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ISOLATION AND IDENTIFICATION OF BACTERIA ASSOCIATED WITH THE SPOILAGE OF TOMATO (*Lycopersicon esculentum*) FRUITS

***JUNAIDU SANUSI; & **IBRAHIM SULEIMAN**

**School of Postgraduate Studies, Department of Biological Science, Usmanu Danfodiyo University Sokoto. **Department of Biology, Isa Kaita College of Education Dutsinma, Katsina State*

ABSTRACT

Tomato is one of the most important staple fruits in Nigeria that provides essential nutrients for maintaining good health and curing nutritional disorders. Tomato production has been facing serious challenges to its sustenance due to economic loss resulting from spoilage by microorganisms which makes its storage and transportation difficult. In this

INTRODUCTION

Tomato fruit is one of the most important staple in Nigeria that provides essential nutrients for maintaining good health and curing nutritional disorders, it provide essential nutrients in human diets by supplying the necessary growth regulating factors and antioxidants for maintaining normal health (Rossato *et al*., 2009). It is widely distributed in nature and is considered commercially important and nutritionally essential food commodity (Hayes, 2005). The development of fruit industries has stimulated large scale production of the fruits and enhanced diversification of entrepreneurship in the subsector (Ibeawuchi *et al*., 2015). This improve quality of life of some rural population thus reduced the rate of rural-urban migration. Despite the attempts being put in place for large-scale tomato production and profit maximization, poor handling and quality deterioration have

study, isolation and identification of bacterial associated with the spoilage of tomato fruits were carryout using standard laboratory procedures. Tomato samples were collected from Dundaye, Gidan Siminti and Asare Fadama's, thus, major Fadama's that supply tomato in Sokoto metropolis. Bacterial isolated were characterized and identified base on colonial, microscopic and biochemical test. The Bacterial counts of the tomato cultivars showed that DanEka has the highest bacterial counts of 5.39CFU/g, UTC 4.56CFU/g and Bahaushe 4.26CFU/g respectively. Results of biochemical test of isolated bacteria showed that two (2) species of bacteria were isolated in DanEka namely: Entrobacter cancerogenus and Bacillus coagulance, E. cancerogenus is the most frequent species that was isolated. Bahaushe cultivar was found to be infected by four (4) different species of bacteria namely Staphylococcus carnosus, Pseudomons trivialis Enterococcus casseli flavus and Raoutella terrgena. Also four (4) different species were also isolated from UTC namely Morganella morganii, Bacillus mycoides, Erwinia mallotivora, and Burkhoderia glumae. The findings showed that Bahaushe and UTC culitars were more susceptible to bacterial spoilage than DanEka. Researchers recommends precautionary measure of handling postharvest tomato fruits such as proper sanitary conditions, treatment with antimicrobial agents (chlorinated water) and refrigeration are necessary not only in reducing microbial toxins to human health, but also enhanced the fruits' shelf-life, there is also need to develop spoilage-resistant cultivars for effective prevention and control of tomato fruits spoilage.

Key words: *Spoilage, Cultivars and Bacteria*

always been a major problem that causes post-harvest losses (Zewter *et al*., 2012).

In Nigeria, large quantities of tomatoes are produced in million tones, 1.8 million tones is estimated as annual production (Ugonna *et al*., 2015). Its production is believed to be a multimillion business and generates a lot of employment in Nigeria and world at large. Tomato production in Nigeria is an important business because it provides a means of

livelihood for the people. Consequently, tomato fruits contribute to economy as it serves as important source of income of both the rural (farmers) and urban (traders) dwellers. The industrial potential of tomatoes available in Nigeria is on the increased and as a result, people have embarked on massive production of tomatoes not only for its nutritive value but for enhancing the establishment of processing industries (Adebamiji and Omotola, 2009).

Statement of the Problem

Tomato production has been facing serious challenges to its sustenance due economic loss. Available data from Food and Agricultural Organization (FAO) estimated that one-third of tomato fruits produced for human consumption are lost globally, which amounts to about 1.3 billion tons per year (FAO, 2016; Vilariño *et al*., 2017). The estimated total loss in Nigeria due to these constraints is about 45% (FAO, 2016), this means that huge amounts of resources used in food production and marketing are lost in worthless circumstances. Despite the advancement in food production and storage, the problem of tomato spoilage has become persistent. Opadokun (2010) reported that 21% of tomato harvested in Nigeria is lost to rot in the field and additional 20% to poor storage system, transportation and marketing.

