Isolation and Characterization of Endophytic Bacteria from *Moringa oleifera*

Abstract

Moringa oleifera (commonly called drumstick) is a plant having many medicinal uses and high nutritional value. Medicinal and anti-microbial properties of the plant are proven and are associated with its various secondary metabolites such as flavonoids, alkaloids & phenols. An innovative approach is to explore properties of the endophytic bacteria present in the plant for medicinal purpose. The aim of this research is to isolate and explore whether the endophytic bacteria present in the stem of Moringa Oleifera contribute to the antimicrobial activity of the plant.

Introduction

- The world of traditional medicine has long used Moringa for the treatment of various diseases, like liver disease and is often used in patients with chronic illness including AIDS and HIV.
- Parts of moringa plants, such as leaves, fruits, flowers and roots are used for various purposes such as food, medicine, dyes, animal feed and also wastewater purifier.
- Moringa plant parts such as leaves, seeds, flowers, roots, and stem bark have been shown to be a source of antimicrobial ingredients in medicine.
- Endophytic bacteria are bacteria living in host tissue without causing symptoms .
- It is likely that endophytic bacteria present in the plant can also synthesize the same antibacterial compounds and thus have antibacterial effects
- Earlier studies have shown that endophytic bacteria isolated from various medicinal plants have the ability to inhibit the growth of pathogenic bacteria.

Materials and Methods

Isolation of endophytic bacteria

- Fresh stem's bark of *Moringa oleifera* was obtained from authentic source.
- The surface of the bark was washed with running water and then cut into pieces , sterilized with 70% alcohol, 4% NaOCl, rinsed with sterile distilled water.
- Sterilized samples were aseptically transferred to sterile Nutrient Agar plates, incubated at 32^{0}C for 72 hours.
- The endophytic bacteria grown from the plant were subcultured on sterile Nutrient Agar and incubated at 32^{0}C for 24 hours.
- The endophytic bacterial colonies were isolated on sterile Nutrient Agar plates to obtain pure isolates .

Materials and Methods

Antibacterial activity test of the endophytic bacteria

- Fresh culture of *Escherichia coli* was inoculated on sterile nutrient agar plate using spread plate method.
- Spot inoculation of endophytic bacterial isolates was done on the plates containing E.Coli under sterile conditions.
- The plates were incubates at 37^{0}C for 24hrs.

Figure 1: Growth of endophytic Bacterial colonies

Results

- **Eight endophytic bacterial isolates** were obtained from the stem of Moringa plant.
- Screening of endophytic bacterial isolates capable of inhibiting the growth of pathogenic E.coli bacteria was performed using spot inoculation method.
- **Two endophytic bacterial** isolates showing clear zones i.e. **having antibacterial activity** against *E.coli* are selected.
- Identification of the endophytic bateria is under process.

Figure 2: Endophytic bacteria were isolated using 5 quadrant method

Figure 3 : Endophytic bacteria were sub-cultured

Figure 4 : Pure isolates obtained from the endophytic bacteria

Figure 5: E.Coli on EndoAgar (Test organism)

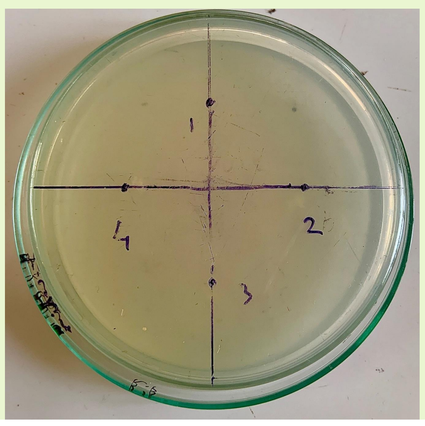

Figure 6: Spot inoculation of endophytic bacteria on Nutrient agar plate spread with E.coli culture

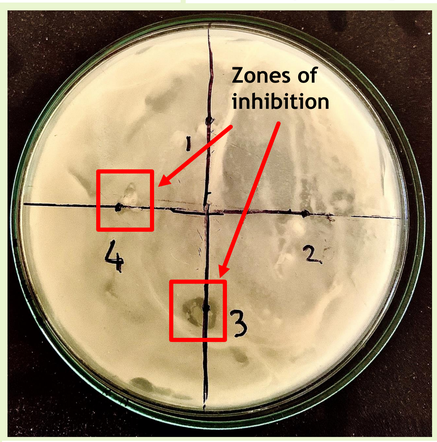

Figure 7: Zone of clearance after 24hrs of incubation indicating anti-microbial activity

Conclusions

- Endophytic bacteria were isolated from stem of *Moringa oleifera* .
- Out of the eight endophytic bacterial isolates obtained from the Moringa plant, two isolates demonstrated anti-bacterial activity when tested against *E.coli.*
- Thus, endophytic bacteria can be contributing to the anti-microbial and medicinal activity of the plant.
- Further detailed studies are required for identification of the endophytes and their antibacterial activity against other Gram positive and Gram negative test organisms to determine the anti-microbial spectrum.

Future Aspects

- Molecular identification of the endophytic bacteria showing antibacterial activity can be done by 16srRNA Sequencing.
- Characterization of the antibacterial compounds obtained from the Endophytes can open a new vista for commercial production of pharma products.
- Similar studies can be done on various plants mentioned in Ayurveda to evaluate potential of endophytes.

References

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Acknowledgements

We are very thankful to the Principal and management of our college for providing us all the requirements for the experiment and our mentor for supporting and guiding us throughout the experiment. Without their help this project would not had been possible.