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MICROBIAL SPOILAGE AND PATHOGENS IN VEGETABLES COLLECTED FROM VEGETABLE MARKET

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ABSTRACT:

Vegetable products have dramatically increased in India during the past few decades. It is also estimated that about 20 % vegetables produced is lost each year due to spoilage by insects and microorganisms. This present research work addressed the characteristics of spoilage microorganisms associated with vegetable categories including spoilage mechanisms, spoilage defects, prevention and control of spoilage and methods for detecting spoilage microorganisms. In this present study, an attempt was made to isolate and identify the bacteria and fungi from the spoiled vegetables like brinjal, bitterguard, onion, carrot, tomato, potato, beetroot and radish. The vegetable samples were collected from Chidambaram vegetable market area, Tamil Nadu. Isolation was carried out using Pour plate technique. The bacterial isolates were identified as *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Vibrio cholerae* and *Salmonella typhi* by using preliminary tests, plating on selective media and biochemical tests. The fungal isolates were identified as *Aspergillus niger, Penicillium expansum* and *Mucor racemosus* based on Lactophenol cotton blue staining and plating on Sabouraud's dextrose agar.

KEY WORDS: Vegetables, Spoilage, Bacteria and Fungi.

INTRODUCTION :

Vegetables are related to outbreaks of food borne illness in several countries and also the organisms concerned embrace microorganism, viruses and parasites. These outbreaks vary in size from some persons affected to several thousands. Contamination of vegetables could happen the least bit stages throughout pre and post-harvest techniques (De Roever, 1999). Cultivation and operation or preparation the vegetables area unit to blame for this contamination (Sumner and Peter, 1997). Unsafe water used for rinse the vegetables and sprinkling to stay them contemporary is additionally a supply of contamination (Mensah et al., 2002). different potential sources of microorganisms embrace soil, ordure (human and animal origin), water (irrigation, cleaning), ice, animals (including insects and birds), handling of the merchandise, gather and process instrumentation and transport (Johannessen et al., 2002). Microorganisms capable of inflicting human ill health et al. whose food borne illness potential is unsure, like Aeromonas hydrophila, Citrobacter freundii, Enterobacter cloacae and enteric bacteria sp. has been isolated in lettuce and dish vegetables (Francis et al., 1999).

Foodborne microorganism pathogens ordinarily detected in contemporary vegetables were coliform microorganism, E. coli, staph aureus and enterobacteria sp. (Tambekar and Mundhada, 2006). Coliforms area unit facultative anaerobic, Gram negative, non-spore forming rods that ferment milk sugar with gas formation at intervals forty eight hrs once grownup in milk sugar broth at thirty five °C. {they area unit|they're} commonly-used microorganism indicator of hygienical quality of foods associate degreed water and thought of as an indicator of microorganism pollution and that they are common inhabitants of animal and human guts (Tortora, 1995). carboxylic acid microorganism were detected in nearly each fresh product, as well as honeydew melon, papaya, pineapple, cantaloupe, cabbage, carrots, chicory, celery, bell peppers, and varied dish mixes (Jacxsens et al., 2003). mould spoilage of contemporary turn out, particularly contemporary fruit is caused by species of genus Penicillium, Phytophthora, Alternaria, Botrytis, Fusarium, Cladosporium, Phoma, Trichoderma, genus Aspergillus, Alternaria, Rhizopus, Aureobasidium and Colletotrichum. The symptoms embrace visible growth, rots and discoloration, like blue mould rot, grey mould rot, botrytis rot, and plant disease. Like yeasts, mould populations are reportable in varied varieties of fresh fruits and vegetables (Tournas, 2005), mould populations are reportable in varied varieties of fresh fruits and vegetables (Hagenmaier and Baker, 1998) and visual molds have resulted in uneatable fresh fruits, like strawberry, honeydew, pineapple, and cantaloupe (O'Connor-Shaw et al., 1996; Ukuku and Fett, 2005).

