Uttar Pradesh Journal of Zoology

Volume 45, Issue 17, Page 633-638, 2024; Article no.UPJOZ.3931
ISSN: 0256-971X (P)

Characterization of Bacterial Population Associated with Deterioration of Stored Kinnow Fruits (*Citrus nobilis* x *Citrus deliciosa*)

Rogeshwori Toijam [a] and Gagan Preet Kour Bali [a*]

[a] Department of Biosciences, Chandigarh University, Gharuan, Mohali 140413, Punjab, India.

Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.56557/upjoz/2024/v45i174408

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://prh.mbimph.com/review-history/3931

Original Research Article

Received: 22/06/2024
Accepted: 26/08/2024
Published: 31/08/2024

ABSTRACT

Fresh and spoiled Kinnow fruits were collected from local market in Kharar, Punjab, India to isolate and identify the microbial factors that causes deterioration of stored Kinnow (*Citrus reticulata*), as well as to study the impact of temperature to fruits at different storage conditions. Several microorganisms, including bacteria and fungus, may thrive in ideal condition present within the fruits. Using the pour plate method, the infected portion of the fruit was utilised to isolate the bacteria by serial dilution. Healthy colonies were selected and sub-cultured. Two types of bacteria have been isolated. Following morphological analysis and biochemical characterization, *Staphylococcus* spp. and *Escherichia coli* were determined to be the responsible organism.

Keywords: Citrus nobilis x Citrus deliciosa; spoilage; Staphylococcus; E. coli; shelf life.

**Corresponding author: Email: gaganviren@gmail.com;*

Cite as: *Toijam, Rogeshwori, and Gagan Preet Kour Bali. 2024. "Characterization of Bacterial Population Associated With Deterioration of Stored Kinnow Fruits (Citrus Nobilis X Citrus Deliciosa)". UTTAR PRADESH JOURNAL OF ZOOLOGY 45 (17):633-38. https://doi.org/10.56557/upjoz/2024/v45i174408.*

1. INTRODUCTION

The nutrient-rich interior of fruits provides ideal environments for a variety of microorganisms to flourish. They provide an adequate source of nourishment. Sincefruits are packed with proteins, minerals, vitamins and carbohydrates, they are a perfect food source for a variety of parasitic and saprophytic microbes [1]. Tissues become softer due to pectin degradation brought by bacterial spoiling and the fruit may finally turn into a slimy mass. After starch and sugars are break down, lactic acid and ethanol are produces, along with undesirable flavours and odours [2]. Spoilage is any change in the condition of food that renders it unsuitable for human consumption [3]. Pathogenic and contaminating microorganisms can reduce the fruit's acceptability and shelf life [4].

Fruits are prone to microbial deterioration due to their composition; thus, quality and safety are essential [5]. India is the world's largest fruit producer [6] but nearly 20%-30% of the harvested fruit is wasted due to putrescible nature [7]. This amounts a substantial loss of irreplaceable food. Therefore, it's crucial to both grow more and protect what has been produced at considerable cost.

Kinnow (*Citrus noblilis* x *Citrus deliciosa*) is a hybrid mandarin group of citrus fruit that was developed from the king and willow leaf (*Citrus noblilis* and *Citrus deliciosa*), created in 1935 at the Citrus Experiment Station at the University of California, and in the early 1940s, it was first shown in India. It is the most productive and largest citrus fruit in Punjab and belongs to Rutaceae family [8,9]. Kinnow exhibit robust growth, characterized by a standard orange-yellow hue of both peel and pulp. They are exceptionally juicy, boasting a flavour aroma and a wealth of nutrients [10]. Major Kinnow growing region in India include Haryana, Punjab, Himachal Pradesh, Rajasthan, Uttar Pradesh, and Jammu [11].

Kinnow is renowned for its abundance of primary metabolites, including amino acids and essential vitamins - ascorbic acid, pro-vitamin-A, and folate, alongside secondary metabolites like bioactive compound such as limonoids, flavones, phenolics, flavonoids, and carotenoids [12]. Kinnow fruit is very rich in Vitamin C, high content of β-carotene, reducing sugars and non-reducing sugars [13].

The fruit's quality - weight, colour, taste, total soluble solids, acidity, and sugar have been affected by storage. Kinnow fruits exhibit changes in fruit texture, colour, and aroma, and biochemical characteristics. These changes lead to poor fruit quality and post-harvest losses. The main causes limiting the shelf life of Kinnow are post-harvest losses from fungal infections and physiological activities (respiration, ethylene liberation, and enzyme activity [14]. The cumulative harvest and post-harvest losses in Kinnow are estimated by Singh et al. was found to be between 25% and 30% [15].

