

# **BACTERIAL FLORA OF VEGETABLES COLLECTED FROM THE SUPERMARKET AND VEGETABLE MARKET**

## **Abstract**

To prevent food borne diseases, microbiological control is very important in the food industry. Therefore, the present investigation was undertaken to conduct a microbiological quality assessment of fresh vegetables that were collected from several regions of Chandigarh. The purpose of this study was to determine the bacterial load and safety of vegetables presented for sale in the marketplace. Vegetable samples (tomato and capsicum) collected from two different sites (vegetables market and supermarket), were analyzed for their bacteriological contaminants following different procedures. The maximum bacterial count present in the vegetable market was compared to that of the super market. The micro biota of these vegetable samples was found to be dominated by *Staphylococcus* and *Bacillus*. *Bacillus* and *Staphylococcus* were detected in tomato and Capsicum respectively. *Bacillus* and *Staphylococcus* were included as the most abundant species and as pathogens of concern when working to improve the microbial quality of fresh vegetables. Our study demonstrated that the vegetables, sold in the vegetable market, were found to contain a higher microbial load that may represent a greater risk for human health. Hence, for many microorganisms, vegetables may act as a reservoir colonize and infect the host.

## **Keywords**

Microorganism, Identification, Biochemical test, Microbial load, Vegetables.

## **Introduction**

There are several pathogenic microbes that have found their way into fresh vegetables and fruits over the years leading to spoilage (Balali et al, 2020). Spoilage is the process in which fruits and vegetables (a great source of human healthy diet) are contaminated to the point that the product becomes unacceptable for human consumption. Some spoilage organisms such as yeast target fruits and vegetables due to their low pH which allows the bacteria to grow on the surface of the produce. Human infection and outbreaks are associated with the consumption of raw vegetables (Beuchat et al, 2022). Bacterial spoilage is the primary cause of tissue softening as the pectin is degraded. Micro biota is dominated by spoilage bacteria such as *Bacillus cereus*, *Salmonella*, *E.coli*, *Clostridium*, *Staphylococcus* and (Tambekar et al, 2006) and *Thiomargarita magnifica* (Volland et al, 2022). Food poisoning can become a major problem when items are not stored properly. Common bacteria that causes food poisoning are *Salmonella*, *Staphylococcus aureus* and *E.coil*. Bacteria are often present on the surface of vegetable and fruits like *Staphylococcus*, *Bacillus*, *E.coli*, *Clostridium*, and *Agrobacterium*.

Nutrients are necessary for the growth of these microbes. The substrate on which bacterial cultures are grown is known as the medium which provides both nutrition and pH to the organism. There are generally two different types of media utilized solid media and liquid media. Solid media are used for studying the cloning character of the

bacteria and liquid media are used for profuse growth such as with a blood culture. Chemical composition studies require two types of media a routine laboratory media and a synthetic media. Autoclave, dry heat, and radiation methods are common laboratory sterilization method.

Human pathogens commonly associated with fruits and vegetables include *Bacillus*, *Staphylococcus*, *E.coli*, and *Salmonella*. Microbes found on kitchen surfaces may have originated from a produce source. Fruits and vegetable contamination may occur at any stage from production to consumption. Fresh apples, peppers, tomatoes and capsicum are known to harbor large bacterial populations. The presence of *Staphylococcus* in retail food has already been reported in China (Shubo et al, 2015).

This study evaluates the diversity of the bacterial community found on the surface of vegetables found in the marketplace utilizing microbial plate counts and biochemical testing

## **Materials and Methods**

### **Location of samples**

Bacteria samples were cultured from fruits and vegetables purchased in the vegetable market sector 40 Chandigarh and a supermarket SCF4 sector 9D Chandigarh. *Bacillus* collected from the surface of tomato was cultured from the vegetable market fruit and *Staphylococcus* falling microorganisms were collected from the surface of a capsicum from the supermarket. Bacteria were collected from the surface of vegetables by using the cotton swabs. After swabbing, a zig zag line was drawn on agar petri plates. Close the lid with plastic wrap. After that petri dishes were incubated in inverted position. Temperature for incubation was 32°C. Bacterial colonies were appears after 2-3 days. Total 6 plates were prepared, 3 plates for the supermarket and 3 plates for the vegetable market.

