Current **A**pplied **S**cience and **T**echnology e-ISSN: 2586-9396

Curr. Appl. Sci. Technol. ….., Vol... (No...), e0270010

Research article

Isolation and Characterization of Multifunctional Seed-Borne Endophytic Bacterium *Lysinibacillus sphaericus* YEBEVIA for Enhancing Maize Growth

Bartholomew Saanu Adeleke[1,2]*

[1]Department of Microbiology, Faculty of Natural and Applied Sciences, Federal University of Technology and Environmental Sciences, PMB 001, Iyin-Ekiti, Nigeria
[2]Food Security and Safety Focus Area, Faculty of Natural and Agricultural Sciences, North-West University, Mmabatho 2735, South Africa

Received: 28 November 2025, Revised: 12 March 2026, Accepted: 31 March 2026, Published: 7 July 2026

Abstract

The endosphere biology attracts increasing interest from researchers seeking to understand plant-microbe synergism and the bio-efficacy of microbial inoculants in enhancing plant growth and soil health. This study was designed to isolate and identify a novel seed-borne endophytic *Lysinibacillus sphaericus* YEBEVIA with plant growth-promoting capabilities and bioinoculation potential. Morphological assessment revealed that isolate D3 was a Gram-positive, rod-shaped bacterium with a large cell size and undulated colony margins. All the isolates fermented glucose, fructose, sucrose, and maltose, producing only acid from lactose. PGP screening demonstrated that all isolates exhibited beneficial traits, although at varying intensities. *Lysinibacillus sphaericus* YEBEVIA displayed remarkable zinc-solubilizing ability (3.0 cm), siderophore production (2.5 cm), and high HCN activity (2.87 µg/mL). Phosphate solubilization (17.46 µg/mL) and IAA production (14.26 µg/mL) were also notably high in *L. sphaericus* YEBEVIA. Heavy-metal tolerance assays revealed that *L. sphaericus* YEBEVIA responded positively to increasing concentrations of zinc and lead, with the highest growth observed at 2 g supplementation. Enhanced tolerance to chromium was also recorded, whereas cadmium showed no significant effect compared with the control. Minimum inhibitory concentration (MIC) effects were observed only with lead sulphate, producing inhibitory zones of 5.1 cm, 2.5 cm, and 6.8 cm. Bioinoculation studies showed significant improvements in maize growth. Under field conditions, inoculated plants developed 13 leaves, 41 roots, and a fresh weight of 256.9 g, while greenhouse trials produced comparable enhancements. These findings highlight the strong potential of *L. sphaericus* YEBEVIA as a biofertilizer for sustainable crop improvement.

Keywords: bioinoculation; cereal crops; heavy-metal tolerance; maize growth enhancement; plant growth promotion; seed-borne beneficial bacteria

*Corresponding author: E-mail: bartholomew.adeleke@futes.edu.ng; microbade@yahoo.com
https://doi.org/10.55003/cast.2026.270010

1. Introduction

Growing maize as a staple cereal crop has continued to attract the interest of farmers due to its rich nutritional profile and economic value (Adeleke & Fakoya, 2025). It serves as a major source of food for humans and animals, provides livestock feed, and functions as an important raw material in various industries (Abiodun et al., 2021). Globally, and particularly in Nigeria, maize cultivation has expanded significantly, contributing to agricultural and economic development across diverse agro-ecological regions. While increasing maize production to ensure food security remains a key priority, the heavy reliance on agrochemicals has made this approach unsustainable because of the associated environmental and health consequences (Lipková et al., 2021). Therefore, improving maize productivity through environmentally friendly strategies requires innovative biorational approaches that utilize potent microorganisms of agricultural importance.

Maize seeds harbor diverse microorganisms within their endosphere, including endophytic bacteria that colonize internal seed tissues without harmful consequences (Wallace, 2023). These seed-borne endophytes have gained scientific attention due to their remarkable attributes in plant growth promotion, nutrient bioavailability and acquisition, enhancement of plant resilience to environmental stresses, and biocontrol of phytopathogens (Hu et al., 2025). Their vertical transmission from seed to seedling makes them particularly valuable, as they ensure early colonization of emerging plants, thereby supporting healthy growth and long-term plant health (Zhang et al., 2017). Consequently, seed-associated bacteria represent a promising research frontier in sustainable agriculture (Walitang et al., 2017).

Among the plant growth-promoting endophytic bacteria, *Lysinibacillus sphaericus* has attracted considerable interest because of its multifunctional qualities, including biocontrol and bioremediation potentials (Shabanamol et al., 2018). Although *Lysinibacillus fusiformis* and *Bacillus cereus* have been reported as plant growth-promoting bacteria (Aguilar et al., 2025). Recent studies have demonstrated their ability to enhance plant growth through the production of indole-3-acetic acid (IAA), exopolysaccharides, siderophores, ammonia, and the solubilization of essential soil nutrients (Eke et al., 2019; Shabanamol et al., 2018).

However, while several studies have focused on soil-associated strains (Lebrazi et al., 2020; AlAli et al., 2021), research on seed-borne endophytic *L. sphaericus* YEBEVIA remains limited. Compared with rhizobacterial strains inhabiting the soil environment, endophytic *L. sphaericus* with distinct functional traits can readily colonize internal plant tissues and establish synergistic interactions with their host (Chen et al., 2024). These interactions influence the biochemical and physiological processes of plants through the synthesis of phytohormones, enhanced nutrient mobilization, and the mitigation of biotic and abiotic stresses (Adeleke & Fakoya, 2025). While soil-associated *L. sphaericus* primarily contributes to nutrient cycling, bioremediation, and pollutant control that sustain soil health, endophytic strains often promote plant growth more directly through their intimate association with plant tissues and their potential for vertical transmission from seed to seedling (Sun et al., 2023). Despite these advantages, the mechanisms employed by plant growth-promoting bacteria associated with maize seeds and soil remain poorly understood, thereby limiting their broader application as bioinoculants for improving maize productivity (Sun et al., 2023). Consequently, the identification of indigenous seed-borne endophytes is essential for bridging the gap between research and agricultural practice, particularly in promoting eco-friendly agro-inputs that are compatible with local ecosystems. Understanding these microbial communities will support the development of

sustainable agricultural practices and reduce dependence on chemical fertilizers (Abdelfattah et al., 2023).

Therefore, the objective of this study is to isolate and molecularly identify the endophytic bacterium *Lysinibacillus sphaericus* YEBEVIA from the maize seed endosphere and to evaluate its novelty and potential role as a plant growth-promoting bacterium. This investigation seeks to expand current knowledge of beneficial endophytic bacteria and their relevance to biorational agricultural practices and sustainable maize production.

2. Materials and Methods

2.1 Sample collection, preparation, and microbial analysis

Approximately 70 g of healthy maize seeds (SUWAN) purchased from Premier Seed Nigeria Limited were weighed and prepared for surface sterilization by putting them into sterile distilled water to remove the traces of dirt and floating materials, washed thoroughly, and soaked in sterile distilled water for 1 h. Then, the maize seeds were further surface sterilized using 70% ethanol for 1 min, followed by 3.5% hypochlorite for 30 s, and then 70% ethanol was added for 1 min, and rinsed with distilled water 5 times. The sterilized samples were macerated inside a mortar and pestle by adding a little sterile distilled water. Then, 1 g of the slurry obtained was weighed and aseptically dispensed into Falcon tubes containing 9 mL sterile distilled water and mixed properly. Afterwards, 1.0 mL of the diluent was aseptically pipetted and serially diluted up to 10^6 dilutions. From dilutions 10^2 and 10^3, 0.1 ml of the suspension was withdrawn and gently dispensed into a labelled sterile Petri dish in triplicate using a 5 mL sterile pipette and poured with sterilized molten nutrient agar. The Petri plates were incubated at 37ºC for 24 h and observed for bacterial growth. Distinct bacterial colonies on the plates were counted, recorded, and selected based on morphological characteristics. The streaking method was employed to obtain pure bacterial cultures, which were stored for further analysis. Gram staining and various biochemical tests, such as catalase, hydrogen sulfide production, starch hydrolysis, indole production, gelatin and sugar fermentation test, were performed on the bacterial isolates (Adeleke et al., 2022).

