



International Journal of Research in Agronomy

E-ISSN: 2618-0618
P-ISSN: 2618-060X
© Agronomy
NAAS Rating (2026): 5.20
www.agronomyjournals.com
2026; 9(7): 754-757
Received: 19-04-2026
Accepted: 23-05-2026

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A comparative analysis of endophytic bacterial diversity in maize across distinct agroecosystems

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DOI: <https://www.doi.org/10.33545/2618060X.2026.v9.i7j.6007>

Abstract

The present study investigated culturable endophytic bacterial populations associated with maize (*Zea mays* L.) under natural and conventional farming systems in Rangareddy district, Telangana. Higher bacterial populations and greater diversity were observed under natural farming than conventional farming. Aziznagar recorded the highest population (8.67 log₁₀ CFU g⁻¹ tissue), while roots harboured the highest bacterial population, followed by stems and leaves. The findings indicate that farming practices and plant tissue type influence endophytic bacterial abundance and diversity. These endophytes may serve as potential candidates for sustainable plant growth promotion and biocontrol.

Keywords: Endophytic bacteria, *Zea mays* L., natural farming, conventional farming

1. Introduction

Maize is one of the important cereal crops cultivated throughout the world and serves as a major source of food, feed and industrial raw materials. The productivity and sustainability of maize cultivation are strongly influenced by plant associated microbial communities, which play a crucial role in nutrient cycling, plant growth promotion and stress tolerance. In recent years, increasing emphasis has been placed on understanding beneficial plant-microbe interactions as a means of improving agricultural productivity while reducing dependence on chemical inputs (Verma *et al.*, 2021) ^[10].

Endophytic bacteria are microorganisms that colonize the internal tissues of the plants without causing visible disease symptoms. They inhabit roots, stems, leaves, seeds and other plant organs, establishing intimate association with their host plants (Johnston-Monje *et al.*, 2014) ^[6]. Hallmann *et al.* (1997) ^[5] defined bacterial endophytes as bacteria that can be isolated from surface-sterilized plant tissues and do not cause harm to the host plant. These microorganisms are considered ubiquitous in agricultural crops and represent an important component of plant microbiomes.

Endophytic bacteria contribute to plant growth and development through several direct and indirect mechanisms. These include biological nitrogen fixation, phosphate solubilization, siderophore production, phytohormone synthesis, nutrient mobilization and suppression of plant pathogens. Because of their close association with plant tissues, endophytes can exert a greater influence on plant metabolism and physiology than many free living soil microorganisms. Their role in enhancing plant fitness and adaption to environmental stresses has been widely documented (Silva *et al.*, 2014) ^[3].

The relation ship between plants and endophytic bacteria is generally mutualistic. Plants provide carbon sources and protective niches for bacterial colonization, while endophytes enhance nutrient acquisition, stimulate growth and improve resistance to biotic and abiotic stresses. Several bacterial genera, including *Bacillus*, *Pseudomonas*, *Enterobacteria*, *Burkholderia* and *Azospirillum*, have been reported as beneficial endophytic in maize and other crops. Consequently, these microorganisms are increasingly being explored as potential biofertilizers and biocontrol agents for sustainable agriculture (Verma *et al.*, 2021) ^[10].

Agricultural management practices play a significant role in shaping the structure and diversity of plant associated microbial communities. Conventional farming systems often depend on synthetic

fertilizers and pesticides to maximize crop productivity, whereas natural farming systems rely on ecological processes, organic inputs and biological interactions for nutrient management and crop protection. Differences in these management practices can influence soil health, microbial diversity and the recruitment of beneficial endophytes by plants (Correa-Galeote *et al.*, 2018) [2]. Natural farming systems are generally characterized by greater biological activity and enhanced microbial diversity due to reduced chemical disturbances and increased organic matter inputs. In contrast, intensive conventional farming practices may alter microbial community composition through repeated fertilizer and pesticide applications. Such changes can affect the abundance and diversity of endophytic bacterial populations residing within plant tissues. Therefore, comparing endophytic communities under contrasting agroecosystems provides valuable information on the ecological consequences of different farming practices (Correa-Galeote *et al.*, 2018; Silva *et al.*, 2014) [2, 3].

