



Seed coat treatment by plant-growth-promoting rhizobacteria *Lysobacter antibioticus* 13–6 enhances maize yield and changes rhizosphere bacterial communities

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Abstract

The plant rhizosphere is a major habitat for diverse microorganisms because of its heterogeneous microenvironment. It is well known that an imbalance in rhizosphere microorganisms has direct and indirect effects on soil fertility and plant health. In this study, we investigated the impact of *Lysobacter antibioticus* 13–6 seed coat treatment on the soil physicochemical properties, plant growth, and bacterial community composition of maize plants in both in vivo and in vitro experiments, using high-throughput amplicon sequencing of 16S rRNA. Under in vitro conditions, we determined that *L. antibioticus* 13–6 has the ability to solubilize P, hydrolyze cellulose, and synthesize indole acetic acid. Furthermore, under in vivo conditions, *L. antibioticus* 13–6 significantly improved the soil physicochemical properties and enhanced the root length, stalk height, dry weight of root and stalk, grain yield, and chlorophyll contents by successful colonization in the rhizosphere of maize plants. The results of high-throughput amplicon sequencing of 16S rRNA demonstrated that *L. antibioticus* 13–6 significantly changed the diversity and composition of the rhizosphere bacterial communities. The relative abundance of Gammaproteobacteria, Gemmatimonadetes, and Bacteroidetes at the phylum level and *Streptomyces*, *Lysobacter*, and *Nitrospira* at the genus level significantly increased in the rhizosphere of *L. antibioticus* 13–6 seed coat-treated plants. Co-occurrence networks analysis revealed that the rhizosphere of *L. antibioticus* 13–6 seed coat-treated plants had fewer negative correlations and less competition for resources among bacterial communities. Genome analysis of *L. antibioticus* 13–6 revealed that the genome of *L. antibioticus* 13–6 encodes genes related to indole acetic acid synthesis, chitinase decomposition, and P solubilization, making it one of the most potent plant growth-promoting bacteria. Overall, this study demonstrated the potential of *L. antibioticus* 13–6 as a promising seed coat bioagent for sustainable agriculture and to minimize the utilization of agrochemicals.

Keywords *Lysobacter antibioticus* 13–6 · Maize · High-throughput sequencing · Rhizosphere bacterial community · Plant growth promotion

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Introduction

Maize (*Zea mays* L.), also known as corn, is a major industrial crop worldwide, accounting for 38% of the world's cereal production (Chen et al. 2021). Several biotic and abiotic factors affect maize production, including insect pests, diseases, weeds, and drought (War et al. 2016). Currently, high production of maize is mainly based on the cultivation of hybrid varieties that are resistant to biotic and abiotic stresses (Messina et al. 2011; Van Bueren et al. 2011). In contrast, agrochemicals have been extensively used to attain high-yield goals (Liu et al. 2015). Many studies have shown that soil microbial diversity, health, and climatic conditions are the main factors contributing to high crop yield and quality production (Cai et al. 2021; Olowe et al. 2018). Diseases such as root rot, stem rot, and ear rot caused by *Fusarium* spp. and root rot caused by *Rhizoctonia* spp. pose a significant threat to high yield and improved quality crop production, owing to the presence of pathogens with a broad host range and highly persistent nature (Gai et al. 2018; Olowe et al. 2018). The excessive use of agrochemicals adversely affects soil microbial diversity and develops resistance in pathogens (Kumar and Kumar 2019). Thus, it is essential to shift from chemical-based to nature-based cures, whereby biological control offers an environmentally friendly alternative in integrated disease management (Ahmed et al. 2022).

The plant rhizosphere is a main habitat for variety of microorganisms and is considered one of the most complex ecosystems on earth (Raaijmakers 2015). In recent years, many studies have shown that plant rhizospheres are enriched with a large number of plant-growth-promoting rhizobacteria (PGPR) (Bhattacharyya and Jha 2012). A mutualistic relationship was found between PGPR and plants. PGPR help plants with nutrient availability, stress protection, plant growth promotion (PGP), and antagonism to many phytopathogens through the production of volatile organic compounds and secondary metabolites (Wei et al. 2021). In response, they obtain carbon as an energy source from plants (Cordovez et al. 2019; Mehmood et al. 2018). PGPR decompose rock phosphorus and potassium to make them available to the plant, which directly enhance plant growth and develop resistance in plants against biotic and abiotic stresses (Basu et al. 2021; Etesami and Adl 2020).

With advancements in science and modern agriculture tools, many studies have reported the use of rhizobacteria as biocontrol agents and plant growth promoters to improve crop health and yield (Kumari et al. 2019). *Lysobacter antibioticus* belongs to the family *Xanthomonadaceae* and is a new type of biocontrol bacteria with antimicrobial and PGP properties (Liu et al. 2022). Lyase and myxin produced by *L. antibioticus* HS124 and *L.*

antibioticus OH13 reportedly inhibit the growth of *Phytophthora* and DNA synthesis of the pathogen, respectively (Jiang et al. 2018; Ko et al. 2009). *L. antibioticus* 13–1 was found to significantly reduce the incidence of bacterial blight in rice by up to 78% (Ji et al. 2008).

Our previous studies demonstrated that *L. antibioticus* 13–6 has the ability to produce antimicrobial compounds (phenazine substances) (Liu et al. 2022), and in bacterial consortia it significantly suppressed clubroot disease in Chinese cabbage (Wei et al. 2021) and root rot disease of *Panax notoginseng* (Zhang et al. 2020), while also serving as plant growth promoter. However, the PGP potential of *L. antibioticus* 13–6 as the sole strain and its interactions with local bacterial communities are still unknown. In this study, we investigated the PGP potential of *L. antibioticus* 13–6 as the sole strain through the seed coat treatment on maize plants under greenhouse and field conditions. We assumed that the application of PGPR (*L. antibioticus* 13–6) as a seed coat treatment improves soil fertility and enhances plant growth, and the interaction between *L. antibioticus* 13–6 and local bacterial communities play an important role in the realization of high-yield goals.

Materials and methods

Experimental sites, design descriptions, and plant material

All greenhouse experiments were performed at State Key Laboratory for Conservation and Utilization of Bio-Resources in Yunnan, Yunnan Agricultural University, Kunming (24.8801° N, 102.8329° E), China. The greenhouse conditions were maintained at day/night temperature of 28 °C/22 °C with 60% relative humidity. Field experiments were conducted in Xuanwei County, Qujing City (26.06° N 104.03° E) from May to September 2019 and Mangshi County, Dehong City (24.15° N 98.16° E) from October 2019 to January 2020 in Yunnan Province, China. The average annual temperature and rainfall in Dehong and Qujing were recorded at 19.8 °C, 14.4 °C and 1300–1653 mm, 1200–1400 mm, respectively. Greenhouse experiments were conducted using a completely randomized design, whereas field experiments were conducted using a randomized complete block design. The soil used in the greenhouse experiments and in Xuanwei field was sandy loam, whereas that in Mangshi field was clay. The maize (*Zea mays* L.) variety (Xuan Huang No. 18) was used as the plant material in all greenhouse and field experiments.

Bacterial strain, growth media, and culture conditions

Lysobacter antibioticus 13–6 (Accession no. NZ_JMTZ00000000.1), a potent biocontrol strain with broad-spectrum antimicrobial activity against plant pathogens, was previously isolated from the Konjac plant rhizosphere soil and stored in our laboratory. *L. antibioticus* 13–6 was cultured on King's B (KB) medium plates (Peptone 20 g L⁻¹, MgSO₄·7H₂O 1.5 g L⁻¹, K₂HPO₄ 1.5 g L⁻¹, Glycerol 10 ml L⁻¹, Agar 20 g L⁻¹, and pH 7.0) and incubated at 28 °C for 36 h (Liu et al. 2022). Before each experiment, *L. Antibiotics* 13–6 was cultured in KB broth at 28 °C and 160×g for 24 h, and the optical density was adjusted to OD_{600nm} ≈ 0.5 (10⁷ CFU mL⁻¹) using a spectrophotometer (GE Ultrospec 2100 pro). The pure culture of the bacterial strain was stored in 50% (v/v) glycerol solution at –80 °C for future use.

