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Determination of Microbial Load Associated with Spoilage of Tomato (*Solanum lycopersicum*) under Storage

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Tomato contains large amount of water which makes them more susceptible to spoilage by the action of various microorganisms. This makes the storage of this vegetable difficult. The objective of this study is to determine microbial load of tomatoes including microbial species it constitute under storage with particular reference to low temperature storage. Pour plate techniques were used to enumerate microbial load of the samples. Discrete isolated colonies from these sources were sub-cultured using streak plate method to get purified cultures for the study. Standard microbiological methods (morphologically, microscopically and biochemical tests) were subsequently used to identify the tomatoes microflora. Microorganisms generally encountered include seven bacterial species such as, *Acidovorax facilis*, *Aminobacter aminovorans*, *Acidiphilium facilis, Burkholderia cepacia, Azomonas* sp., *Xanthobacter flavus* and *Azomonas insignis*. Similarly, three fungal species were encountered during the study. These include *Alternaria alternata lycopersi, Alternaria tenuissima* and *Phycomyces microspores*. The investigation revealed that up to 90% of the samples were infected with one or more fungal and bacterial species. The most predominant pathogenic microbes isolated from the samples were, *A. alternata lycopersi* (42.0%), *A. tenuissima* and *X. flavus* (35.0%), others include *A. insignis* (32%), *A. facilis* (25%) and *B. cepacia* (20%). Proper handling from the farm as well as during storage and the avoidance of mixing of diseased ones with the healthy ones were identified as important factors in preventing loss.

Key words: Bacteria, fungi, identification, microorganism, spoilage, tomato.

Microorganisms are associated in various ways to all types of food that human beings consume. Presence of microorganisms may greatly influence the quality of food. Naturally occurring foods like vegetables and fruits sometimes may be contaminated during picking and transportation by means of handling (Bukar *et al.,* 2009). Food contents may provide a suitable medium for the growth of microorganisms that may result in production of toxic substances, spoilage and poisoning of food. All these factors results in transmission of diseases (Pelczar *et al.*, 1993). Tomato (*Solanum lycopersicum*) is herbaceous plant of the Solanaceae family. Tomato is widely consumed as fresh vegetable and also valuable in the food industry (Njoku, 1991). It is most frequently used vegetable that's way always available as freshly stored and processed vegetable. Due to valuable food source all over the world makes it fourth mostly commonly consumed fresh vegetable (Ajayi, 2013). Various species of microorganism can alter adversely the nutritional quality of tomatoes. Microorganisms that cause spoilage like moulds, yeast and bacteria. Principle spread of microbial infection in fruits and vegetables revealed that a single infected cell of tomato can be the source of infection to other tomatoes during storage (Jay, 2003). Common air

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mould and bacteria may gain entry into the susceptible tissue and cause loss of nutritional value of tomato. Main purpose of this research work was set up to isolate and identify fungal and bacterial species associated with deterioration and spoilage of tomatoes.

MATERIALS AND METHODS

Sterilization of Materials and Media Preparation

All glass ware were sterilized in the hot air oven at 160ºC for two hours. The inoculating needle and wire were sterilized by flaming in the Bunsen burner until red hot, working surface were sterilized with 95% ethanol (Buchann, 2008). Malt extract agar (MEA) Luria Bertani Agar (LBA) media were used for microbial isolation. Culture media were sterilized at 121ºC and 15 lb/in^2 for 15minutes in an autoclave.

Sample collection

A total of ten (10) tomato fruits (*S. lycopersicum*) were collected from storage house were used in this study. All the samples collected were placed in sterile polythene bags separately and labeled appropriately and transported to Fungal Systematic Laboratory of First Fungal Culture Bank of Pakistan, Institute of Agricultural Sciences, University of the Punjab Lahore, Pakistan for microbial analysis.

Isolation of Microbes from the Study Samples

The infected tomato fruits were surface sterilized with 1% sodium hypochlorite and cut from the advancing edges of rotten part. The cut portion of the rotten parts was then rinsed in three different changes of distilled autoclaved water. The segments of the infected fruits were then plated on solidified Malt Extract Agar (MEA) plates for fungal isolation and Luria Bertani Agar (LBA) media plates used for bacterial isolation. The inoculated plates were labeled properly and then incubated at 26 ±3ºc for 5-7 days for fungal growth while bacterial growth occurred at 37ºC for 24hours (Pelczar, 1993). The isolated colonies were subsequently sub cultured in order to obtain pure cultures.

