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Research article

Colonization of Potassium-Solubilizing Purple Nonsulfur Bacteria and Their Role in Promoting the Growth of Hybrid Maize

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Abstract: The use of biofertilisers to replace conventional chemical fertilisers has been widely investigated. However, the colonization of microbes in plants and their effects on plant growth are still unclear. The experiment aimed to (i) determine the ability of potassium-solubilizing purple nonsulfur bacteria (Ksol-PNSB) to colonize in hybrid maize and (ii) measure their capacity to promote plant growth. A randomised complete block design experiment was conducted in undeposited alluvial soil collected from An Phu, An Giang, under nethouse conditions with five treatments and six replications. The treatments were: (i) the control, (ii) *Cereibacter sphaeroides* M-Sl-09, (iii) *Rhodopseudomonas thermotolerans* M-So-11, (iv) *Rhodospeudomonas palustris* M-So-14, and (v) a combination of the three strains. Results revealed the presence of bacteria in soft tissues of the cortex and medulla in the root hair region of hybrid maize plants. Supplying one or three strains of Ksol-PNSB increased crop height (20.7–34.4%), root length (17.0–40.5%), root biomass (20.9–154.0%), and stem-leaf biomass (73.8–173.8%) compared to the control. The current study has successfully revealed the inhabitation locations of the beneficial bacteria within plant tissues, along with the improved growth characteristics of the hybrid maize. The current study is a good reference for studies investigating the works of beneficial microbes in plants. However, further molecular investigations should explore the potassium-solubilizing traits of the bacteria.

Keywords: Biofertilizer; *Cereibacter sphaeroides*; *Rhodospeudomonas palustris*; *Rhodopseudomonas thermotolerans*; root anatomy.

1. Introduction

Maize (*Zea mays* L.) globally provides food for human beings and livestock [1]. In Vietnam, maize is highly important in agricultural production and livelihoods [2]. Sandhu *et al.* [3] estimated that approximately 80% of maize is consumed in animal husbandry. Recently, the rice-rice-maize cropping system has been developed in the Mekong Delta. However, maize cultivation faces different difficulties due to the excessive use of chemical fertilizers, leading to nutrient imbalances, reduced efficiency, soil degradation, and biodiversity loss [4].

Potassium (K) is vital in photosynthesis, water storage, and stomatal control [5]. Deficiency in K reduces photosynthesis efficiency and leaf size [6] and affects osmotic regulation [7]. Thus, K fertilization in maize can improve crop nutrient uptake and crop yield [8]. Potassium exists in soil under soluble, exchangeable, non-exchangeable, and structural forms [9]. Nevertheless, a large portion of K is immobilised in clay minerals, making it unavailable to plants. Sun *et al.* [10] suggested exploring biological nutrient sources to replace chemical fertilisers, solubilise soil K, and enhance plant growth. For hybrid maize cultivation, using biofertiliser is a sustainable approach to improve productivity and to remediate the environment [11]. The microorganisms within biofertilisers can improve soil nutrient availability and facilitate plant nutrient uptake [12].

Biofertilisers, containing beneficial microorganisms, can improve plant nutrition through multiple mechanisms, such as nitrogen fixation, phosphate and potassium solubilization, organic acid production, siderophore release, and phytohormone synthesis [12–14]. Among them, purple nonsulfur bacteria (PNSB) are a metabolically versatile group capable of photosynthesis, nitrogen fixation, and nutrient solubilization [15,16]. PNSB has been successfully applied as biofertilisers to enhance potassium availability for various crops [17,18]. Several PNSB species, such as *Rhodopseudomonas* and *Cereibacter* spp., have been reported to enhance soil fertility and plant growth through their potassium-solubilizing capacity and plant growth–promoting (PGP) traits [17–19]. PNSB may promote crop development not only by increasing nutrient bioavailability but also by synthesizing auxins and gibberellins, regulating reactive oxygen species, and improving chlorophyll formation and root development [20–22]. In Vietnam, different potassium-solubilizing PNSB (Ksol-PNSB) strains have been isolated from hybrid maize rhizosphere soils, including *Cereibacter sphaeroides* M-Sl-09, *Rhodopseudomonas thermotolerans* M-So-11, and *Rhodopseudomonas palustris* M-So-14 [23]. However, limited information exists regarding their colonization patterns within maize tissues and their effects on plant growth under early vegetative stages.

Therefore, this study aimed to: (i) assess the colonization potential of Ksol-PNSB during hybrid maize growth stages and (ii) determine their plant growth-promoting capabilities. The findings are expected to provide insight into the interaction between beneficial PNSB and maize roots and support the development of microbial biofertilizers for sustainable maize cultivation in alluvial soils.

2. Materials and Methods

2.1 Materials

Location and duration: The experiment was conducted from September 21 to October 6, 2024, in a greenhouse at the Faculty of Crop Science, College of Agriculture, Can Tho University.

