

Research Article



Study of Microbial Contamination in Local Fruits and Vegetables Samples of Solan, Himachal Pradesh

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ABSTRACT

Food Microbiology is the study of food micro-organisms. In the present study, we have chosen to isolate and identify bacteria and fungus from various fruits and vegetables. The various fruits had been collected from local market of Solan (H.P). After the complete identification, the isolated microorganism was identified as bacteria i.e. *Enterobacter*, *Xanthomonas*, *Erwinia*, *Acidovoraxavenae*, *Pseudomonas*, *Staphylococcus*, *Bacillus*, *Streptococcus* and fungus i.e. *Cercospora mamaoms*, *Colletotrichum*, *Fusarium*, *Alternaria alternate*, *Aspergillus niger*. Chances of contamination can be reduced by the good hygienic conditions.

Keywords: Microorganism, Bacteria, Fungus, contamination, food.

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INTRODUCTION

Consumption of fruits and vegetables products has dramatically increased in India by more than 30% from past two decades. It is also estimated that about 20% of all fruits and vegetables produced is lost each year due to spoilage. Pasteur is known as the founder of food microbiology and microbial science¹. The focus of this project is to provide a general background on microbiological spoilage of fruit and vegetables products that are organized in three categories. Fresh whole fruits and vegetables, fresh cut fruits and vegetables and fermented or acidified fruits and vegetables. Foods is any substance, usually composed primarily of carbohydrates, fats, water and proteins, which can be eaten or drunk by mammals for nutrition or pleasure². During the period 1970-2004 US per capita consumption of fruit and vegetables increased by 19.9% to 694.3 pounds per capita per year. Fresh fruits and vegetables consumption increased 25.8% and 32.6% respectively. According to USDA- Economic Research service study in 1995 18.9 billion pond of fresh fruits and vegetables were lost annually due to spoilage. Most microorganism that are initially observed on whole fruit and vegetable surface are soil inhabitants members of very large and diverse community of microbes that collectively are responsible for maintaining a dynamic ecological balance with most

agricultural systems³. There are many microorganism and fungi that cause spoilage of fruits and vegetables⁴. Fruits and vegetables are generally contaminated by bacteria including species of *Bacillus*, *Enterobacter*, *Pseudomonas*, *Staphylococcus*, *Streptococcus* etc⁵. The main pathogenic bacteria associated with spoilage of fruits and vegetables are: *Salmonella*, *Bacillus*, *Staphylococcus*, *Xanthomonas*, *Streptococcus*, *Pseudomonas sp.*, *Coccus*, *E.coli*, *Aeromonas*, and *Clostridium*. The fungi that associated with spoilage of fruit and vegetable are *Yeast*, *Penicillium*, *Rhizopus*, *Mucor* etc⁶. The present study for the examination of fruits and vegetables are collected from Solan city of Himachal Pradesh of pathogenic bacteria. Microorganisms may cause the food to undergo decomposition which results in spoilage and beneficial product may yield⁷.

MATERIALS AND METHODS

Samples (Fruits and vegetables) swab, Petri dish and others glass wares, burner, Autoclave, hot air oven, Incubator, Laminar air chamber, sterile needle, sterile inoculation loop, Cotton, Glass slides, cover slips, sterile test tubes, Durham tubes, micropipette. Different types of media like nutrient agar, peptone water, Glucose phosphate broth, Simmon's citrate agar, Christensen's medium, Nitrate broth, Lactose broth, Glucose broth, Mannitol broth, Sucrose broth were used.

Methods

Collection of samples

The samples (i.e. spoiled fruits and vegetables) were collected from the market in sterile, disposable, air tight plastic containers. The same were picked up from market with gloved hands. Then isolation of microorganism from



the sample is done. Gram's staining is performed to classify the bacteria. Different biochemical test like Indole test, Methyl red test, Voges Proskauer, citrate utilization test, catalase test, urase test, nitrate reduction test, oxidase test, lactose fermentation, glucose fermentation, mannitol, Sucrose fermentation were performed

RESULTS AND DISCUSSION

Collection of food samples

The Samples of Vegetables and Fruits were taken from Solan. The total 10 samples were collected. The samples include various rotten fruits like Grapes, Banana, Orange,

Papaya, Mango, Lemon and rotten vegetables like Ladyfinger, Onion, Potato, Bitterguard. Samples were taken from college mess and local market (Sabji Mandi). The presence of *Coliform* and *Staphylococci* in kinnow and mandarin juices and fruits in Patiala city were reported⁸.