In vitro assays to investigate the plant-growth-promoting traits of *Lysobacter antibioticus* 13–6

Salkowski's reagent was used to assess the auxin production ability of *L. antibioticus* 13–6 following the methodology of He et al. (2021) by measuring the light absorbance at OD_{535 nm} using a spectrophotometer (GE Uitrospec 2100 pro). The cellulose production ability was assessed on Congo red medium using the methodology described by Cui et al. (2019). Phosphate solubilization ability was determined as a clear circle around the bacterial colony on Pikovskaya solid agar medium (PVK) (Cui et al. 2019), and the available phosphorus content was determined by the molybdenum blue calorimetry method (Abdelmoteleb and Gonzalez-Mendoza 2020).

Greenhouse assays

Analyzing the effect of different concentrations of *Lysobacter antibioticus* 13–6 on the growth of maize plant

A pot experiment was conducted to evaluate the effect of different bacterial concentrations of *L. antibioticus* 13–6 on the growth of maize plants. *L. antibioticus* 13–6 was grown in KB broth at 28 °C and 160×g for 24 h, and cell pellets were collected by centrifugation at 12,000×g for 10 min. The cell pellets were then adjusted to 10⁵, 10⁶, 10⁷, 10⁸, and 10⁹ colony-forming units·mL⁻¹ (CFU mL⁻¹) using sterilized distilled water (sdH₂O). Briefly, maize seeds were surface-sterilized with 75% alcohol for 30 s, washed thrice with sdH₂O, and treated with different concentrations of *L. antibioticus* 13–6 for 30 min. The experiment was performed under six different conditions: maize seeds treated with sdH₂O as

the control (CK), maize seeds treated with 10⁵ CFU mL⁻¹ (T1), maize seeds treated with 10⁶ CFU mL⁻¹ (T2), maize seeds treated with 10⁷ CFU mL⁻¹ (T3), maize seeds treated with 10⁸ CFU·mL⁻¹ (T4), and maize seeds treated with 10⁹ CFU mL⁻¹ (T5). Treated seeds were planted (three seeds/pot) in pots (20 cm × 15 cm) containing 3 kg of sterilized soil, and the pots were placed in the greenhouse. After 45 days, the plants were uprooted and washed under tap water to remove extra soil from the roots, and the root length, stalk height, and dry weight of the roots and stalks were measured. The experiment was repeated thrice with 15 plants per replicate for each treatment.

Determining the effect of *Lysobacter antibioticus* 13–6 seed coat treatment on the growth of maize plant

Taking 10⁷ CFU mL⁻¹ as the ideal concentration from the preliminary experiment, a subsequent pot experiment was conducted to further evaluate the PGP potential of *L. antibioticus* 13–6 through seed coating and other treatments. *L. antibioticus* 13–6 cell pellets were collected by centrifugation and adjusted to 10⁷ CFU·mL⁻¹ as described above. The experiment was performed under three different conditions: *L. antibioticus* 13–6 + additives (T1), 2% tebuconazole + additives (T2), and sdH₂O + additives as control (CK). The additive solution was prepared as described by Rahim et al. (2019) and added to each treatment at a 1:1 (v/v) ratio (Table S1). Approximately 3 mL of the coating agent was used for 50 g of seeds (80% of the seed surface was coated), and coated seeds were air-dried naturally for later use. As described above, coated seeds (three seeds/pot) were planted in pots (20 cm × 15 cm) containing 3 kg of disease-free fresh soil, and the pots were placed in the greenhouse. Forty-five days after planting, the root length, stalk height, and dry weight of the root and stalk were measured. The predecessor was used for leaf area measurements, as described by Amanullah et al. (2009). The experiment was repeated three times, with 15 plants per treatment used as replicates.

Lysobacter antibioticus 13–6 population dynamic in the maize plant rhizosphere

The colonization ability of *L. antibioticus* 13–6 was determined in the rhizosphere of maize plants treated with *L. antibioticus* 13–6 (T1) and CK plants by quantitative-PCR (qPCR). Rhizosphere soil samples were collected from each treatment (T1 and CK) 1 day after planting to the end of the experiment, with an interval of seven days. Total soil DNA was extracted from 0.5 g soil using a Power Soil® DNA isolation kit, following the manufacturer's instructions, and the extracted DNA was stored at –20 °C for further experiments. *L. antibioticus* 13–6 gene copy number (fresh soil·g⁻¹) was determined by qPCR using the primer

pair phzNO1 F1 (5'-GTCGGAAGAACGCCAGA-3') and phzNO1 R1 (5'-ATAGTCGGTGCAGACCG-3') (Lina et al. 2018). PCR amplification conditions were as follows: initial denaturation at 95 °C for 10 min, followed by 45 cycles of denaturation at 95 °C for 10 s, annealing at 52 °C for 10 s, and final extension at 72 °C for 10 s.

Analysis of *Lysobacter antibioticus* 13–6 plant growth-promoting related genes

The genome of *L. antibioticus* 13–6 (accession number: JMTZ00000000) is available in the NCBI database (<https://www.ncbi.nlm.nih.gov/>) (Zhou et al. 2014). Total genomic DNA of *L. antibioticus* 13–6 was extracted using a TIANamp Bacteria DNA isolation kit (TIANGEN® Co. Ltd.), according to the manufacturer's instructions. The extracted DNA was processed using the Nextera XT library preparation kit (Illumina, USA) and sequenced using an Illumina HiSeqTM2500 instrument at Shanghai OE Biotech Co., Ltd. (Shanghai, China). The ambiguous base (N) was detected and deleted using Trimmomatic software v0.36, to prepare paired-end reads (Bolger et al. 2014). De novo assembly was performed using software Velvet 1.2.09 with a K-mer of 45, as described by Chu et al. (2020). Gene detection and genome annotation were performed using BlastKO-ALA v2.1 (<http://www.kegg.jp/blastkoala/>) (Kanehisa et al. 2016), and the annotated genes related to PGP pathways were investigated.

Field experiment

Two field experiments were performed in Xuanwei County, Qujing City and Mangshi County, Dehong City, Yunnan Province, China, during the growing season from May to September 2019 and from October 2019 to January 2020, respectively. Briefly, *L. antibioticus* 13–6 was cultured in KB broth for 24 h, and bacterial cells were collected via centrifugation and adjusted to $OD_{600\text{ nm}} \approx 0.5$ (10^7 CFU mL^{-1}). The experiment was performed under three different conditions: *L. antibioticus* 13–6 + additives (T1), 2% tebuconazole + additives (T2), and sdH₂O + additives as control (CK). Chlorophyll content was measured in the leaves of maize plants at the heading stage using a SPAD-502 leaf chlorophyll meter, as described by Ling et al. (2011). In addition, to overcome the nutrient deficiency, fertilizers were applied in the form of compound (N – P₂O₅ – K₂O; 200 kg hm⁻²) and nitrogen (160 kg hm⁻²) fertilizers as top (before sowing) and base (at seedling stage) fertilizers, respectively. At the end of the experiment (R6 stage/physiological maturity), cobs were harvested and air-dried naturally to a moisture level of $20 \pm 1\%$ (Li et al. 2017), and the maize yield (kg ha⁻¹) was recorded. The experiment was conducted in replicates, and each treatment was repeated three times with 90 plants/plot

in Xuanwei County and 120 plants/plot in Mangshi County fields, planted in six rows per replicate.