Pots: Plastic containers with a dimension of top diameter x bottom diameter x height at 14 x 11 x 12 (cm). At the bottom, there were 5 five drainage holes.

Soil: Undeposited alluvial soil collected from An Phu - An Giang (0-20 cm depth), air-dried and cleaned from plant debris. Each pot contained 2 kg of prepared soil. Before the experiment, the soil was analyzed for key physicochemical properties: pH = 5.33, electrical conductivity = 0.27 mS cm^{-1}, total N = 0.07%, total P = 0.06 % P_2O_5, and exchangeable K = 0.21 meq 100 kg^{-1}. These values represent moderately fertile, slightly acidic conditions typical of in-dyked alluvial soils in the Mekong Delta.

Plant material: Hybrid maize seeds, NK7328 (Bayer company, Germany).

Bacterial strains: Three Ksol-PNSB strains, including *Cereibacter sphaeroides* M-Sl-09, *Rhodopseudomonas thermotolerans* M-So-11, and *Rhodospeudomonas palustris* M-So-14 [19], were obtained from the Faculty of Crop Science, College of Agriculture, Can Tho University.

2.2 Methods

The experiment followed a randomised complete block design with five treatments: (i) the control, (ii) M-Sl-09, (iii) M-So-11, (iv) M-So-14, and (v) a combination of the three strains. The control group was treated identically to the inoculated groups except that seeds were soaked and watered with sterilized distilled water instead of bacterial suspension. This ensured that any observed differences were due solely to bacterial effects. No fertilizer was used in this experiment. Every day, 100 mL of water was applied to each pot at 5:00 p.m.

Bacterial inoculation: Maize seeds were surface-sterilised using 70% ethanol and 1% sodium hypochlorite before being rinsed with sterilised deionised water. Sanitised seeds were germinated in darkness for 24 hours, then immersed in the corresponding Ksol-PNSB solution suspension (1 x 10^8 CFU mL^{-1}) for 1 hour before sowing, achieving a final bacterial load of 2 x 10^8 CFU per seed as 2 seeds × 1 × 10^8 from 50 seeds/50 mL = 2 × 10^8 [20].

Biofertiliser application: Biofertilisers of *C. sphaeroides* M-Sl-09, *R. thermotolerans* M-So-11, and *R. palustris* M-So-14 were applied into the soil at a rate of 10 mL pot^{-1} on days 5, 10, and 15 after planting (DAP). For the three-strain treatment (v), 3.33 mL of each strain was used.

Tissue microtomy was conducted following the slicing method and cell wall staining by Carmin aluné –vert d' iode for vegetative organs (stem-leaf) at 15 DAP. Samples were soaked with Javen solution (3%) and acetic acid (5%) before being dyed [21]. In particular, carmine made cellulose-made cell walls pink, and vert d' iode turned lignin-made cell walls green.

Anatomic positions: Root samples with zones of division, elongation, and maturation were randomly selected. Stem samples were collected from the middle of the second internode from the ground. Leaf samples were mature leaves at the vegetative positions, including leaf sheath (the third leaf from the tip), leaf blade (0.5 cm from the leaf margin), and midrib (a large bump in the middle of the leaf). All samples were cut in cross-section. The anatomical characteristics of the vegetative organs of maize had the general structure of a monocotyledonous plant (a big herbaceous form).

The presence of bacteria within plant organs: An anatomic method without bleaching and fuchsine staining was applied. In brief, samples were surface-sterilised with alcohol 70°. Then, the samples were quickly dissected, cut in cross-section, and stained with fuchsine to detect tissue and cell structures. This was the scientific evidence to determine the habitat and movement of bacteria among treatments. The samples were observed and sized by the light microscope (Olympus CX23) and Toupview software.

Vegetative parameters were determined on two plants per pot at 15 DAP. Plants were harvested at 15 DAP to focus on the early vegetative stage, when initial colonization and root–microbe interactions occur. Although longer trials could reveal yield responses, this short-term evaluation aimed to visualize colonization patterns and detect early physiological responses before external nutrient limitations or senescence occur in pots.

- Plant height (cm): Measure the height of the plant in each pot, from the ground to the tip of the tallest leaf on top.
- Stem diameter (cm): Measure at the top, middle, and base to calculate the average.
- Leaf length (cm): Measure from the leaf collar to the leaf tip of the top leaf.
- Leaf width (cm): Measure the widest part of the top leaf.
- Root length (cm): Measure from base to root tip.
- Root biomass (g): Cut and weigh the roots.
- Stem-leaf biomass (g): Weigh the whole plant.
- SPAD: The chlorophyll content was measured by SPAD 520 at the $^2/_3$ position of the leaf from the petiole to the leaf tip [22].
- The chlorophyll a and b: Leaf samples were cut into 1.0 cm-wide circles. The leaf samples were let react with 3 mL of NN-Dimethyl Formamidecho in a 50 mL test tube. The tube was kept from light for 24 hours. The solution was measured by spectrophotometry at 663 and 647 nm. The chlorophyll a and b contents were calculated according to Porra *et al.* [23] as follows:

Chlorophyll a = $12.00*A_{663.2} - 3.11*A_{646.8}$ µg Chl/mL

Chlorophyll b = $20.78*A_{663.2} - 4.88*A_{646.8}$ µg Chl/mL

Chlorophyll a+b = $17.67*A_{663.2} + 7.12*A_{646.8}$ µg Chl/mL

- Proline content: Samples (0.5 g) were ground in 10 mL of sulfosalicylic acid 3%. The ground sample was added into a centrifugal tube, shaken by a reciprocal shaker for 30 min, centrifuged at 3,500 rpm for 10 min, and collected for the clear extract. The extracted solution was reacted with 2 mL of Ninhydrin and 2mL of glacial acetic acid, boiled in a double boiler at 90 ℃ for 1 h, cooled by ice, and then 4 mL of toluene, shaken for 15 - 20 seconds, and measured by spectrophotometry at 520 nm [24].

2.3 Statistical analysis

The SPSS 13.0 software was applied to compare differences between means by the Duncan test at 5% significance.

3. Results and Discussion

3.1 Colonization patterns of the Ksol-PNSB in hybrid maize

Microscopic observation of maize tissues revealed clear anatomical differentiation across root, stem, and leaf sections (Figure 1–3).

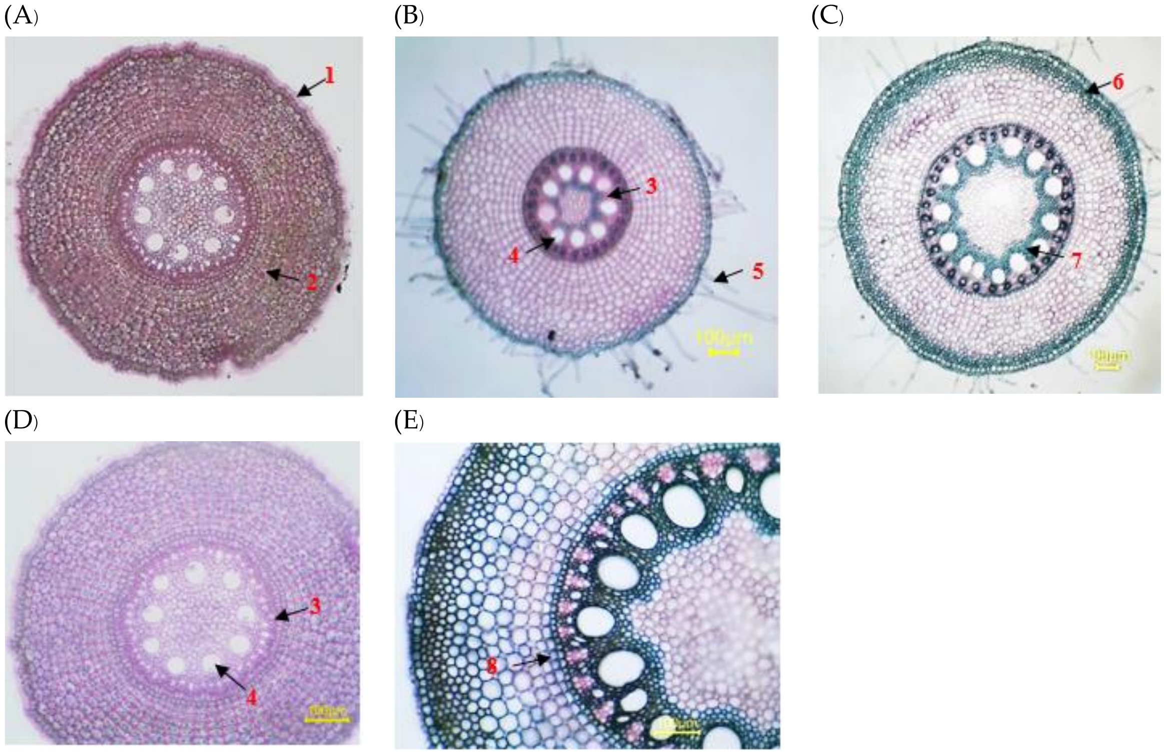

Figure 1. Cross-section anatomy of maize root. A, D: division zone, B: elongation zone, C, E: maturation zone; 1: epidermis; 2: cortex parenchyma; 3: phloem; 4: xylem; 5: root hairs; 6: suberin-impregnated zone; 7: sclerenchyma surrounding xylem; 8: endodermis with U-shaped suberine frame.

