Evaluation of Probiotic Potential of Endophytic Microflora from Tomato, Chilli, Cucumber and Capsicum

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ABSTRACT:
Microorganisms that colonize internal plant tissues without endangering the host plant are known as endophytic microflora. These microbes are well-known for their advantageous qualities, including probiotic potential, stress tolerance, plant growth promotion, and antibacterial activity. Endophytic microorganisms from tomatoes (Solanum Lycopersicon), chilies (Capsicum annuum), Cucumis sativus, and capsicum (Capsicum annuum var. grossum) are isolated, characterized, and their probiotic potential is assessed. Nutrient agar and potato dextrose agar media were used for microbial isolation after healthy plant tissues, including roots, stems, leaves, and fruits, were gathered and surface sterilized. The morphological, microscopic, and biochemical traits of the isolated endophytes were used to identify them. Acid tolerance, bile salt tolerance, antibacterial activity, antibiotic susceptibility, and adhesion ability were among the tests used to assess the isolates' probiotic qualities. According to the findings, a number of isolates showed notable antibacterial activity against common pathogenic microorganisms as well as strong resistance to bile salts and acidic pH. These results imply that endophytic bacteria from frequently eaten vegetables could be viable probiotic candidates for use in the food, pharmaceutical, and agricultural industries. The study advances the investigation of sustainable and environmentally beneficial microbial resources.

Keyword: Endophytic microflora , probiotic , antibacterial activity , Nutrient agar , potato dextrose agar , Acid tolerance, bile salt tolerance.

1. INTRODUCTION

1.1 Overview of Endophytic Microflora

Microorganisms known as endophytes live inside plant tissues without harming the host plant. These microbes, which form symbiotic interactions with plants, include fungi, bacteria, and actinomycetes. Endophytic microflora contribute significantly to plant growth and health through nitrogen fixation, phytohormone production, nutrient solubilization, and resistance against pathogens. In recent years, scientific interest in endophytes has increased due to their ability to produce biologically active compounds such as enzymes, antibiotics, antioxidants, and antimicrobial substances.

The genera Bacillus and Pseudomonas are common examples of endophytic bacteria, which are present in several phyla, including Firmicutes, Bacteroidetes, Proteobacteria, and Actinobacteria. Endophytes live inside the host's tissue and provide several benefits to the plant, such as increased nutrient availability, improved nutrient uptake, and improved nitrogen fix action (Chaudhary et al., 2022). About 40–80% of bacteria are known to be able to mechanize the production of biofilms on a variety of surfaces, including manufactured and natural ones. (Flemming and Stefan Waerts 2019).

The role of endophytes as potential probiotics has emerged as an important field of research. Probiotics are live microorganisms that provide health benefits. Probiotic organisms belong to genera such as Lactobacillus and Bifidobacterium. Furthermore, bacterial endophytes can create two plant growth hormones, indole-3-acetic acid and side-rophores (Gaiero et al., 2013; Rosenblueth, 2006), which improve nutrient uptake, phosphate solubilization, and nitrogen fixation (Singh et al., 2017). This review aims to advance knowledge of endophytes and their complex mechanisms in improving plant systems by developing a conceptual framework. Endophyte bioactive chemicals may be investigated further for potential use in the pharmacological, medicinal, agricultural, and industrial sectors.In both humans and plants, endophytes are crucial in the fight against infections. Certain endophytic bacteria has the capacity to generate antimicrobial substances that offer defense by preventing the growth of infections.

1.2 The significance of probiotics

Probiotics are good bacteria that enhance the balance of microorganisms in the intestines and support general health. They improve immunity, lessen gastrointestinal issues, aid in digestion, and prevent infections. Probiotic products are in greater demand due to growing antibiotic resistance and increased knowledge of natural health-promoting substances. Endophytic microorganisms isolated from vegetables may possess probiotic properties due to their adaptation to diverse environmental conditions.

2. MATERIAL AND METHODS

2.1 Collection of Plant Samples

For isolation of endophytic bacteria, The roots of the selected samples of tomato, chilli, cucumber, and capsicum, were carefully cut out, tagged and stored in plastic bags . Within 5-6 hours, these plant materials were used for the isolation of endophytes.