Justification

Tomato fruit is very rich in mineral, vitamins, and carbohydrate that provide dietary fluids and fibres necessary for digestion and essential for maintaining health and curing nutritional disorders. Lycopene is one of its constituent antioxidant carotenoid compounds occurring in it. With the increasing demand of tomato due to rapid increase in human population, production of large quantity of tomato while avoiding loss to spoilage has become necessary, investigation of microorganisms spoiling the tomato fruits (bacteria). If we can demonstrate that it will enable us have an improved understanding of the bacteria involved in the deterioration of tomato fruits.

Aim and Objectives

The aim of this study is isolation and identification of bacterial associated with the spoilage of tomato cultivars (*Lycopersicon esculentum*) fruit with the following specific objectives:

i. Isolate of bacteria associated with the spoilage of tomato cultivars in Sokoto

ii. Identification of bacteria associated with the spoilage of tomato cultivars in Sokoto

Materials and method

Sample Collection and Preparation

Ten (10) samples of each healthy tomato fruits of different cultivar were sourced from Fadama within sokoto metropolis, Sokoto state. Dundaye, Gidan Siminti and Asare Fadama were the major fadamas that supplied most of the tomatoes in sokoto metropolis. The ripened tomato fruits selected were fresh, undamaged, firm and healthy. Samples were taken to the laboratory, washed with distilled water and drained. The tomatoes samples were kept from dust and insects free at room temperature for up to 14 days to undergo a natural process of deterioration before being used for study. Samples were collected in a separate sterile polythene bag. Prior to isolation, samples were surface sterilized by dipping completely in 0.1% mercuric chloride solution for less than 1 minute and immediately rinsed in sterile distilled water.

Sterilization of Glassware

The glassware were washed with liquid soap and sufficiently rinsed with tap water and distilled water respectively, air dried and sterilized in hot air oven at 160°C for 1hour, while the conical flask were autoclaved.

Preparation of Media

Preparation of Nutrient Agar (NA)

Nutrient agar (NA) was prepared according to manufactures instructions; 28g NA was dissolved in 1000ml of distilled water, the suspension were mixed until completely homogenized. The conical flask containing the media was plugged with cotton wool and capped with aluminum foil. The flask was sterilized using lender autoclave at 121°C for 15 minutes, cooled to 30°C and poured into sterile plates Biotech Laboratories ltd (Cheesebrough, 2009).

Enumeration of Microbial Load

A ten-fold serial dilution of each of the samples was carried out. Spread plate technique was employed by inoculating 0.1ml aliquot aseptically from the 10^{-7} and 10^{-4} dilutions onto nutrient and MacConkey agar plates. The agar plates were incubated at 37°C for 28 hours. Sample was inoculated in duplicate and the mean values of bacterial counts were recorded as colony forming unit per ml (cfu/g) Obunukwu *et al*. (2018).

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Isolation of Bacteria

Bacterial population of the fruit samples was enumerated by serially diluting 1g of spoilt fruits. From the seventh dilution, (10^{-7}) 1 ml were transferred into sterile molten nutrient agar plates using a dropper pipette, spread using bend glass rod. The plates were incubated at 30°C for 24 h (Chikere *et al*., 2009). The number of viable bacteria, in the samples was estimated from the number of colonies formed using a colony counters and expressed as CFU/g (Obunukwu *et al*, 2018).

Purification of Bacterial Isolates

Colonies from the primary plates were aseptically picked with a sterile wire loop and transferred onto freshly prepared sterile nutrient agar plate, with a streaking technique such that discrete colonies appear at the end of streaked lines after incubation. The subculture plates were incubated at 37°C for 24 hours. Discrete colonies from the subculture plates were aseptically transferred and streaked on slant and incubated for another 24 hours at 37°C which were stored at 4°C and used subsequently for microscopic characterization and biochemical analyses (Obunukwu *et al*. 2018).

Characterization and Identification of Bacterial Isolates

All bacterial isolates were characterized and identified based on their cultural, morphological, microscopic examination and biochemical characteristics following the methods prescribed by (Cheesbrough, 2005). Biochemical test conducted include the following: Gram stain, Catalase test, Oxidase test, Motility test, Methyl red test, Citrate test and Urease test (Obunukwu *et al*, 2018).