2. MATERIALS AND METHODS

2.1. Collection of samples

The spoiled vegetable samples were collected from vegetable market at Chidambaram, Tamil Nadu, India for the isolation and identification of bacterial and fungal isolates. The collected samples were stored in the refrigerator for further experimental work.

2.2. Isolation of bacterial and fungal population

The bacterial and fungal species were isolated by Serial dilution technique. One gram of spoiled vegetables were aseptically transferred into 100 ml of sterile distilled water and shaked well. The dilutions were made into 10-1. From this dilution, again 1 ml of sample was transferred into 10 ml of sterile distilled water and the dilutions were made into 10^{-2}. Likewise, the samples were serially diluted upto 10^{-6}. Pour plate technique was used to isolate bacterial and fungal isolates in Nutrient agar and Rose Bengal agar respectively. The inoculated plates were incubated at 37 °C for 24 hours (in Nutrient agar for bacteria) and room temperature for 3 days (in Rose Bengal agar for fungi). The bacterial and fungal species were enumerated by using Qubec colony counter and expressed as cfu/ml. The isolated colonies from Nutrient agar plates and Rose Bengal agar plates were sub-cultured into agar slants and stored at 4 °C for further identification.

2.3. Identification of bacterial and fungal isolates

Identification of the different bacterial isolates was carried out by the routine bacteriological methods i.e., By the colony morphology, Preliminary tests like Gram staining, Capsule staining, Endospore staining, Motility, Plating on selective media and by performing biochemical tests. Identification of purified fungal cultures were characterized by their morphology, hyphal characteristics, presence or absence of asexual spores, arrangement of conidia and reproductive structures (Alexopoulos and Mims, 1979, Beisher, 1991) and by performing Lactophenol cotton blue mount.

3. RESULT AND DISCUSSION

Vegetables could also be contaminated with morbific microorganisms throughout growing within the field or throughout harvest, post harvest, handling, process and distribution. Therefore, vegetables might act as a reservoir for several microorganisms from that they're going to be colonised within these vegetables and infect vulnerable host. nearly any able to eat vegetables that are contaminated with pathogens either from the setting or from human or animal excretory product or through storage, process and handling may doubtless cause unwellness (Beuchat, 2002). the massive variety of Aerobic Plate Count (APC), indicator organisms (coliforms and enterics coli) and pathogens (Staphylococcus aureus) detected within the food samples surveyed during this investigation unconcealed that the contamination of those foods by morbific organism might gift a possible risk to shoppers in Chidambaram particularly in Annamalai Nagar space.

In several countries, waste used for irrigation of vegetables is predicated on the worth of its content and constituents that square measure used as fertilizers. additionally, waste conjointly contains salts, noxious gold-bearing compounds and morbific organisms which can be harmful to the soil, crops, grazing animals and human health (Rosas and Coutino, 1984). The study of Halablab et al. (2010) unconcealed that the most important cause for contamination of vegetables in Bekaa is that the irrigation with waste wherever the transported pathogens from waste might survive in soil and crops which is able to successively be transported to shoppers might doubtless cause varied diseases.

during this gift study, the morbific bacterium and fungi were isolated from spoiled vegetables collected from Chidambaram vegetable market. 5 microorganism isolates and 3 fungous isolates were known by Pour plate technique. the whole microorganism and fungous population gift within the vegetable sample was calculable and therefore the results were showed in Table - one. The fungous population was terribly high in tomato (31 × 10-3 and twenty eight × 10-4 cfu/g) followed by onion, brinjal, bitterguard, carrot, potato, beet root and radish. The microorganism population was terribly high in carrot (24 × 10-5 and seventeen × 10-6 cfu/g) followed by bitterguard, onion, tomato and

Solanum melongena.

