

Microbial Contamination of Raw Fruits and Vegetables

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Abstract

India is currently producing over 77 million tone fruits and over 150 million tone vegetables per year, and is one of the leading country in horticulture. The cases of food borne illness are also increasing every year, which needs our special attention. Raw fruits and vegetables were collected from market to identify the presence of any pathogenic microbes (Bacteria and fungi). As a result, some highly infectious bacterial genera like *Corynebacterium*, *Streptococcus* and *Staphylococcus* were found to be dominantly present on outer surface of these fresh produces.

Key words: Food borne illness, Fruits and Vegetables, Bergey's Manual of Systematic Bacteriology

Introduction

Recent improvement of the standard of life in India is associated with diversification of food needs including availability of fresh produce everywhere (mainly big cities) and throughout year. All of these require proper transport and storage facility. India is the second largest producer of fruits and vegetables in the world and accounts for about 15% of the world's total production. Fruits and vegetables plays key role in growth and development of body and in prevention of many diseases due to high nutritive value.

Contamination of fresh produce during handling process is a common problem and it is usually ignored the use appropriate techniques of decontamination. If consumed, contaminated fruits and vegetables can lead to food poisoning, because of the presence of intestinal infectious microbes on the outer surfaces. The surrounding environment is always changing with more drug resistant microbes in air, soil and water. Such changes can directly affect food poisoning patterns, such as frequency of incidents, number of patients involved, and mortalities.

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The majority of diseases associated with fresh fruits and vegetables are primarily those transmitted by the fecal-oral route, and therefore, are a result of contamination at some point in the process (De Roeber, 1998). Gastroenteritis, Giardiasis, Hepatitis A, Hepatitis E, Shigellosis (bacillary dysentery), Typhoid fever, *Vibrio parahaemolyticus* infections and Cholera are very evident examples of fecal-oral route transmitted diseases. Microbes can cause food poisoning in two different ways. Some infect the intestines, causing inflammation and difficulty absorbing nutrients and water, leading to diarrhea. Other produce chemicals in foods (toxins) those are poisonous to the human digestive system. When eaten, these chemicals can lead to nausea and vomiting, kidney failure, and even death.

Cultivation of trees/plants of fruits and vegetables in the areas with a presence of potentially harmful microbes like sewage, sludge, animal feces, and toxic weeds can lead to the contamination during growth, harvesting and storage; also the places where operations with livestock or birds are made can cause the contamination.

In India, the presence of Coliforms and Staphylococci in kinnow and mandarin juices in Patiala city were reported (Ganguli et al. 2004). Similarly Coliforms were

observed in fresh fruit and vegetable juices sold by the street vendors of Nagpur city (Titarmare, Dabholkar and Godbole, 2009). Viswanathan and Kaur,(2001) examined different salad vegetables such as carrots, radishes, tomatoes, lettuce, cabbage, cucumbers, coriander and reported presence of *S.aureus*, *E. coli*, *Enterobacter* spp., *Klebsiella* spp., *S. typhi*, *Serratia* spp., *Providencia* spp. and *P. aeruginosa*.

The present study focus on Superficial extraction of microbes (particularly bacteria and fungi) from fresh fruits and vegetables, which can help in observing common causes of contamination of fruits and vegetables and also the presence of any pathogenic microbe associated with human diseases.

Materials and Methods

For collection of raw vegetables and fruits to identify dominating microbial (bacterial and fungal) communities, the main fruits and vegetables market of Bikaner city (Rajasthan, India) was selected. It is centrally located big sale market of Bikaner, here we could observe fruits and vegetables grown nearby Bikaner regions as well as imported from other states of the country.

Varieties of different fresh produce like Apple, Mango, Papaya, Lemon, Spinach, Tomato, Onion, Luffa, Chili, Cucumber and Potato were selected, which are a very common part of our daily meals. To isolate bacteria and fungi from the samples, all samples were chopped up and placed into a sterile water blank followed by serially dilution and inoculation on separately on Nutrient agar triple sugar agar media

Bacterial colonies in pure form were collected using streak plate method. Based on phenotypic colony characters on media plates, total 112 different bacterial colonies were isolated in pure forms, which were further identified and characterized using different techniques.