Maize hosts a diverse range of endophytic bacterial species that vary according to plant genotype, soil type, environmental conditions and cultivation history. Previous studies have demonstrated that soil management practices and cultivation history significantly influence the diversity and composition of the maize-associated endophytic bacteria. These findings suggest that agricultural systems may determine the persistence of beneficial microbial populations within maize tissue (Johnston-Monje *et al.*, 2014; Correa-Galeote *et al.*, 2018) [6, 2].

Isolations and enumeration of endophytic bacteria constitute the first step toward understanding their ecological significance and potential agricultural applications. Isolation allows the recovery of culturable bacterial endophytes from internal part tissues, whereas enumeration provides quantitative information regarding their population density and distribution. Such investigations facilitate the identification of promising isolates for subsequent evaluation of plant growth-promoting and biocontrol attributes (Hallmann *et al.*, 1997; Liu *et al.*, 2013) [5, 9].

Although several studies have reported the occurrence of endophytic bacteria in maize, comparative information regarding their abundance under natural and conventional farming systems remains limited, particularly under Indian agroecological conditions. Understanding how different agroecosystems influence endophytic bacterial populations is essential for developing microbial-based technologies that support sustainable crop production and soil health management (Verma *et al.*, 2021) [10].

Therefore, the present study was undertaken to isolate and enumerate endophytic bacteria associated with maize cultivated under natural and conventional farming systems. The study aims to compare the endophytic bacterial populations present in these contrasting agroecosystems and to generate baseline information for the identification and utilization of beneficial endophytic bacteria in sustainable maize production systems.

2. Materials and Methods

2.1 Collection of plant samples

Healthy maize (*Zea mays* L.) plants were collected from fields managed under natural farming and conventional farming systems. Samples were collected at vegetative stage from natural and conventional farms located in Rangareddy district. Plants were carefully uprooted and transported to laboratory in sterile polythene bags under refrigerated conditions for further analysis. Similar sampling procedures have been employed in studies investigating endophytic bacterial diversity in maize (Correa-Galeote *et al.*, 2018) [2].

2.2 Surface sterilization of plant tissues

Plants were washed thoroughly under running tap water to remove adhering soil particles and debris. The tissues were cut into small segments (approximately 1 cm) and subjected to surface sterilization. The segments were immersed in 70% ethanol for 1 min followed by 2% sodium hypochlorite solution for 3-5 min and then rinsed five times with sterile distilled water. The final rinse water was plated on nutrient agar medium to verify the effectiveness of surface sterilization. Absence of microbial growth from the rinse water was considered evidence of successful elimination of epiphytic microorganisms. This procedure was adopted from (Hallmann *et al.*, 1997; Zinniel *et al.*, 2002) [5, 13].

2.3 Isolation of endophytic bacteria

Surface-sterilized plant tissues were aseptically macerated using a sterile mortar and pestle containing 9 ml sterile physiological saline (0.85% NaCl). One gram of tissue was homogenized thoroughly to obtain a uniform suspension. Serial dilutions were prepared up to 10^6 dilution. Aliquots (0.1 ml) from 10^4 , 10^5 and 10^6 dilutions were spread onto nutrient agar and King's B agar plates using a sterile glass spreader. The inoculated plates were incubated at 28 ± 2 °C for 24-48 h. Distinct bacterial colonies differing in morphology, size, shape, colour and texture were selected and repeatedly streaked on fresh nutrient agar plates until pure cultures were obtained. Similar procedures have been reported for maize endophytic bacterial isolations by (Moturu *et al.*, 2023) [8].

2.4 Enumeration of endophytic bacterial population

The population density of endophytic bacteria was determined using the standard serial dilution plate count technique. Colonies appearing on nutrient agar plates were counted after incubation and the bacterial population was expressed as colony-forming units per gram (CFU g⁻¹) of fresh plant tissues using the formula:

$$\text{CFU g}^{-1} = \frac{\text{Number of colonies} \times \text{Dilution factor}}{\text{Amount plated}}$$

The values were transformed into logarithmic units [$\log_{10}$ (CFU g⁻¹)] before statistical analysis. The serial dilution and plate count method is widely used for quantitative estimation of culturable endophytic bacterial populations in maize tissues (Marag *et al.*, 1985) [7].

2.5 Purification and Maintenance of isolates

Pure bacterial cultures were maintained on nutrient agar slants and stored at 4°C for preservation. Periodic subculturing was carried out to maintain the viability of isolates throughout the study. For long-term preservation cultures are stored in nutrient broth supplemented with glycerol at -20°C.