2.2 Screening of pure bacterial isolates for plant growth-promoting traits

2.2.1 Production of indole-3-acetic acid

The bacterial isolates were screened for IAA production in IAA-production medium supplemented with L-tryptophan. 2 g peptone, 1 g NaCl, and 0.2 g tryptophan were weighed and dispensed into 200 mL sterile distilled water. Ten mL of the solution was dispensed into Falcon tubes and sterilized at 121ºC for 15 min and allowed to cool down. After cooling, a loopful of the bacteria was inoculated and incubated for 48 h at 37ºC. The broth cultures were centrifuged at 4000 rpm for 10 min. After centrifugation, 3 drops of orthophosphoric acid and 4 mL Salkowski reagent (0.05L of 35% perchloric acid and 1 mL of 0.5M $FeCl_2.6H_2O$) were added to 2 mL of the cell supernatant. The mixture was incubated in the dark for 30 min. The development of pink coloration indicated IAA production. A tube without bacterial inoculation served as a control. The quantity of the IAA produced in the inoculated IAA-production medium was determined using a spectrophotometer at 530 nm (Adeleke et al., 2022).

2.2.2 Hydrogen cyanide production test

The hydrogen production ability of bacterial isolates was tested by weighing 0.8 g of glycine and 3.2 g of nutrient broth into 200 mL of distilled water. Ten mL of the solution was dispensed into each Falcon tube and sterilized at 121ºC for 15 min. After sterilization, the tubes were allowed to cool. Whatman paper cut into sizes was soaked inside with picric acid, and sodium carbonate solution (1 g of sodium carbonate was dissolved in 50 mL of distilled water). A loopful of the bacteria was inoculated into each Falcon tube with soaked Whatman filter paper plugged with cotton wool into the tubes just above the broth solution and incubated at 37ºC for 7 days. After incubation, the change in color of the Whatman paper from yellow to deep orange indicated the production of hydrogen cyanide. The uninoculated tube served as a control. The quantity of the HCN produced in the inoculated HCN-production medium was determined using a spectrophotometer at 300 nm (Adeleke et al., 2022).

2.2.3 Phosphate solubilization

Phosphate solubilization test was carried out in a modified Pikovskaya agar composed of 2.5 g of tricalcium phosphate, 5 g of glucose, 0.001 g of $MnSO_4 \cdot H_2O$, 0.1 g of NaCl, 0.1 g of KCl, 0.05 g of $MgSO_4$, and 0.25 g of yeast extract, 7.5 g of agar, and 500 mL of distilled water. The medium was sterilized at 121ºC for 15 min. The sterilized medium was allowed to cool before pouring plating under aseptic conditions. The plates were spot inoculated at the center with the 24-h-old bacterial culture and incubated at 37ºC for 7 days. The zone of clearance around the colony on the cultured plates indicated positive results. A plate without bacterial inoculation served as a control (Adeleke et al., 2022). The quantity of phosphorus produced was determined in a Pikovskaya broth medium inside Falcon tube inoculated with a loopful of the bacterial cultures and incubated at 37ºC for 5 days. The absorbance of the broth cultures was measured at 600 nm using a spectrophotometer (Adeleke et al., 2022).

2.2.4 Zinc solubilization

For zinc solubilization screening, 10 g of dextrose, 1 g of ammonia sulphate, 0.2 g of potassium chloride, 0.1 g of dipotassium hydrogen phosphate, 0.2 g of magnesium sulphate, zinc carbonate 0.1%, and 7.5 g of agar were weighed into 500 mL of sterile distilled water and sterilized at 121ºC for 15 min. The solution was allowed to cool and poured plated under aseptic conditions. Bacterial cultures (24-h-old) were spot inoculated at the center of each plate and then incubated at 37ºC for 48 h. The formation of a clearance zone around the colonies indicated zinc. A plate without bacterial inoculation served as a control for solubilization (Adeleke et al., 2022). The quantity of zinc produced in the inoculated zinc-production broth medium was determined using a spectrophotometer at 600 nm (Adeleke et al., 2022).

2.2.5 Ammonia production

Ammonia production was performed by weighing 3 g of peptone water into 200 mL of sterile distilled water in a 500 mL conical flask. Ten mL of the solution was dispensed into each Falcon tube and autoclaved at 121ºC for 15 min. After autoclaving, the tubes were allowed to cool, and a loopful of the bacterial culture was inoculated and incubated at 37ºC for 48 h. After the incubation, 0.5 mL of Nessler's reagent was dropped into each Falcon tube

and was left for 5 min and allowed to stand. A color change from slight yellow to brownish indicated ammonia production. A tube without bacterial inoculation served as a control. The quantity of the ammonia produced in the inoculated ammonia-production medium was determined using a spectrophotometer at 425 nm (Adeleke et al., 2022).

2.2.6 Exopolysaccharide production

For the exopolysaccharide production test, 14 g of nutrient agar was prepared into 500 mL of sterile distilled water. The medium was amended with 12.5 g of sucrose and sterilized at 121ºC for 15 min. After cooling, sterile Whatman No 1 paper cut into pieces of equal diameter was placed inside the plates containing solidified media. Then, a loopful of bacterial suspension was dropped on the sterile Whatman paper and incubated at 37ºC for 48 h. The cloudy colony growth around the filter paper indicated a positive test. Uninoculated plates serve as a control. The quantity of the exopolysaccharide produced in the inoculated exopolysaccharide nutrient broth medium was determined using a spectrophotometer at 600 nm (Adeleke et al., 2022).

2.2.7 Nitrogen fixation

The medium of nitrogen fixation is composed of 10 g of sucrose, 0.5 g of K_2HPO_4, 0.25 g of $MgSO_4$, 0.25 g of NaCl, 0.05 g of $FeSO_4 \cdot 7H_2O$, 1 g of $CaCO_3$, 7.5 g of agar, 0.0025g of $NaMoO_4$, and 500 mL of distilled water. The medium was sterilized at 121ºC for 15 min. The solution was allowed to cool and poured plated under aseptic conditions. 24-h-old bacteria were spot inoculated at the center of each plate and then incubated at 37ºC for 7 days. The clear zone around the colony indicated a positive result. A plate without bacterial inoculation served as a control. The quantity of the nitrogen produced in the inoculated nitrogen-production broth medium was determined using a spectrophotometer at 600 nm (Adeleke et al., 2022).

2.2.8 Siderophore production

For the siderophore production test, the bacterial isolates were inoculated in a medium containing Chrome Azurol S (CAS) following the method of Adeleke et al. (2022) with little modification. After a streak inoculation with a 24-h-old bacterial culture, the Petri plates were incubated for 72 h at 37ºC. The appearance of yellow or orange coloration around the colonies implied siderophore production. The quantity of the siderophore produced in the inoculated siderophore-production medium was determined using a spectrophotometer at 530 nm.

2.3 Effect of heavy metals on bacterial growth and minimum inhibitory concentration (MIC) determination

The bacterial response to heavy metals, such as lead, zinc, chromium, iron, and cadmium, was determined in a nutrient broth supplemented with heavy metals. Briefly, 80 mL of sterile distilled water was measured into 4 different conical flasks, each containing 1.04 g of nutrient broth. Different concentrations of heavy metals, 2, 4, 6, and 8 g of lead, zinc, chromium, iron, and cadmium sulphates were measured separately and mixed. The solution in each of the conical flasks was dispensed into 10 mL Falcon tubes with appropriate labelling. The Falcon tubes were sterilized at 121ºC for 15 min and then

allowed to cool. After cooling, the tubes were inoculated separately with the isolated bacteria and incubated at 37ºC for 24 h. The bacterial growth response to lead, zinc, chromium, iron, and cadmium was measured using a spectrophotometer at 600 nm.