Identification of Fungal Isolates

The isolated pure fungal colonies were subjected for macro & microscopic identification.

Colonial morphology

Pure and fresh cultures of fungal isolates were identified using cultural and morphological features such as; colony growth pattern, color, texture and growth elevation. From the incubated plates different fungal isolates with different coloration was observed includes; (a) White (b) Brown (c) Grey black, which signifies the %age occurrence of different fungal colonies (Emory, 2007; Mirza *et al.*, 1979).

Cellular morphology

A small piece of mycelia from representative culture was placed on the glass slide containing lectophenol cotton blue stain for colored cultures and trypan blue for white culture colonies using a sterile inoculating needle. The morphological characteristics and appearance of various fungal isolates from rotten tomato fruits were confirmed and authenticated with the help of Identification manual (Emory, 2007; Mirza *et al.*, 1979).

Biochemical Test and Identification of Bacterial Isolates

For bacterial isolation the surface sterilized sample was cut in to small portions and placed on LBA (Luria Bertani Agar) media plates under aseptic conditions. Inoculated plates were incubated at 37ºC for 24hours. Different bacterial isolates were observed from inoculated plates, that signifies the %age occurrence of various bacterial isolates. Bacterial isolates were re-cultured in order to obtain pure bacterial isolates. Identification of bacteria is carried out following the standardized procedure starting with the colony and cell morphology followed by Gram staining and finally testing the metabolic activities of unknown strain. Identification of bacterial species was done by recording colony morphological features (Beishir, 1991) (color, shape, size, texture, margins and odor etc.) and cell microscopic characters (color, cell wall, contents, shape, arrangement, material, Gram stain, spore stain, capsular material and motility). The pure colonies were differentiated by biochemical tests (Holt *et al.*, 2000; Benson, 1996).

Pathogenicity Test

Pathogenicity test was conceded out in order to confirm that the isolated microorganisms were really responsible for the rotting of tomato fruits. Healthy fruits were surface sterilized with 1% sodium hypochlorite solution. Agar disc containing microbial inoculums were placed in the holes created on the healthy fruit with the help of

sterilized cork borer. The process was repeated separately for each microbial strain; i.e. both bacterial and fungal isolates. The inoculated and control samples were incubated for 7 days at 26 ±2ºC. The inoculated region of each type of fungus and bacteria was finally examined and compared with the initial isolates.

RESULTS

Present study shows that various types of microorganisms were bump into stored rotten tomatoes and analyzed in the laboratory. The organisms that were isolated include seven bacterial and three fungal species. Morphological and microscopic characteristics of fungal and bacterial isolates were indexed in Table 1 & 2.

On the basis of microscopic and biochemical characterization the bacterial species were categorized in to six different genera such as *Acidovorax* species, *Aminobacter* species, *Acidiphilium* species, *Burkholderia* species, *Azomonas* species, *Xanthobacter* species. Three different fungal species were identified during study belonging to two different genera; *Alternaria* species and *Phycomyces* species. Frequency occurrence of various types of fungal and bacterial isolates from rotten tomato fruits were shown in Figure 1. The present study exihibited that up to 90% of the samples were infected with one or more fungal and bacterial species. The most predominant pathogenic microbes isolated from the samples were, *A. alternata lycopersi* (42.0%), *A. tenuissima* and *X. flavus* (35.0%) and *A. insignis* (32%). The pathogenicity of the isolated bacteria and fungi from the tomato fruit after seven days of incubation shown in Table 4. Identified bacterial and fungal species were deposited in First Culture Bank of Pakistan (FCBP). All species versus their reference number as well as their FCBP accession numbers are given in Table 3.

DISCUSSION

The present investigation was aimed to isolate and identify the microbial load associated with tomatoes. Observations made under this context signify the presence of certain active pathogens i.e., seven bacterial species were categorized while three different fungal species