2.6 Statistical analysis

The experiment was conducted using a completely randomized Design (CRD). Endophytic bacterial population data obtained from different plant tissues and farming systems were subjected to analysis of variance (ANOVA). Mean comparisons were performed using the least significant difference (LSD) test at 5% probability level. Bacterial counts were log-transformed before analysis to normalize variance. Statistical procedure followed standard microbiological and agricultural experimental methods.

3. Results and Discussion

3.1 Endophytic bacterial population in maize under different agroecosystems

The population of culturable endophytic bacteria varied significantly between maize plants grown under natural farming and conventional farming systems. The population of endophytic bacteria isolated from maize tissues under natural and conventional farming systems is presented in the (Fig. 3.2) as $\log_{10}$ CFU g^{-1} tissue. Under the natural farming system, the highest bacterial population is observed in Aziznagar ($8.67 \log_{10}$ CFU g^{-1}), followed by Shabad ($8.54 \log_{10}$ CFU g^{-1}) and Maheshwaram ($8.41 \log_{10}$ CFU g^{-1}). Among the natural farming locations, Aziznagar recorded the highest bacterial counts. Similarly, under the conventional farming system the highest population was recorded in Chevella ($8.58 \log_{10}$ CFU g^{-1}) followed by Moinabad ($8.44 \log_{10}$ CFU g^{-1}) and Rajendranagar ($8.26 \log_{10}$ CFU g^{-1}). Among the conventional farming locations Chevella exhibited the highest bacterial abundance across all tissues. Higher endophytic bacterial populations were observed in plants collected from the natural farming system compared to those from conventional farming system.

The increased abundance of endophytic bacteria in natural farming systems may be attributed to reduced chemical inputs, greater organic matter availability and favourable ecological conditions that support microbial colonization and persistence within plant tissues. Similar observations were reported by Correa-Galeote *et al.* (2018) [2], who demonstrated that soil cultivation history significantly influenced maize endophytic bacterial diversity and abundance, with sustainability managed soils harbouring richer endophytic communities than intensively managed soils. Furthermore, long-term agricultural practices were identified as important drivers of endophytic colonization and bacterial community structure in maize roots. Xia *et al.* (2019) [12] reported significantly higher abundance and diversity of culturable endophytic microorganisms in organically managed crop fields than in conventionally managed fields, suggesting that sustainable farming practice promote the establishment and persistence of beneficial endophytes. Similarly, Xia *et al.* (2015) [11] observed that organically cultivated crops harboured a greater number of culturable bacterial endophytes than conventional grown crops.

3.2 Distribution of endophytic bacteria in different plant parts

Among the plant tissue examined, roots recorded the highest endophytic bacterial population followed by stem and leaves. The root tissues contained $8.67 \log_{10}$ CFU g^{-1} tissue, while stem and leaf tissues contained $8.57 \log_{10}$ CFU g^{-1} tissue and $8.45 \log_{10}$ CFU g^{-1} tissue, respectively.

The highest population in roots may be due to their direct contact with the soil environment, which serves as the primary reservoir of microbial communities. Root exudates provide nutrients that facilitate bacterial colonization and subsequent entry into the internal plant tissues. Similar findings were reported by Correa-Galeote *et al.* (2018) [2], who isolated diverse endophytic bacterial populations from maize roots and observed that roots act as the principal site of bacterial populations from maize roots and observed that roots act as the principal site of bacterial entry and establishment. Studies on maize microbiomes have consistently shown greater microbial richness and diversity in root tissues compared with aerial plant parts. Bourceret *et al.* (2022) [1] demonstrated that maize roots harbour complex and dynamic microbial communities recruited from the surrounding soil throughout plant development. The

study showed that roots constitute a major microbial habitat, supporting a rich and diverse microbiota that contributes to plant growth and health. In addition, Gao *et al.* (2024) [4] observed substantial diversity of endophytic bacterial communities in maize germinating roots, further confirming that root tissues are important reservoirs of endophytic microorganisms.

Therefore, the significantly higher endophytic bacterial population recorded in root compared to stem and leaves in the present study may be due to close association of roots with the rhizosphere, continuous exposure to soil microbial inoculum and the availability of exudates that favour bacterial recruitment, colonization and persistence within plant tissues.