The MIC of heavy metals on bacterial growth was achieved in a heavy-metal-containing medium. Briefly, 11.2 g of nutrient agar was weighed and dispensed into conical flasks containing 400 mL of sterile distilled water with amended different concentrations of heavy metals, lead, zinc, chromium, iron, and cadmium at 2 g, 4 g, 6 g, and 8 g, respectively, poured into different conical flasks separately. Then, the solution was sterilized, allowed to cool, and pour plated and allowed to solidify. Then, plates were point-inoculated with the pure bacterial cultures and incubated at 37ºC for 48 h. The zone of clearance around the colony on the culture plate indicated a positive result. The results obtained were compared with the control, uninoculated (Adeleke & Fakoya, 2025).

2.4 Indole 3-acetic acid production in a medium supplemented with carbon sources

To check for optimization of IAA using tryptophan as a sole substrate in the medium, varied concentrations of glucose, lactose, and fructose at 2 g, 4 g, 6 g, and 8 g were supplemented into the IAA medium composed of 1.0 g of tryptophan in each of the sugar solutions. The medium was dispensed into Falcon tubes and sterilized at 121ºC for 15 min. After cooling, the tubes were inoculated with bacterial cultures and incubated at 37ºC for 24 h. The bacterial growth response was measured using a spectrophotometer at 600 nm. Uninoculated Falcon tubes serve as control (Adeleke et al., 2022).

2.5 Bacterial identification by molecular analysis

The genomic DNA was extracted from the bacterial cultures using Quick-DNA™ Fungal/Bacterial Miniprep Kit (Zymo Research, Catalogue No. D6005) following the manufacturer's protocol (Altschul et al., 1997). The quality and quantity of the extracted DNA were measured using a nanodrop (Thermo Scientific™ NanoDrop™ One Microvolume UV-Vis Spectrophotometer). The system was blanked using 1 µl of DNA Elution Buffer. Afterwards, 1 µl of the DNA was placed on the pedestal and measured. The concentration (ng/µl), A260/280 ratio, and A260/230 ratio of the sample were subsequently recorded (Altschul et al., 1997).

Polymerase chain reaction (PCR) amplification:
The target region of the PCR product was amplified using OneTaq® Quick-Load® 2X MasterMix (NEB, Catalogue No. M0486). The identified isolate was subjected to PCR using the primers: forward, 27F - (5' AGAGTTTGATCMTGGCTCAG 3') and reverse, 1492R - (5'CGGTTACCTTGTTACGACTT 3'). The primers were purchased from Inqaba Biotechnological Industrial (Pty) Ltd, Ibadan, Nigeria. A total of 25 µL reaction mixture composed of 1 µL of template DNA, 0.5 µL (10 nM) of 10 µM Forward primer, 0.5 µL (10nM) of 10 µM Reversed primer, 12.5µL for One Taq Quick Load 2X Master Mix with standard buffer, 10.5 µL Nuclease-free water. The samples were then subjected to the following thermal cycling conditions using the Eppendorf Mastercycler Nexus gradient 230. The PCR conditions were set at initial denaturation at 94ºC for 5 min, denaturation1 at 94ºC for 30 s, annealing1 at 50ºC for 30 , extension1 at 68 ºC for 1 min and 30 s, denaturation2 at 94ºC for 1 min and 30 s, annealing2 at 53ºC for 30 s, extension2 at 68ºC for 1 min and 30 s, denaturation3 at 94ºC for 30 s, annealing3 at 55ºC for 30 s, extension3 at 68 ºC for 1 min

and 30 s, final extension at 68ºC for 10 min, and hold at 4ºC till infinity. The PCR underwent 35 cycles of amplification (Altschul et al., 1997).

Gel electrophoresis:
After PCR amplification, 2 ul of each PCR product was run on 1% agarose gel, stained with SafeView Red (5 uL), and photographed using a gel documentation system (E-BOX, Vilber Lourmat, Italy) (Altschul et al., 1997).

Post-PCR purification:
Firstly, the PCR products were cleaned using an enzymatic method (ExoSAP) as follows: the ExoSAP master mix was prepared by adding the following to a 0.6 ml micro-centrifuge tube: a. 50 µL of 20U/µL exonucylease I (Catalogue No. NEB M0293L) and b. 200 µL of 1U/µl of shrimp alkaline phosphatase (Catalogue No. NEB M0371). Secondly, the reaction mixture was prepared by mixing the following and incubating the resulting mix at 37ºC for 15 min and at 80ºC for 15 min. a. Amplified PCR product 10 µL and b. ExoSAP Mix (step 1) 2.5 µL (Altschul et al., 1997).

Gene sequencing:
The fragments/PCR products were sequenced using the Nimagen, Brilliant Dye™ Terminator Cycle Sequencing Kit V3.1, BRD3-100/1000, and purified (Zymo Research, ZR-96 DNA Sequencing Clean-up Kit™, Catalogue No. D4050) following the manufacturer's instructions. The sequencing reaction was done using internal sequencing primers: 785F: (5'-GGATTAGATACCCTGGTA-3') forward, and 907R: (5'-CCGTCAATTC MTTTRAGTTT-3') reverse. The labelled products were then cleaned with the ZR-96 DNA Sequencing Clean-up Kit (Catalogue No. D4053). The cleaned DNA products of each sample were injected and analyzed on the Applied Biosystems ABI 3500XL Genetic Analyser (Applied Biosystems, ThermoFisher Scientific) with a 50 cm array, using POP7 and sequence data collected (Altschul et al., 1997). The NCBI links to the sequenced data can be accessed at BioProject: https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1422676.

2.6 Bioinoculation attributes of identifiable seed-borne endophytic bacterial isolates

The bioinoculation efficacy of the bacterial isolates was tested on maize seeds according to the method of Adeleke and Fakoya (2025). Briefly, the inoculum preparation was achieved in a sterilized nutrient broth inoculated with the bacterial isolates. After incubation for 5 days, the broth culture was centrifuged at 4000 rpm for 10 min, and the pelletized bacterial cells were washed with 0.85% normal saline solution. The inoculum was standardized using a spectrophotometer and stored in a McCartney bottle before use (Adeleke et al., 2022).

Greenhouse and field experiments using bacterialized maize seeds:
The effect of the bacteria on the maize growth was tested under experimental conditions using sterile and non-sterile soil collected at Research Farm, Olusegun Agagu University of Science and Technology, Okitipupa farmland. The soil was sterilized using autoclaved at 121ºC for 15 min and allowed to cool. Approximately 30 kg of sterilized soil samples were placed inside sterile plastic pots and randomly arranged at 10 cm intervals.

The maize seeds (SUWAN) were surface sterilized and dispensed into bacterial suspension containing CMC, and allowed to stand for 30 minutes with gentle shaking. The

solution was decanted, and 10 bacterialized seeds were planted in each bag, randomly arranged. Each of the seeds was about 5 cm away from the others. For the field experiment, ridges were made on the farmland and bacterialized seeds were planted directly into the sterilized-amended soil with a spacing of 10 cm in row and column. After seven days of seed germination, the seedlings were inoculated with the bacterial suspension directly to the maize roots and monitored for 52 days after planting. The growth parameters measured include the number of roots, the number of leaves, plant length, leaf width, and leaf length. The uninoculated maize plants served as a control.

2.7 Statistical analysis

The triplicate data obtained were analyzed using analysis of variance (ANOVA), followed by Duncan's Multiple Range Test (DMRT) using the Statistical Package for the Social Sciences (SPSS), version 17.0, and presented as mean±standard deviation. Summary data for individual variables were illustrated using simple and clustered bar graphs, showing the mean values of the tests and the control.

3. Results and Discussion

3.1 Bacterial counts, cellular morphology, and microscopic observations

The cellular morphology and microscopic observations of the bacterial isolates presented in Table 1 showed that the three isolates designated D1, D2, and D3 exhibited similar microscopic characteristics, appearing as large rod-shaped cells under the light microscope and Gram reaction, positive. The creamy pigmentation and opaque optical appearance were observed in all the isolates. Isolates D1 and D3 exhibited raised elevations, whereas D2 showed a flat elevation. In terms of colony margin, D1 displayed an entire margin, D2 showed an irregular margin, while D3 exhibited an undulate margin. The seed endosphere represents a unique niche for diverse microorganisms of agricultural importance, particularly those capable of stimulating growth factors that enhance seedling vigor, plant development, nutrient utilization, stress tolerance, and pathogen suppression (Jha et al., 2025). In this study, the isolation and characterization of seed-borne endophytic bacteria revealed substantial plant growth-promoting (PGP) attributes, morphological traits, and biochemical capabilities. The bacterial isolate (D3) demonstrated remarkable functional and physiological characteristics.