Total coliform counts is thought of as a hygiene indicator, particularly for soiled contamination. Their presence indicates that pathogens can be gift thanks to soiled contamination by human, animal or irrigation water (Vishwanathan and Kaur, 2001). during this investigation, the extent of coliforms altogether vegetable samples ranged from a pair of.0 to 10.71 log10 cfu g-1. Similar findings had been according by Viswanathan and Kaur (2001) wherever the coliform counts of dish vegetables ranged from half dozen.0 - 9.0 log10 cfu g-1. moreover, Fang et al. (2003) obtained a variety from a pair of.3 to 7.55 log10 cfu g-1 of coliforms altogether vegetable samples in Taiwan. Nguz et al. (2005) in Republic of Zambia found a variety of coliform counts on vegetable merchandise between a pair of.2 and 5.9 log10 cfu g-1 and Aycicek et al. (2006) obtained a variety of total count of coliforms on vegetable samples from three.0 to 6.9 log10 cfu g-1. However, the whole coliform according by Soriano et al. (2000) ranged from zero.47 to 3.38. moreover, Joseph Eggleston Johnston et al. (2005) according a coliform level for all inexperienced greens and herbs of but one to four.3 log10 cfu g-1 that is in agreement with Joseph Eggleston Johnston et al. (2006) that according a coliform forecast vegetable turn out collected from u. s. were but one.0 to 4.5 log10 cfu g-1.

In this present study, five different bacteria were isolated from the spoiled vegetables. Based on preliminary tests, plating on selective media and biochemical tests, they were identified as *Vibrio cholerae* (Onion, Carrot and Tomato), Pseudomonas aeruginosa (Brinjal, Carrot, Potato, Beet root and Radish), *Salmonella typhi* (Brinjal, Bitter guard, Onion and Tomato), *Staphylococcus aureus* (Brinjal, Bitter guard, Carrot, Tomato and Radish) and Escherichia coli (Brinjal, Bitter guard, Onion, Carrot, Tomato, Potato, Beet root and Radish). The characteristics of the isolated bacterial isolates were given in the Table - 2 to Table - 6. Three different fungi were isolated from the spoiled vegetables. Based on Lactophenol cotton blue staining and colony morphology on Sabouraud's dextrose agar, they were identified as *Aspergillus niger* (Onion, Tomato, Potato and Radish), *Penicillium chrysogenum* (Brinjal, Carrot, Potato, Beet root and Radish) and Mucor racemosus (Brinjal, Bitter guard, Carrot and Beet root) (Table - 7).

Table - 1: Total bacterial and fungal population present in spoiled vegetable samples

Sample	Fungal population (cfu/g)		Bacterial population (cfu/g)	
	10^{-3}	10^{-4}	10^{-5}	10^{-6}
Brinjal	28	26	20	17
Bitterguard	27	24	23	16
Onion	30	28	22	15
Carrot	26	24	24	17
Tomato	31	28	21	18
Potato	25	22	18	15
Beet root	23	19	15	12
Radish	21	17	14	10

Table - 2: Characterization of *Vibrio cholerae*

Test	Results
Gram staining	Gram negative, comma shaped rods
Motility	Motile
Catalase	Positive
Oxidase	Positive
Nutrient agar	Circular, moist, smooth, translucent and bluish tinge colonies
MacConkey agar	Smooth, gloosy and late lactose fermenting colonies
TCBS agar	Small yellow colonies
Glucose fermentation	Acid and gas produced
Lactose fermentation	Acid and gas produced
Sucrose fermentation	Acid and gas produced
Mannitol fermentation	Acid and gas produced
Indole	Positive
Methyl Red Test	Negative
Voges Proskauer Test	Positive
Citrate utilization	Negative
Urease	Negative
TSI	Acid butt, alkaline slant, No H_2S and no gas produced

Table - 3: Characteristics of *Pseudomonas aeruginosa*

Test	Results
Gram staining	Gram negative slender rods
Motility	Actively motile
Catalase	Positive
Oxidase	Positive
Nutrient agar	Blue coloured diffusible pigment producing colonies
MacConkey agar	Non-lactose fermenting colonies
Glucose fermentation	Not fermented
Mannitol fermentation	Not fermented
Dextrose fermentation	Not fermented
Sucrose fermentation	Not fermented
Indole	Negative
Methyl Red Test	Negative
Voges Proskauer Test	Negative
Citrate utilization	Positive
Urease	Positive
TSI	Alkaline butt, alkaline slant. No H_2S and No gas production
O-F test	Oxidative
Casein hydrolysis	Positive

