

Comparative Analysis of the Growth-promoting Effects of Three Endophytic Bacteria on the Medicinal Plant *Emilia prenanthoidea* DC.

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Abstract [**Objectives**] To assess the effects of endophytic bacteria on the growth, antioxidant responses, and the production of key secondary metabolites in *Emilia prenanthoidea* DC. [**Methods**] Three endophytic strains (H1, H2, L1) were inoculated onto tissue-cultured seedlings and cultivated for 20 d under greenhouse conditions. Growth traits, reactive oxygen species (ROS) indicators, antioxidant enzyme activities, and the content of chlorogenic acid and quercetin were analyzed using one-way ANOVA followed by Tukey's test. [**Results**] Bacterial inoculation significantly enhanced plant performance. Treatment H2 increased plant height by 27%, chlorophyll content by 73%, and fresh weight by 31%. Levels of ROS (O_2^- , H_2O_2) and MDA decreased markedly, whereas the activities of POD and CAT increased. Additionally, the content of chlorogenic acid and quercetin increased by up to 67% and 64%, respectively, with both H2 and L1 treatments showing the most pronounced effects. [**Conclusions**] Endophytic bacteria markedly improve growth, redox balance, and phenolic accumulation in *E. prenanthoidea*. Strain H2 represents a promising bioinoculant for improving the medicinal quality of this species.

Key words Endophytic bacteria, *Emilia prenanthoidea* DC., Plant growth promotion, Flavonoid metabolism, Antioxidant enzymes, ROS homeostasis, Medicinal plant improvement

1 Introduction

Emilia prenanthoidea DC. is a medicinal herb belonging to the Asteraceae family, native to southern China and Southeast Asia. It is traditionally employed in herbal medicine for its heat-clearing and anti-inflammatory properties. Chemical fingerprinting analysis has demonstrated the presence of multiple flavonoids and phenolic acids in *E. prenanthoidea*. In particular, chlorogenic acid, hyperoside, and quercitrin have been identified as markers for quality control^[1–2]. These compounds are considered to play a significant role in the antioxidant and anti-inflammatory properties of the species.

Endophytic bacteria are increasingly recognized as effective promoters of plant growth^[3]. Their beneficial effects are mediated through various mechanisms, including IAA synthesis, phosphate solubilization, nitrogen fixation, siderophore production, and ACC deaminase activity^[4–5]. In addition, the antioxidant defenses of the host plant can be enhanced. After inoculation, increases in the activities of SOD, POD, and CAT, along with reductions in MDA accumulation, have frequently been observed^[6–7]. These physiological changes often result in improved chlorophyll content, more robust root systems, and enhanced biomass^[8].

In medicinal plants, endophytes play a significant role in modulating secondary metabolism and stress responses. For example, *Artemisia annua* harbors diverse endophytic bacteria that produce IAA and organic acids^[9–10]. Certain bacterial strains are capable of colonizing other medicinal plants while retaining their plant-growth-promoting traits^[11]. Indeed, numerous species with

in the Asteraceae family exhibit pronounced positive responses to PGPB, including increased chlorophyll content, enhanced root activity, and elevated antioxidant capacity^[9].

However, little is known about endophytic bacteria in *E. prenanthoidea*. Previous research has focused mainly on pharmacological or antimicrobial properties of these endophytes, with limited attention given to their potential roles in promoting plant growth^[12]. Furthermore, no comprehensive investigation has systematically compared the effects of endophytes derived from *A. annua* and *E. prenanthoidea* on parameters such as plant growth, chlorophyll accumulation, root vitality, or antioxidant enzyme activity under controlled conditions^[13–14].

In this study, we assessed three endophytic bacterial isolates—two derived from *A. annua* and one from *E. prenanthoidea*. Greenhouse experiments were conducted to measure various plant growth parameters, including plant height, root length, fresh/dry biomass, chlorophyll content, root activity, and antioxidant responses (SOD, POD, CAT, MDA). The objective was to identify effective endophytes for *E. prenanthoidea* and to develop a microbial strategy to enhance its cultivation.