### **Nutrient agar medium**

Bacteria falling on the surface of vegetables were measured via two types of agar media (nutrient agar media and peptone dextrose agar media) and two types of growth media (Mannitol salt agar and Luria Bertani). Nutrient agar media was utilized for the bacterial growth counts as it can effectively grow a variety of bacteria as well as fungi. Peptone dextrose agar was a general media utilized for the fungal isolation from plant samples. Organic compounds were used as a source of energy. Yeast extract peptone dextrose was used as a growth medium to grow Yeast. Mannitol salt agar media contains a high salt concentration and was used as a selective and differential growth medium in the laboratory. The identification of *Staphylococcus aureus* in clinical isolates requires a battery of tests which are costly in resources in a limited setting. Luria Bertani (LB) is a nutritionally rich medium for culturing bacteria. LB media allows for the plating of bacterial cultures and the growth of colonies.

### **Identification of bacteria**

Identification of bacteria was conducted via various physical and chemical methods.

## **A. Physical methods utilized**

### **1. Shape of bacterial cell- rods or cocci**

### **2. Gram staining**

It was used to distinguish between gram negative and gram positive groups by coloring the cell violet or red. This technique is useful for bacterial sample contamination assessment and observation of morphological features of bacteria that were isolated from mixed cultures (Moyes et al, 2009).

## **B. Biochemical testing included the**

### **1. Catalase test**

Catalase is enzymes that detoxify hydrogen peroxide into molecular oxygen and water (Gagnon et al, 1959). A Catalase test is positive if bubbles are observed after adding hydrogen peroxide. It should appear as if simple water was dropped on the smear. Catalase test is used for gram positive bacteria and can utilize to distinguish *Staphylococcus* and *Micrococcus*.

### **2. Citrate test**

Citrate test is used to determine the bacterial ability to use sodium citrate as the source of carbon. The citrate test is possible for those organisms which are capable of fermenting citrate. Positive test occurs when a particular color change is observed in the Simmons citrate tube and negative test was observed in case of no color change. The citrate test was used to distinguish between *Bacillus*, *Citrobacter*, *E.coil* and *Staphylococcus*.

### **3. Vogues -Proskauer (VP) test**

The ability of bacteria to convert glucose to acetoin creates a red/purple color near top of tube. Bacteria producing a negative Voges-Proskauer result exhibit no color change (Hasan et al, 2015). When potassium hydroxide is added into acetoin then acetoin is converted to diacetyl that later reacts with alpha-naphtol and make pink color compound.

### **4. Oxidase test**

This test is used to determine the presence of bacterial enzyme cytochrome oxidase (Tarrand et al, 1982). Isolated colony was picked up by using the loop from the fresh bacterial plate and rub it into filter paper and add 2 drop of oxidase reagent on the organism smear and observe for color change. In Positive test, there was dark purple color and no color in case of negative test.

### **5. Urease test**

Urease test identifies that organism that is capable of hydrolyzing urea to produce ammonia and carbon dioxide (Brink et al, 2019). Urease test is a biochemical test that detect the alkaline fermentation of urine with the resultant production. Rapids positive organism turned the entire medium pink within 24 hours. Negative organism produced no color change or yellow as a react of acidic production.

### **6. Methyl red test**

Many bacteria have ability to utilize glucose and converted into a stable acidic acid like acetic acids, formic acid or lactic acid, as the end product. Methyl red changes the color immediately and help to predict rather

it's basic or acidic. Bacteria metabolize the glucose in pyruvic acid and later pyruvic acid metabolize through the mixed acid pathway to produce stable acids. The acid production is different from species to species and depends on the enzymatic pathway present in the bacteria. Red color and yellow color or colorless appeared in case of positive and negative test respectively (In Atlasal oral microbiology).

#### **7. Nitrate reduction test**

Nitrate test is used to determine the presence and absence of nitrate in the solution. Nitrate test differentiate those bacteria that have ability to produce nitrate reductase enzymes. This enzyme hydrolyze nitrate to nitrite and nitrite further degrade into various nitrogen product like nitrogen oxides, nitrous oxides and ammonia. It can be performed by adding sulfanilic acid that will react with the nitrous acid and produce diazotized sulfanilic acid. This diazotized sulfanilic acid react with alpha-naphthylamine and form a red color compound in positive test. While no color was detected in case of negative test (Tlaieakhon et al, 2018).

#### **8. Carbohydrate test**

The microbes utilize carbohydrate to produce energy in form of ATP. Degradation of the monosaccharide, disaccharides and polysaccharides by microorganism under anaerobic condition is carried out in the fermentation. Its end product can either be organic acids like formic, lactic, or organic acids. The fermentation medium contain carbohydrate with PH indicator. After fermentation, organic acid product turns into yellow color with the reduction of PH. When the medium changes to yellow color, it means organisms are able to ferment carbohydrate. If it retains red color, means organism do not utilize the carbohydrate (Weldezgina et al, 2016).