Table 1. Biochemical characteristics of bacterial isolates encountered

Code of Isolate	Colony characters	Gram stain	Spore	Motility	Biochemical tests							Probable identity of isolates
					Catalase	Citrate Utilization	Oxidase	Indole	Glucose	Methyl red	Hydrogen Sulfide	Nitrate reduction	
B-1	Raised smoothspherical entirecreamy opaque	-	-	+	-	-	+	-	-	-	-	-	*Acidovorax facilis*
B-2	Raised smoothSpherical entire Yellowishtranslucent	-	-	+	+	-	+	-	-	-	-	-	*Aminobacter aminovorans*
B-3	Flatsmoothsphericalentire creamyopaque	-	-	+	-	+	-	-	-	-	-	-	*Acidiphilium facilis*
B-4	Flat smoothlong rodsrhizoid creamyopaque	-	-	-	-	-	-	+	-	-	-	+	*Burkholderia cepacia*
B-5	Raised, smooth,spherical, entirecreamy,translucent	-	-	+	+	-	-	-	-	-	-	+	*Azomonas* sp.
B-6	Slimy smoothshort rodsentire yellowishopaque	-	-	-	+	-	-	-	-	-	-	+	*Xanthobacter flavus*
B-7	Flatrough sphericalrhizoid creamyopaque	-	-	+	+	-	-	-	+	-	-	+	*Azomonas insignis*

were identified during study belonging to two different genera. Some of these pathogens have been reportedly isolated from Pawpaw fruits in Nigeria (Baiyewu *et al.,* 2007; Chukwuka *et al.,* 2010).

Frequency of occurrence of various types of fungal and bacterial isolates was also exhibited from rotten tomato fruits i.e., *A. alternata lycopersi* (42.0%), *A. tenuissima* and *X. flavus* (35.0%), *A. insignis* (32%), *A. facilis* (25%) and *B. cepacia* (20%). Some however, did not cause spoilage on re-infection. This could be due their inability of independent growth and survival on the tomato and might be dependent on the breakdown product of other spoilage organisms or conditions for survival. *A. alternate lycopersi* and *A. tenuissima* were able to cause spoilage on re-infection, whereas *X. flavus* and *A. insignis* have about serious occurrence rate on re-infection, as well as *A. facilis, A. aminovorans, B. cepacia* and *Azomonas* sp. also showed infection on tomato fruit. The occurrence of these organisms may be attributed to their ability to produce resistant spores, as reported by Fawole and Oso, (1995) that spores of *Alternaria* spp. are more resistant to high temperature. Despite the fact that *P. microspores* and *A. facilis* showed no growth by testing pathogenicity on tomato. This is however in agreement with Ifeanyi, (1995) and Bello (2010) whom both isolated about seven different fungal genera from different fruits including sweet orange fruits and when these isolates were aseptically inoculated into healthy susceptible fruits, the characteristic symptoms originally observed were also noticed. All the four organisms were successfully taking part in the decay and are thus confirmed as the causal organism of fruit decay (Baiyewu *et al.,* 2007; Chukwuka *et al.,* 2010).Thus these microbes were also found to be associated with the deterioration of tomato fruits, All the ten organisms isolated were confirmed to cause spoilage on the tomato fruits but in varying degrees. The differences in storage conditions and varieties of these products in the different areas where they are produced may account for the variation in the isolates detected by different workers (Bukar *et al.,* 2009). The presence of the microbes or their resistant spores is most likely to have originated from the farms where the fruits were harvested and some from the stores due to

Table 2. Microbiological characterization of fungi isolates encountered

Code of isolates	Colony morphology	Mycelium	Conidial color and shape	No. of septa	Conidial size	Identity of isolates
F-1	Greenish brown, smooth edge, sporulation on surface. Reverse; creamy white	Branch chained, having 4-6 conidia	Brown, ovoid, ellipsoid	Transverse septa; 4-6 Longisepta; 0-2	20-32 x 6-12µm	*Alternaria alternate lycopersci*
F-2	Brownish black, cottony texture, 2-3 concentric growth rings, sporulatd surface, smooth edges, reverse black	Branched hyphae 7-12 conidia	Golden brown, Narrow tapered upper half, ovoid, rough surface	Mostly 4-5 transverse septa,1 or no longi septa	40-60x14-18µm, Maximum length with a developed narrow tapered beak is near 75 µm transverse septa, 1 or no longi septa	*Alternaria tenuissima*
F-3	White, reverse creamy white	Aerial ,phototrophic, hyline, non-septate, having single sporangia Sporangium:17-23 µm Color: light yellow, ovoid	Sporangio-spores: yellow, globose,	No	2-5 µm	*Phycomyces microsporous*