2 Materials and methods

Sterile seedlings of *E. prenanthoidea* were obtained via tissue culture. Uniform seedlings were propagated in NAA (0.4 mg/L) + GA₃ (0.8 mg/L) medium for 15 d. Three endophytic bacterial strains were used: two isolated from *A. annua* (H1 identified as *Arthrobacter pokkali* and H2 as *Pedobacter* sp.) and one from *E. prenanthoidea* (L1 identified as *Brevibacillus nitrificans*). Bacterial cultures were cultivated on NA at 28 °C or 24 h and adjusted to a concentration of 10⁸ CFU/mL.

After 10 d of subculture, seedlings were inoculated with 10 mL of bacterial suspension, while control plants received sterile NA. The plants were cultivated in a greenhouse (25 ± 2 °C;

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16/8 h light/dark) for 20 d. Growth parameters, including plant height, root length, and fresh biomass, were measured. The apical shoots were harvested, extracted, and analyzed using HPLC with a Shim-pack CLC-ODS column (150 mm × 6.0 mm) maintained at 40 °C. The mobile phases consisted of acetonitrile:methanol (11 : 5, *v/v*) (A) and 0.1% formic acid in water (B). Detection was performed at a wavelength of 345 nm. The gradient elution program was as follows: 0–8 min, B 94% → 82%; 8–12 min, B 82% → 50%; 12–15 min, B 50% → 0%; 15–25 min, B 0%; 25–26 min, B 0% → 94%; 26–36 min, B 94%. The flow rates ranged from 1 to 1.4 mL/min. Leaf samples were collected for the determination of SOD, POD, and CAT activities following standard spectrophotometric protocols.

Three biological replicates were employed in the study. Data were expressed as mean ± SD and were analyzed via one-way ANOVA followed by Duncan's test ($P < 0.05$).

3 Results and analysis

3.1 Effects of bacterial inoculation on secondary metabolite accumulation Bacterial inoculation significantly influenced the levels of secondary metabolites in *E. prenanthoidea* (Fig. 1). Treatments H2 and L1 produced markedly higher chlorogenic acid contents (59.46 and 51.23 µg/g, respectively) compared to CK ($P < 0.05$), whereas treatment H1 did not differ significantly from CK. A similar trend was observed for quercetin content. Both H2 and L1 treatments significantly increased quercetin content (65.43 and 58.21 µg/g, respectively) relative to CK ($P < 0.05$), while treatment H1 showed no significant effect. Overall, treatment H2 exhibited the most pronounced enhancement of medicinally relevant metabolites.

3.2 Effects on plant growth parameters As shown in Fig. 2, all bacterial strains promoted plant growth relative to CK. Plant height increased significantly under H1, H2, and L1 treatments (8.57, 7.70, and 8.03 cm, respectively) compared to CK ($P < 0.05$). Fresh weight was significantly greater in treatments H1 and L1 (1.39 and 1.47 g, respectively) relative to CK (0.89 g) ($P < 0.05$), whereas treatment H2 showed a moderate but non-significant increase (1.17 g). Leaf chlorophyll content increased significantly in treatments H2 and L1 (3.60 and 3.90 mg/g, respectively) compared to CK ($P < 0.05$). Treatment H1 showed an intermediate increase (2.99 mg/g), which was not statistically significant. These results suggest that treatments H2 and L1 exert the most pronounced growth-promoting effects, particularly in terms of biomass accumulation and chlorophyll enhancement.

3.3 Effects on antioxidant enzyme activities As illustrated in Fig. 3, SOD activity was significantly lower in all inoculated treatments compared to CK (733.96 U/g FW/h) ($P < 0.05$), with treatment L1 showing the most pronounced reduction (247.17 U/g FW/h). This decline reflects a reduced demand for O_2^- detoxification under bacterial colonization. Additionally, MDA content decreased after inoculation. Treatments H1 and L1 showed significantly lower MDA levels (2.17 and 1.98 µmol/g, respec-

tively) compared to CK (2.52 µmol/g) ($P < 0.05$), whereas H2 showed a moderate, non-significant reduction. These results indicate a reduction in lipid peroxidation. POD activity increased significantly across all treatments. Treatment H2 exhibited the highest POD activity (1 315.56 U/g FW/min), significantly exceeding CK (513.78 U/g FW/min) ($P < 0.05$). Both L1 and H1 treatments also showed strong increases in POD activity relative to CK. CAT activity increased significantly under treatment H1 (103.60 U/g FW/min) ($P < 0.05$), whereas treatments H2 and L1 showed moderate, non-significant increases compared to CK (68.20 U/g FW/min).