Table - 4: Characteristics of *Salmonella typhi*

Test	Results
Gram staining	Gram negative rods
Motility	Motile
Catalase	Positive
Oxidase	Negative
Nutrient agar	Circular, moist, smooth, translucent colonies
MacConkey agar	Non lactose fermenting colonies
SSA agar	Small black colonies
Glucose fermentation	Acid and gas produced
Lactose fermentation	Acid and gas produced
Sucrose fermentation	Acid and gas produced
Mannitol fermentation	Acid and gas produced
Indole	Negative
Methyl Red Test	Positive
Voges Proskauer Test	Negative
Citrate utilization	Positive
Urease	Negative
TSI	Acid butt, alkaline slant, H_2S and gas produced

Table - 5: Characteristics of *Staphylococcus aureus*

Test	Results
Gram staining	Gram positive cocci, arranged in clusters.
Endospore	No spores present
Motility	Non-motile
Catalase	Negative
Oxidase	Negative
Nutrient agar	Colonies are smooth and golden yellow
MacConkey agar	Lactose fermenting colonies.
Glucose fermentation	Acid produced
Mannitol fermentation	Acid produced
Sucrose fermentation	Acid produced
Dextrose fermentation	Acid produced
Indole	Negative
Methyl Red Test	Negative
Voges Proskauer Test	Positive
Citrate utilization	Positive
Coagulase	Positive
DNAase	Positive
Mannitol salt agar	Golden yellow colonies
TSI medium	No reaction
Urease	Negative

Table - 6: Characteristics of *Escherichia coli*

Test	Results
Gram staining	Gram negative straight rods
Motility	Motile
Catalase	Positive
Oxidase	Negative
Nutrient agar	Circular, smooth and colourless colonies
MacConkey agar	Smooth, gloosy and pink coloured lactose fermenting colonies
EMB agar	Small colonies with greenish metallic sheen
Glucose fermentation	Acid and gas produced
Lactose fermentation	Acid gas produced
Sucrose fermentation	Acid gas produced
Mannitol fermentation	Acid gas produced
Indole	Positive
Methyl Red Test	Positive
Voges Proskauer Test	Negative
Citrate utilization	Negative
Urease	Negative
TSI	Acid butt, alkaline slant, No H_2S and gas produced

Table – 7: Colony morphology of fungi isolated from Vegetables

Microscopic examination	Colony morphology on SDA plate
Aspergillus niger	
Conidiophore stipes smooth-walled, hyaline or pigmented. Vesicles sub-spherical, conidial heads radiate. Conidiogenous cells biseriate. Medulla twice as long as the phialides. Conidia brown, ornamented with warts and ridges. Hyphae was septate.	Colonies black, consisting of a dense felt of conidiophores.
Penicillium chrysogenum	
Septate hyphae with branched or unbranched conidiophores which have secondary branches known as medulla. On the medulla flask shaped sterigmata are arranged that bear unbranched chains of round conidia. Entire structure forms a brush border.	Colony surface at first appears white then becoming powdery bluish green with a white border. Some species differ in gross appearance. Reverse side was white.
Mucor racemosus	
Sporangiophores are long, often branched and bear terminal round sporangia filled with spores. Hyphae are non-septate and no rhizoids.	Colonies quickly covers the agar surface with white fluffy mycelia but later turns grey, reverse side is white.

4. CONCLUSION

From this present study, I concluded that the spoiled vegetables collected from market harbor the pathogenic bacteria *(Pseudomonas aeruginosa, Escherichia coli, Vibrio cholerae and Salmonella typhi*) and fungi (*Aspergillus niger, Penicillium chrysogenum and Mucor sp.*) which cause harmful diseases to human beings. It is must to dispose the spoiled vegetables from the market to prevent the spread of disease causing microorganisms and spoilage of fresh vegetables.

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