	30	49.2
B. cereus	2	3.3
B. aureus	4	6.6
Escherichia coli	5	8.2
Staphylococcus aureus	7	11.5
Klebsiella aerogenes	1	1.6
Pseudomonas aeruginosa	8	13.1
Salmonella typhi	3	4.9
Proteus mirabilis	1	1.6
TOTAL	61	100

Table 3: The mean bacterial counts of tomato fruit samples from different markets.

Bacterial isolates	**Markets CFU/g 10^4**			
	New Benin	**Vegetable**	**Oba**	**Santana**
Bacillus subtilis	54.0 x 10^4	25.3 x 10^4	18.7 x 10^4	43.5 x 10^4
B. cereus	54.0 x 10^4	Nil	Nil	Nil
B. aureus	Nil	5.8 x 10^4	29.3 x 10^4	Nil
Escherichia coli	15.4 x 10^4	34.0 x 10^4	8.0 x 10^4	2.3 x 10^4
Staphylococcus aureus	23.4 x 10^4	7.6 x 10^4	3.5 x 10^4	13.6 x 10^4
Klebsiella aerogenes	35.0 x 10^4	Nil	Nil	Nil
Pseudomonas aeruginosa	19.0 x 10^4	Nil	15.4 x 10^4	3.5 x 10^4
Salmonella typhi	5.0 x 10^4	Nil	Nil	Nil
Proteus mirabilis	Nil	3.3 x 10^4	Nil	Nil

Table 4: Antibiotic sensitivity patterns of bacterial isolates

BACTERIAL ISOLATES	ANTIBIOTICS									TOTAL	
	GE	ST	SE	CH	CP	AY	AU	AX	AN		
	MARKETS										
	A B C D	A B C D	A B C D	A B C D	A B C D	A B C D	A B C D	A B C D	A B C D	S No (%)	R No (%)
Bacillus subtilis	S S S S	S S S S	S S S S	S S S S	S S S S	S S S S	S S S S	S S S S	S S S S	36(100)	0%
B. cereus	R R S S	R S S S R	S R S R	R S S S	R S R S	S S S R	R S S R	S S R R	R R S R	19(53)	17(47)
B. aureus	S S S R	R S R R	S R R R	R R S S	R S S R	S S R R	R S S R	R S S R	S R R R	16(44)	20(56)
Escherichia coli	S S S S	R S S R	S S S S	S R R S	S S S S	S S R R	R R S S	S S R S	S S S S	27(75)	9(25)
Staphylococcus aureus	S S S S	S S R R	R R R S	R S S R	S S S S	S S R R	R S S R	R S R S	R S S R	21(58)	15(42)
Klebsiella aerogenes	S R R R	S R S R	S S R R	R S S R	S S S S	S R R S	S S R R	R R S R	R S R S	18(50)	18(50)
Pseudomonas aeruginosa	S R R R	S R R R	R S R R	R R S R	R R S R	R R R R	S R R S	R R R S	R R S R	9(25)	27(75)
Salmonella typhi	R R R R	R S R R	R R R S	R R S R	R R R S	R S R S	R S R R	R R S R	S R R R	9(25)	27(75)
Proteus mirabilis	S S S R	S S S S	S S S R	R S S S	S S S R	R R R S	S R R R	R S S S	R S R R	22(61)	14(39)

KEY:

Antibiotics: GE= gentamycin ST= streptomycin SE= septrin CH= chlorophenicol CP= Ciprofloxacin AM= amoxycillin AU= Augumentin AX= ampiclox AN= ampicilin

Test results: S= sensitivity, R= resistant

Markets: A= New Benin, B= Vegetable, C= Oba, D= Santana

Table 5: Morphological and Cultural characteristics of Fungal Isolates

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* sp.**	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* sp.**	Initially white and cottony but later develop pink centre with a lighter periphery.	Septate hyphae with canoe-shaped macroconidia, condiophores bear conidia singly or in cluster.
***Penicillium* sp.**	The colonies of *Penicillium* sp. 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.
Sacharomyces cerevisiae	Colonies of *Saccharomyces* sp. 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, especially when grown on V-8 medium, acetate ascospor agar.