Isolation of microorganisms from various food samples

Various microorganisms were isolated from the different samples and cultured on the nutrient agar medium and were incubated at 37°C for 24 to 48 hours. After incubation the colonies of different microorganisms appeared on nutrient Agar medium on the petriplates (Fig.1)

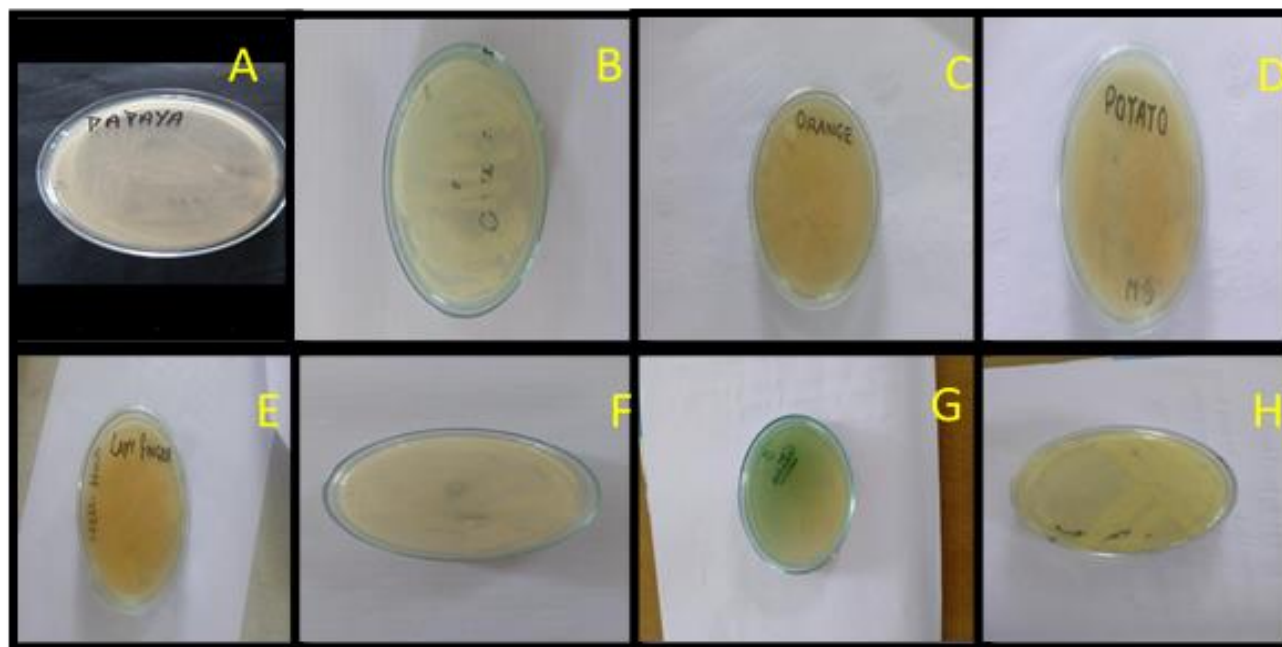


Figure 1: (A-H) Nutrient agar plates for various bacterial cultures from various samples

A) Papaya B) Onion C) Orange D) Potato E) Ladyfinger F) Banana G) Lemon H) Grapes

Identification of microorganisms

Identification of microorganisms was done with the help of gram's staining and biochemical tests. The results

revealed that all the isolates were Gram-ve, rod shaped in Papaya, Orange, Onion, Ladyfinger and Lemon whereas Gram+ve and round shaped in Grapes (Table1).