The objectives of this study are to isolate and identify the bacteria that causes the spoilage of stored Kinnow, characterization of the isolated microbe.

2. METHODOLGY

This study was conducted in the laboratory of the Department of Biosciences, University Institute of Biotechnology, Chandigarh University, Mohali, Punjab in 2023-2024.

2.1 Collection of Samples

Healthy and infected samples of Kinnow fruit were collected from local market in Kharar, Punjab, India. The fruits were then brought inside the laboratory. Before the experiment, the spoiled/infected were left in their natural environment and kept unwashed. And all the fresh samples were surface sterilised by 70% ethanol, followed by 3 rinses in sterile distilled water.

2.2 Preparation of Media

The nutrient media (HIMEDIA Nutrient Agar No.2) was prepared in accordance with the manufacturer's instruction and autoclaved for 15 mins at 121°C to sterilise them.

2.3 Preparation of Sample

The spoiled/deteriorated Kinnow fruit samples were crushed in a sterilized mortar, to make a paste.

2.4 Isolation of Microbes

Pour plate method was used. 1g of the sample was mixed with 9 ml sterile distilled water. A serial dilution was prepared upto10^{-1} to 10^{-6}in

sterile test tubes. Then inoculate 0.1 ml of diluted solution and mix it with the agar and then incubated at 37±2°C in inverted position. After 18-24 hours, plates were checked for growth. Healthy colonies from the media were sub-cultured into freshly manufactured media using aseptic methods to prevent contamination. This enables to attain pure culture of each isolate. The culture colonies were then kept for characterization in nutrient agar slants in a refrigerator (4°C).

2.5 Characterization of the Isolates

Characterization of bacteria is done by morphology characterization, biochemical characterization. This enables us to identify the bacteria properly.

2.6 Morphological Characterization

Culture morphology (Culture, shape, margin, wetness/Dryness, transparency, colour and gram stain) of both bacteria isolate bacteria 1 and bacteria 2 were observed and recorded. The colonial morphology was observed with our naked eyes.

Gram staining: The gram stain procedure was conducted on 24-hour bacterial cultures [16].

2.7 Biochemical characterization

Catalase test: Using a sterile inoculating loop, gather a small sample of each bacterium from a 24-hour culture and deposit onto separate microscope slides. With a dropper, apply a drop of 3% hydrogen peroxide onto each bacteria specimen on the slides. Observe for the prompt formation of bubbles [17].

Oxidase Test: Allow a piece of filter paper to air-dry after soaking in 1% Kovacs oxidase reagent. Pick each isolated bacterium using a sterile loop from 18–24 hours culture plate, then rub it onto a piece of treated filter paper. Keep watching out for colour variations [18].

Indole Test: Infuse a bit of each pure culture to the tryptone broth tube. Incubate for 24 to 48 hours at 35° (+/- 2°C). To check for the formation of indole, put five drops of Kovacs reagent straight into the tube. Check if there is formation of pink to red colour (cherry red ring) on the reagent layer on top [19].

Methyl Red Test: Prepare MR-VP broth. Each tube of MR-VP broth should be inoculated with pure culture of each bacteria isolate (18 to 24 hours). For 48 hours, incubate the cultures at 35° +/- 2°C. Toss five drops of the methyl red solution. Compare the changes in the broth's colour [20].

Voges-Proskauer Test: Each tube of MR-VP broth should be inoculated with pure culture of each bacteria isolate (18 to 24 hours). For 48 hours, incubate the cultures at 35° +/- 2°C.12 drops of Barritt's reagent A, followed by4 drops of Barritt's reagent B is added. To expose the medium to air oxygen, gently shake the tube for 30 sec to 1 min. Give the tube a minimum of 30 min to stand. Check if there are changes in the colour of the broth [20].

Citric acid Test: Keep Simmons Citric medium slant prepared. Use a fresh pure culture bacteria as the inoculation source, and lightly streak the two bacteria on different slant's surface. Since citrate consumption need oxygen, screw caps are positioned loosely on the tube. Incubate for 18 to 48 hours at 35°C (+/- 2°C). Examine the slant surface for any visible growth and any changes in the medium's colour [21].

3. RESULTS AND DISCUSSION

The morphological and biochemical characters of the isolated bacteria are discussed below in Tables 1 and 2. Two types of bacteria have been isolated.