2.2 Isolation of Endophytes

The collected samples were first washed under running tap water and the stems, leaves were cut into small pieces. These small pieces were rinsed in sterile distilled water and after that surface sterilization was performed by treating first with 70% ethanol for 30 sec and then with 0.1% mercuric chloride solution for 2min. After that the plant parts were longitudenally cut with sterile scapel, and inoculated on nutrient agar and potato dextrose agar media. Plates were incubated at suitable temperatures for about 37°C for bacterial and fungal growth. Determination of antibacterial activity through primary screening was done by Streak Plate method . Nutrient agar plates were prepared in which isolated endophytic bacteria were streaked in the centre and these plates were incubated at 37°C for two days.

Later the pathogens (Bacllus subtilis, Pseudomonas sp, were streaked to endophytic strain and incubated at 37°C for 24 hrs. Secondary screening of endophytic bacterial strains was done by agar well diffusion method using Ethyl acetate for extraction. 100 µl of pathogenic microorganisms in nutrient broth was spread onto Nutrient Agar plates. These plates were incubated at 37°C for 24 h.

2.3 Morphological Characterization

The Morphological Characterization is performed for the identification of endophytic bacteria. They were characterized by gram staining, endospore formation .

2.4 Microscopic Examination

Isolated colonies were observed for shape, size, color, texture, elevation, and margin characteristics. Gram staining and microscopic analysis were performed to determine cellular morphology.

2.5 Biochemical Tests

The following biochemical tests were performed:

➢ **Catalase test :**

Principle-

- Detects the presence of the enzyme catalase.
- Catalase breaks down hydrogen peroxide into water and oxygen.

Procedure-

- Place a drop of 3% hydrogen peroxide on a clean slide.
- Transfer a small amount of bacterial culture onto the drop.
- Observe for bubble formation.

Observation-

- Positive: Immediate production of bubbles.
- Negative: No bubbles observed.

➢ **Oxidase test :**

Principle-

- Detects the presence of the enzyme cytochrome c oxidase involved in aerobic respiration.

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Procedure-

- Place oxidase reagent on filter paper.
- Smear a fresh bacterial colony onto the reagent.
- Observe colour change

Observation-

Positive : dark purple/blue color develops within 10-30 seconds.
Negative: No color change.

➢ **Methyl Red (MR)Test :**

Principle-

- Detects the production of stable acidic end products from glucose fermentation.
- Methyl red indicator changes color according to pH.

Procedure-

- Inoculate the isolate into MR-VP broth.
- Incubate for 24–48 hours.
- Add a few drops of methyl red reagent.

Observation-

- Positive: Red color appears.
- Negative: Yellow or orange color appears.

➢ **Voges–Proskauer (VP) Test:**

Principle

- Detects the production of acetoin, a neutral end product of glucose fermentation.

Procedure

- Inoculate the isolate into MR-VP broth.
- Incubate for 24–48 hours.
- Add VP reagents A (α-naphthol) and B (KOH).
- Mix and allow the tube to stand.

Observation-

- Positive: Development of red or pink color.
- Negative: No color change or copper-brown color.

➢ **IMViC Tests:**

IMViC stands for:

I – Indole Test

M – Methyl Red Test

V – Voges-Proskauer Test

C – Citrate Utilization Test

These tests are commonly used for bacterial characterization and identification.

A) Indole Test: It is use to detect the ability of a bacteria to produce indole from tryptophan. As a observation, If a Red ring at the top after adding Kovac reagentis observed , it indicates a positive a test . and if No Red Ring , then result is negative.

B) Methyl Red Test: If a red colour is observed , it indicates a Positive result. And if no colour change, the result is Negative.

C) Voges- Proskauer Test: Pink Red Color Indicates Positive Test

D) Citrate Utilization Test: Determines the ability of the bacteria to use citrate as the sole carbon source. Firstly, we will Streak the bacteria on Simmons Citrate agar and incubate for 24-48 hrs.