Soil samples collection and analysis of soil physicochemical properties

Rhizosphere and bulk soil samples were collected from both experimental sites at the R6 stage/physiological maturity of maize plants (Li et al. 2017), treated with *L. antibioticus* 13–6 (T1) and CK to analyze the bacterial community composition and physicochemical properties, respectively. Bulk soil samples were collected for analysis of soil physicochemical properties at a depth of 10–15 cm, within 15 cm of the plant stem using augers (5 cm in diameter) according to the zig-zag sampling method. Tiny soil particles attached to the root surface of the maize plants (plants used for bulk soil samples) were collected using a brush as rhizosphere soil samples for the analysis of bacterial community composition. To make one replicate, 10 cores of rhizosphere and bulk soil samples per replication per treatment were mixed thoroughly, and 3 biological replicates were collected per treatment. The physicochemical properties of the soil samples were determined using air-dried samples. The contents of available nitrogen (mg kg⁻¹), available phosphorus (mg kg⁻¹), and available potassium (mg kg⁻¹) were measured by the alkali-hydrolyzable nitrogen method, 0.5 mol L⁻¹ NaHCO₃ solution (pH 8.5), and CH₃COONH₄ extraction method, respectively using the methodology described by Cai et al. (2021). Soil pH was determined using a pH meter based on a soil/water ratio of 1:2.5 (w/v). The rhizosphere and bulk soil samples collected from the Mangshi (MS) and Xuanwei (XW) experimental fields for analysis of bacterial community composition and physicochemical properties, respectively were labeled T1=MS_T, CK=MS_CK, T1=XW_T, and CK=XW_CK.

DNA extraction, PCR amplification, and library construction

Total soil DNA was extracted from the rhizosphere soil sample (0.5 g) using the Power Soil® DNA isolation kit according to the manufacturer's instructions, and the extracted DNA was stored at –80 °C for future use. The V3–V4 variable regions of the 16S rRNA gene of bacteria were amplified using the primer pair 343F (50-TACGGRAGGCAGCAG-30) and 798R (50-AGGGTATCTAACCT-30) (Zhang et al. 2020). The amplicon quality was visualized by gel electrophoresis and purified using AMPure XP beads (Agencourt). Quantification of the amplicon was performed using a Qubit DNA Assay Kit (Life Technologies Cat. No. Q328520). Equal numbers of purified amplicons were pooled for subsequent sequencing analysis and sequenced on an Illumina MiSeq platform at Shanghai OE Biotech Co., Ltd. (Shanghai, China).

Bioinformatics analysis

Raw data from the amplicon sequencing of 16S rRNA were collected in FASTQ format and quality-controlled using Trimmomatic software to cut low-quality reads at a 20% cut-off level (Bolger et al. 2014). UCHIME software was used for dual-end splicing, Barcode, Chimera, and to remove the primer sequences (Edgar et al. 2011). The UPARSE pipeline was used to cluster the clean reads into operational taxonomic units (OTUs) at a 97% similarity level (Edgar 2013). All representative reads were annotated and blasted against the Silva database (Greengenes) of bacteria, using the Ribosomal Database Project (RDP) classifier (confidence threshold of 80%) for taxonomic annotation (Quast et al. 2012). Alpha diversity indices (Chao 1, Shannon, and Simpson) and beta diversity based on the Bray – Curtis dissimilarity matrix were calculated using QIIME2, and the results were visualized by bar plots and constrained principal coordinate analysis (CPCoA), respectively. Permutational multivariate analysis of variance (PERMANOVA) was performed using the Adonis function in the vegan package in R (v3.5.0). OTU analysis of inter-group differences, chord diagrams at the phylum level, and redundancy analysis (RDA) analysis were performed using edgeR, Circo package, and Vegan package, respectively, in R software (Version 3.5.5).

The Molecular Ecological Network Analysis pipeline (<http://ieg4.rccc.ou.edu/mena>) was used to construct the microbial co-occurrence network for OTUs at the genus level within phyla for *L. antibioticus* 13–6 seed coat treatment (T1) and CK ($p < 0.01$). The network properties were calculated and visualized using Cytoscape v3.8.2 (<https://cytoscape.org/>). Zi – Pi thresholds were based on the metabolic network method. Briefly, we classified all species into four groups: peripherals ($zi \leq 2.5$; $pi \leq 0.62$), connectors ($zi \leq 2.5$; $pi \geq 0.62$), module hubs ($zi \geq 2.5$; $pi \leq 0.62$), and network hubs ($zi \geq 2.5$; $pi \geq 0.62$) (Zhang et al. 2019b). Data were statistically analyzed using ANOVA, and significant differences among treatments were calculated using the *t*-test and Tukey's test at $p < 0.05$.

Results

In vitro characterization of plant-growth-promoting traits of *Lysobacter antibioticus* 13–6

Several plant growth-promoting traits of *L. antibioticus* 13–6 were tested by in vitro assays (Table S2). In the Salkowski reagent, an orange-red color was produced by the supernatant of *L. antibioticus* 13–6, which indicates that *L. antibioticus* 13–6 has the ability to produce indole acetic acid with a maximum yield of $63.85 \pm 0.26 \mu\text{g mL}^{-1}$. One week after incubation at 28 °C, clear visible halos were observed

on PVK and Congo red agar media, indicating that *L. antibioticus* 13–6 has the potential to decompose rock phosphate and hydrolyze cellulose, respectively. In a liquid medium, P solubilization ability was recorded at $50.61 \pm 3.31 \text{ mg L}^{-1}$.

Assessing the impact of different concentrations of *Lysobacter antibioticus* 13–6 on the growth of maize plant

A greenhouse experiment was conducted to evaluate the effects of *L. antibioticus* 13–6 on the growth of maize. This study showed that the stalk height, root length, and dry weight of stalks and roots of maize plants significantly increased after treatment with different concentrations of *L. antibioticus* 13–6 compared to CK (Fig. 1A and Table S3). Maximum plant growth was recorded when maize seeds were treated with 10^7 CFU mL^{-1} , and plant growth decreased with an increase in the concentration of *L. antibioticus* 13–6. This indicated that a higher concentration of *L. antibioticus* 13–6 ($> 10^7 \text{ CFU mL}^{-1}$) had a negative impact on the growth of maize plants. It was observed that stalk height, root length, dry weight of stalk, and dry weight of roots significantly increased by 24.85%, 63.33%, 121.95%, and 175%, respectively, under 10^7 CFU mL^{-1} *L. antibioticus* 13–6 treatment than that of CK.

Lysobacter antibioticus 13–6 seed coat treatment enhances the growth of maize plant

From the preliminary experiment, we found that 10^7 CFU mL^{-1} of *L. antibioticus* 13–6 significantly enhanced plant growth. We further evaluated the effects of *L. antibioticus* 13–6 (T1), tebuconazole (T2), and sdH₂O (CK) seed coat treatments on the growth of maize plants (Fig. 1B and Table S4). The results showed that under *L. antibioticus* 13–6 treatment (T1) the root length, stalk height, and leaf area of maize plants were significantly increased by 42.77%, 13.43%, and 72.30%, respectively compared with CK, while compared to the tebuconazole seed coat treatment (T2), they were increased by 9.22%, 6.92%, and 32.84%, respectively.

Colonization ability of *Lysobacter antibioticus* 13–6 in the rhizosphere of maize plant

Quantitative-PCR was used to quantify gene copy numbers to investigate the colonization ability of *L. antibioticus* 13–6 in the maize rhizosphere using the cyclohexanone monooxygenase gene (*phzNO1*). The population of *L. antibioticus* 13–6 increased significantly in the maize rhizosphere compared to CK (Fig. 1C). The *L. antibioticus* 13–6 population increased during the first 7 days after treatment; and then the population dynamics began to decline. The population of *L.*