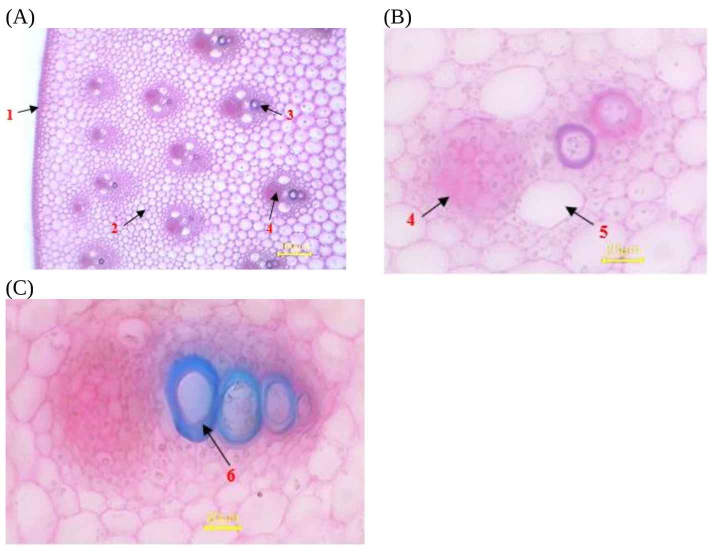

Figure 2. Cross-section anatomy of maize culm. A: stem structure, B, C: vascular bundle development from young to older stages. 1: epidermis; 2: parenchyma; 3: xylem; 4: phloem; 5: newly formed xylem; 6: lignified xylem

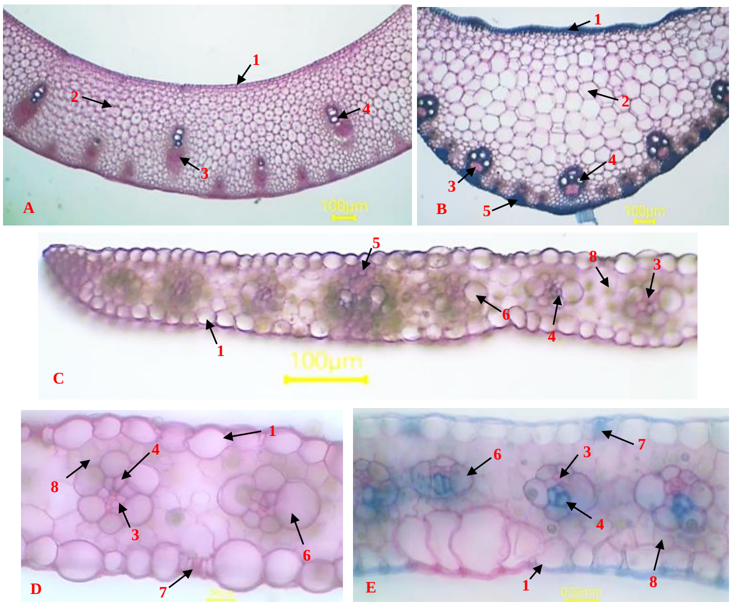

Figure 3. Cross-section anatomy of maize leaf. Leaf sheath structure (A). Anatomical structure of leaf blade: midrib region (B), leaf margin region (C), leaf blade in young leaf stage (D), leaf blade in older leaf stage (E); 1: epidermis; 2: parenchyma; 3: phloem; 4: wood; 5: sclerenchyma; 6: bundle sheath cells; 7: stomata; 8: anabolic tissue

Root anatomy: The root exhibited distinct zones in cross-section, including division (uppermost zone from root tip with active cell generation) (Figure 1A, 1D), elongation (Figure 1B), and maturation zones (Figure 1C, 1E). Cells and tissues were arranged in concentric layers from exterior to interior. The root epidermis consisted of thin-cell-wall cells closely packed together to form a layer that covers the inner parts, along with elongated unicellular root hairs (Figure 1). The thickness of the suberised cork layer varied with tissue maturity. The following layer was the soft cortical tissue that occupied a large area with mostly soft tissues. The endodermis was a layer of rectangular cells arranged in a characteristic U-shaped pattern with suberised cell walls. Numerous vascular bundles formed a ring, with centripetal xylem arrangement alternating with phloem tissues.

Stem anatomy: The cross-section of the maize stem was circular. The tissue arrangement from outside to inside. The outermost epidermis was a layer of small cells packed closely together. In the maize stem, the cross-section of the maize did not distinguish between the cortex and the medulla. The vascular bundles were scattered, and the parenchyma occupied all the remaining space. A few layers of thick tissue (hypodermis) laid just below the epidermis. For the bundle area, the more peripheral positions were, the smaller the bundles (closer to the epidermis) became in size but more numerous, while the bundles in the central region were larger and farther apart. The vascular bundles were arranged in a superimposed pattern, phloem above and xylem below. The xylem resembled a 'Y' shape, but the two xylem (sides) usually had delayed lignification (Figure 2).

Leaf anatomy: Different positions had different structures, with a distinct patterns in the leaf sheath, midrib, and blade regions.

Leaf sheath: The vascular bundles were arranged in parallel rows of different sizes. Each vascular bundle contained both xylem and phloem tissues. The remaining space was filled with parenchyma tissue. (Fig. 3A).