3.1 Morphological Character of Isolated Bacteria

Isolates 1 i.e., Bacteria 1 has circular and convex shaped cultural colony where the margin of the colony is smooth. It's showing wet and slightly yellow color colony. They exhibit gram positive nature. In case of isolates 2 i.e., Bacteria 2 also display circular, convex and smooth margin colony. It's flaunting wet and white color colony. In case of this bacteria, they are gram negative.

3.2 Biochemical Characters

Isolate 1 in Fig. 1 (Bacteria 1) when undergo catalase taste it show positive catalase which are evident by instantaneous bubble formation (effervescence). Thus, this signifies the presence of enzyme catalase in the isolated bacteria [17]. But in case of oxidase test, the color did not change even after 2 minutes which denotes the absence of cytochrome oxidase. Thus, this isolate 1 is oxidase negative [18]. Moreover,

during indole test, this reagent layer did not change and remain yellow and slightly cloudy, which signifies the absence of indole in the isolated bacteria. Thus, it is an indole negative bacterium [19]. Followed by methyl red test, which exhibits a red coloring due to increased acid generation after adding methyl red reagent. This is due to fermentation of glucose and increase acid production which decreases the pH of the culture media, which marks methyl red positive test. Next test is Voges-Proskauer, in this case we observe the broth showed red coloration on top of the culture media after 30 minutes, this indicate that the bacteria 1 is Voges-Proskauer positive [20]. When undergo citrate test, the medium becomes a vivid Prussian blue, and there is discernible growth on the slant surface. The medium's pH rises to over 7.6 due to the alkaline carbonates and bicarbonates that are byproducts of citrates catabolism, which causes the bromothymol blue to become blue instead of green [21]. Thus, explains that these bacteria give positive result in citrate test. This indicates the utilization of citrate as carbon and energy source. Thus, according to the observed morphological and biochemical characteristics, this bacterium 1 is identified as Staphylococcus *spp*.

Isolate 2 in Fig. 2, when undergo catalase taste it show positive catalase which are evident by instantaneous bubble formation (effervescence). Thus, this signifies the presence of enzyme catalase in the isolated bacteria [17]. But in case of oxidase test, the color did not change even after 2 minutes which denotes the absence of cytochrome oxidase. Thus, this isolate, Bacteria 2 is oxidase negative [18]. While in case of indole test, within seconds of introducing the reagent, a red color began to appear in the reagent layer on top of the agar. This denotes the presence of in dole and thus it is an indole positive bacterium [19]. Again, in case of methyl red test, which exhibits a red coloring due to increased acid generation after adding methyl red reagent. This is due to fermentation of glucose and increase acid production which decreases the pH of the culture media, which marks methyl red positive test. Followed by Voges-Proskauer, in this case we observe the broth media has a yellowish color and did not change in the culture media even after 1 hour, this indicate that the bacteria 2 is Voges-Proskauer negative [20]. Again bacterium 2 undergo citrate test, the medium becomes a vivid Prussian blue, and there is discernible growth on the slant surface. The medium's pH rises to over 7.6 due to the alkaline carbonates and bicarbonates that are byproducts of citrates catabolism, which causes the bromothymol blue to become blue instead of green [21-23]. Thus, explain that this bacterium gives positive result in citrate test. This indicates the utilizaton of citrate as it's carbon and energy source. As per the morphological and biochemical characters, the isolated bacteria2 is identified as *Escherichia coli*.

Table 1. Morphological Characteristics of the bacteria isolates

Characteristics	Bacteria 1	Bacteria 2
Culture	Circular	Circular
Culture Shape	Convex	Convex
Margin	Smooth	Smooth
Wetness/Dryness	Wet	Wet
Transparency	Opaque	Opaque
Colour	Yellow	White
Gram stain	+ve	-ve

Fig. 1. Isolated bacteria 1

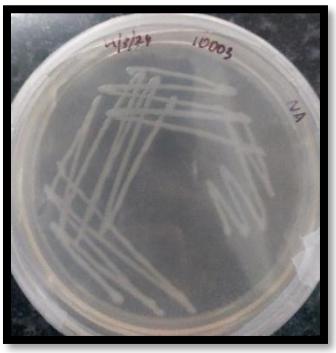

Fig. 2. Isolated bacteria 2

Table 2. Biochemical character of bacteria isolates

Test	Bacteria 1	Bacteria 2
Catalase Test	+ve	+ve
Oxidase Test	-ve	-ve
Indole Test	-ve	+ve
Methl Red Test	+ve	+ve
VP Test	+ve	-ve
Citrate Test	+ve	+ve