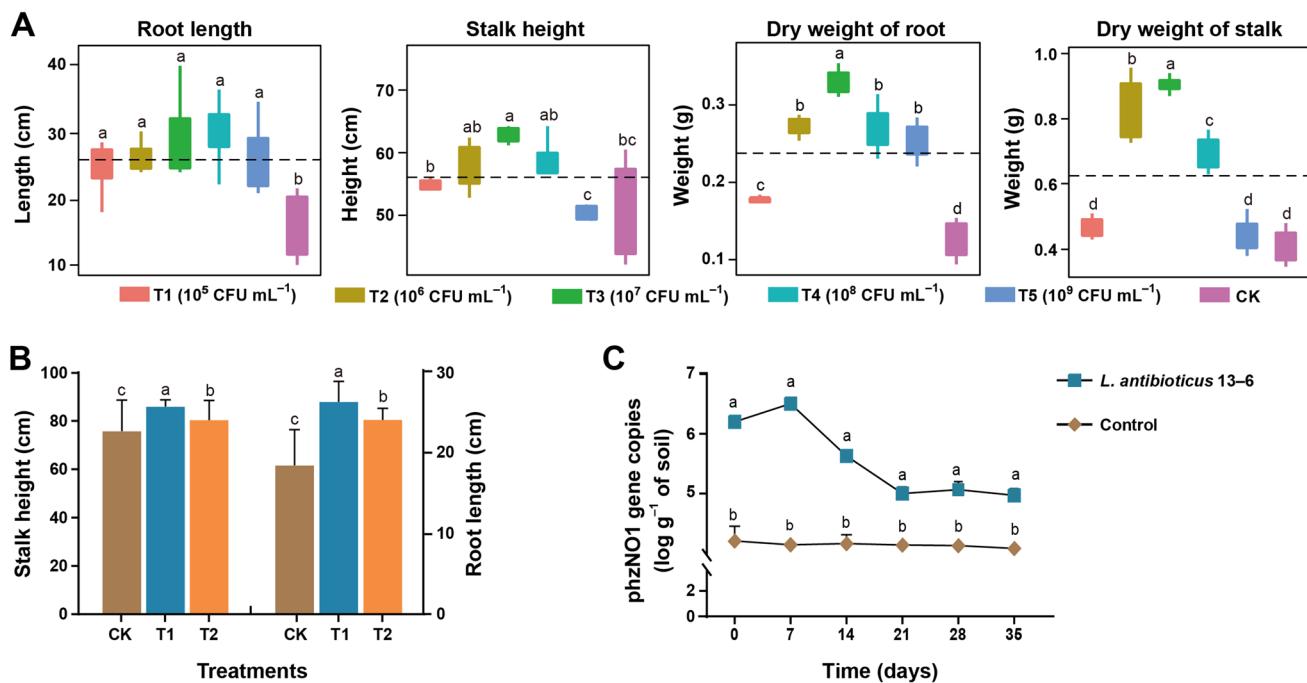


Fig. 1 Growth promotion traits and colonization dynamics of *Lysobacter antibioticus* 13–6 in the greenhouse assay. **A** Effect of different concentrations (10^5 – 10^9 CFU mL $^{-1}$) of *L. antibioticus* 13–6 on the growth of maize plant. **B** Impact of different seed coat treatments on

maize growth. **C** Colonization ability of *L. antibioticus* 13–6 in the rhizosphere of maize plant after specific days of treatment. *L. antibioticus* 13–6 + additives (T1), 2% tebuconazole + additives (T2), and sdH₂O + additives as control (CK)

antibioticus 13–6 was at a stable level 21 days after treatment, remaining between $10^{4.98}$ – $10^{5.16}$ soil g $^{-1}$.

Effect of *Lysobacter antibioticus* 13–6 seed coat treatment on grain yield of maize

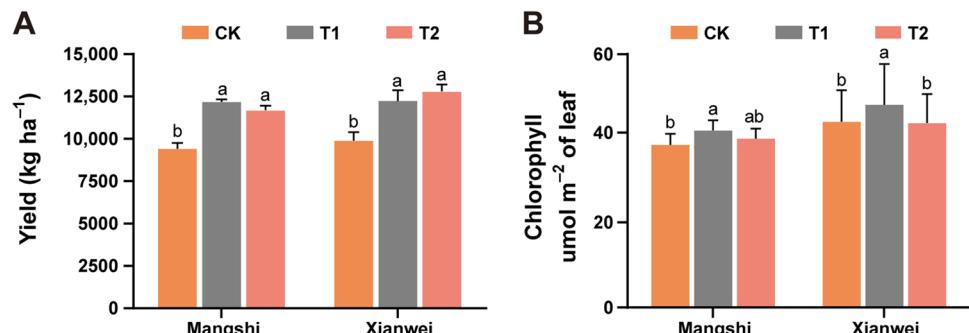
Field experiments were conducted in 2019–2020 in Mangshi and Qujing Counties, Yunnan Province, to explore the effect of *L. antibioticus* 13–6 seed coat treatment and other treatments on the grain yield of maize plants (Fig. 2 and Table S5). Maize yield significantly increased by 29.32% (Mangshi County) and 23.77% (Xuanwei County) when treated with *L. antibioticus* 13–6 compared to CK. However, compared with chemical treatment T2, the maize

yield increased by 4.17% in the Mangshi County field experiment and decreased by 4.39% in Qujing County (Fig. 2A). The chlorophyll content in the leaves of maize plants treated with *L. antibioticus* 13–6 was higher than that of CK and T2 at both experimental sites (Fig. 2B).

Soil physicochemical analysis

Soil physicochemical properties (available nitrogen, available phosphorus, available potassium, and soil pH) were determined in the soil associated with maize plants treated with *L. antibioticus* 13–6 and CK at both experimental sites (Mangshi and Xuanwei) (Table 1). The level of available nitrogen, available phosphorus, and available potassium

Fig. 2 Effect of *Lysobacter antibioticus* 13–6 seed coat treatment on grain yield of maize in the field experiments. **A** Yield and **B** Chlorophyll contents. *L. antibioticus* 13–6 + additives (T1), 2% tebuconazole + additives (T2), and sdH₂O + additives as control (CK)



were higher in the soil of Mangshi County than in Xuanwei County. However, in Mangshi County, the soil was acidic ($\text{pH } 4.42 - 4.76$) and neutral to weakly acidic ($\text{pH } 6.85 - 7.54$) in Xuanwei County. The level of available nitrogen, available phosphorus, and available potassium were significantly increased by 11.74%, 13.11%, and 32.03%, respectively in Mangshi County, and 9.74%, 18.60%, and 15.19%, respectively in Xuanwei County in the soil of maize plants treated with *L. antibioticus* 13–6 compared to CK. However, pH was decreased in the soil of maize plants treated with *L. antibioticus* 13–6 than CK.

Identification of genes related to plant-growth-promoting traits in the genome of *Lysobacter antibioticus* 13–6

A total of 4,015,365 raw reads (250 bp in length) were generated from the genome sequencing of *L. antibioticus* 13–6. These raw reads were trimmed, filtered with a low-quality average ($\leq Q20\%$), and used for de novo assembly. Ultimately, 153 scaffolds were generated, with a total length of 5,527,310 bp. The genome of *L. antibioticus* 13–6 contains 4464 putative coding sequences (CDS), 3 rRNAs, 48 tRNAs, and 4 ncRNAs, with an overall guanine – cytosine content of approximately 76.1% (Table S6). *L. antibioticus* 13–6 can solubilize inorganic phosphate, decompose cellulose, and produce phytohormone indole acetic acid (IAA) which is directly involved in plant growth, with a maximal yield of $17.54 \pm 0.61 \mu\text{g mL}^{-1}$. Genes related to IAA biosynthesis were found in the genome of *L. antibioticus* 13–6, such as *Indole-3-glycerol phosphate synthase* (EX88_RS0108065), *aldehyde dehydrogenase* (EX88_RS0120140, EX88_RS0118055), *indole pyruvate ferredoxin oxidoreductase* (EX88_RS0119430), *tryptophan synthase* (EX88_RS0104920, EX88_RS0104925), and *tryptophan 2,3-dioxygenase* (EX88_RS0114510) (Table S7). Several genes related to chitinase decomposition (EX88_RS0110110, EX88_RS0122520, EX88_RS0100085, and EX88_RS24300) and phosphate solubilization, including *pyrophosphate phospho-hydrolase* (EX88_RS0102010), *phosphate transporter* (EX88_RS0115770, EX88_RS0102685,

EX88_RS0102675, EX88_RS0102670, EX88_RS0102680), and *polyphosphate kinase* (EX88_RS0119910, EX88_RS0118955), have been identified in the genome of *L. antibioticus* 13–6 (Table S7).