### **Result**

The Petri plates exhibited assorted colonies of bacteria that were present on the surface of vegetables. The bacterial growth on the Vegetable Market plates was higher compared to that of the supermarket plates. The sector 40D vegetable markets produced 20 bacterial colonies on NAM media while the Supermarket plates produced 15 bacterial colonies (Fig. 1, Table 1). We found *Bacillus* bacteria on tomato from the vegetable market and *Staphylococcus* bacteria was observed on the surface of capsicum annum from the supermarket.

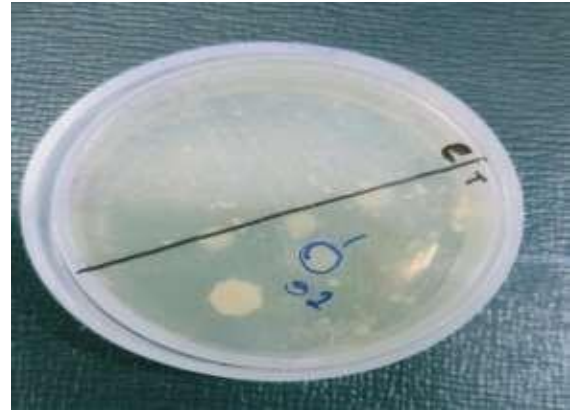
**Table 1**

Sample	Bacterial species	Bacterial Load Count
Capsicum (Supermarket)	Staphylococcus	15 colonies
Tomato (Vegetable Market)	Bacillus	20 colonies

**FIG.1 Bacterial colonies present on the Petri plates**



***Bacillus* bacteria collected from vegetable market (tomato)**



**Staphylococcus bacteria collected from the supermarket (Capsicum)**

**1. Gram staining**

In our experiment, plates (vegetable market and supermarket) containing *bacillus* and *staphylococcus* respectively were found to be Gram positive as they exhibited a purple color (Fig. 2).

**Fig.2 Gram staining experiments**



**Sample A (*Bacillus*)**



**Sample B (*Staphylococcus*)**

**2. Catalase Test**

The Catalase test is used to differentiate bacteria. Bacteria produce the enzyme Catalase by using oxygen, to protect themselves from the toxic by-products of oxygen metabolism. In the *Bacillus* and *Staphylococcus* samples bubbles present confirmed that the bacteria were Catalase positive (Fig. 3)

**Fig.3 Catalase test**



**Sample A (*Bacillus*)**



**Sample B (*Staphylococcus*)**

### 3. Citrate test

When an organism utilizes citrate then it exhibits a positive reaction but not necessarily as the sole carbon source. The pH turns the pH indicator from green to blue. The blue color appears which indicate the test is positive. Both the *Bacillus* and *Staphylococcus* samples were found to be positive as shown in fig 4a. The observed color change was green to blue.

**FIG.4 (a) Citrate test**



**Sample A (*Bacillus*)**



**Sample B (*Staphylococcus*)**

### 4. Nitrate test

When nitrate is present in the medium, then a red color will be produced. In our experiment the *Bacillus* and *Staphylococcus* cultures did not exhibit a color change so both samples were found to be negative (Fig 4b).

**FIG.4 (b) Nitrate test**



**Sample A (*Bacillus*)**



**Sample B (*Staphylococcus*)**

### 5. Urease method

The product of decarboxylation of amino acids is urea. After the hydrolysis process, urea produces Carbon dioxide and Ammonia. In our experiment, both *Bacillus* and *Staphylococcus* sample showed a positive reaction by developing an intense magenta to bright pink color in 15 min to 24 h (Fig 5).

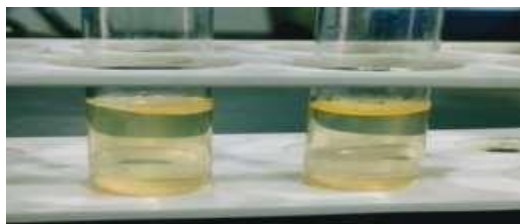
**FIG.5 Urease test**



**6. Methyl red test (MR)**

In this test, the test bacteria were grown in a broth medium that contained glucose. As we know, bacteria has the ability to utilize glucose via the production of acid, a color change was observed from yellow to red in the broth cultures. *Staphylococcus* has the ability to utilize glucose and *Bacillus* does not have ability to utilize glucose, thus, the bacillus test was positive and the staphylococcus was negative (Fig 6).