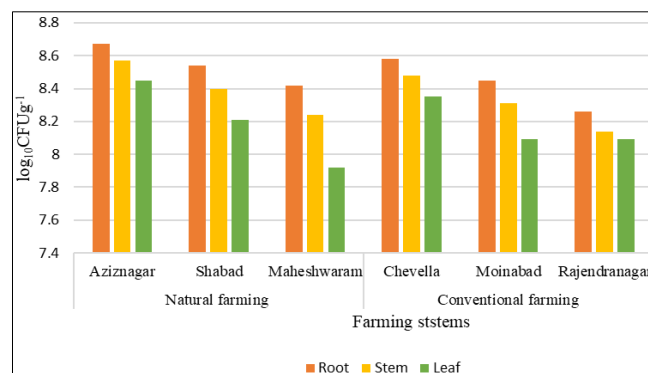


Fig 1: Endophytic bacterial population ($\log_{10}$ CFU g^{-1} tissue) in root, stem and leaf samples of maize collected from natural farming locations (Aziznagar, Shabad and Maheshwaram) and conventional farming locations (Chevella, Moinabad and Rajendranagar). Higher bacterial populations were observed in roots followed by stems and leaves, with natural farming locations generally showing greater endophytic bacterial abundance than conventional farming locations.

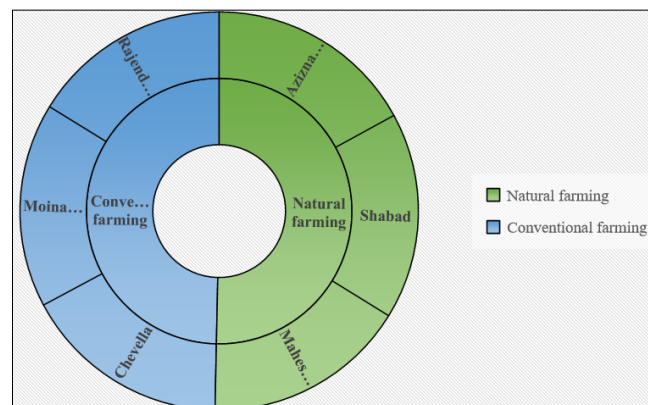


Fig 3.1: Distribution of endophytic bacterial isolates recovered from maize cultivated under natural farming (Aziznagar, Shabad and Maheshwaram) and conventional farming (Chevella, Moinabad and Rajendranagar) systems. The inner ring represents the farming system, while the outer ring shows the distribution of isolates among individual locations.

3.3 Effect of farming system on endophytic diversity

Morphological characterization of the isolates revealed substantial variation in colony morphology, Pigmentation, texture, elevation and margin characteristics. A greater number of morphologically distinct bacterial isolates were recovered from maize plants grown under natural farming than from conventional farming.

The greater diversity observed under natural farming may be associated with enhanced microbial interactions and reduced disturbance caused by synthetic agrochemicals. Sustainable and ecologically managed soils often promote microbial richness,

which subsequently influences the recruitment of beneficial endophytes by host plants. Correa-Galeote *et al.* (2018) ^[2] reported that maize plants cultivated under different soil management histories exhibited distinct endophytic bacterial communities, indicating that agricultural practices play a crucial role in shaping endophytic diversity.

3.4 Ecological significance of endophytic bacterial colonization

The presence of abundant endophytic bacterial populations in maize tissues suggests their potential role in supporting plant growth and health. Endophytic bacteria are known to promote plant development through biological nitrogen fixation, phosphate solubilisation, phytohormone production, siderophore synthesis and suppression of phytopathogens.

The higher populations observed under natural farming systems indicate that these agroecosystems may favour the establishment of beneficial plant-microbe associations. Endophytes are recognized as important contributors to nutrient acquisition, stress tolerance and overall plant fitness. Their ability to colonize internal tissues allows them to interact directly with host physiological processes, thereby enhancing crop performance and resilience under varying environmental conditions.

4. Conclusion

The study demonstrated that maize plants cultivated under natural farming systems harboured higher populations and greater diversity of culturable endophytic bacteria than those grown under conventional farming systems. Root tissues supported the highest bacterial populations compared with stems and leaves. These findings suggest that farming practices strongly influence endophytic bacterial colonization and that natural farming systems may provide favourable conditions for the establishment of beneficial microbial communities. The isolated endophytic bacteria may serve as potential candidates for further evaluation of plant growth-promoting and biocontrol traits.

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