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# Dynamic movement of endophytic bacteria in sugarcane during the crop lifecycle

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**Abstract**
This study investigates the colonization and movement of endophytic bacterial consortia within sugarcane (*Saccharum officinarum* L.) across different crop growth stages under varying nitrogen fertilizer regimes. Total viable counts (TVC) of six endophytic genera-Acetobacter diazotrophicus, Agrobacterium, Azoarcus, Azospirillum, Burkholderia, and Herbaspirillum-were assessed in leaves, stems, and roots at multiple crop stages. Results revealed distinct organ- and stage-specific colonization dynamics: leaves showed higher bacterial counts in early stages (30–60 days), stems during mid-growth (120–240 days), and roots at harvest (~360 days). These findings provide insights into bacterial migration patterns and suggest optimized inoculation strategies for sugarcane biofertilization.

**Keywords:** Endophytic bacteria, bacterial consortia, sugarcane (*Saccharum officinarum* L.)

## Introduction
Sugarcane is a major agro-industrial crop with high nitrogen demand. Endophytic bacteria play a significant role in enhancing sugarcane growth, nitrogen fixation, and stress tolerance. While their presence in different plant organs is documented, the spatial and temporal movement of endophytic bacteria within the crop lifecycle remains underexplored. Understanding these dynamics is crucial for developing effective biofertilizer strategies and reducing chemical nitrogen dependency.
The objective of this study was to evaluate the population dynamics of six key endophytic bacterial genera across sugarcane plant parts (leaf, stem, root) under varying nitrogen and microbial consortia treatments, and to characterize their movement during the crop lifecycle.

## Materials and Methods
- **Experimental Setup:** Sugarcane plants were grown under different nitrogen levels (0%, 25%, 50%, and 100% NPK) with or without bacterial consortia.
- **Sampling:** Plant samples (leaf, stem, root) were collected at key growth stages: 30–60 days (early), 120–240 days (mid), and harvest (~360 days).
- **Bacterial Isolation:** Tissue samples were surface-sterilized and homogenized. Serial dilutions ($10^2$–$10^8$) were plated on selective media for each bacterial genus.
- **Enumeration:** Colony forming units (CFU $\times 10^8$) were recorded for each tissue and treatment.
- **Data Analysis:** Comparative trends were analyzed across treatments and growth stages to assess spatial and temporal colonization.

## Results
### Organ-wise colonization
- **Leaves:** High bacterial counts at early stages (30–60 days). Herbaspirillum and Agrobacterium dominated leaf colonization.
- **Stems:** Maximum colonization during 120–240 days. Agrobacterium and Azospirillum showed strong systemic presence.
- **Roots:** Dominant colonization at harvest stage (~360 days), with Azoarcus and Azospirillum as key colonizers.

**Treatment effects**

- Reduced nitrogen treatments (25% N + consortia) supported higher bacterial counts than 100% NPK, indicating successful replacement potential of microbial inoculants.
- Omission of specific genera from the consortia reduced colonization efficiency, suggesting synergistic roles.

**Temporal colonization dynamics**

- **30–60 days (early growth):** Leaf tissues harbored maximum microbial populations, highlighting initial phyllosphere colonization.
- **120–240 days (mid growth):** Stem tissues showed peak colonization, reflecting systemic migration via xylem.
- **At harvest (~360 days):** Roots had the highest bacterial counts, confirming preferential rhizosphere-endophyte associations in late growth.

**Discussion**

The study demonstrates that endophytic bacterial colonization in sugarcane is dynamic and organ-specific, varying with crop age. Initial colonization in leaves may occur through stomatal or aerial entry, followed by systemic movement into stems during vigorous growth, and eventual dominance in roots at maturity. These results align with the hypothesis that sugarcane endophytes actively migrate according to host physiology and nutrient allocation.

Importantly, nitrogen reduction treatments enhanced endophyte proliferation compared to full NPK, confirming that microbial consortia can partially substitute chemical fertilizers. The findings suggest that timing of inoculation and tissue targeting are critical to maximize microbial efficiency.

**Table 1:** Representative data table

| Stage | Dominant Tissue | Key Colonizers |
|---|---|---|
| 30–60 days | Leaf | Herbaspirillum, Agrobacterium |
| 120–240 days | Stem | Agrobacterium, Azospirillum |
| Harvest (~360 days) | Root | Azoarcus, Azospirillum |

**Conclusion**

This research highlights the dynamic movement of endophytic bacteria within sugarcane throughout its lifecycle. Colonization shifts from leaves in the early stage to stems in mid-growth and roots at harvest. Such insights can guide the design of stage-specific biofertilizer application strategies, contributing to sustainable sugarcane cultivation.

**Declarations**

**Ethics approval and consent to participate**

Not applicable. This study did not involve humans or animals requiring ethical approval.

**Consent for publication**

Not applicable.

**Availability of data and material**

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

**Competing interests**

The author declares that there are no competing interests.

**Funding**

No funding was received for conducting this study.

**Authors' contributions**

Avinash Salunke designed the study, conducted the experiments, analyzed the data, and prepared the manuscript.

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**Abbreviations**

- CFU – Colony Forming Unit
- TVC – Total Viable Count
- NPK – Nitrogen, Phosphorus, Potassium fertilizer
- DNA – Deoxyribonucleic Acid
- PCR – Polymerase Chain Reaction
- L – Liter
- g – Gram
- ml – Milliliter
- h – Hour

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