Pathogenicity Test of the Isolates

The procedures of Onuorah *et al*. (2015),Chukwuka *et al*. (2010) and Baiyewu *et al*. (2007) was used. Ten (10) healthy tomatoes cultivars (DanEka, Bahaushe and UTC) were properly washed with tap water, rinsed with distilled water and surface-disinfected with ethanol. Injection syringe was used to inoculate the bacterial colony in each of the tomato fruits. One tomato of each of the cultivars was also being given same treatment but without inoculating the isolates, these serve as the control. The inoculated tomato fruits and the controls were placed in sterile transparent polythene bag (one fruit per bag). Each of the fruits was moistened with wet balls of absorbent cotton wool to create a humid condition. The fruits were incubated at 28°C for five days and

observed for spoilage. The microbes were re-isolated from the fruits and compared with the original isolates.

RESULTS AND DISCUSION

Pathogenicity test of Bacterial Isolates

Bacterial counts of the tomato cultivars showed that DanEka has the highest bacterial counts of 5.39CFU/g, followed by UTC 4.26CFU/g and Bahaushe cultivar has the least with 4.56CFU/g as indicated in figure 1 and Figure 2:

Characterization and Identification of Bacterial Isolates

All bacterial isolates were characterized and identified base on colonial and microscopic appearance by Cheesbrough, (2005) as indicated in Table 2 and Biochemical test in Table 3.

Table 1: Pathogenicity testing of the Isolates

Cultivars	Symptoms of Spoilage
DanEka	i. Fruit appeared water soaked and soft, total tissue rot and exudation of liquids ii. Soft rot spoilage and infected area turns brown and water soaked iii. Soft rot in the affected area iv. The lesion produced distension in the fruits surface and changed in colour in the affected area. v. Blue black coloration and distorted smell vi. Loss of weight
Bahaushe	i. Soft rot spoilage and infected area turns brown and water soaked ii. Soft rot in the affected area iii. The lesion produced distension in the fruits surface and changed in colour in the affected area. iv. Blue black coloration and distorted smell v. Loss of weight and change in colour vi. Tomato appeared to be soft, total tissue rot and exudation of liquids
UTC	i. Soft rot spoilage and inoculated area turns brown ii. Soft rot in the affected area.

iii. Blue black coloration and distorted smell
iv. White mold appearance and watery discharge

CONTROL Watery discharge and loss of size

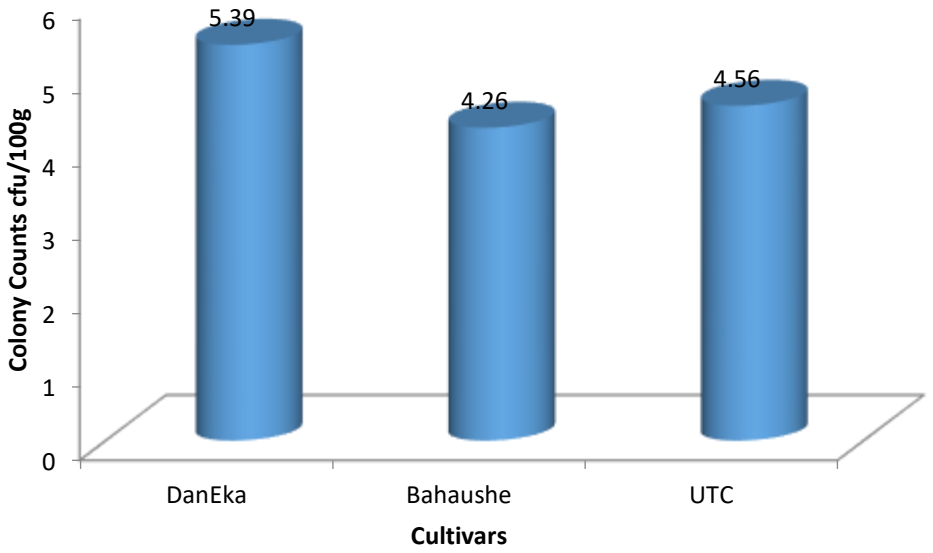

Figure 1: Pathogenicity test of bacterial load of tomatoes cultivars

Table 2: Cultural Morphology and Grams Reaction of Pathogenicity Bacterial Isolate

Cultivars	Morphology	Grams Reaction
DanEka	SCW	- R
	SCM	+ R
	LCW	- R
	LCM	- R
Bahaushe	LCW	+ CC
	SCM	+ CC
	VSM	+ R
	LCM	- SR
UTC	LCW	- R
	SCM	+ R
	LCM	- R
	SCW	- R