The dominant endophytic bacteria genera, *Bacillus* and *Lysinibacillus,* in seed tissues have been reported by Pantoja-Guerra et al. (2023) and Nandana and Anith (2025), who noted that their dominance is often linked to their endospore-forming ability, desiccation tolerance, and resistance to environmental stress during seed maturation and storage.

High abundance in seed endophytes is frequently linked to ecological competitiveness and vertical transmission (Walitang et al., 2017). Recent studies also highlight the dominance of *Lysinibacillus, Bacillus, Pantoea,* and *Enterobacter* in seed endosphere niches due to their resilience and versatility in nutrient utilization (Sharma et al., 2023; Soumya et al., 2026). These findings reinforce the critical role of seed endophytes in early plant establishment and improved crop performance.

Table 1. Cellular morphology and microscopic observations

IC	Shape	GRN	Size	Pigment	Elevation	Margin	Optical
D1	Rod	+	Large	Creamy	Raised	Entire	Opaque
D2	Rod	+	Large	Creamy	Flat	Irregular	Opaque
D3	Rod	+	Large	Creamy	Raised	Undulate	Opaque

Key: IC - isolate code

The morphological and microscopic characteristics of the bacterial isolates observed in this study were consistent with traits commonly associated with beneficial endophytic bacteria (Adeleke et al., 2022; Aguilar et al., 2025; Van Chuong et al., 2026). These observations also support their identification, particularly isolate D3, which was identified as *L. sphaericus* YEBEVIA with distinctive functional growth traits among the screened isolates (Table 3). The bacterial isolates exhibiting similar morphological features may also share related physiological characteristics. In many studies, rod-shaped and Gram-positive endophytic bacteria, *Pseudomonas* spp., *Enterobacter hormaechei*, and *Enterobacter* spp. have frequently been isolated and identified from nodules, roots, and seeds of *Vigna radiata* (Bhagya et al., 2019). Abd Elhakeem et al. (2024) have also reported rod-shaped and Gram-positive endophytic bacteria with plant growth-promoting traits from the seeds of *Moringa oleifera*. The variations observed in the colony morphology of the isolates may be attributed to their diverse phenotypic traits, which play important roles in ecological adaptation and functional interactions within host plants (Romero-Gutierrez et al., 2020). Morphological characterization is often employed as a preliminary approach in distinguishing endophytic bacterial isolates before biochemical and molecular identification, and it can provide early indications of potential functional capabilities (Ikeda et al., 2013). Furthermore, the presence of similar morphological features among some isolates suggests that they may belong to closely related taxonomic groups, whereas differences in colony margin, elevation, and texture may reflect strain-level variations that could influence their functional attributes. *Lysinibacillus* and *Bacillus* spp. possess structural adaptations, such as endospore formation, that facilitate survival during seed development, enabling vertical transmission (Rutkowska et al., 2025; Rocha et al., 2026). Recent evidence shows that these structural traits promote colonization and persistence within seed tissues (Khan et al., 2025). Seed endophyte abundance is strongly influenced by plant genotype, seed nutrient composition, and environmental conditions during seed formation (Petrosyan et al., 2026). The isolation of *L. sphaericus* YEBEVIA from maize seeds aligns with these ecological observations.

3.2 Biochemical characterization of isolated bacteria

Isolates D2 and D3 exhibited positive reactions to all biochemical tests conducted. All isolates fermented glucose, fructose, sucrose, and maltose, but produced only acid during lactose fermentation (Table 2). Biochemically, the isolates showed broad metabolic versatility, including the fermentation of glucose, fructose, sucrose, and maltose, and acid

production in lactose fermentation. This is consistent with endophytes that rely on plant-derived substrates within seeds (Mishra et al., 2018). The absence of gas production is characteristic of *Bacillus*-like endophytes (Mehta et al., 2014). Metabolic diversity is an essential adaptive trait influencing colonization and survival in nutrient-limited seed environments (Cross, 2022). Sugar fermentation ability plays a key role in tolerating fluctuating moisture and nutrient conditions (Omomowo & Babalola, 2022). The broad biochemical reactivity of isolates D2 and D3 aligns with earlier reports on multifunctional seed endophytes such as *Lysinibacillus, Bacillus, Serratia,* and *Enterobacter* (Sharma et al., 2023). These metabolic traits underpin their PGP functions, including nutrient solubilization, hormone production, and stress mitigation (Lastochkina & Allagulova, 2023).

Table 2. Biochemical characterization of isolated bacteria

IC	Biochemical tests						Sugar fermentation tests				
	CT	HSP	Indole	SHT	MR	GL	Glucose	Fructose	Sucrose	Lactose	Maltose
D1	+	+	-	+	+	+	Ag	Ag	Ag	Ag	Ag
D2	+	+	+	+	+	+	Ag	Ag	Ag	Ag	Ag
D3	+	+	+	+	+	+	Ag	Ag	Ag	Ag	Ag

Key: + (positive), - (negative), IC - Isolate code, CT - Catalase test, HSP - Hydrogen sulfide production, SHT - Starch hydrolysis test, MR - Methyl red test, GL - Gelatin test, A - Color changes, a - No color changes, G - Gas production, and g - No gas production. The combination of these letters indicates the specific results for each sugar and sample.

3.3. Plant growth-promoting screening of the isolated bacteria

Table 3 shows the plant growth-promoting (PGP) activities of the bacterial isolates, all of which displayed PGP traits with varying activities. Isolate D3 produced a zone of clearance of 3.0 cm for zinc solubilization and 2.5 cm for siderophore production. It also exhibited high HCN production (2.87) compared with the control (Table 4). Similarly, phosphate-solubilizing activity (17.46) and IAA activity of 14.26 were recorded for isolate D3 (Figure 1).

Table 3. Plant growth-promoting screening of the isolated bacteria

Isolate Code	Plant Growth-promoting Screening							
	IAA(cm)	EXP	ZS(cm)	HCN	AP	NF(cm)	PS(cm)	SP(cm)
D1	+	+	+(2.5)	+	+	+(1.6)	+(1.4)	+(1.2)
D2	+	+	+(2.9)	+	+	+(1.0)	+(1.6)	+(1.8)
D3	+	+	+(3.0)	+	+	+(1.8)	+(1.8)	+(2.5)

Key: + (positive), - (negative), IAA- Indole-3-acetic acid, EXP- Exopolysaccharide production, PS- Phosphate solubilization, ZS- Zinc solubilization, AP- Ammonia production, NF- Nitrogen fixation, HCN- Hydrogen cyanide, SP- Siderophore production.

Table 4. Quantitative screening of plant growth promoting bacteria

IC	HCN (µg/mL)	NF (µg/mL)	ZS (µg/mL)	AP (µg/mL)	SP (µg/mL)	EXP (µg/mL)
Control	2.72±0.02^{a}	1.18±0.01^{a}	1.19±0.01^{a}	2.27±0.01^{a}	1.19±0.01^{a}	1.12±0.01^{a}
D1	2.85±0.03^{a}	2.83±0.02^{b}	1.87±0.02^{b}	2.42±0.01^{a}	1.82±0.01^{b}	1.96±0.02^{b}
D2	2.81±0.01^{a}	2.86±0.01^{b}	1.91±0.01^{b}	2.23±0.02^{a}	2.22±0.02^{b}	1.81±0.01^{b}
D3	2.87±0.02^{a}	2.79±0.01^{b}	2.04±0.00^{b}	2.56±0.01^{a}	2.20±0.00^{b}	1.66±0.00^{b}

Key: IC - Isolate code, HCN: Hydrogen cyanide, NF: Nitrogen fixation, ZS: Zinc solubilization, AP: Ammonia production, SP: Siderophore production test, EXP: Exopolysaccharide production. Values of triplicate readings were presented as mean±standard deviation (n = 3). Designated alphabets down the column revealed the significant difference determined by one-way ANOVA with Duncan's Post Hoc test (p<0.05).