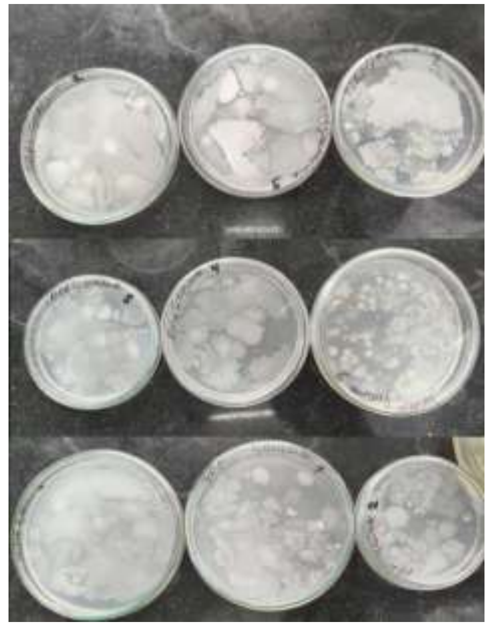
Positive Result - Shows growth with blue Coloration of medium. Negative Result- No Growth, medium remains green.

3. Result and Observation

Here are the test of methodology used:

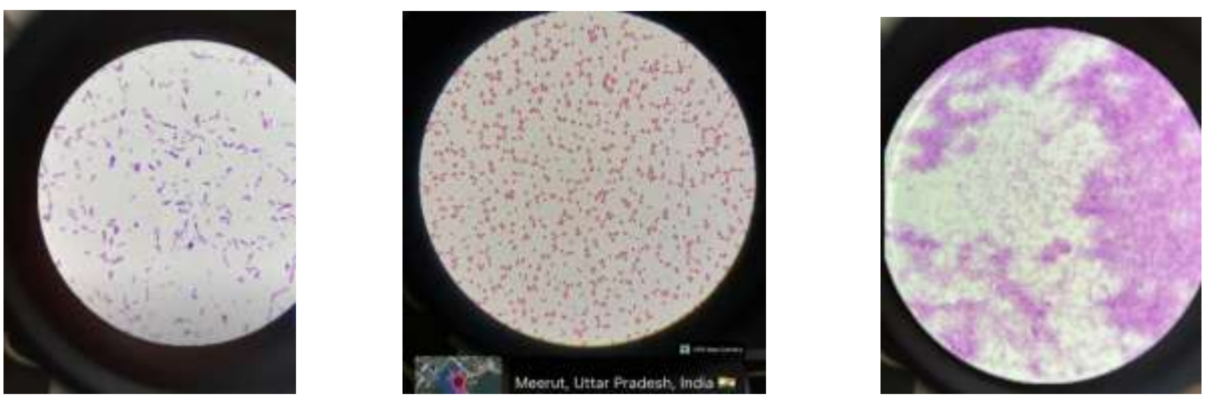
3.1 Biochemical Identification of Sample from healthy plants

The samples like chili, tomato, cucumber and capsicum which harbor diverse endophytic bacterial populations with significant probiotic potential. These endophytic bacteria can tolerate acidic pH and bile salts, which are essential probiotic characteristics and also contribute in maintaining a healthy gut microbiota.