Table 6: The occurrence of fungal isolates from the different markets.

Fungi genera	Number of occurrence	Percentage of occurrence
Mucor	29	52.7
Aspergillus	10	18.2
Penicillium	8	14.5
Fusarium	3	5.5
Sacharomyces	5	9.1
Total	55	100

Table 7: The mean fungal counts of tomato fruit samples obtained from different markets.

Fungal isolates	Markets cfu/g 10^4			
	New Benin	**Vegetable**	**Oba**	**Santana**
***Mucor* sp.**	42.5×10^4	70.1×10^4	29.5×10^4	23.0×10^4
Aspergillus niger	45.0×10^4	13.8×10^4	28.3×10^4	Nil
***Penicillium* sp.**	17.0×10^4	10.3×10^4	13.0×10^4	14.0×10^4
***Fusarium* sp.**	43×10^4	9.0×10^4	5.0×10^4	Nil
Sacharomyces cerevisiae	Nil	7.8×10^4	Nil	8.4×10^4

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 bacterial and fungal diseases.

REFERENCES

1. Maddox DA; Implications of new technologies for seed health testing and the worldwide movement of seed. Seed Science Research, 1998; 8:277-284.
2. Butler EE; Pathogenicity and taxonomy of *Geotrichum candidum*. Phytopathology, 1992; 50:665-672.
3. Wokoma ECW; Preliminary report on disease of tomatoes in Choba, Rivers State. J. Appl. Sci. Environ., 2008; 12(3):178-121.
4. Eni AO, Ibokunoluwa O, Oranusi U; Microbial quality of fruits and vegetables. African Journal of Food Science, 2010; 4(5): 291-296.
5. Holt JG, Krieg NR, Sneath PHA, Stanley JT, Williams ST; Bergey's Manual of Determinative Bacteriology, 1994; 9th Edn., Williams and Wilkins, Baltimore.
6. Bauer AW, Kirby WM, Strerris JC, Turk M; Antibiotic susceptibility testing by a standard

single disk method. American Journal of Clinical Pathology, 1999; 45: 493-496.

7. Clinical and Laboratory Standards Institute (CLSI); Performance standards for antimicrobial susceptibility testing, 2012; 4(1) Wayne, PA. USA.
8. Beuchat LR; Ecological factors influencing survival and growth of human pathogens on raw fruits and vegetables. Microb. Infect., 2002; 4:413-423.
9. Goldoni JS, Cavestre RC, Kurozawa C, Roca RC, Bonassi IA; Occurrence of spoilage bacteria on tomato fruit (*Lycopersicum esculentum* Mill). Acta Hort., (ISHS), 1992; 301: 285-292.
10. Ibrahim AD, Musa K, Sani A, Aliero AA, Yusuf BS; Microorganisms associated with the production of volatile compounds in spoilt tomatoes. Research in Biotechology, 2011; 2(2): 82-89.
11. Jushi AP, Patel SP; Microbiological analysis of fresh fruits and vegetables and effects of antimicrobial agent on microbial load. Journal of Food Protection, 2008; 14:23-24.
12. Ghosh A; Identification of microorganisms responsible for spoilage of tomato (*Lycopersicum esculentum*) fruit. Journal of Phytology. 2009; 1(6): 414-416.
13. Akinmusire OO; Fungal species associated with spoilage of some edible fruits in Maiduguri, north eastern Nigeria. Adv. Environ. Biol., 2011; 5 (1): 157-161.
14. Al-Hindi RR, Al-Najada AR, Mohamed SA; Isolation and identification of some fruit spoilage fungi: screening of plant cell wall degrading enzymes. African Journal of Microbial Research, 2011; 5(4): 443-448.
15. Dennis C, Davis RF; Fungal spoilage of fresh tomatoes. Biennial Report of Field Research Institute, 1980; 78:44-45.