Effect of *Lysobacter antibioticus* 13–6 seed coat treatment on diversity and composition of rhizosphere bacterial community

High-throughput sequencing was used to study the effect of *L. antibioticus* 13–6 seed coat treatment on the diversity and composition of maize plant rhizosphere bacteria (Fig. 3). A total of 946,549 high-quality sequences were obtained from 12 samples (average reads: 78,879 per sample). Through the analysis of high-quality reads using QIIME2, a total of 14,519 operational taxonomic units (OTUs) (2087 OTUs/sample) were obtained (Table S8). A significant difference was observed in the α -diversity (Shannon, Observed OTUs, and Chao1) indices of *L. antibioticus* 13–6 seed coat treatment compared to CK (t test, $p < 0.05$; Fig. 3A, B and Table S9). This suggests that the *L. antibioticus* 13–6 seed coat-treated rhizosphere was enriched in the bacterial communities compared to CK.

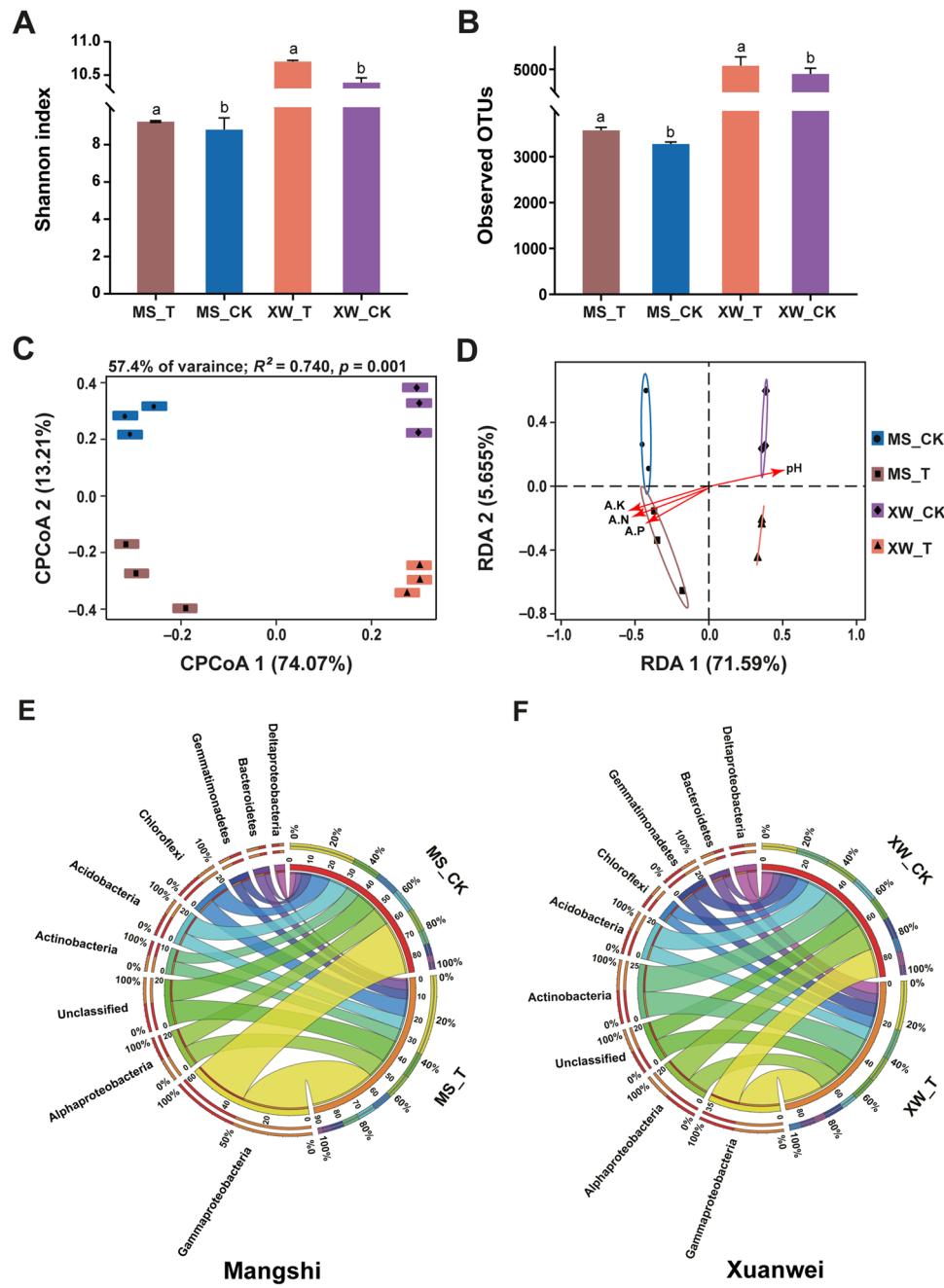
A constrained principal coordinate analysis (CPCoA) based on the Bray–Curtis dissimilarity matrix was performed to analyze the differences among the bacterial communities in the rhizosphere of *L. antibioticus* 13–6 seed coat treated and CK maize plants for both experimental sites (Mangshi and Xuanwei). According to the CPCoA results, the first two axes explain 74.07% and 13.21% of the total variation in the bacterial community in both fields (Fig. 3C), indicating that *L. antibioticus* 13–6 treatment significantly changed the composition of the rhizosphere bacterial community associated with maize plants (PERMANOVA; $R^2 = 0.740$, $p < 0.001$). Canonical correlation analysis showed that at both experimental sites, soil physicochemical properties were affected by soil bacterial communities (Fig. 3D). Soil available nitrogen, available phosphorus, and available potassium were positively correlated with *L. antibioticus* 13–6 seed coat treatment bacterial community composition ($R^2 = 0.93$, 0.76, and 0.82,

Table 1 Basic physicochemical properties of maize soil under different treatments

Location	Year	Treatments	Soil texture	A•N (mg kg^{-1})	A•P (mg kg^{-1})	A•K (mg kg^{-1})	pH
Mangshi	2019	T	Clay	$225.30 \pm 5.44\text{a}$	$141.63 \pm 7.47\text{a}$	$282.76 \pm 8.01\text{a}$	$4.42 \pm 0.75\text{a}$
		CK		$201.63 \pm 3.31\text{b}$	$125.21 \pm 5.18\text{b}$	$214.16 \pm 7.50\text{b}$	$4.76 \pm 0.23\text{a}$
Xuanwei	2020	T	Sandy loam	$163.63 \pm 3.15\text{a}$	$76.14 \pm 0.66\text{a}$	$103.90 \pm 1.47\text{a}$	$6.85 \pm 0.03\text{a}$
		CK		$149.10 \pm 0.36\text{b}$	$64.20 \pm 2.10\text{b}$	$90.20 \pm 0.57\text{b}$	$7.54 \pm 0.05\text{b}$

T *Lysobacter antibioticus* 13–6 seed coat treatment, CK non-seed coat treatment, A•N available nitrogen, A•P available phosphorus, and A•K available potassium. Significance difference among treatments is shown by different small letters within a column according to Tukey's HSD-test at $p < 0.05$

Fig. 3 Impact of *Lysobacter antibioticus* 13–6 seed coat treatment on diversity and composition of rhizosphere bacterial communities. **A** Shannon index, **B** Observed OTUs, **C** CPCoA based on Bray–Curtis dissimilarity matrix showing the changes in rhizosphere bacterial communities, **D** RDA used to explore the relationships between the rhizosphere bacterial microbial communities and soil physicochemical properties, and chord diagram represents the relative abundance of bacterial phylum under different treatments at two experimental locations, **E** Mangshi and **F** Xuanwei. Significant differences among treatments are shown by different small letters on the error bars according to the *t* test at $p < 0.05$. The arrows indicate the lengths, and the angles between explanatory and response variables reflect their correlations. MS = Mangshi County, XW = Xuanwei County, T = *L. antibioticus* 13–6 seed coat treatment, and CK = non-seed coat treatment



respectively) (Table S10). However, a negative correlation was found between *L. antibioticus* 13–6 seed coat treatment bacterial community composition and soil pH. The bacterial community composition of *L. antibioticus* 13–6 seed coat-treated maize plants differed significantly at the phylum level than that of CK (Fig. 3E, F). In both experimental sites (Mangshi and Xuanwei), the relative abundances of Gammaproteobacteria, Gemmatimonadetes, and Bacteroidetes were significantly higher in the rhizosphere of maize plants under *L. antibioticus* 13–6 seed coat treatment than in CK (*t* test, $p < 0.05$). The relative abundances of Acidobacteria, Chloroflexi, and Deltaproteobacteria

were significantly decreased in the rhizosphere soil of *L. antibioticus* 13–6 seed coat-treated maize plants compared to CK (*t* test, $p < 0.05$; Table S11).