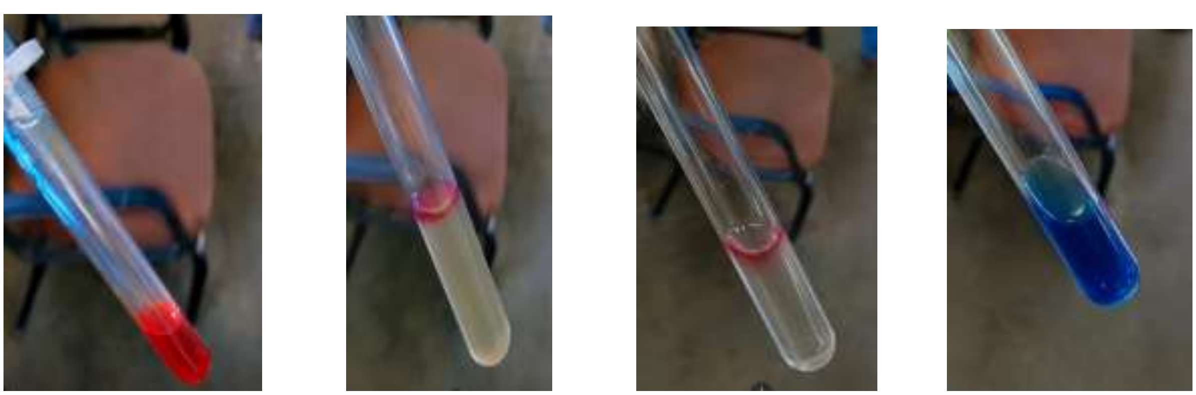


A) Colecion of Endophytic bacteria from chili, Tomato, capsicum and cucumber.

B) Isolation of colonies from Spread plate



C) Microscopic Examination of Isolated Bacteria



D)

Organisms	**Indole**	**Methyl Red**	**Voges-Proskauer**	**Citrate**
Enterobacter aerogene	-	-	+	+
Pseudomonas fluorescens	-	-	-	+
Bacillus subtilis	-	+	+	+

Fig3.1 Biochemical Test For Identification of bacterial isolates

Conclusion

The current study on the "Evaluation of Probiotic Potential of Endophytic Microflora from Tomato, Chilli, Cucumber, and Capsicum" showed that endophytic bacterial isolates from various vegetable crops have probiotic microbes. The isolates had advantageous physiological and biochemical characteristics, including biochemical tests, demonstrating their metabolic flexibility and adaptability. These traits imply that endophytic bacteria may be advantageous microbes for human and plant health as well as contribute to microbial balance.

Bacillus, Pseudomonas, and Enterobacter species were among the isolated endophytes that showed encouraging probiotic characteristics, making them viable candidates for additional research. Their symbiotic nature and potential significance in promoting plant growth, nutritional availability, and pathogen resistance are highlighted by their existence within plant tissues without causing illness.

The results show that the endophytic microflora linked to tomatoes, chillies, cucumbers, and capsicums are an important source of good bacteria with probiotic potential. To confirm their use in agriculture, food biotechnology, and probiotic formulations, more research involving molecular characterisation, safety evaluation, antibacterial activity, and in vivo testing is advised.

References

1. Chaudhary, P., Agri, U., Chaudhary, A., Kumar, A., & Kumar, G. (2022). Endophytes and their potential in biotic stress management and crop production. Frontiers in Microbiology, 13, 933017

2. Flemming, H. C., & Wuertz, S. (2019). Bacteria and archaea on Earth and their abundance in biofilms. Nature Reviews Microbiology, 17(4), 247–260.

3. Gaiero, J. R., McCall, C. A., Thompson, K. A., Day, N. J., Best, A. S., & Dunfield, K. E. (2013). Inside the root microbiome: bacterial root endophytes and plant growth promotion. American Journal of Botany, 100(9), 1738–1750

4. Rosenblueth, M., & Martínez-Romero, E. (2006). Bacterial endophytes and their interactions with hosts. Molecular Plant-Microbe Interactions, 19(8), 827–837.

5. Singh, R., Singh, P., & Sharma, R. (2017). Microorganism as a tool of bioremediation technology for cleaning environment: A review. Proceedings of the International Academy of Ecology and Environmental Sciences, 7(1), 1–6.

6. Santoyo, G., Moreno-Hagelsieb, G., del Carmen Orozco-Mosqueda, M., & Glick, B. R. (2016). Plant growth-promoting bacterial endophytes. Microbiological Research, 183, 92–99.

7. Jasim, B., Anish, M. C., Shimil, V., Jyothis, M., & Radhakrishnan, E. K. (2015). Studies on plant growth promoting properties of fruit-associated bacteria from Elettaria cardamomum and molecular analysis of ACC deaminase gene. Applied Biochemistry and Biotechnology, 177(1), 175–189.Supports ACC deaminase, IAA production, and phosphate solubilization.