3.4 Effects on reactive oxygen species (ROS) accumulation

As presented in Fig. 4, all inoculated plants exhibited significantly reduced levels of O_2^- relative to CK (1.43 nmol/g). Treatment H2 showed the greatest reduction (0.55 nmol/g), followed by treatments L1 (0.76 nmol/g) and H1 (1.00 nmol/g), with all treatments showing statistically significant differences from CK ($P < 0.05$). Additionally, H_2O_2 content also decreased after inoculation. Treatment H2 significantly reduced H_2O_2 level to 2.94 nmol/g compared to CK (4.54 nmol/g) ($P < 0.05$), whereas treatments H1 and L1 showed moderate, non-significant reductions (3.74 and 3.62 nmol/g, respectively).

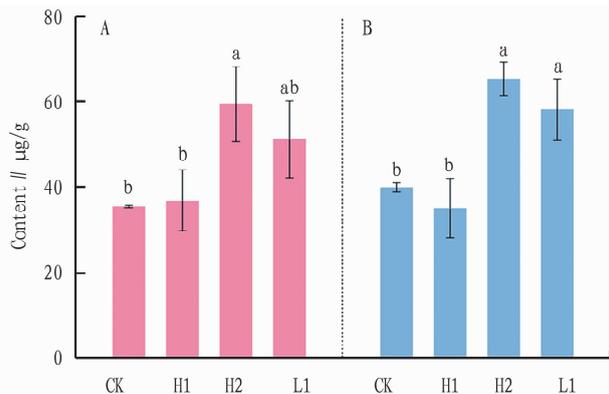
4 Discussion

In this study, three endophytic bacterial strains (H1, H2, and L1) significantly improved the growth, antioxidant capacity, and secondary metabolite accumulation of *E. prenanthoidea* under controlled greenhouse conditions. The findings demonstrate that plant-microbe interactions play a crucial role in modulating the physiological and biochemical characteristics of medicinal plants, consistent with recent observations in other species within the Asteraceae family.

4.1 Enhancement of plant growth by endophytic bacteria The three bacterial strains promoted plant height, biomass accumulation, and chlorophyll content to varying degrees, with strains H2 and L1 showing the most pronounced growth-promoting effects. Similar trends have been observed in endophyte-mediated growth enhancement in *A. annua*, *Chrysanthemum morifolium*, and *Saussurea involucreata*, where growth promotion is often associated with phytohormone production (IAA, GA₃), improved nutrient uptake, or root system modification^[15–16].

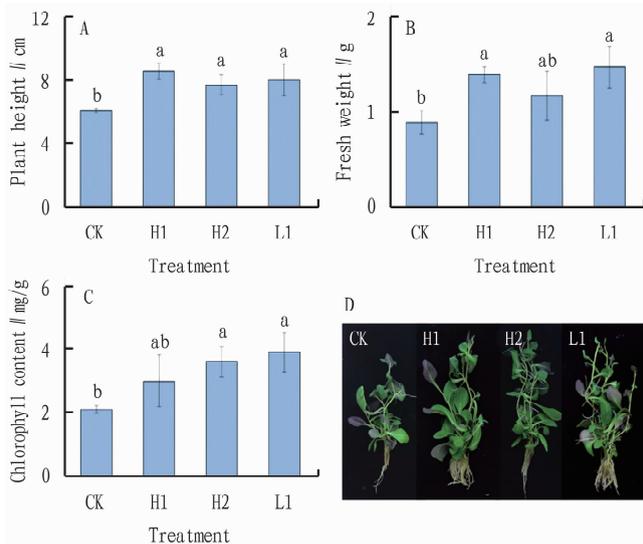
Treatments L1 and H1 significantly increased fresh weight and chlorophyll content, suggesting that bacterial colonization may stimulate chloroplast development or nitrogen assimilation pathways. Such mechanisms have been frequently observed in beneficial endophytes capable of producing siderophores or nitrogen-related metabolites^[17].