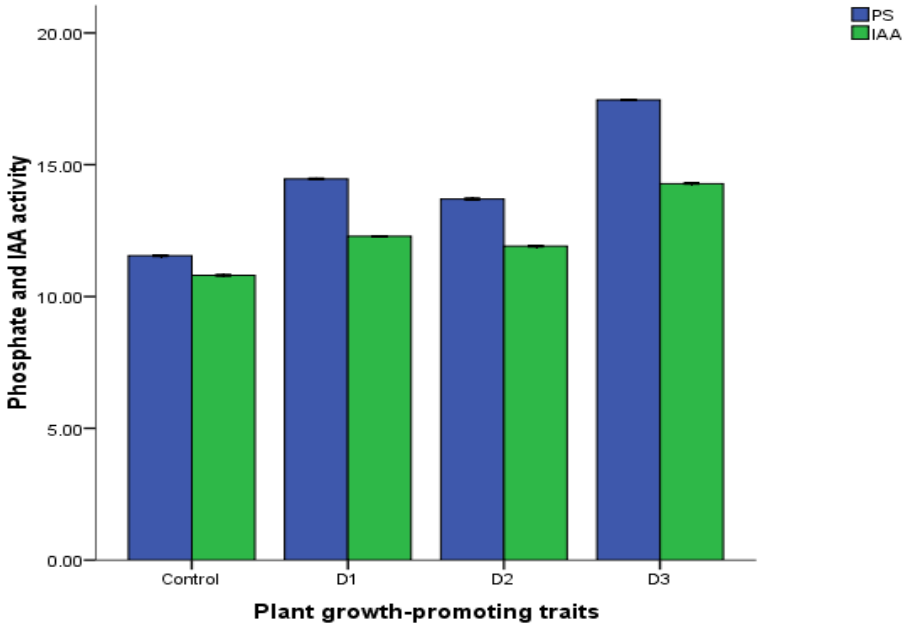

Figure 1. Quantitative screening of phosphate solubilization (µg/mL) and IAA production (µg/mL) by the isolated bacteria. Key: PS - phosphate solubilization, IAA - indole-3-acetic acid

The PGP traits exhibited by the isolates, particularly D3, suggest strong potential for enhancing plant growth and soil health. *L. sphaericus* YEBEVIA displayed substantial zinc solubilization (3.0 cm zone), consistent with reports that zinc-solubilizing endophytes are abundant in cereals (Yahaghi et al., 2025). Its nutrient-solubilizing abilities correspond with findings that *Bacillus*-like endophytes release organic acids and chelators that mobilize micronutrients (Escalante-Beltrán et al., 2026). The high siderophore production (2.5 cm)

observed suggests improved iron availability, an essential trait for plant health and pathogen suppression (Walitang et al., 2017). *L. sphaericus* YEBEVIA also showed high HCN production, a characteristic associated with biocontrol activity, consistent with reports by Etminani and Harighi (2018). Low-level HCN production helps suppress soil-borne pathogens without harming plants (Saadaoui et al., 2023). Additionally, the isolate demonstrated strong phosphate-solubilizing activity (17.46), aligning with recent reports documenting *Lysinibacillus* spp. as efficient phosphate solubilizers in soybean (Vitorino et al., 2024). IAA production (14.26 µg/mL) by *L. sphaericus* YEBEVIA is consistent with earlier observations that seed endophytic *Bacillus* and *Lysinibacillus* spp. enhance root architecture and plant growth by synthesizing IAA (Bhutani et al., 2018; Pal et al., 2024). This phytohormone promotes root elongation, lateral root formation, nutrient uptake, and early seedling vigor (Zhao et al., 2020; Shukla & Pathak, 2025).

3.4 *Lysinibacillus sphaericus* growth response to heavy metals

The growth response of *L. sphaericus* YEBEVIA to different heavy metal concentrations is shown in Figure 2. The highest growth responses to zinc (1.58) and lead (1.93) occurred at 2 g supplementation in the growth medium. Increased chromium tolerance was observed with increasing chromium concentrations compared to the control. No significant differences in cadmium tolerance were observed between media supplemented with 4 g of chromium and the control. The MIC effects were observed only in media supplemented with lead sulphate, as the other heavy metals did not inhibit bacterial growth. Inhibitory zones of 5.1 cm, 2.5 cm, and 6.8 cm were recorded at 2 g lead sulphate supplementation (Table 5). The heavy metal tolerance exhibited by *L. sphaericus* YEBEVIA aligns with studies describing *Lysinibacillus* spp. as highly resistant to lead, zinc, cadmium, and chromium (Eke et al., 2019; Jinal et al., 2021; Adeleke & Fakoya, 2025). Growth stimulation at 2 g concentrations of zinc (1.58) and lead (1.93), and increased chromium tolerance, reflects robust resistance mechanisms. Similar tolerances have been linked to endophyte-mediated phytoprotection and bioremediation (Yongpisanphop et al., 2020; Yadav et al., 2025).

Reduced sensitivity to cadmium indicates intrinsic resistance associated with bioadsorption, metallothioneins, and enzymatic detoxification (Alotaibi et al., 2021). MIC responses to lead sulfate also align with metal-specific tolerance mechanisms described by Jinal et al. (2021). Kumar and Rani (2026) highlighted that metal-tolerant endophytes support plant growth under contamination by immobilizing or transforming toxic metals. Thus, the isolate's heavy-metal tolerance reinforces its potential for dual use as a plant growth promoter and bioremediation agent.

3.5 Bioinoculation efficacy of the endophytic bacterium

The bioinoculation effects of the endophytic bacterium *L. sphaericus* YEBEVIA on maize growth under field and greenhouse conditions are presented in Table 6. Under field conditions, a total of 13 leaves and 41 roots were recorded after 50 days of planting, with a leaf length of 101.5 cm and a plant fresh weight of 256.9 g. Similar trends were observed under greenhouse conditions, with 11 leaves, 28 roots, a leaf length of 67.5 cm, and a plant fresh weight of 92.2 g. Maize inoculation with *L. sphaericus* YEBEVIA significantly enhanced growth under both field and greenhouse conditions, increasing leaf number, leaf length, root proliferation, and plant biomass.

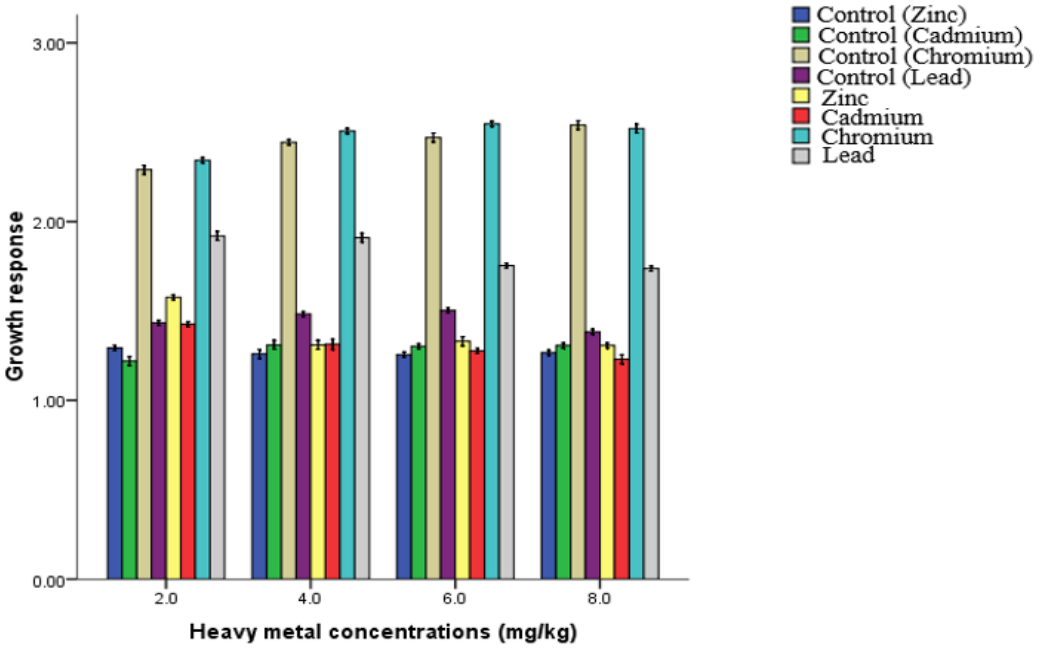

Table 5. Minimum inhibitory concentration (MIC) of heavy metals on bacterial growth