The characterization and identification of bacteria was done with the help of Bergey's Manual of Systematic Bacteriology, It is a very useful tool for identifying bacteria. It is grouped by families of bacteria and lists common features of bacteria in a given family. It can eventually help narrow characteristics down to a single genus, if not species. It is very useful in microbiology lab classes when trying to determine the genera/species of unknowns. Primary characterization of bacteria was done by visual colony characters, microscopic characterization (Gram and Endospore staining) and some other tests like motility test, oxygen tolerance test, catalase test and the secondary identification of the isolates was carried out on the basis of biochemical tests (IMViC tests) and carbohydrate utilization test.

The isolation of fungi was done with Potato Dextrose Agar media, after isolation of all fungal colonies in pure culture, all of them were visually examined for

phenotypic characters like color, texture, exudates, growth zones, aerial/submerged hyphae, and macroscopic structures such as ascocarps, pycnidia, sclerotia, sporodochia, and synnemata.

The final identification of fungi was performed by morphological examination of microscopic structures, particularly the spores and the conidia, using lactophenol and cotton blue.

Result and Discussion

In search of finding any pathogenic microbes (bacteria and fungi) on fruits and vegetables purchased from market, which could be responsible for food poisoning, Different techniques were used for isolation and characterization was done with help of phenotypic and biochemical characters. The total number of isolated bacterial colonies from all fruits and vegetables (Apple, Mango, Papaya, Lemon, Spinach, Tomato, Onion, Luffa, Chili, Cucumber and Potato) was 112. Which were further subjected for identification with the use of Bergey's Manual of Systematic Bacteriology. The following table reveals the number of isolated microbial colonies from different fruits and vegetables.

* To see all results of phenotypic and biochemical tests please check supplementary table 1 and 2

Name of fruits and vegetables involved in study	Number of associated bacterial colonies found	Number of associated fungal colonies found
Apple	7	1
Mango	10	2
Papaya	14	2
Potato	6	3
Spinach	15	3
Lemon	7	2
Tomato	8	1
Onion	8	1
Luffa	18	2
Chili	9	1
Cucumber	10	2

Table 1. Number of bacterial and fungal colonies isolated from all selected fruits and vegetables

According to the data present in Table 1, the papaya and luffa were found to be most contaminated with bacterial contamination and potato and spinach were with highest

fungus colonies. All isolated microbial communities identified using various methods. The subsequent table reveals the total number of bacterial genera found on fruits and vegetables.

Bacterial Genus	No. of strains identified
<i>Bacillus</i>	14
<i>Lactobacillus</i>	8
<i>Corynebacterium</i>	35
<i>Streptococcus</i>	12
<i>Staphylococcus</i>	25
<i>Micrococcus</i>	6
<i>Pseudomonas</i>	5
<i>Enterobacteriaceae</i>	7

Table 2. Number of bacterial genus found to be present on fruits and vegetables

All identified bacterial genus were also tested for their intrinsic antibiotic resistance pattern (IPR) against variable concentrations (10 g ml⁻¹ to 20 g ml⁻¹) of five antibiotics viz., Tetracycline, Kanamycin, Chloramphenicol, Ampicillin and Vancomycin, and all of them were found to be resistant from these antibiotics.

The identification of isolated fungi was done with help of microscopic characterization and all dominated fungal communities were found to be *A.flavus*, *Rhizopus*, *Drechslera*, *A.niger*, *Fusarium*, *Absidia*, *Saccharomyces cerevisiae*, *Penicillium*, *Phoma pycnidia*, *Chaetomium*, *Trichoderma*, *Candida albicans*, *Mucor* and *Arthrospore*.

The result of following study reveals the presence of some highly pathogenic bacteria and fungi on raw fruits and vegetables purchased from market. These microbes are generally associated with some acute to chronic diseases. For example *Pseudomonas* is an opportunistic human pathogen and a common food spoiler. *Micrococcus* can lead to many infections like bacteremia, septic shock, septic arthritis, endocarditis and meningitis.

Corynebacterium is well known pathogen for diphtheria and *Streptococcus* is responsible for pneumonia. *Staphylococcus* is generally related to gastrointestinal infections because of its ability to produce very strong toxins. The group members of *Enterobacteriaceae* are widely known for intestinal infection and diarrhea.

Fungi are commonly not associated with food poisoning but if turned out to be poisonous, it can cause hallucination, nausea, vomiting, abdominal pain and diarrhea.

Conclusions

Raw fruits and vegetables purchased from the market can be associated with pathogenic microbial load, which should be cured before consuming them. The FDA, India (Food and Drug Association) does not recommend using any soap, cleaning agents or detergent to wash fruits and vegetables but thorough washing with fresh water can help to reduce the number of microbial communities. Moreover it can also help in increasing shelf life of fruits and vegetables in fridge.

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