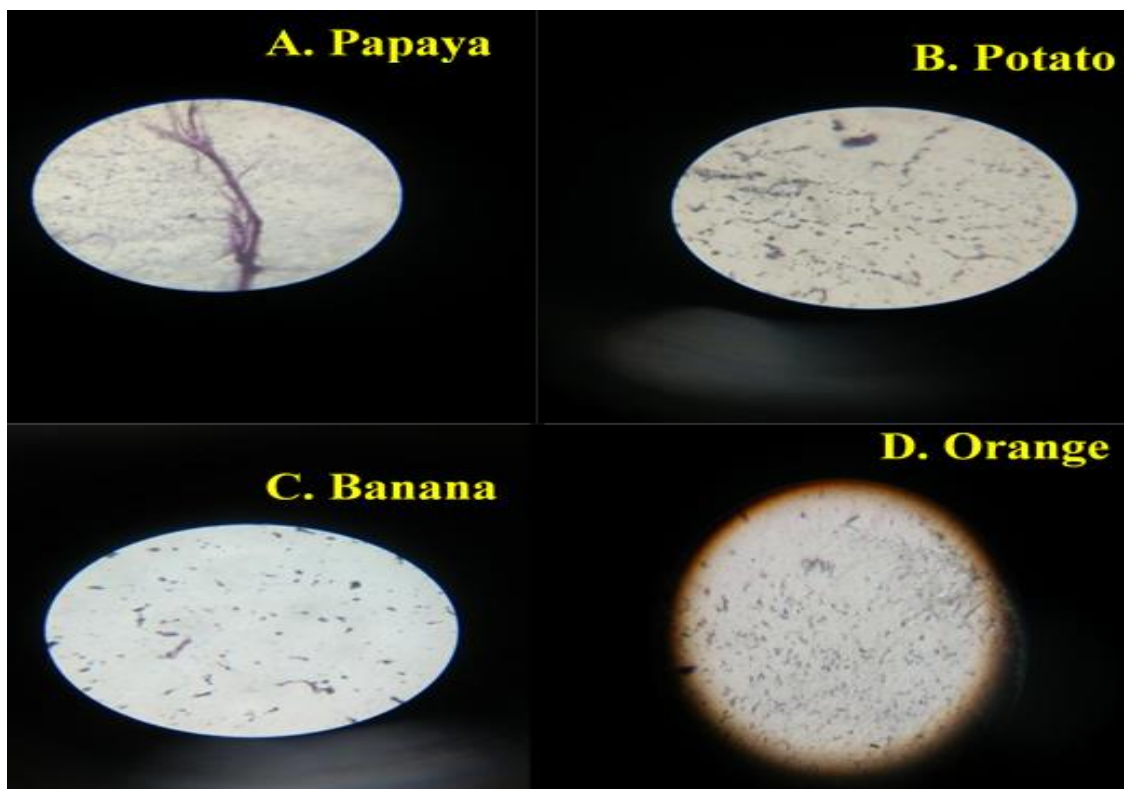
Table 1: Cultural and Staining characteristics of Bacterial Isolates

Sr. No.	Samples	Colony morphology on nutrient agar plate	Gram's staining
1.	Papaya	Yellow pigmented colonies	Gram-ve, rod shaped bacteria
2.	Orange	Mucoid, convex ,yellow type of colonies	Gram-ve, straight rod shaped bacteria
3.	Onion	Mucoid, slightly yellow colonies	Gram-ve, rod shaped bacteria
4.	Ladyfinger	Bright yellow colonies presented as singly or 2-3 rod chains	Gram-ve, rod shaped bacteria
5.	Lemon	Blue to blue-green pigmented colonies	Gram-ve, rod shaped with polar flagella
6.	Grapes	Golden appearance of colonies forming grape like clusters	Gram+ve, rounded, convex shaped bacteria

The staining of fungal is done with the help of LCB (Lactophenol Cotton Blue) stain (Table2)

Table 2: Cultural and staining characteristics of Fungal Isolates

Sr. No.	Organisms	Colony Morphology on Potato Dextrose agar/colonies	LCB (Lactophenol Cotton Blue)
1.	Papaya	Grey colored colony	LCB stained, branched, septate, slender, intercellular mycelium
2.	Potato	Blackish white colony on nutrient agar	LCB stained
3.	Banana	Yellow colored, single cell colony	LCB stained
4.	Orange	Brownish black colony	LCB stained
5.	Onion	White colonies	LCB stained, forming mats of hyphae
6.	Bittergourd	Red/white colored unicellular colonies	LCB stained, globose, ellipsoid to elongate