Test	Concentration (mg/kg)	D1	D2	D3
Lead sulphate	2	+(5.1)	+(2.5)	+(6.8)
	4	+(1.9)	+(1.0)	+(3.0)
	6	+(1.0)	+(2.2)	+(3.9)
	8	+(1.9)	+(1.7)	+(1.6)
Iron, cadmium, and zinc sulphate	2	-	-	-
	4	-	-	-
	6	-	-	-
	8	-	-	-

Key: + = positive, - = negative

These observations agree with studies demonstrating the effectiveness of *Lysinibacillus* and *Bacillus* seed endophytes in improving maize growth, nutrient uptake, and stress tolerance (Massucato et al., 2022; Imran et al., 2023). The improvements recorded in this study parallel earlier findings on *L. sphaericus* in maize (Naureen et al., 2017). Enhanced root and leaf development can be attributed to the isolate's IAA production, siderophore activity, and nutrient-solubilizing abilities (Pal et al., 2024). Increased biomass corresponds with reports by Rahman et al. (2023). The stronger performance under field conditions may reflect richer microbial interactions, higher nutrient mobilization, and greater rhizosphere complexity, as also described by Beevi et al. (2025).

Table 6. Greenhouse and field experiment of maize bioinoculation

Growth Parameters	Greenhouse		Field	
	Control	**Treatment**	**Control**	**Treatment**
No of leaves	9.00±0.01[a]	11.00±0.01[b]	11.00±0.01[a]	13.00±0.01[b]
Plant length (cm)	54.01±0.01[a]	62.01±0.02[b]	189.00±0.00[a]	216.00±0.00[b]
Plant width (cm)	1.00±0.00[a]	2.00±0.00[b]	2.00±0.00[a]	3.00±0.00[b]
Leaf length (cm)	65.40±0.01[a]	86.50±0.01[b]	80.01±0.01[a]	101.50±0.06[b]
Leaf width (cm)	4.00±0.01[a]	5.20±0.01[b]	8.00±0.00[a]	8.97±0.06[a]
Plant fresh weight (g)	74.50±0.01[a]	92.10±0.10[b]	117.10±0.01[a]	256.90±0.02[b]
Number of roots	17.01±0.03[a]	27.99±0.01[b]	28.99±0.01[a]	41.00±0.00[b]

Values from triplicate measurements are presented as mean±standard deviation (n = 3). Different superscripts letters within a column indicate significant differences between the control and treatment under both greenhouse and field conditions, as determined by one-way ANOVA followed by Duncan's Post Hoc test (p<0.05).

4. Conclusions

Overall, the findings demonstrate that the seed-origin *L. sphaericus* YEBEVIA was morphologically characterized as a rod-shaped, Gram-positive bacterium with the biochemical capacity to ferment sugars. Qualitative screening revealed positive reactions for multiple plant growth-promoting traits, such as indole-3-acetic acid, exopolysaccharide production, phosphate solubilization, zinc solubilization, ammonia production, nitrogen fixation, hydrogen cyanide, and siderophore production. The isolate also exhibited high phosphate solubilization and indole-3-acetic acid (IAA) production, along with notable tolerance to chromium and lead sulphate. Maize plants inoculated with the identified bacterium showed significant improvements in growth parameters, including plant length and fresh weight, compared with the control. Therefore, further investigation and exploitation of this bacterium as a biofertilizer may provide an innovative model for sustainable and eco-friendly agricultural development.

5. Acknowledgements

The author gratefully acknowledges Olusegun Agagu University of Science and Technology, Okitipupa, Nigeria, for providing technical assistance and access to laboratory facilities. The author also acknowledges the Federal University of Technology and Environmental Sciences, Iyin-Ekiti, Nigeria. Sincere appreciation is extended to colleagues who participated in this research.

6. Authors' Contributions

Bartholomew Saanu Adeleke designed research; performed research; contributed new reagents/analytic tools; analyzed data; coordinated research; and wrote the paper.

ORCID
Bartholomew Saanu Adeleke https://orcid.org/0000-0001-7707-8816

7. Conflicts of Interest

The author declares that no conflicts of interest exist.

References

Abd Elhakeem, F. G., El-Amier, Y. A., & Elsayed, A. A. (2024). Isolation and characterization of endophytic bacteria isolated from seeds of *Moringa oleifera* (Moringaceae). *Mansoura Journal of Biology*, *68*, 29-34. https://doi.org/10.21608/mjb.2024.446679

Abdelfattah, A., Tack, A. J. M., Lobato, C., Wassermann, B., & Berg, G. (2023). From seed to seed: The role of microbial inheritance in the assembly of the plant microbiome. *Trends in Microbiology*, *31*(4), 346-355. https://doi.org/10.1016/j.tim.2022.10.009

Abiodun, A., Olayinka, A., & Omotola, A. (2021). Production of complementary food for infants made from sweet potatoes, soybeans, maize and guinea corn. *European Journal of Nutrition and Food Safety*, *13*(2), 59-64. https://doi.org/10.9734/ejnfs/2021/v13i230375

Adeleke, B. S., & Fakoya, S. (2025). Isolation and molecular characterization of potential plant growth-promoting bacteria from groundnut and maize. *International Journal of Plant Biology*, *16*(3), Article 102. https://doi.org/10.3390/ijpb16030102

Adeleke, B. S., Ayangbenro, A. S., & Babalola, O. O. (2022). *In vitro* screening of sunflower associated endophytic bacteria with plant growth-promoting traits. *Frontiers in Sustainable Food Systems*, *6*, Article 903114. https://doi.org/10.3389/fsufs.2022.903114

Aguilar, C., Herrera, R., Causadías, J. L., Bernal, B., Chavarria, O., González, C., Gondola, J., Moreno, A., & Martínez, A. A. (2025). Genomic insights into plant growth-promoting traits of *Lysinibacillus fusiformis* and *Bacillus cereus* from rice fields in Panama. *Microbiology Research*, *16*(5), Article 95. https://doi.org/10.3390/microbiolres16050095

AlAli, H. A., Khalifa, A., & Al-Malki, M. (2021). Plant growth-promoting rhizobacteria from *Ocimum basilicum* improve growth of *Phaseolus vulgaris* and *Abelmoschus esculentus*. *South African Journal of Botany*, *139*, 200-209. https://doi.org/10.1016/j.sajb.2021.02.019

Alotaibi, B. S., Khan, M., & Shamim, S. (2021). Unraveling the underlying heavy metal detoxification mechanisms of *Bacillus* species. *Microorganisms*, *9*(8), Article 1628. https://doi.org/10.3390/microorganisms9081628

Altschul, S. F., Madden, T. L., Schäffer, A. A., Zhang, J., Zhang, Z., Miller, W., & Lipman, D. J. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research*, *25*(17), 3389-3402. https://doi.org/10.1093/nar/25.17.3389

Beevi, S., Thampi, M., Moideen, J. M. S., & Rahman, S. (2025). Colonization and endophytic establishment of diazotrophic *Lysinibacillus sphaericus* in *Oryza sativa* and its efficacy in plant growth promotion. *Journal of Plant Interactions*, *20*(1), Article 2523278. https://doi.org/10.1080/17429145.2025.2523278

Bhagya, M., Nagaraju, K., Biradar, B. J. P., Santhosh, G. P., & Gundappagol, R. C. (2019). Isolation and characterization of endophytic bacteria from nodule, root and seeds of *Greengram (Vigna radiata* L.). *Indian Journal of Pure and Applied Bioscience*, *7*(4), 319-328. https://doi.org/10.18782/2320-7051.7737