4.2 Regulation of oxidative stress and ROS homeostasis A notable finding is the substantial decline in superoxide radicals (O_2^-) and H_2O_2 following bacterial inoculation, particularly in H2-treated plants. The reduced ROS levels were consistent with a notable increase in POD activity and, to a lesser extent, CAT ac-



NOTE A. Chlorogenic acid content; B. Quercetin content under four treatments (CK, H1, H2, L1). Values are presented as mean \pm SD ($n = 3$). Different letters above the bars indicate statistically significant differences among treatments at $P < 0.05$.

Fig. 1 Effects of endophytic bacteria on chlorogenic acid and quercetin contents in *Emilia prenanthoidea* seedlings

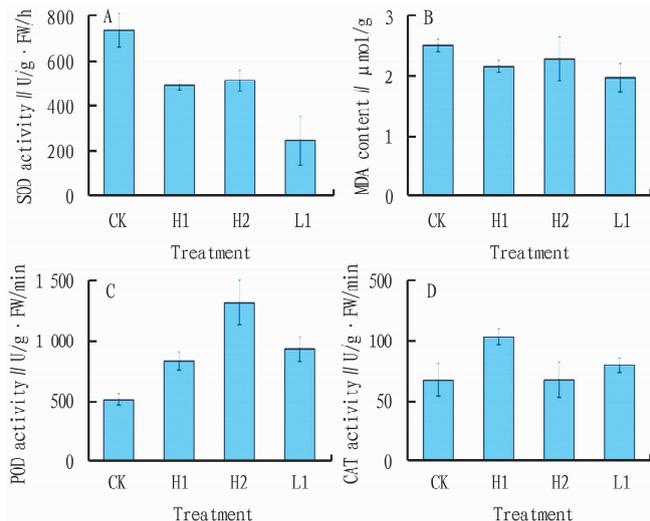


NOTE A. Plant height; B. Fresh weight; C. Leaf chlorophyll content under four treatments (CK, H1, H2, L1); D. Representative plant morphology under each treatment. Bars represent mean \pm SD ($n = 3$). Different letters above the bars indicate statistically significant differences among treatments at $P < 0.05$.

Fig. 2 Effects of inoculation with various rhizobacterial strains on plant growth and chlorophyll content

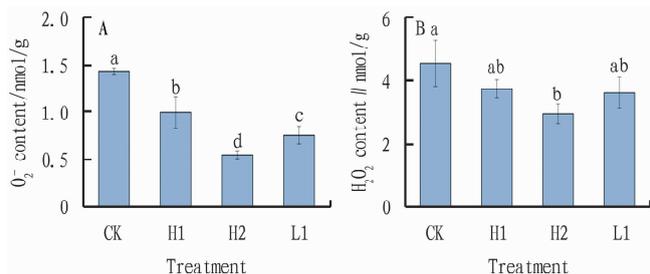
tivity. This pattern indicates that endophytic bacteria contribute to maintaining a more favorable redox balance in *E. prenanthoidea*. The observed decrease in SOD activity in inoculated plants suggests a reduction in the overall oxidative burden, thereby lowering the need for SOD-mediated detoxification of O_2^- . This transition from reliance on SOD to enhanced POD/CAT activity is widely documented in microbe-stimulated ROS reprogramming^[18–19].

Lower MDA content observed in H1 and L1 treatments further confirms that endophytes alleviate lipid peroxidation and membrane damage. The suppression of oxidative stress by microbial agents has been shown to improve photosystem stability and metabolic efficiency in various medicinal plants^[13, 20].



NOTE A. Superoxide dismutase (SOD) activity; B. Malondialdehyde (MDA) content; C. Peroxidase (POD) activity; D. Catalase (CAT) activity in plants under four treatments (CK, H1, H2, L1). Values represent mean \pm SD ($n = 3$). Different letters above the bars indicate statistically significant differences among treatments at $P < 0.05$.