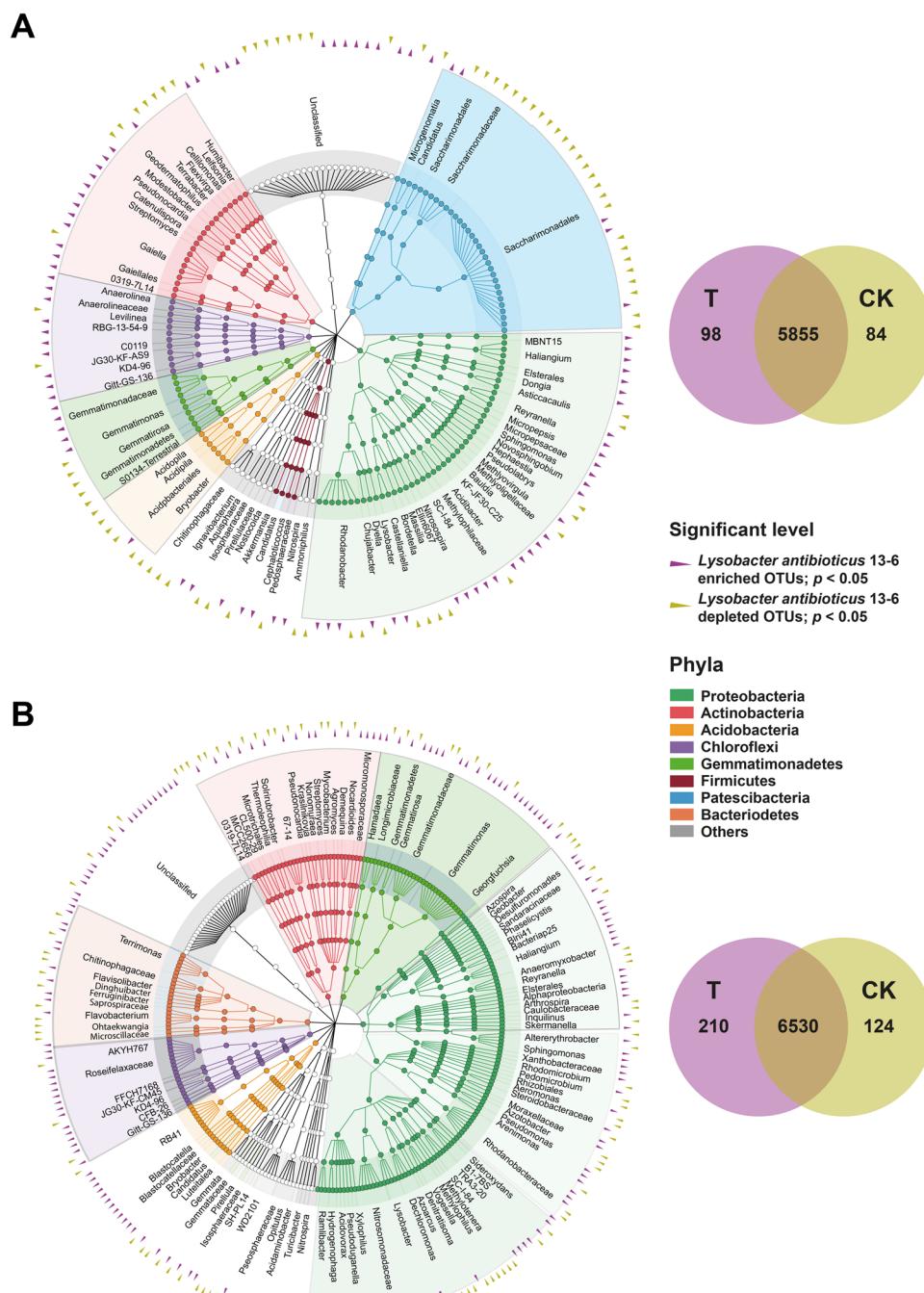
Microbial community composition and differentially abundant taxa

The results of microbial community composition and differentially abundant taxa at the OTU level for inter-genus differences in the rhizosphere of *L. antibioticus* 13–6 seed coat-treated maize plants and CK are shown in Fig. 4. A total of 6037 and 6864 OTUs were found in the rhizospheres of

the experimental sites at Mangshi and Xuanwei, respectively. Furthermore, analysis of 6037 OTUs at the Mangshi site showed that 98 and 84 OTUs were significantly enriched in the rhizosphere of *L. antibioticus* 13–6 seed coat-treated plants and CK, respectively (Fig. 4A and Table S12). The rhizosphere of *L. antibioticus* 13–6 seed coat-treated plants was significantly enriched in bacterial genera such as *Streptomyces*, *Chitinophagaceae*, *Roseiflexaceae*, *Chloroflexi* KD4-96, *Chloroflexi* Gitt-GS-136, *Pyrinomonadaceae* RB41, *Blastocatella*, *Bryobacter*, *Gemmamimonas*,

Nitrospira, *Lysobacter*, *Betaproteobacteriales* SC-I-84, *Rhodanobacteraceae*, *Methylotenera*, *Sideroxydans*, *Nitrosomonadaceae*, *Xanthomonadaceae*, *Sphingomonas*, *Myxococcales* BIrii41, and *Haliangium*. At both experimental sites bacterial genera including *Streptomyces*, *Chloroflexi* KD4-96, *Chloroflexi* Gitt-GS-136, *Gemmamimonas*, *Nitrospira*, *Lysobacter*, and *Betaproteobacteriales* SC-I-84 were widely distributed in the rhizosphere of *L. antibioticus* 13–6 seed coat-treated plants. Similarly, analysis of 6864 OTUs at the Xuanwei experimental site revealed that 210 and 124

Fig. 4 OTUs analysis of inter-genus differences at phylum level under *Lysobacter antibioticus* 13–6 coat treatment and control. **A** Mangshi County and **B** Xuanwei County. Here, T = *L. antibioticus* 13–6 seed coat treatment and CK = non-seed coat treatment



OTUs were significantly enriched in the rhizosphere of *L. antibioticus* 13–6 seed coat-treated plants and CK, respectively (Fig. 4B and Table S13). Bacterial genera such as *Gaiella*, *Streptomyces*, *Catenulisporea*, *Anaerolinea*, *Chloroflexi* C0119, *Chloroflexi* KD4-96, *Chloroflexi* Gitt-GS-136, *Gemmimonas*, *Nitrospira*, *Ammoniphilus*, *Lysobacter*, *Nitrosospira*, *Massilia*, *Betaproteobacteriales* SC-I-84, and *Micropepsis* were widely distributed in the rhizosphere of *L. antibioticus* 13–6 seed coat-treated maize plants. The OTUs that were significantly enriched in genus *Lysobacter* at both experimental sites under *L. antibioticus* 13–6 seed coat treatment are shown in Figure S1.

Characteristics of co-occurrence network between rhizosphere microorganisms of *Lysobacter antibioticus* 13–6-treated plants and control

A microbial co-occurrence network analysis was performed for OTUs at the genus level within phyla for each sample type (*L. antibioticus* 13–6 seed coat-treated and CK) by excluding the relative abundance of less than 0.001 (Fig. 5). The total number of nodes (409) and edges (4555) were significantly higher in *L. antibioticus* 13–6 seed coat treatment than in control (total nodes: 289 and total edges: 2752) (Fig. 5A, B). The *L. antibioticus* 13–6 seed coat treatment was divided into four modules, and CK was divided into five modules, which indicated that the microbial network of *L. antibioticus* 13–6 seed coat treatment was more complex and the internal members were closer. The number of negatively correlated edges was significantly decreased between genera in *L. antibioticus* 13–6 seed coat treatment (46.59%) compared with CK (65.68%). This indicates that *L. antibioticus* 13–6 seed coat treatment had a lower competitive relationship than CK. Network hubs were not found in either treatment; however, module hubs (4.48%) in *L. antibioticus* 13–6 seed coat treatment were significantly higher than in CK (0.69%) (Fig. 5C, D), which is the opposite of the connectors.