Leaf blade: The leaf blade had an isosurface structure (the upper and lower surfaces of the leaf cannot be distinguished). The microscopic observations revealed that the leaf blade cross-sections displayed specialised cellular organization characteristics of C4 photosynthesis. The mesophyll tissue contained prominent bundle sheaths with regularly spaced vascular bundles. Each vascular bundle showed well-defined xylem and phloem tissues. The bundles maintained consistent spacing and size throughout the leaf blade sections examined (Figure 3B, 3C).

Lead midrib: Cross-sections of the midrib region demonstrated distinct anatomical features dominated by a large central vascular bundle. Ground tissue surrounded the vascular elements, which maintained typical organization with adaxial xylem and abaxial phloem arrangement (Figure 3D, 3E).

Bacterial colonization: Presumed bacterial cells, stained red with fuchsine, were visible in the cortex and medulla of maize roots supplied with *C. sphaeroides* M-Sl-09, *R. thermotolerans* M-So-11, and *R. palustris* M-So-14 (Figure 4–7), but not in the control (Figure 8). This could be assumed that according to the treatment description, there was the presence of M-Sl-09 (Figure 4), M-So-11 (Figure 5), M-So-14 (Figure 6), and a mixture of three strains M-Sl-09, M-So-11, and M-So-14 (Figure 7) compared to the control case (Figure 8). The microorganisms were localized primarily in thin-walled parenchyma cells and near the xylem and phloem vessels. These regions are known to facilitate endophytic movement through apoplastic flow. The bacteria likely entered through root hairs and epidermal micro-cracks, then migrated via intercellular spaces and xylem sap.

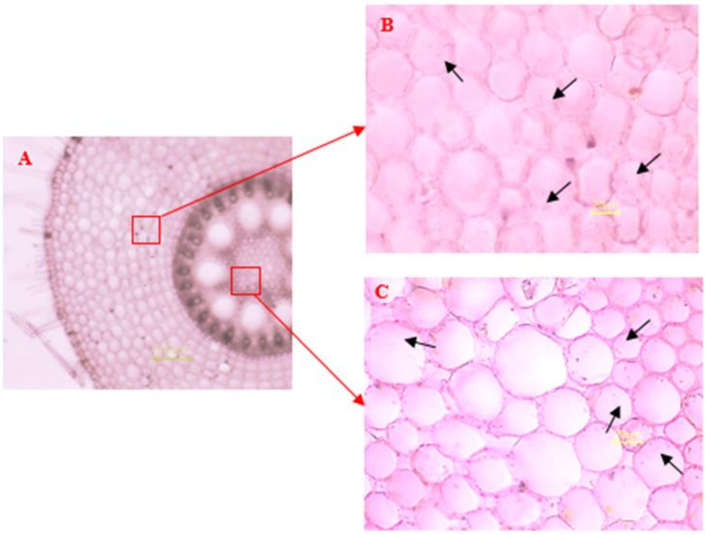

Figure 4. Presence of bacteria in maize supplied with potassium-solubilizing purple nonsulfur bacteria M-Sl-09. A: structure of elongation zone; B: cortex region; C: stele region.

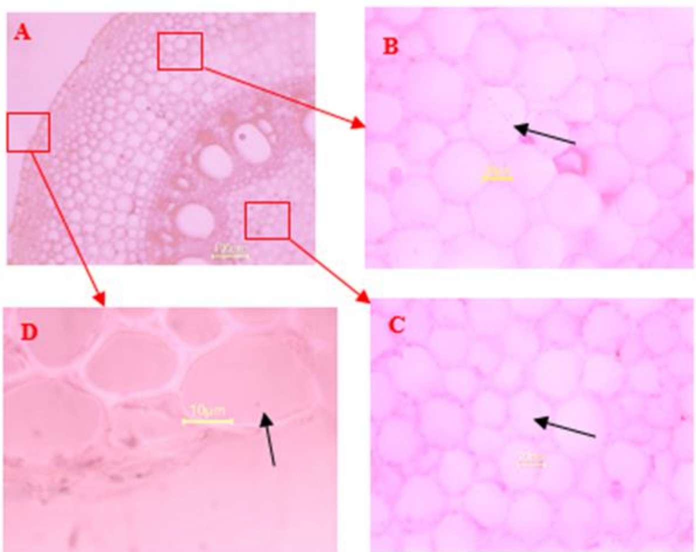

Figure 5. Presence of bacilli in maize supplied with potassium-solubilizing purple nonsulfur bacteria M-So-11. A: structure of maturation zone; B: cortex region; C: stele region; D: Epidermis