Fig. 3 Effects of different treatments on antioxidant enzyme activities and membrane lipid peroxidation



NOTE A. Superoxide anion (O_2^-) content; B. Hydrogen peroxide (H_2O_2) content under four treatments (CK, H1, H2, L1). Values represent mean \pm SD ($n = 3$). Different letters above the bars indicate statistically significant differences among treatments at $P < 0.05$.

Fig. 4 Effects of different treatments on ROS accumulation in plants

4.3 Promotion of chlorogenic acid and quercetin biosynthesis

A primary objective of this study was to examine whether endophytic bacteria can enhance the production of pharmacologically important secondary metabolites. Both H2 and L1 treatments significantly increased the content of chlorogenic acid and quercetin, which are two major antioxidant constituents of *E. prenanthoidea*. These findings suggest that endophytic colonization activates the phenylpropanoid and flavonoid pathways. Similar results have been reported in *Artemisia argyi*, *Echinacea purpurea*, and *Scutellaria baicalensis*, where endophytic bacteria enhance the expression of PAL, CHS, and other critical enzymes involved in phenolic biosynthesis^[21–22]. Recent studies indicate that microbially derived signaling molecules (*e. g.*, volatile organic compounds, indole derivatives, quorum-sensing molecules) can directly stimu-

late secondary metabolic networks in plant cells^[23].

The superior performance of strain H2 suggests a more robust elicitation capability, possibly related to strain-specific metabolites or enhanced colonization efficiency. Enhanced phenolic accumulation often correlates with moderate ROS fluctuations and POD activation, consistent with the redox-linked regulation of phenylpropanoid metabolism^[24].

4.4 Mechanistic considerations in microbe-medicinal plant interactions The integration of the physiological and biochemical responses suggests several mechanisms through which the isolates promote growth and metabolite accumulation in *E. prenanthoidea*. These mechanisms include redox-dependent metabolic activation, reduction of ROS, and increased POD activity leading to the activation of phenolic biosynthesis. Improved photosynthetic performance and increased chlorophyll content may enhance the availability of carbon skeleton necessary for secondary metabolite production. The effect of microbial elicitors, particularly endophytic bacteria, likely involves the production of signaling molecules that induce the chlorogenic acid and flavonoid pathways. Additionally, stress alleviation, as evidenced by reduced oxidative damage (low levels of MDA and ROS), generally facilitates the reallocation of energy toward growth and secondary metabolism.

These mechanisms are consistent with emerging frameworks describing endophytic microorganisms as metabolic "co-regulators" in medicinal plants^[15].

4.5 Implications for medicinal plant cultivation The observed improvements in biomass, antioxidant capacity, and bioactive compound accumulation highlight the potential application of these bacterial strains as bioinoculants for the sustainable cultivation of *E. prenanthoidea*. Particularly, strain H2 appears to be a promising candidate due to its superior effects on metabolite accumulation and antioxidant balance. Future investigation should prioritize genome-level analysis of bacterial metabolites involved in the regulation of plant secondary pathways, transcriptomics of phenylpropanoid and flavonoid metabolism, field-scale validation under variable environmental conditions and interactions with soil microbiota and nutrient cycling. Such approaches will contribute to the development of microbe-based strategies for enhancing the medicinal quality of species within the Asteraceae family.

5 Conclusions

This study demonstrates that inoculation with endophytic bacteria significantly enhances the growth, antioxidant defense, and secondary metabolite accumulation in the medicinal plant *E. prenanthoidea*. Among the strains tested, strain H2 exerted the most pronounced beneficial effects, including increased plant height, chlorophyll content, and fresh weight, as well as a marked elevation in POD activity and a reduction in ROS levels (O_2^- , H_2O_2) and MDA accumulation. These physiological adjustments indicate that H2 markedly improves cellular redox homeostasis. Moreover, both H2 and L1 strains substantially enhanced the content of chlorogenic acid and quercetin, thereby confirming that the selected endo-

phytes can stimulate phenolic biosynthesis and enhance medicinal quality. Collectively, these findings reveal that bacterial endophytes regulate growth and specialized metabolism through coordinated modulation of antioxidant systems and ROS signaling, providing a promising microbial strategy for improving the yield and pharmacological value of *E. prenanthoidea*.

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