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 amicrobiological quality assessment of fresh vegetables that were collected from several regions of Chandigarh.The purpose of this studywas 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 inthe vegetable market,were found to contain ahigher microbial load that may represent a greater risk for human health. Hence, for many microorganisms, vegetables may act as a reservoircolonizeand 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 outbreaksareassociated 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 *Thiomargaritamagnifica* (Volland et al, 2022).Foodpoisoning can become a major problem when itemsare not stored properly. Common bacteria that causes food poisoning are*Salmonella, Staphylococcusaureus* 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 ofmedia utilized solid media and liquid media.Solid media areused for studying the cloning character of the bacteria and liquid mediaare 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 arecommon 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 foundon 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 c*ollected from the surface of tomato was cultured from the vegetable market fruit and *Staphylococcus* falling microorganisms were collected from the surface of a capsicumfrom 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 32ºC. 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.

Nutrientagar 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 LuriaBertani)**.** Nutrient agar mediawasutilized for the bacterial growth counts asit can effectively grow a variety of bacteria as well as fungi.Peptone dextrose agarwas 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.LuriaBertani (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 viavariousphysical 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 thecell violet or red.This technique is useful for bacterial sample contamination assessment andobservation 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 potassiumhydroxide 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(Tarrandet 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.Theacid 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 sulfanilicacid. 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 productturns 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 etal,2016).

Result

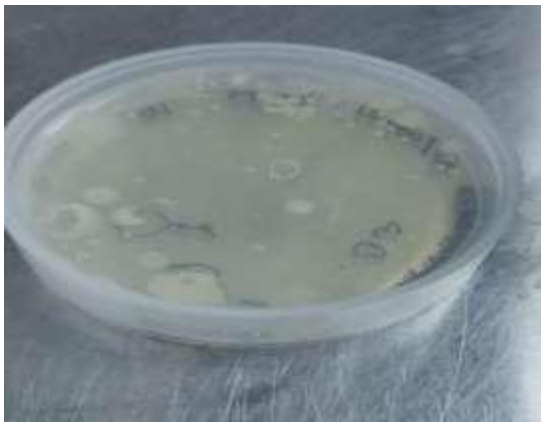
The Petri plates exhibited assorted colonies of bacteria that were present on the surface of vegetables. The bacterial growth on the Vegetable Market plates was highercompared to that of the supermarket plates. The sector 40D vegetable markets produced 20 bacterial colonies on NAM media while the Supermarket 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.

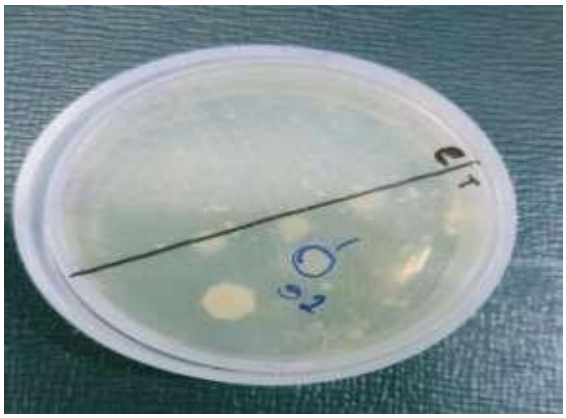
Table 1

Sample	Bacterial species	Bacterial Load Count

Capsicum (Supermarket)	Staphylococcus	15 colonies
Tomato (Vegetable Market)	Bacillus	20 colonies

FIG.1Bacterialcoloniespresentonthe Petri plates





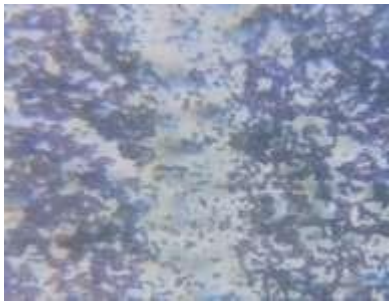
***Bacillus*bacteriacollectedfrom vegetable market(tomato)**

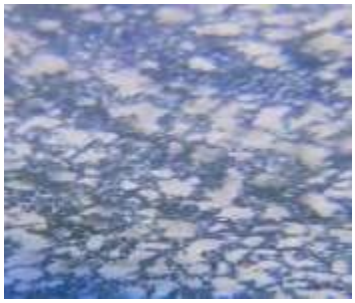
Staphylococcus bacteriacollected fromthe supermarket (Capsicum)

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.2Gramstaining experiments





SampleA(*Bacillus*)

SampleB(*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.3Catalasetest

SampleA(*Bacillus*) **SampleB(*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 bepositive as shown in fig 4a. Theobserved color change was green to blue.

FIG.4(a)Citratetest

SampleA(*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 notexhibit a color change so both sampleswere found to benegative (Fig 4b).

FIG.4(b)Nitratetest

Sample A(*Bacillus*) **SampleB(*Staphylococcus*)**

5. **Ureasemethod**

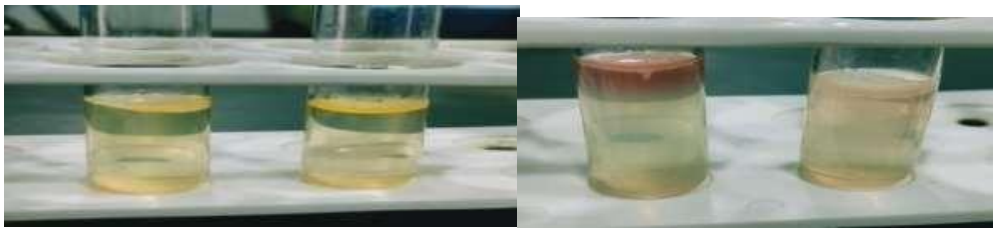
The product of decarboxylation of amino acids is urea. After the hydrolysis process, urea produces Carbon dioxide and Ammonia. In our experiment, both *Bacillus* and *Staphylococcus* sample showeda positive reaction by developing an intense magenta to bright pink color in 15 min to 24 h (Fig 5).