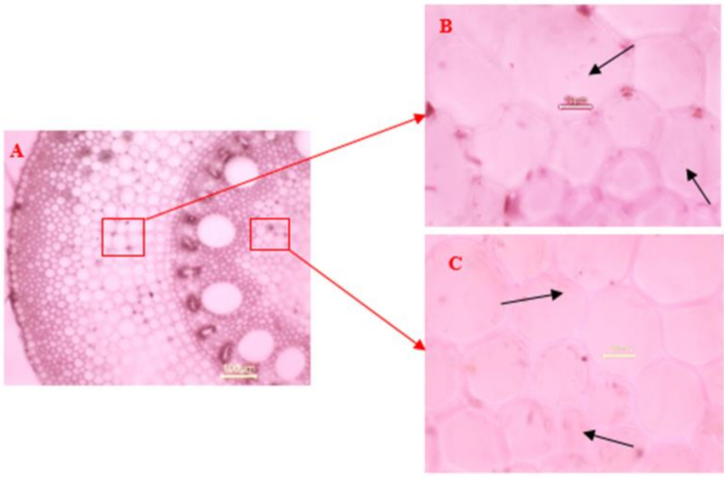

Figure 6. Presence of bacilli in maize supplied with potassium-solubilizing purple nonsulfur bacteria M-So-14. A: structure of maturation zone; B: cortex region; C: stele region

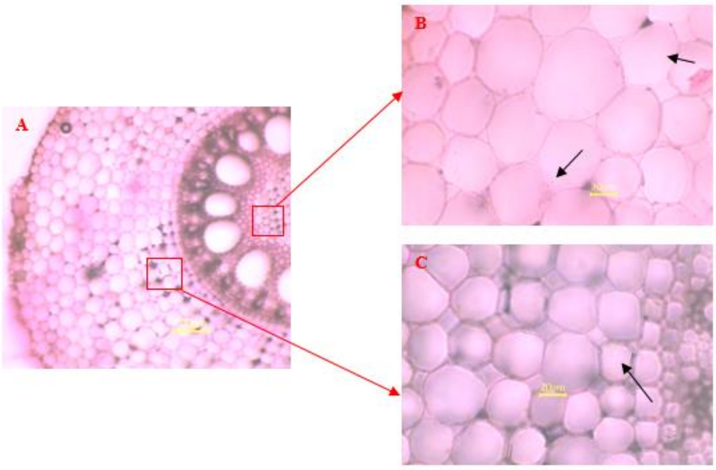

Figure 7. Presence of bacteria in maize supplied with potassium-solubilizing purple nonsulfur bacteria M-Sl-09, M-So-11, and M-So-14. A: structure of maturation zone; B: cortex region; C: stele region

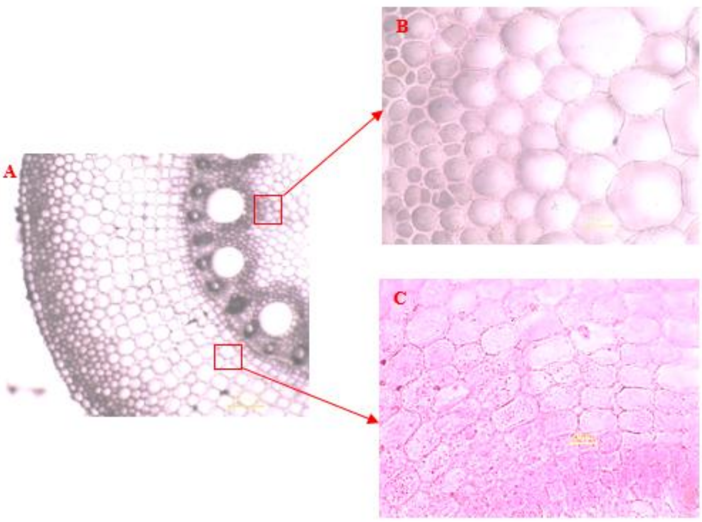

Figure 8. Presence of bacilli in maize without potassium-solubilizing purple nonsulfur bacteria. A: structure of maturation zone; B: cortex region; C: stele region

In conclusion, the soft tissue surrounding the xylem and the medullary parenchyma gradually became impregnated with the xylem, causing the cell walls to thicken and turn green when stained. The medullary parenchyma cells were loosely arranged and contained numerous starch granules. Microsamples at different locations also revealed a development trend that increased the rigidity of the root, the thin-walled tissues made of cellulose (living cells) were gradually replaced by thicker-walled cells impregnated with xylem/lignin (becoming dead cells) (Figure 1-3). Bacteria were observed in the cortex and medulla, areas with thin-walled cellulose structures (Figure 4-7). Normally, the soft tissues contain many storage substances, which are the environment for bacteria to grow. Some bacteria appeared in the epidermal area (in small numbers); however, they did not penetrate deeply due to the presence of suberin-impregnated cells that form a protective barrier. Bacteria inside the plant likely followed the water transport route, entering through the root hairs and moving through the internal structures. However, because only anatomical staining was used, the bacterial cells observed cannot be definitively confirmed as the inoculated PNSB strains. Other naturally occurring microorganisms could exhibit similar morphology. Therefore, molecular or fluorescence-based confirmation, such as PCR amplification of species-specific genes, is recommended for future studies.