**Fig.6 Methyl red test (MR)**



**Sample A (*Bacillus*)**



**Sample B (*Staphylococcus*)**

**7. Voges–Proskauer**

Voges–Proskauer or VP is a test used for the detection of acetoin in a bacterial broth culture. This test is performed by adding potassium hydroxide and alpha-naphthol to the bacterial inoculated broth. Both samples *bacillus* and *staphylococcus* showed a negative reaction via lack of a pink-red color (Fig 7).



**Fig.7 Voges–Proskauer**

**Sample A (*Bacillus*)**

**(*Staphylococcus*)**

**Sample B**

#### 8. Oxidase test

The presence of the cytochrome oxidase pathway was detected via the oxidase test. It catalyzes the transport of electrons between electron donors and a redox dye tetramethyl-p-phenylene-diamine in the bacteria. This dye is reduced to a purple color. Both *Bacillus* and *Staphylococcus* were negative because of no color changes (Fig 8).

**Fig.8 Oxidase test**



#### 9. Carbohydrate test

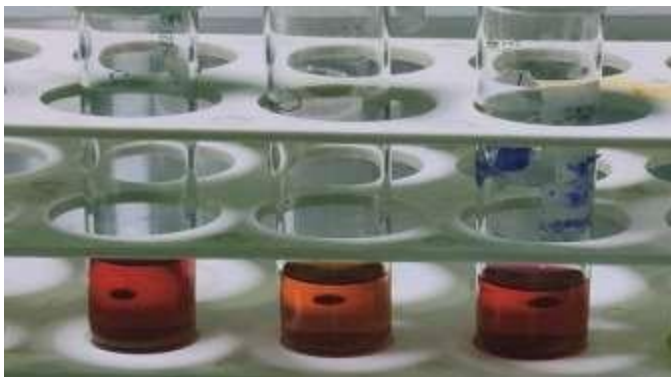
During the Carbohydrate test, the medium changed to a yellow color due to the production of acid. Microbes produce organic acids following fermentation of the carbohydrates. Both *Bacillus* and *Staphylococcus* were positive (Fig 9).

**Fig 9 Carbohydrates test**

**Fig 9.1 Nutrient Media with carbohydrates for LB**



**Fig 9.2 Nutrient Media with Carbohydrates for MSA**



**Fig 9.3 Following inoculation, color changes occurred in sample A and sample B**



In the end, it was concluded that different tests were commonly used to identify *Staphylococcus* and *Bacillus* bacteria. *Staphylococcus* were confirmed by the shape, size, gram staining experiment, catalase, citrate, urea and carbohydrate tests. *Bacillus* were confirmed by shape, size, gram staining, catalase, citrate, VP and carbohydrate tests. Results of these tests were found to match with properties of that particular bacteria.

## **Discussion**

The Vegetable market samples contained a greater abundance of bacterial flora compared to that of the supermarket. Thus, more contamination was evident in the Vegetable market samples. Observations from the surface growth showed that the supermarket samples of *Staphylococcus* growth were 50 percent, which was less numerous than the vegetable market samples, where the *Bacillus* count was 90 percent. Further, additional experimentation was conducted to identify the microbes from the environment of different places. Mostly bacteria of human and soil origin were found. The microbes from humans were mostly from the skin and respiratory tract, disbursed by sneezing or talking whereas the soil microbes were released via dust. These infectious bacteria make us unhealthy by releasing toxins that damage our tissues, such as *Streptococcus*, *Staphylococcus*, *Bacillus*, and *E.coli*. Both *Bacillus* and *Staphylococcus* microbes are gastrointestinal pathogens, *Vibrio cholera* causes cholera, *E.coli* causes cystitis and *Bacillus* pathogen is capable of causing disease due to the production of an enterotoxin or non-ribosomal peptide synthetase toxin. Tambekar and Mundhada in 2006 also reported pathogens detected from fresh vegetables included *Bacillus*, *Staphylococcus* and *E.coli*. Several studies have reported the presence of pathogenic bacteria in vegetables and fruits, however the bacterial counts were rarely documented (Olaimat et al, 2012). Kuramoto et al, 2006 reported that *Staphylococcus aureus* was the causal agent of a food poisoning outbreak in Japan in 2005 where pickled radish was one of its sources (Kuramoto et al, 2006). It was also observed that 50% of all vegetable samples that were collected from 39 major cities in China contained *B. cereus*. The level of contamination was more or less similar as to those of previous surveys in other countries, 57% in Mexico City, 20–48% in Korea (Chon et al, 2015), and 52% in the southeast of Spain (Flores-Urban et al, 2014). These reports, together with our study, indicate that *Bacillus* and *Staphylococcus* contamination in vegetables is very common. This result suggests that the consumption of vegetables contaminated with pathogenic bacteria is a potential health hazard. Treatment of bacterial infections includes good nutrition and intensive care. People who have had close contact with a person with bacterial disease may also need to be treated for the disease. Thus, during the preparation of vegetables some precautions may be required like washing your hands, kitchen utensils, chopping boards, and countertops before and after preparing fruits and vegetables. Moreover, it is very important to clean the vegetables and fruits prior to consumption.