4. CONCLUSION

The fruit's strong metabolic activity led to deterioration soon after harvest. At the post-harvest stage, microbial pathogens are the main underlying causes of fresh fruits damage. Several microbes infect fruits through surface damage. The external factors that could possibly cause damage include temperature, relative humidity, and oxygen balance, especially while the product is being stored. Thus, from this study we isolate and identify two bacteria. After conducting morphology study and biochemical characteristics, it was identified as *Staphylococcus* spp. and *E. coli*.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Authors hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

ACKNOWLEDGEMENT

Authors extend sincere thanks to Department of Biosciences, UIBT, Chandigarh University for providing continuous support.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Thiyam B, Sharma GD. Isolation and identification of fungi associated with local fruits of Barak valley, Assam. Current World Environment. 2013;8(2):319-322.
   DOI:10.129944/CWE.8.2.20.
2. Rawat S. Food Spoilage: Microorganisms and their prevention. Asian Journal of Plant Science & Research. 2015;5.
3. Akinmusire OO. Fungal species associated with the spoilage of some edible fruits in Maiduguri Northern Eastern Nigeria. Advances in Environmental Biology. 2011;157-162.
4. Obetta S, Nwakonobi, Theresa, Adiwu O. Microbial effects on selected stored fruits and vegetables under Ambient Condition in Makurdi, Benue State, Nigeria. Research Journal of Applied Sciences, Engineering and Technology; 2011.
5. Slavin JL, Loyd B. Health benefits of fruits and vegetables Advances innutrition. 2012;3(4):506-516.
   DOI: 10.3945/an.112.002154.506.
6. Ranjan J. Post-harvest storage structures for fruits and vegetables in India. Ropan. 2023;11(3): CHH-BIL/2020/79641.
7. Basediya A, Samuel DVK,Beera V. Evaporative cooling system for storage of fruits and vegetables- A Review. Journal of food science and technology. 2013;50 (3):429-442.
8. Anonymous. Area and production of fruits. Package of practices for cultivation of fruits, Directorate of communication, Punjab Agricultural University Ludhiana, India. 2012;1.
9. Frost HB. Four new citrus varieties- The Kara, Kinnow, and Wilking mandarins and the Trovita orange; 1935.
10. Singh S.K. Characteristics of Kinnow mandarin fruit stored under incubator. Annals of Biology. 2018;34(2):126-129.
11. Purewal, Sukavinder Singh, Kawaljit Singh Sandhu. Nutritional profile and health benefits of Kinnow: an updated review. International Journal of Fruit Science. 2020;20(sup3):S1385-S1405.
12. Matsumoto, Hikaru, YoshinoriaIkoma. Effect of different postharvest temperatures on the accumulation of sugars, organic acids, and amino acids in the juice sacs of satsuma mandarin (*Citrus unshiu* Marc.) Fruit.). Journal of Agricultural and Food Chemistry. 2012;60(39):9900–9909.
    DOI: 10.1021/jf303532s.

13. Khan MM, Asim M, Mahmood A, Asharf T, Anjum N, Hayat F, Salik R, Jamil A, Qamar R. Characterization and comparative nutritional study of three strains of Kinnow mandarin (*Citrus reticulata* Blanco). Asian Journal of Agriculture and Biology. 2020;8(3):299-307.
14. Singh D, Mandal, G. Improved control of Rhizopus stolonifera-induced storage rot of peach with hot water and antagonistic yeast, *Debaryomyces hanselli*. Indian Phytopathology. 2006;59:168-173.
15. Singh SV, Jain RK, Gupta A.K. Adsorptive reduction of Naringin from Kinnow mandarin juice with non-ionic microporous adsorbent resin. Indian Chemical Engineer. 2016;58(2):136-156.
16. Tripathi N, Sapra A. Gram Staining. Treasure Island Stat Peals Publishing; 2023.
17. Reiner K. Catalase test protocol. American society for microbiology. 2010;1(1):1-9.
18. Shields P, Cathcart L. Oxidase test protocol. American Society for Microbiology. 2010;1-9.
19. MacWilliams MP. Indole test protocol. American Society for Microbiology, Washington, DC; 2012.
20. McDevitt S. Methyl red and Voges-proskauer test protocols. American Society for Microbiology. 2009;8.
21. MacWilliam MP. Citrate test protocol. American Society for Microbiology. 2009; 1-7.
22. Mahajan BVC, Dhillon WS, Kumar M. Effect of surface coating on the shelf life and quality of Kinnow fruits during storage. Journal of Postharvest Technology. 2013;1(1):8-15.
23. Zagory D, Kader AA. Modified atmosphere packaging of fresh produce. Food Technology. 1988;42:70-77.

Peer-review history:
The peer review history for this paper can be accessed here:
https://prh.mbimph.com/review-history/3931