Table 3. Isolated bacterial and fungal species with FCBP accession numbers

Microbial group	Code of Isolates	Species	Accession nos. of Isolates
Bacteria	B-1	*Acidovorax facilis*	FCBP-373
	B-2	*Aminobacter aminovorans*	FCBP-374
	B-3	*Acidiphilium facilis*	FCBP-378
	B-4	*Burkholderia cepacia*	FCBP-526
	B-5	*Azomonas* sp.	FCBP-527
	B-6	*Xanthobacter flavus*	FCBP-528
	B-7	*Azomonas insignis*	FCBP-529
Fungi	F-1	*Alternaria alternata lycopersici*	FCBP-1315
	F-2	*Alternaria tenuissima*	FCBP-1316
	F-3	*Phycomyces microspores*	FCBP-1317

Table 4. Pathogenecity test on healthy tomato fruits

Species Name	Pathogenecity result
Alternaria alternata lycopersici	+
Alternaria tenuissima	+
Phycomyces microspores	-
Acidovorax facilis	+
Aminobacter aminovorans	+
Acidiphilium facilis	-
Burkholderia cepacia	+
Azomonas sp.	+
Xanthobacter flavus	+
Azomonas insignis	+

Key:
+ = Isolates grow with a similar growth characteristic features to the original diseased samples
-= Isolates not able to grow on the sample

horizontal contamination by the already spoilt fruits as Jay (2003) observed that most spoilage organisms may be present on fruits and vegetables from the farm, during harvest operations, and this may result in post harvest contamination and spoilage of these fruits and vegetables. The present and subsequent spoilage due to these fungi, if not checked could lead to serious economic loss and possible health hazards when these fruits are consumed. Various forms of microorganism were isolated from exposed sample source after some weeks of this study. This shows the presence of some spoilage microorganisms such as *X. flavus*, *A. insignis*, *A. facilis* that were associated with the tomato fruit. This is consistent with the study of Herson and Hullard (1980) which demonstrated the

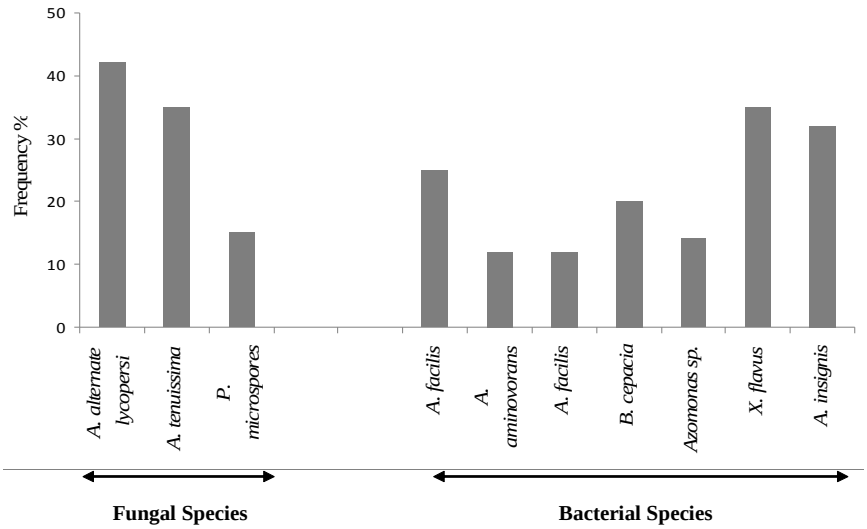

Fig. 1. Types of bacterial and fungal species obtained and their percentage frequency

involvement of this group of organisms in spoilage. Similarly, various pathogens can invade tomato based on some environmental conditions. Bacterial diseases of tomatoes can be some of the most serious and destructive diseases affecting both field- and greenhouse grown crops (Ajayi, 2013). Based on some routine laboratory measures as prescribed by Cheesebrough (2004) the sample were examined for the microbial load. Problems of food spoilage and deterioration can be curtailed by adequate processing of tomato and sterilization of some facilities on industrial basis (Todar, 2012).

CONCLUSION

The microbial load including fungal and bacterial isolates from stored tomatoes are at close range to those isolated from the tomatoes sources which still shows the natural protective lactic acid components of this food source. So, tomatoes should be well protected from contaminants to limit proliferation of some spoilage microbes that can use tomatoes as vehicle of infection. Also the technology of packing and storage should be improved by taking adequate precautions to ensure safety during the process and will enhance nutritive consumption of food source to promote health. Moreover, tomato fruits should be properly refrigerated and should be discarded of there are any changes notice in the color or taste of the fruit as will be hazardous to human health.

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