Discussion

Maize (*Zea mays* L.) is an important industrial crop worldwide, including China (Chen et al. 2021). Studies have shown that plant growth-promoting rhizobacteria (PGPR) play an important role in sustainable agriculture by enhancing plant growth and suppressing the incidence of diseases (Backer et al. 2018; Zhang et al. 2022). In this study, we evaluated the effect of a PGPR strain *Lysobacter antibioticus* 13–6 on maize plant growth, yield, and microbial diversity through seed coat treatment in both in vitro and in vivo studies. In two field experiments, *L. antibioticus* 13–6 significantly enhanced maize yield, making it a

valuable seed-coating agent with plant-growth-promoting traits. Many studies have reported that genus *Lysobacter* has the ability to promote plant growth (Puopolo et al. 2010), and *L. gummosus* OH17 and *L. antibioticus* HS124 significantly reduce disease incidence and enhance the yield of many crops, including rice and wheat (Kim et al. 2019; Laborda et al. 2018).

It is well known that efficient colonization is a prerequisite for plant-growth-promoting strains to perform their function at potential infection sites (Compant et al. 2010). Postma et al. (2011) studied the population dynamic of three *Lysobacter* spp. in the soil, and the population was found as $\log_{10}^{4.0} - \log_{10}^{6.95}$ copy numbers/gram of soil. The colonization dynamics of PGPR in the rhizosphere of host plants are responsible for better crop yield. It has been reported that *Bacillus subtilis* Beb-LSBS and *B. amyloliquefaciens* FZB42 have strong colonization ability in the rhizosphere of tomato and cotton crops, respectively, significantly increasing the yield (Alavo et al. 2015; Mena-Violante and Olalde-Portugal 2007). The results of qPCR demonstrated that *L. antibioticus* 13–6 successfully colonized in the rhizosphere of maize plants with copy numbers of $\log_{10}^{4.98} - \log_{10}^{5.16}$ per gram of soil. Our results are similar to the findings of Fu et al. (2018) and Postma et al. (2011) that *L. antibioticus* is widely distributed in natural soil and can colonize the rhizosphere. However, the detailed plant-growth-promoting mechanism of *L. antibioticus* 13–6 remains unclear and requires further in-depth study.

Plant-growth-promoting rhizobacteria enhance plant growth and regulate root development through nutrient acquisition and synthesis of plant hormones (cytokinin and auxin) and volatile organic compounds (Basu et al. 2021). PGPR decomposes rock phosphate and solubilizes potassium minerals in soil through organic acid secretion and biosynthesis of extracellular polysaccharides (Cui et al. 2019). The results of the in vitro assay demonstrated that *L. antibioticus* 13–6 can synthesize auxins, solubilize P, and hydrolyze cellulose. However, the use of tricalcium phosphate to determine P solubilizers has been criticized by Bashan et al. (2013), which suggested an additional test in a liquid medium to determine the P dissolution ability of PGPR. In this study, the P solubilization ability of *L. antibioticus* 13–6 was initially determined as a halo on PVK, which was further confirmed by the dissolution of P in the liquid medium.

The application of PGPR having a function of N fixation, P decomposition, and K solubilization improved the soil physicochemical properties. However, the application of N-fixing and P-solubilizing bacteria in soil decreases the pH of the soil by creating an N – P-offering micro-area around the plant rhizosphere (Adesemoye et al. 2010; Rana et al. 2012). Our results confirmed that the soil nutrient contents of available nitrogen, available phosphorus, and

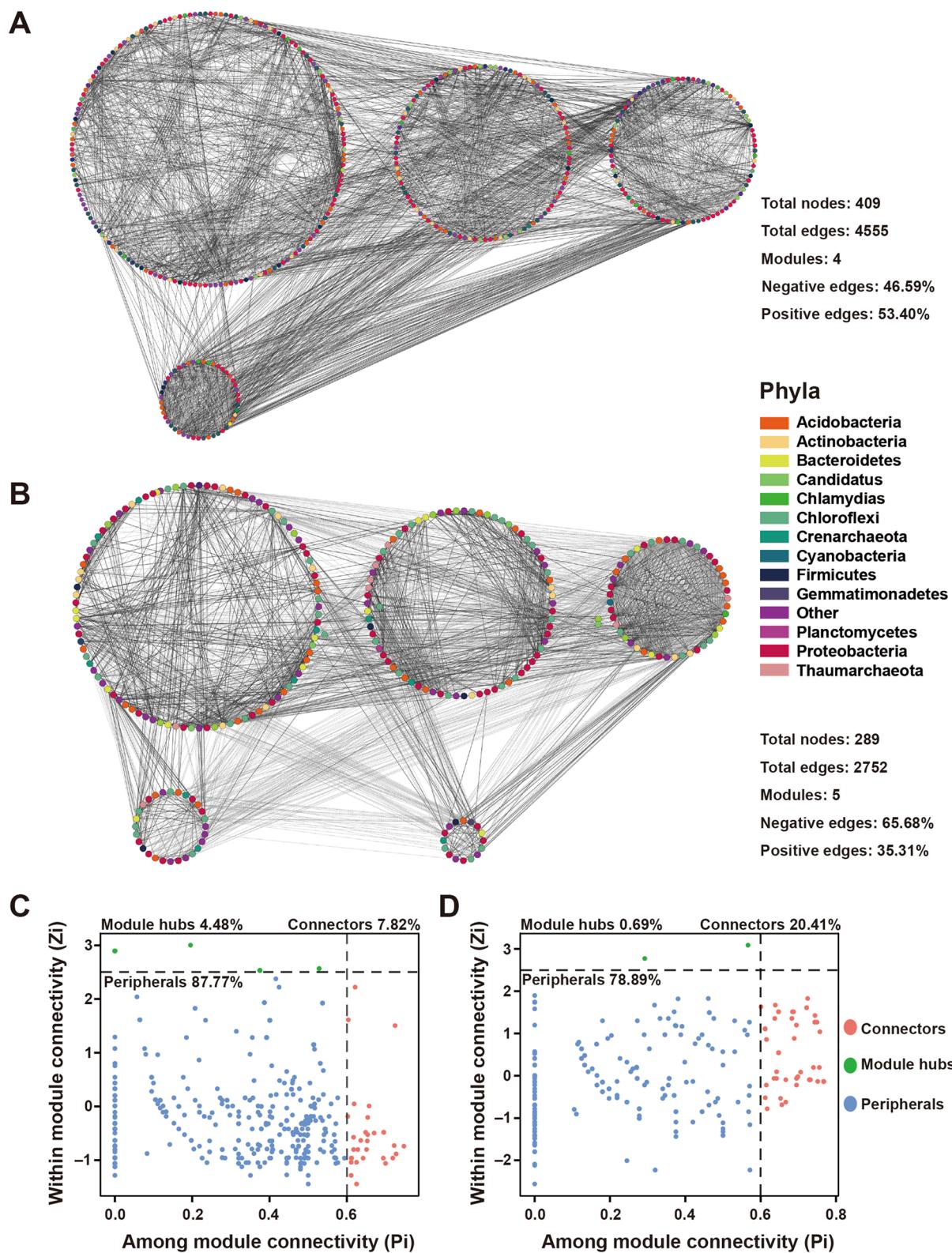


Fig. 5 Co-occurrence network analysis of maize plant rhizosphere bacterial community network under different treatments. Co-occurrence network of **A** *Lysobacter antibioticus* 13–6 seed coat-treated

maize plants and **B** non-seed coat-treated maize plants. Zi-Pi plot for co-occurrence network of **C** *L. antibioticus* 13–6 seed coat-treated maize plants and **D** non-seed coat-treated maize plants

available potassium were significantly increased, and pH decreased in the rhizosphere of *L. antibioticus* 13–6 seed coat-treated plants compared to CK. This is due to the soil nutrient cycling ability of *L. antibioticus* 13–6, or perhaps *L. antibioticus* 13–6 enhances the abundance of bacterial taxa involved in P solubilization and soil nutrient cycling. An increase in nitrogen and phosphorus uptake results in lavish vegetative growth, phosphorus translocation, and photosynthesis, which enhances plant growth, biomass production, and yield (Ahmed et al. 2020). Maize crops demand a high amount of macro-elements for better yield production, and a lack of these elements reduces maize yield (Messina et al. 2011). *L. antibioticus* 13–6 successfully colonized the rhizosphere of maize plants, enhancing plant nutrient availability, and yield.