Bhutani, N., Maheshwari, R., Negi, M., & Suneja, P. (2018). Optimization of IAA production by endophytic Bacillus spp. from *Vigna radiata* for their potential use as plant growth promoters. *Israel Journal of Plant Sciences*, *65*(1-2), 83-96. https://doi.org/10.1163/22238980-00001025

Chen, Q., Song, Y., An, Y., Lu, Y., & Zhong, G. (2024). Mechanisms and impact of rhizosphere microbial metabolites on crop health, traits, functional components: A comprehensive review. *Molecules*, *29*(24), Article 5922. https://doi.org/10.3390/molecules29245922

Cross, A. T. (2022). Nutrient-acquisition strategy influences seed nutrient concentration and seed-to-seedling transition in ecological restoration in a regional dryland flora. *Plant and Soil*, *476*(1), 653-668. https://doi.org/10.1007/s11104-021-05198-z

Eke, P., Kumar, A., Sahu, K. P., Wakam, L. N., Sheoran, N., Ashajyothi, M., Patel, A., & Fekam, F. B. (2019). Endophytic bacteria of desert cactus (*Euphorbia trigonas* Mill) confer drought tolerance and induce growth promotion in tomato (*Solanum lycopersicum* L.). *Microbiological Research*, *228*, Article 126302. https://doi.org/10.1016/j.micres.2020.126689

Escalante-Beltrán, A., Morales-Sandoval, P. H., Montoya-Martínez, A. C., Cubedo-Ruíz, E. A., Félix-Gastélum, R., Parra-Cota, F. I., & de Los Santos-Villalobos, S. (2026). Draft genome sequence of *Bacillus* sp. strain 11b20, a promising plant-growth promoting bacterium associated with maize (*Zea mays* L.) in the Yaqui Valley, Mexico. *Microorganisms*, *14*(2), Article 485. https://doi.org/10.3390/microorganisms14020485

Etminani, F., & Harighi, B. (2018). Isolation and identification of endophytic bacteria with plant growth promoting activity and biocontrol potential from wild pistachio trees. *The Plant Pathology Journal*, *34*(3), Article 208. https://doi.org/10.5423/ppj.oa.07.2017.0158

Hu, H., Geng, S., Zhu, Y., He, X., Pan, X., & Yang, M. (2025). Seed-borne endophytes and their host effects. *Microorganisms*, *13*(4), Article 842. https://doi.org/10.3390/microorganisms13040842

Ikeda, A. C., Bassani, L. L., Adamoski, D., Stringari, D., Cordeiro, V. K., Glienke, C., Steffens, M. B. R., Hungria, M., & Galli-Terasawa, L. V. (2013). Morphological and genetic characterization of endophytic bacteria isolated from roots of different maize genotypes. *Microbial Ecology*, *65*(1), 154-160. https://doi.org/10.1007/s00248-012-0104-0

Imran, M., Mpovo, C. L., Aaqil Khan, M., Shaffique, S., Ninson, D., Bilal, S., Khan, M., Kwon, E. H., Kang, S. M., Yun, B. W., & Lee, I. J. (2023). Synergistic effect of melatonin and *Lysinibacillus fusiformis* L.(PLT16) to mitigate drought stress via regulation of hormonal, antioxidants system, and physio-molecular responses in soybean plants. *International Journal of Molecular Sciences*, *24*(10), Article 8489. https://doi.org/10.3390/ijms24108489

Jha, R., Manonmani, V., Sundaralingam, K., Vanitha, S., Gnanachitra, M., & Kalaiselvi, T. (2025). The seed microbiome: microbial hashes for plant wellbeing. *Open Journal of Environmental Biology*, *10*, 7-22. https://doi.org/10.17352/ojeb.000046

Jinal, H. N., Gopi, K., Kumar, K., & Amaresan, N. (2021). Effect of zinc-resistant *Lysinibacillus* species inoculation on growth, physiological properties, and zinc uptake in maize (*Zea mays* L.). *Environmental Science and Pollution Research*, *28*(6), 6540-6548. https://doi.org/10.1007/s11356-020-10998-4

Khan, D., Shaw, R., Kabiraj, A., Paul, A., & Bandopadhyay, R. (2025). Microbial inheritance through seed: a clouded area needs to be enlightened. *Archives of Microbiology*, *207*(1), Article 23. https://doi.org/10.1007/s00203-024-04225-8

Kumar, A., & Rani, R. (2026). Isolation and characterization of heavy metal-tolerant, plant growth-promoting bacteria and evaluation of the impact of lead [Pb (II)] on their functional traits. *Archives of Microbiology*, *208*(4), Article 206. https://doi.org/10.1007/s00203-026-04762-4

Lastochkina, O. V., & Allagulova, C. R. (2023). The mechanisms of the growth promotion and protective effects of endophytic PGP bacteria in wheat plants under the impact of drought. *Applied Biochemistry and Microbiology*, *59*(1), 14-32. https://doi.org/10.1134/S0003683823010039

Lebrazi, S., Niehaus, K., Bednarz, H., Fadil, M., Chraibi, M., & Fikri-Benbrahim, K. (2020). Screening and optimization of indole-3-acetic acid production and phosphate solubilization by rhizobacterial strains isolated from *Acacia cyanophylla* root nodules and their effects on its plant growth. *Journal of Genetic Engineering and Biotechnology*, *18*(1), Article 71. https://doi.org/10.1186/s43141-020-00090-2

Lipková, N., Cinkocki, R., Maková, J., Medo, J., & Javoreková, S. (2021). Characterization of endophytic bacteria of the genus *Bacillus* and their influence on the growth of maize (*Zea mays*) *in vivo*. *Journal of Microbiology, Biotechnology and Food Sciences*, *10*(5), Article e3602. https://doi.org/10.15414/jmbfs.3602

Massucato, L. R., Almeida, S. R. D. A., Silva, M. B., Mosela, M., Zeffa, D. M., Nogueira, A. F., de Lima Filho, R. B., Mian, S., Higashi, A. Y., Teixeira, G. M., & Shimizu, G. D. (2022). Efficiency of combining strains Ag87 (*Bacillus megaterium*) and Ag94 (*Lysinibacillus* sp.) as phosphate solubilizers and growth promoters in maize. *Microorganisms*, *10*(7), Article 1401. https://doi.org/10.3390/microorganisms10071401

Mehta, P., Walia, A., Kakkar, N., & Shirkot, C. K. (2014). Tricalcium phosphate solubilisation by new endophyte Bacillus methylotrophicus CKAM isolated from apple root endosphere and its plant growth-promoting activities. *Acta Physiologiae Plantarum*, *36*(8), 2033-2045. https://doi.org/10.1007/s11738-014-1581-1

Mishra, A., Singh, S. P., Mahfooz, S., Singh, S. P., Bhattacharya, A., Mishra, N., & Nautiyal, C. S. (2018). Endophyte-mediated modulation of defense-related genes and systemic resistance in *Withania somnifera* (L.) Dunal under *Alternaria alternata* stress. *Applied and Environmental Microbiology*, *84*(8), Article e02845-17. https://doi.org/10.1128/aem.02845-17

Nandana, M. S., & Anith, K. N. (2025). Endospore-producing endophytes: Structural adaptations, functional roles, and their significance in plant growth and stress management. *International Journal of Plant and Soil Science*, *37*(8), 298-312. https://doi.org/10.9734/ijpss/2025/v37i85631

Naureen, Z., Rehman, N. U., Hussain, H., Hussain, J., Gilani, S. A., Al Housni, S. K., Mabood, F., Khan, A. L., Farooq, S., Abbas, G., & Harrasi, A. A. (2017). Exploring the potentials of *Lysinibacillus sphaericus* ZA9 for plant growth promotion and biocontrol activities against phytopathogenic fungi. *Frontiers in Microbiology*, *8*, Article 1477. https://doi.org/10.3389/fmicb.2017.01477

Omomowo, O. I., & Babalola, O. O. (2022). Genomic insights into two endophytic strains: *Stenotrophomonas geniculata* NWUBe21 and *Pseudomonas carnis* NWUBe30 from Cowpea with plant growth-stimulating attributes. *Applied Sciences*, *12*(24), Article 12953. https://doi.org/10.3390/app122412953