3.2 Influencing capacity of Ksol-PNSB on growth and biochemical traits of hybrid maize

3.2.1 Growth traits

The treatments with Ksol-PNSB showed significant increases by 10.1 - 16.8 cm in plant height, by 1.01 - 1.58 cm in stem diameter, by 9.70 - 15.3 cm in leaf length, by 1.02 - 1.69 cm in leaf width, by 8.00 - 19.0 cm in root length, by 0.41 - 3.02 g in root biomass, and by 4.80 - 11.3 g in stem-leaf biomass (Table 1).

Table 1. Effectiveness of potassium-solubilizing purple nonsulfur bacteria in improving hybrid maize growth in undeposited alluvial soil in An Phu, An Giang, under nethouse conditions

Treatment	Crop height	Stem diameter	Leaf length	Leaf width	Root length	Root biomass	Culm-leaf weight
			(cm)				(g)
Control	48.7^{c}	1.92^{c}	33.7^{c}	1.98^{d}	46.8^{c}	1.96^{c}	6.50^{d}
M-Sl-09	64.4^{a}	3.50^{a}	45.3^{b}	3.40^{ab}	62.7^{a}	4.27^{b}	17.8^{a}
M-So-11	64.5^{a}	3.30^{a}	45.3^{b}	3.25^{bc}	57.2^{b}	4.31^{b}	13.9^{b}
M-So-14	58.8^{b}	2.93^{b}	43.4^{b}	3.00^{c}	54.8^{b}	2.37^{c}	11.3^{c}
Mix	65.5^{a}	3.30^{a}	49.0^{a}	3.67^{a}	65.8^{a}	4.98^{a}	17.7^{a}
Significance	*	*	*	*	*	*	*
CV (%)	3.07	7.33	3.12	6.12	4.18	7.65	8.56

Note: In the same column, numbers followed by different letters are significantly different at 5% (*); ns: no significance. M-Sl-09: *Cereibacter sphaeroides* M-Sl-09, M-So-11: *Rhodopseudomonas thermotolerans* M-So-11, M-So-14: *Rhodospeudomonas palustris* M-So-14. Mix: the mixture of three strains *Cereibacter sphaeroides* M-Sl-09, *Rhodopseudomonas thermotolerans* M-So-11, *Rhodospeudomonas palustris* M-So-14.

Table 1 shows that strains of Ksol-PNSB significantly improved hybrid maize growth compared to the control. Previous studies have demonstrated the ability of PNSB strains, such as *R. palustris*, to enhance the growth of different crops, *e.g.*, Chinese cabbage Pak choi (*Brassica chinensis* L.) [25], stevia (*Stevia rebaudiana* Bert.) [26], urad bean (*Vigna mungo* L.) [27], and rice (*Oryza sativa* L.) [28–30]. In this study, *C. sphaeroides* M-Sl-09, *R. thermotolerans* M-So-11, and *R. palustris* M-So-14 significantly increased crop height, stem diameter, leaf length, leaf width, root length, root biomass, and stem-leaf weight, resulting in significantly higher fresh biomass of hybrid maize (Table 1). Ksol-PNSB releases organic acids (e.g., citric, malic, and gluconic acids) that chelate K from insoluble minerals such as mica and feldspar, increasing soluble K concentration in the rhizosphere [31,32]. Moreover, PNSB synthesise indole-3-acetic acid (IAA) and gibberellins that promote cell elongation and root development, leading to enhanced nutrient uptake [33]. Unlike other microorganisms, PNSB can function under both aerobic and microaerobic light conditions, contributing to improved root oxygenation and carbon metabolism [34]. These combined effects indicate that Ksol-PNSB stimulates maize growth primarily through physiological activation rather than simple nutrient supplementation.

3.2.2 Biochemical traits

By improving nutrient balance and metabolic efficiency, PNSB increases chlorophyll content and photosynthetic capacity, as supported by higher SPAD values observed in this study. Chlorophyll content (SPAD index) significantly increased under Ksol-PNSB treatments (37.5 - 45.5) compared to the control (35.9) (Table 2). The SPAD index, a reliable indicator of the N nutritional status in leaves, is positively correlated with leaf N content [35]. A SPAD index below 35 indicates that the plant is in an N-insufficient status [36]. Table 2 revealed that Ksol-PNSB M-Sl-09, M-So-11, and M-So-14 improved the SPAD index. The a and a+b chlorophyll contents peaked in the M-So-14 treatment (8.26 μg g^{-1} and 13.1 μg g^{-1}, respectively). The a and a+b chlorophyll in the Ksol-PNSB treatments were significantly greater than those of the control at 15 DAP. The b chlorophyll ranged from 4.71 to 4.87 μg g^{-1} (Table 2). The enhancement of chlorophyll accumulation reflects improved N assimilation and photosynthetic performance. Similar findings were reported by Xu et al. [26,27] and Sundar et al. [37], who demonstrated that PNSB inoculation improved photosynthetic pigments and plant vigor in *Stevia rebaudiana* and rice, respectively.