Stained fungal colonies from different samples (Fig 2)**Figure 2:** (A-D) Showing different types of fungal colonies**Biochemical tests for various identified microorganisms (Bacteria)****Table 3:** Biochemical tests for various isolated microorganisms (Bacteria) from various samples

Sr. No.	Samples	Indole	MR	VP	Citrate	Catalaze	Oxidase	Nitrate	L	G	S	M
1.	Papaya	+ve	+Ve	+ve	+ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve
2.	Orange	+ve	+ve	+ve	+ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve
3.	Onion	-ve	+ve	-ve	+ve	-ve	+ve	-ve	-ve	+ve	+ve	-ve
4.	Ladyfinger	+ve	+ve	-ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
5.	Citrus	-ve	-ve	-ve	+ve	+ve	+ve	-ve	-ve	-ve	-ve	-ve
6.	Grapes	-ve	-ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	-ve
7.	Mango	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
8.	Bittergourd	+ve	-ve	+ve	+ve	+ve	+ve	-ve	-ve	-ve	-ve	-ve

AG= Acid gas formation, A= Acid only, NA= No acid gas formation, MR= Methyl red, VP= Voges Proskauer, L= Lactose, G= Glucose, S= Sucrose, M= Mannitol.

Identified microorganisms

In the present microbiological auditing of food, total 10 samples were taken from the (Sabji Mandi) Solan. Mainly

microorganisms present in food were *Enterobactor*, *Erwinia*, *Bacillus*, *Staphylococcus*, *Xanthomonas*, *Stpeptococcus*, *Pseudomonas*, *Coccus*. The fungi that associated with spoilage of fruit & vegetable are

Saccharomyces cerevisiae, *Alternaria alternate*, *Fusarium oxysporium*, *Aspergillus niger*, *Cercospora*, *Colletotrichum*.

The purpose of this study was to isolate and examine the fruits and vegetables products for the presence of Bacteria and Fungus that spoils fruits and vegetables. This study may help us to determine the extent of food contamination at Solan City and to understand resistance

pattern of bacteria against various antibiotics. Keeping this in view, the total no. of isolation of rotten fruits and vegetables were collected from local market of Solan city of (H.P) which includes 6 rotten fruits and 4 rotten vegetables. Similar results were reported by, they also isolate *Staphylococcus* from grapes and isolate control of green mould of lemons with *Pseudomonas* ⁹.

Table 4: Identification of various microorganisms in various samples:

Sr. No.	Samples	Isolated Bacteria	Isolated Fungus
1.	Papaya	<i>Enterobacter</i>	<i>Cercospora mamaoms</i>
2.	Potato	<i>Xanthomonas campestris</i>	<i>Colletotrichum coccodes</i>
3.	Banana	–	<i>Fusarium oxysporium</i>
4.	Ladyfinger	<i>Acidovorax avenae</i>	–
5.	Onion	<i>Erwinia</i>	<i>Aspergillus niger</i>
6.	Orange	<i>Xanthomonas campestris</i>	<i>Alternaria alternate</i>
7.	Grapes	<i>Staphylococcus</i>	–
8.	Lemon	<i>Pseudomonas</i>	–
9.	Mango	–	<i>Saccharomyces</i>
10.	Bitterguard	–	<i>Saccharomyces</i>

It is evident from table that various food samples contained large number of contaminating bacteria.

CONCLUSION

In the present study a total 10 samples of 5 fruits and 5 vegetables were collected from local market of Solan (H.P).The collected samples were processed through enrichment technique. Enriched cultured were streaked on selective media i.e. nutrient agar, then incubated for (24-48) hours at 37 °C. All the isolates were further identified on the basis of culture characteristics, Gram's staining, LCB and the Biochemical tests which showed the presence of Bacteria and Fungus. Out of 10 samples 7 showed the presence of Bacteria and 6 shows the presence of Fungus. After the complete identification the isolated microorganism were identified as Bacteriae: *Enterobacter*, *Xanthomonas*, *Erwinia*, *Acidovorax avenae*, *Pseudomonas*, *Staphylococcus*, *Bacillus*, *Streptococcus* and Fungus: *Cercospora mamaoms*, *Colletotrichum*, *Fusarium*, *Alternaria alternate*, *Aspergillus niger*. A good hygienic condition can reduce the chances of contamination. Such studies should be carried out to reduce the chances of contamination from the food.

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