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SCW= small colony white, SCM = small colony milk, LCW = large colony white, LCM = large colony milk, VCM = very small colony milk, R= rod, CC = cocci, SR = short rod and SL= slow

Bacterial load of spoilt tomatoes stored at ambient temperature

Bacterial counts of the tomato cultivars showed that UTC has the highest bacterial counts of 4.56CFU/g, followed by DanEka 4.36CFU/g and Bahaushe cultivar has the least with 3.76CFU/g and shown in the figure 3:

Characterization and Identification of Bacterial Isolates

Table 8 is a result of biochemical test of isolated bacteria, *Entrobacter cancerogenus* and *Bacillus coagulance* is found in DanEka, and *E. cancerogenus* is the most abundance species that is responsible for the spoilage of DanEka cultivar. Bahaushe cultivar was found to be infected by four different species of bacteria namely *Staphylococcus carnosus, Pseudomons trivialis Enterococcus casseliflavus* and *Raoutella terrgena*. Also four different species were also isolated from UTC namely *Morganella morganii, Bacillus mycoides, Erwinia mallotivora,* and *Burkhoderia gluma.*

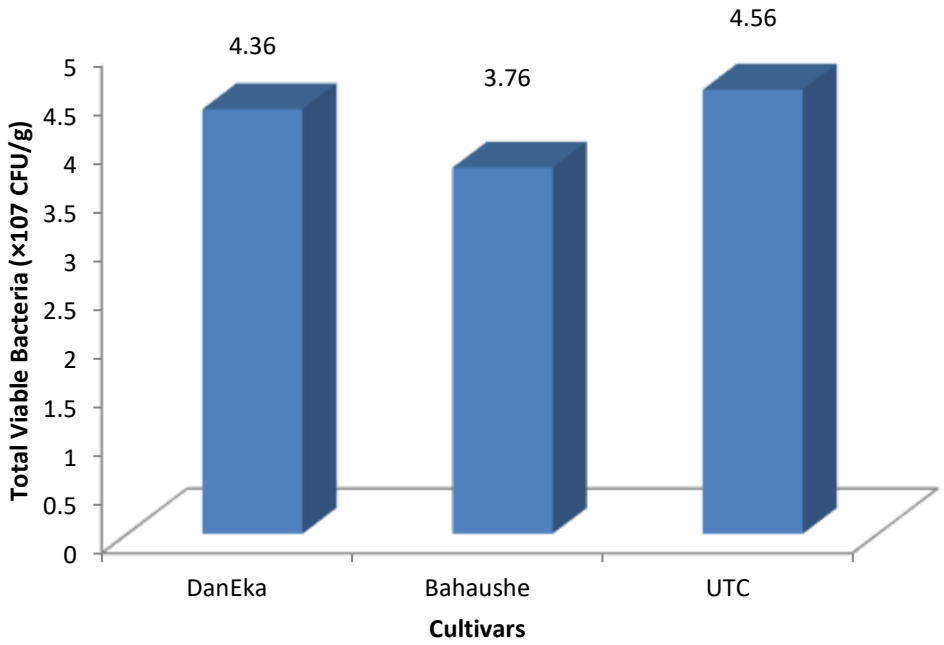

Figure 2: Bacterial loads of tomato cultivars obtained in Sokoto

Table 3: Biochemical characteristics of bacterial isolates

ISOLATE	MOP.	GRA.	H_2S	CAT	GAS	M.R	V.P	MOT	CIT.	IND	COA	GLU	LAC	SUC	STH	UR	POSSIBLE ORG
DanEka																	
1	SCW	- R	-	+	+	-	+	+	+	-	NA	+	-	-	-	-	*Entrobacter cancerogenus*
2	SCM	+ R	-	+	-	-	+	+	-	-	NA	+	-	-	+	-	*Bacillus coagulance*
3	LCW	- R	-	+	+	-	+	+	+	-	NA	+	-	-	-	-	*Entrobacter cancerogenus*
4	LCM	- R	-	+	+	-	+	+	+	-	NA	+	-	-	-	-	*Entrobacter cancerogenus*
Bahaushe																	
1	LCW	+ CC	-	+	+	-	+	+	+	-	+	+	-	-	-	-	*Staphylococcus carnosus*
2	SCM	+ CC	-	-	-	-	+	+	+	-	NA	+	+	+	-	-	*Enterococcus casseliflavus*
3	VSM	+ R	-	-	-	-	+	+	+	-	NA	+	-	-	-	-	*Pseudomonsdrivialis*
4	LCM	- SR	-	+	-	-	+	-	-	-	NA	+	+	+	+	+ SL	*Raoutella terrgena*
UTC																	
1	LCW	- R	-	+	+	+	-	+	-	+	NA	+	-	-	+	+	*Morganella morganii*
2	SCM	+ R	+	-	-	-	+	-	+	-	NA	+	-	-	+	+	*Bacillus mycoides*
3	LCM	- R	-	+	-	-	+	+	+	-	NA	+	-	-	-	-	*Erwinia mallotivora*
4	SCW	- R	-	+	-	-	+	+	+	-	NA	+	-	-	+	+	*Burkhoderia glumae*