FIG.5 Ureasetest

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 viathe production of acid, a color change was observed from yellow to red inthe broth cultures. *Staphylococcus* has the ability to utilize glucose and *Bacillus* does not have ability to utilize glucose,thus, the bacillus testwas positive and the staphylococcus was negative (Fig 6).

Fig.6 Methylredtest(MR)



SampleA(*Bacillus*) **SampleB(*Staphylococcus*)**

7. **Voges–Proskauer**

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

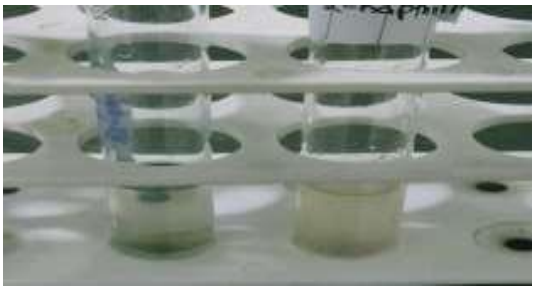


Fig.7Voges–Proskauer

SampleA(*Bacillus*)

SampleB(*Staphylococcus*)

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.8Oxidasetest

9. **Carbohydrate test**

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

Fig9Carbohydratestest

Fig9.1NutrientMediawithcarbohydratesforLB

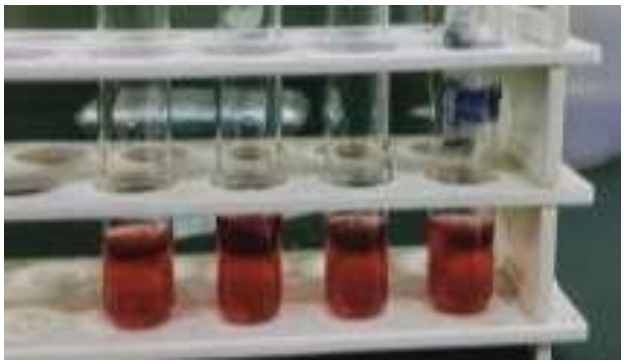


Fig9.2NutrientMediawithCarbohydratesforMSA

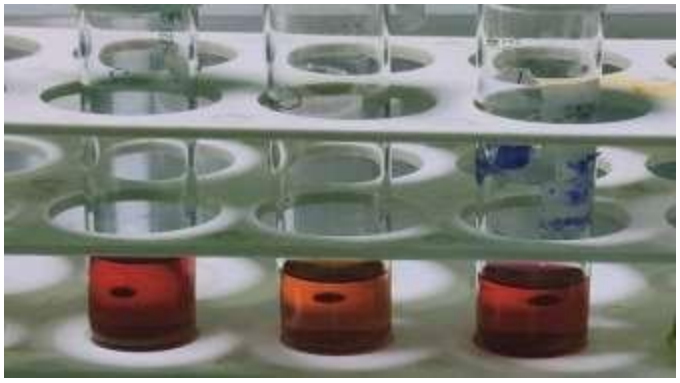
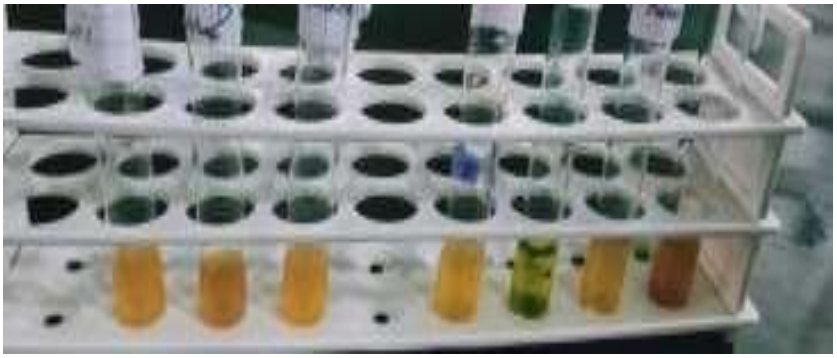


Fig9.3Followinginoculation, color changes occurred in sampleAandsampleB



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 numerousthan the vegetable market samples, where the*Bacillus* count was 90 percent. Further, additional experimentationwas 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 toxinsthat damage our tissues, such as *Streptococcus*, *Staphylococcus*, *Bacillus, and E.coil*. 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. Tambekarand Mundhada in 2006 also reportedpathogens detected from fresh vegetables included *Bacillus,Staphylococcus*and *E.coil*. 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 alsoobserved 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 othercountries, 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 bacteriais a potential health hazard. Treatment of bacterial infections includes good nutrition and intensive care. People who have had closecontact 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, choppingboards, 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 studyreported 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 higherthan the *Staphylococcus* bacteria present in the supermarket.At last, we want to suggest that washing vegetables and thenstoring them in food grade antibacterial chemicals may assist with reducing pathogens and other microbial flora. Thus, new and improved agricultural practicesare requiredalong 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- VSconceived 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.

Ethics approval- Not applicable

Consent to participate- Not applicable

Consent for publication- Not applicable

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