## **Conclusion**

This study reported pathogenic bacteria found on vegetables collected from the vegetable market and super market in Chandigarh. The hygienic quality of the vegetables and water in the area was very poor due to the higher mean bacterial counts found (many recorded were beyond the standard safe limits). The Vegetable micro biota was dominated by *Staphylococcus* and *Bacillus*. The presence of *Bacillus* bacteria in the vegetable market was higher than the *Staphylococcus* bacteria present in the supermarket. At last, we want to suggest that washing vegetables and then storing them in food grade antibacterial chemicals may assist with reducing pathogens and other microbial flora. Thus, new and improved agricultural practices are required along with various safety measures during the production of fresh fruits and vegetables in the region.

**Acknowledgements-** Poonam is thankful to DayaNand College, Hisar (India), for providing any kind of assistance.

**Author contribution-** VS conceived the research, P conducted the experiment, P and AR wrote the manuscript. RS, TC, KK, RR and PB analyzed the data.

#### **Declarations**

**Conflict of interest-** The authors declare that they have no competing interests.

#### **Reference**

1. Aryal S. Oxidase test, principle, procedure, result. Microbiology; 2018; 31.
2. Balali GI, Yar DD, Adjeikusi P. Microbial contamination, an increasing threat to the consumption of fresh fruits and vegetables in today world. Int J Microbiol. 2020. 13.
3. Beuchat RL. Ecological factor influencing growth pathogens on raw vegetables. Microbes and infect; 2022; 4; 413-423
4. Brink B. Urease test protocol. ASM; 2019;236
5. Chon, JW, Yim, JH et al. Quantitative prevalence and toxin gene profile of *Bacillus cereus* from ready-to-eat vegetables in South Korea. Foodborne Pathog; 2015; 12; 795–799.
6. Coico R. Gram staining. Curr Protoc Microbiol; 2005; 32(4).
7. Flores-Urban KA, Natividad-Bonifacio, I. Detection of toxigenic *Bacillus cereus* strains isolated from vegetables in Mexico City. J Food Pro; 2014
8. Gagnon M, Hunting WM, Esselen WB. New method for Catalase determination. Anal chem; 1959; 31(1); 144-146.
9. Hasan NA, Zulkahar MI. Isolation and identification of bacteria from spoiled fruits. Biodivers J; 2015.
10. Kuramoto S, Kodama H, Yamada K. Food poisoning attributable to *Staphylococcus aureus* deficient in all of the staphylococcal enterotoxin gene so far reported. Jap J Infect Dis; 2006; 59(5); 347.
11. Moyes RB, Reynolds J. Differential staining of bacteria gram stain. Curr Protoc Microbiol; 2009; 32(3)
12. Olaimat N and Holley RA. Factors influencing the microbial safety of fresh produce: a review. Food Microbiol; 2012; 32(1); 1–19.

13. Shubo Yu, Wang J, Chao M, Yu S A. Study on prevalence and characterization of bacillus in foods. *Front. microbiol*; 2020.
14. Tambekar DH, Mundhada RH. Bacteriological quality of salad vegetables sold in city. *Biol sci*; 2006; 6(1).
15. Tarrand JJ, Groschel DH. Rapid modified oxidase variable bacterial isolates. *J clin Microbiol*; 1982; 16(4); 772-774.
16. Tlaieakhonzani A, Alae S, Ponraj M. Guidelines for quick application of Biochemical test to identify unknown bacteria. *J Biotech Res*; 2018; 2(2); 065-082.
17. Weldezgina D and Muleta D. Bacteriological contaminants of some fresh vegetables. *J adv biol*; 2016; 11.
18. Volland, JM, Rizzo SG, Gros O, Tysl T, Ivanova N, Schulz F, Goudeau D, Elisabeth NH, Nath N, Udway D, Malmstrom RR, Rontani CG, Kluge SB, Davies KM, Jean MR, Mansot JL, Mouncey NJ, Angert E, Woyke T, Date SV. A centimeter-long bacterium with DNA compartmentalized in membrane-bound organelles. 2022. bioRxiv preprint doi: <https://doi.org/10.1101/2022.02.16.480423>.