Another focus of our study was the comparison of bacterial communities in the rhizosphere of *L. antibioticus* 13–6 seed coat-treated maize plants and CK plants. Plant-associated rhizosphere microbial communities act as a plant's second genome and are crucial for plant health and productivity (Vessey 2003). In the rhizosphere, plants are directly exposed to many disease-causing agents and beneficial microorganisms (Bai et al. 2015; Zhang et al. 2019a); the application of PGPR changes the diversity and composition of the rhizosphere microbiota (Olowe et al. 2018). Studies have shown that rhizosphere microbial diversity increases with an increase in aboveground (vegetative) plant parts (Bai et al. 2022; Xiao et al. 2016). We observed a significant shift in the diversity and composition of rhizosphere bacterial communities at both experimental sites under *L. antibioticus* 13–6 seed coat treatment compared to CK. We assumed that this shift was due to the increase in maize plant growth (root length and stalk height) under seed coat treatment of *L. antibioticus* 13–6 over that of CK and that *L. antibioticus* 13–6 enhanced the abundance of other bacterial taxa in the rhizosphere of maize plants. An increase in alpha diversity indices and CPCoA based on the Bray–Curtis dissimilarity matrix showed a clear separation among samples from both experimental sites, indicating that the diversity and composition of rhizosphere bacterial communities were significantly changed in the presence of *L. antibioticus* 13–6. Gammaproteobacteria, Gemmatimonadetes, and Bacteroidetes were the dominant phyla in the rhizosphere soil of *L. antibioticus* 13–6 seed coat-treated maize plants. Previous studies have reported that many members of Bacteroidetes and Proteobacteria are closely involved in the N cycle. Furthermore, Gemmatimonadetes are known as facultative bacteria and have been positively correlated with soil N content (Chen et al. 2016; Leff et al. 2015). Interestingly, the relative abundance of bacterial consortia, such as *Streptomyces*, *Nitrospira*, and *Lysobacter* was also increased in the rhizosphere of *L. antibioticus* 13–6 seed coat-treated plants compared to CK. Members of *Streptomyces*, *Nitrospira*, and *Lysobacter*

are known to be involved in PGP, endosymbionts, nitrite-/ammonia oxidization, and antagonistic processes, respectively (Beeckman et al. 2018; Vurukonda et al. 2018; Wei et al. 2021). However, it is still unknown how long the *L. antibioticus* 13–6 microbial effect lasts and requires further study, and varying environmental factors also have negative or positive effects on microbes.

The abundance of a single beneficial strain causes non-linear changes in the composition of the microbiome (Erlacher et al. 2014). *L. antibioticus* 13–6 increased the relative abundance of a specific subset of bacteria in the maize rhizosphere bacterial community, which may have enhanced the positive microbe–microbe interaction. Indole acetic acid (IAA) produced by PGPR changes the root morphology and provides a habitat necessary for beneficial bacteria (Chu et al. 2020). Some bacteria use IAA as a signal molecule to produce antibiotics and enhance their ability to compete with other microorganisms for limited resources (Duca et al. 2014). They also degrade IAA as a source of nitrogen and carbon, which helps them to better colonize rhizosphere (Scott et al. 2013). Our study demonstrated that *L. antibioticus* 13–6 can produce IAA, and qPCR results revealed that it successfully colonized the rhizosphere of *L. antibioticus* 13–6 seed coat-treated plants compared to CK. Analysis of co-occurrence networks showed fewer negative correlations in the rhizosphere of *L. antibioticus* 13–6 seed coat-treated plants indicating that *L. antibioticus* 13–6 can reduce interspecies competition. Our results are similar to previous findings that the availability of high resources reduces competition between microbial communities (Costello et al. 2012).

In vitro and in vivo experiments, we found that *L. antibioticus* 13–6 enhanced the supply of macro elements that directly improved the growth, biomass production, and yield of maize plants. The genome of *L. antibioticus* 13–6 was analyzed using high-throughput sequencing to understand the PGP mechanism at the molecular level. Previous studies have reported that the genomes of *L. antibioticus* HS124, ATCC29479, and 76 encode genes related to chitin decomposition (de Bruijn et al. 2015; Gardener et al. 2014). Puopolo et al. (2014) and de Bruijn et al. (2015) performed a comparative genomic analysis of the genus *Lysobacter*, including *L. capsici* 55, AZ78, *L. enzymogenes* C3, *L. antibioticus* ATCC29479, 76, and *L. gummosus* 3.2.11. They found that the genomes of these *Lysobacter* species contain a large number of genes encoding extracellular enzymes, including chitinases, glucanases, lipases, peptidases, and xylanases. The above studies demonstrate that the genus *Lysobacter* has broad-spectrum antimicrobial activity and can produce several secondary metabolites. However, to date, there has been no report (based on the above study) that the genome of the genus *Lysobacter* encodes genes related to P solubilization. Interestingly, in our study, through genome analysis of *L. antibioticus* 13–6 we found seven genes related

to auxin synthesis, four genes related to chitin decomposition, and eight genes related to P solubilization. However, gaps are still present, and further studies related to molecular mechanisms involving these genes could provide insight to unravel the in-depth PGP mechanism of *L. antibioticus* 13–6. Based on the overall discussion, we suggest that *L. antibioticus* 13–6 enhanced the relative abundance of the rhizosphere bacterial community and positively affected the bacterial community composition and soil nutrient content in the rhizosphere of the maize plant, which promoted the growth, biomass, and yield of the maize plant.

Conclusions

In summary, we concluded that as a member of a well-characterized PGPR, *L. antibioticus* 13–6 has the potential for P solubilization, hydrolysis of cellulose, biosynthesis of IAA, soil health improvement, and shifting the rhizosphere bacterial community composition toward a positive trend. Through seed coat treatment, *L. antibioticus* 13–6 successfully colonizes the rhizosphere of maize plants and improves growth and grain yield. *L. antibioticus* 13–6 can change the diversity and composition of rhizosphere bacterial microbial communities by increasing the α - β diversity indices and the relative abundance of bacterial consortia, such as *Lysobacter*, *Nitrospira*, and *Streptomyces*. The application of *L. antibioticus* 13–6 as a seed coat treatment reduced the competition for resources between competitive bacterial communities. This study provides theoretical and technical knowledge for the efficient use of microbial resources for sustainable agriculture. However, in the future, detailed molecular mechanisms involving gene cloning related to PGP and disease suppression through seed coat treatment will provide new in-depth insights into the PGP and biocontrol mechanisms of *L. antibioticus* 13–6.

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Authors' contributions G.J. and L.W. conceived and designed the experiments. Z.D., W.A., X.Y., and J.Z. performed the experiments and collected the data. Z.D., W.A., and J.Y. analyzed the data. Z.D. and W.A. illustrated the figures and wrote the first draft of the manuscript. W.A. and G.J. revised the manuscript. All authors contributed to the final draft of the manuscript. All authors read and approved the final manuscript.

Data availability The raw data related to 16S rRNA sequencing is deposited in the sequence read archive (SRA) in NCBI public database with accession No. PRJNA615109.

Declarations

Ethics approval and consent to participate Not applicable.

Consent for publication Not applicable.

Conflict of interest The authors declared that they have no conflict of interest.

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