Table 2. Effectiveness of potassium-solubilizing purple nonsulfur bacteria in improving hybrid maize biochemistry in undeposited alluvial soil in An Phu-An Giang, under nethouse conditions

Treatment	SPAD	Proline (μmol g^{-1} DW)	Chlorophyll a	Chlorophyll b	Chlorophyll a+b
				(μg g^{-1})	
Control	35.9^{d}	19.7^{c}	6.80^{c}	4.85	11.7^{c}
M-Sl-09	42.5^{a}	21.2^{bc}	8.06^{ab}	4.87	12.9^{ab}
M-So-11	37.5^{c}	23.2^{ab}	7.57^{b}	4.71	12.3^{bc}
M-So-14	39.9^{b}	26.5^{a}	8.26^{a}	4.87	13.1^{a}
Mix	40.4^{b}	17.5^{bc}	7.64^{ab}	4.83	12.5^{ab}
Significance	*	*	*	ns	*
CV (%)	2.22	14.7	5.44	4.09	3.90

Note: In the same column, numbers followed by different letters are significantly at 5% (*); ns: no significance. M-Sl-09: *Cereibacter sphaeroides* M-Sl-09, M-So-11: *Rhodopseudomonas thermotolerans* M-So-11, M-So-14: *Rhodospeudomonas palustris* M-So-14. Mix: the mixture of three strains *Cereibacter sphaeroides* M-Sl-09, *Rhodopseudomonas thermotolerans* M-So-11, *Rhodospeudomonas palustris* M-So-14.

Proline content, an indicator of stress tolerance, was highest in the M-So-14 treatment (26.5 μmol g^{-1} DW) and decreased progressively in the treatments with M-Sl-09 (24.2 μmol g^{-1} DW), M-So-11 (21.2 μmol g^{-1} DW), and the combination of M-Sl-09, M-So-11, and M-So-14 (17.5 μmol g^{-1} DW). In comparison, the proline content in the control was 19.7 μmol g^{-1} DW (Table 2). Furthermore, the significantly increased proline content in maize treated with Ksol-PNSB compared to the control suggested that supplying Ksol-PNSB enhanced maize tolerance against stresses. Proline is synthesised to maintain cell osmosis and protect enzymes, proteins, membranes, and cell structures under water scarcity [38]. Proline is commonly used as a biomarker for stress response and osmotic regulation in plants [39]. However, since no abiotic stress was imposed in this experiment, the elevated proline level is more likely linked to enhanced metabolic activity and amino acid turnover during rapid vegetative growth rather than to stress tolerance [40]. The lower proline level in the mixed treatment may reflect a more balanced physiological state, where the combined bacterial consortium improved osmotic stability and nitrogen assimilation efficiency, reducing the need for proline accumulation [41]. The proline accumulation increases crop tolerance and yield [42]. Thus, the increased proline production by the Ksol-PNSB application improved crop growth and yield and minimised the adverse effects on plants.

Overall, these findings suggest that the PNSB strains may contribute to maize vigor through coordinated effects on nutrient mobilization, photosynthesis, and amino acid metabolism rather than by inducing stress-related pathways. The presence of presumed PNSB in maize tissues corresponds with enhanced morphological and biochemical performance. However, colonization alone does not directly prove causality for growth promotion. It is plausible that the bacterial metabolites and solubilised nutrients exerted systemic effects on plant physiology independent of internal colonization. Future studies should integrate molecular detection with metabolomic profiling to verify bacterial persistence and identify the specific bioactive compounds responsible for growth enhancement.

4. Conclusions

This study demonstrated that potassium-solubilizing purple nonsulfur bacteria (*Cereibacter sphaeroides* M-Sl-09, *Rhodopseudomonas thermotolerans* M-So-11, and *Rhodopseudomonas palustris* M-So-14) are presumed to colonize the soft tissues of hybrid maize roots, particularly in the cortex and medulla regions near the vascular system. Their application significantly enhanced early vegetative growth, including plant height, leaf expansion, root elongation, and biomass accumulation, compared with the uninoculated control. The positive effects of Ksol-PNSB on maize growth are likely associated with multiple plant growth–promoting mechanisms such as potassium solubilization, phytohormone synthesis, and improved chlorophyll formation, rather than from direct bacterial colonization alone. Increased proline content observed in certain treatments reflected enhanced metabolic activity during active growth rather than a stress-related response, while the lower proline level in the mixed inoculation suggested a balanced physiological state under optimal nutrient conditions. These results support the potential use of Ksol-PNSB as promising biofertilizer candidates to improve nutrient efficiency and promote sustainable maize cultivation in alluvial soils of the Mekong Delta. However, because this experiment focused on short-term colonization, further studies are required to confirm bacterial identity within plant tissues using molecular or fluorescence-based methods; evaluate their persistence and physiological influence throughout the full growth cycle; and examine the interaction between Ksol-PNSB and potassium fertilizers under field conditions.

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