KEY = negative, + = positive, SCW= small colony white, SCM = small colony milk, LCW = large colony white, LCM = large colony milk, VCM = very small colony milk, R= rod, CC = cocci, SR = short rod, SL= slow, GRA= Grams Reaction and NA= not applicable

DISCUSSION

Total of seven different species of Bacteria were isolated in which *Entrobacter* spp. is the most dominant among the isolates in tomato cultivars. All identified bacteria occurred in all rotten tomatoes are in conformity with the work of (Onuorah and Orji, 2015). Ugwu *et al*. (2014) also discovered 4 species of bacteria; *Escherichia coli, Klebsiella* spp., *Salmonella* spp*. and Pseudomonas aeruginosa*. Bashir *et al*. (2016) was able to identified two bacteria species; *Staphylococcus aureus* and Bacillus on spoilt tomatoes fruits. Pathogenicity of tomato fruits during the ten days deterioration processes. Bacteria pathogens *Entrobacter cancerogenus*, *Pseudomons trivialis*, *Bacillus coagulance* and *Staphylococcus carnosus* were recorded slow growth at the first 24 hours of culturing until the third day, after which the symptoms of fruit colour change appeared on the fruit skins, and followed by wrinkled textures. *Pseudomons trivialis* in tomato fruits contamination has been reported to be associated with poor human handling processes as reported by Ghosh, (2009). Many researchers had earlier isolated various strains of bacteria in tomato fruits. Some earlier researcher observed bacteria

Bacillus megaterium and *Bacillus Laterosporus*, while on the other hand some researchers found bacteria *Bacillus subtilis*, *Bacillus cereus*, *Bacillus Aureu, Lactobacillus fermenti, Pseudomonas stutzeri, Leuconostoc spp* and *Rothia spp, Escherichia coli*, *Mucor mucedo, Monilia spp, Klebsiella aerogenes*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Proteus mirabilis* and *Staphylococcus aureus, Rhizopus stolonifer, Botrytis cineria, Saccharomyces spp, Rhodotorula spp* and *Verticillium albo-atrum* from tomato fruits by (Wogu and Ofuase, 2014 and Chuku *et al*. 2008). The discoloration associated with bacterial infection on tomato fruits had been reported earlier by Miedes and Lorences (2004). Furthermore, *Rhizopus stolonifer* which possibly caused the most rapid rot on stored tomato fruits in Nigeria reported by Okoli and Erinle (1990).

Conclusion

The findings revealed that tomato fruits were more susceptible to bacteria attack, *Entrobacter cancerogenus* was found to be the most active of all pathogens associated with tomato post-harvest spoilage in DanEka. General public should be enlightened on the potential health hazards bedeviling consumption of relatively cheaper ripen-spoilt tomato fruits, as these may be the mediators in food borne fungal and bacterial diseases. Market wastes and refuse should also be properly disposed of at designated sites to reduce microbial contaminations. Precautionary measures of preservation and handling processes of fruits such as proper sanitary conditions, treatment with antimicrobial agents (chlorinated water) and refrigeration are necessary not only in reducing microbial toxins deleterious to human health, but also enhanced the fruits' shelf-life.

Recommendations

The findings recommend the following:

i. There is need to improve storage and preservation methods which could increase the fruits shelf life.
ii. There is also need to develop spoilage-resistant cultivars for effective prevention and control of tomato fruits spoilage.
iii. There is need to divert attention to discovering new organisms associated with tomato spoilage using most recent and most advanced technology since not all microorganisms are cultivable on conventional laboratory media.

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