Pal, G., Saxena, S., Kumar, K., Verma, A., Kumar, D., Shukla, P., Pandey, A., White, J., & Verma, S. K. (2024). Seed endophytic bacterium *Lysinibacillus* sp. (ZM1) from maize (*Zea mays* L.) shapes its root architecture through modulation of auxin biosynthesis and nitrogen metabolism. *Plant Physiology and Biochemistry*, *212*, Article 108731. https://doi.org/10.1016/j.plaphy.2024.108731

Pantoja-Guerra, M., Burkett-Cadena, M., Cadena, J., Dunlap, C. A., & Ramírez, C. A. (2023). *Lysinibacillus* spp.: an IAA-producing endospore forming-bacteria that

promotes plant growth. *Antonie Van Leeuwenhoek*, *116*(7), 615-630. https://doi.org/10.1007/s10482-023-01828-x

Petrosyan, K., Thijs, S., Krucon, T., Piwowarczyk, R., Wiśniewska, K., Kaca, W., & Vangronsveld, J. (2026). Seed endophytic bacteria are involved in metal adaptation of *Orobanche lutea*: community dynamics and plant growth promotion traits. *BMC Plant Biology*, *26,* Article 485. https://doi.org/10.1186/s12870-026-08304-4

Rahman, S., Gangaraj, K. P., Muralikrishna, K. S., & Rajesh, M. K. (2023). Isolation and characterization of full-length coding sequences of gibberellic acid biosynthetic genes in coconut (*Cocos nucifera* L.) and their expression analysis. *South African Journal of Botany*, *153*, 297-307. https://doi.org/10.1016/j.sajb.2023.01.011

Rocha, S. M. B., Lopes, Â. C. D. A., da Silva, V. B., Gomes, R. L. F., Costa, M. K. L., de Alcantara Neto, F., de Araujo Pereira, A. P., de Medeiros, E. V., Miranda, A. R. L., Mendes, L. W., & Araujo, A. S. F. (2026). Shifts in seed-associated endophytic bacterial communities from parental lines to advanced generations in lima bean. *Annals of Applied Biology*, *188*(3), 781-788. https://doi.org/10.1111/aab.70091

Romero-Gutierrez, K. J., Dourado, M. N., Garrido, L. M., Olchanheski, L. R., Mano, E. T., Dini-Andreote, F., Valvano, M. A., & Araujo, W. L., (2020). Phenotypic traits of *Burkholderia* spp. associated with ecological adaptation and plant-host interaction. *Microbiological Research*, *236*, Article 126451. https://doi.org/10.1016/j.micres.2020.126451

Rutkowska, N., Daroch, M., & Marchut-Mikołajczyk, O. (2025). Exploring the diversity and genomics of cultivable *Bacillus*-related endophytic bacteria from the medicinal plant *Galium aparine* L. *Frontiers in Microbiology*, *16*, Article 1612860. https://doi.org/10.3389/fmicb.2025.1612860

Saadaoui, M., Faize, M., Bonhomme, L., Benyoussef, N. O., Kharrat, M., Chaar, H., Label, P., & Venisse, J. S. (2023). Assessment of *Tunisian trichoderma* isolates on wheat seed germination, seedling growth and *Fusarium* seedling blight suppression. *Microorganisms*, *11*(6), Article 1512. https://doi.org/10.3390/microorganisms11061512

Shabanamol, S., Divya, K., George, T. K., Rishad, K. S., Sreekumar, T. S., & Jisha, M. S. (2018). Characterization and *in planta* nitrogen fixation of plant growth promoting endophytic diazotrophic *Lysinibacillus sphaericus* isolated from rice (*Oryza sativa*). *Physiological and Molecular Plant Pathology*, *102*, 46-54. https://doi.org/10.1016/j.pmpp.2017.11.003

Sharma, P., Suman, A., Aswini, K., SaiPrasad, J., & Gond, S. (2023). Endophytic bacterial taxonomic and functional diversity in the seeds of wheat genotypes from different agroecologies. *Journal of Plant Interactions*, *18*(1), Article 2227652. https://doi.org/10.1080/17429145.2023.2227652

Shukla, D., & Pathak, S. (2025). Ethylene as a central regulator of seed germination, early seedling development, and stress adaptation. *Methods in Molecular Biology*, *2945,* 229-243. https://doi.org/10.1007/978-1-0716-4650-2_17

Soumya, P., Unnikrishnan, R., Nair, S., Priya, V. K., Jayachandran, K., & Radhakrishnan, E. K. (2026). *In vitro*, in planta and whole genome sequence-based elucidation of plant beneficial effects of endophytic *Enterobacter* sp. P1P. *3 Biotech*, *16*(3), Article 91. https://doi.org/10.1007/s13205-026-04696-0

Sun, Z., Adeleke, B. S., Shi, Y., & Li, C. (2023). The seed microbiomes of staple food crops. *Microbial Biotechnology*, *16*(12), 2236-2249.

Van Chuong, N., Tri, T. L. K., Liem, T. T., Van Thuan, N., Em, T. V. T., & Trang, N. N. P. (2026). Four newly identified endophytic *Bacillus megaterium* strains with biofertilizer potential for enhancing maize growth and productivity. *Trends in Sciences*, *23*(3), Article 11347. https://doi.org/10.48048/tis.2026.11347

Vitorino, L. C., da Silva, E. J., Oliveira, M. S., Silva, I. D. O., Santos, L. D. S., Mendonça, M. A. C., Oliveira, T. C. S., & Bessa, L. A. (2024). Effect of a *Bacillus velezensis* and *Lysinibacillus fusiformis*-based biofertilizer on phosphorus acquisition and grain yield of soybean. *Frontiers in Plant Science*, *15*, Article 1433828. https://doi.org/10.3389/fpls.2024.1433828

Walitang, D. I., Kim, K., Madhaiyan, M., Kim, Y. K., Kang, Y., & Sa, T. (2017). Characterizing endophytic competence and plant growth promotion of bacterial endophytes inhabiting the seed endosphere of rice. *BMC Microbiology*, *17*(1), Article 209. https://doi.org/10.1186/s12866-017-1117-0

Wallace, J. G. (2023). Maize seed endophytes. *Molecular Plant Pathology*, *24*(7), 801-810. https://doi.org/10.1111/mpp.13278

Yadav, S., Kajala, R., Rajpurohit, D., Upadhyay, S. K., Kumar, P., Singh, A., & Jain, D. (2025). Role of newly isolated plant growth-promoting lead-tolerant bacteria in lead bio-remediation and plant growth performance under lead stress. *Annals of Microbiology*, *75*(1), Article 27. https://doi.org/10.1186/s13213-025-01822-w

Yahaghi, Z., Shirvani, M., Nourbakhsh, F., & Pueyo, J. J. (2025). Isolation, identification, and application of zinc-solubilizing bacteria exhibiting beneficial traits to promote plant growth and metal uptake. *International Journal of Environmental Science and Technology*, *22*(5), 3675-3690. https://doi.org/10.1007/s13762-024-06064-y

Yongpisanphop, J., Babel, S., Kurisu, F., Kruatrachue, M., & Pokethitiyook, P. (2020). Isolation and characterization of Pb-resistant plant growth promoting endophytic bacteria and their role in Pb accumulation by fast-growing trees. *Environmental Technology*, *41*(27), 3598-3606. https://doi.org/10.1080/09593330.2019.1615993

Zhang, X., Ma, Y. N., Wang, X., Liao, K., He, S., Zhao, X., Guo, H., Zhao, D., & Wei, H.-L. (2022). Dynamics of rice microbiomes reveal core vertically transmitted seed endophytes. *Microbiome, 10*, Article 216. https://doi.org/10.1186/s40168-022-01422-9

Zhao, T., Deng, X., Xiao, Q., Han, Y., Zhu, S., & Chen, J. (2020). IAA priming improves the germination and seedling growth in cotton (*Gossypium hirsutum* L.) via regulating the endogenous phytohormones and enhancing the sucrose metabolism. *Industrial Crops and Products*, *155*, Article 112788. https://doi.org/10.1016/j.indcrop.2020.112788