The utilization of plant growth-promoting bacteria to enhance stevia (*Stevia rebaudiana*) herb yield at low land

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**Abstract.** Stevia is a native crop of sub-tropical regions; its cultivation is extended to low land tropical regions, which might the growth is not good as in its origin growth areas. Bacteria as growth promoters are expected to enhance stevia growth in low-land tropical regions. This study aimed to determine the effect of bacteria on the growth and yield of stevia. The research was conducted at the Microbiology Laboratory and Greenhouse of the Indonesian Spices and Medicinal Crops Research Institute at 240 m above sea level and was arranged in a complete randomized block design with four replications. The treatments consisted of 1) control, 2) rhizosphere bacteria *Brevibacillus parabrevis* GF13, 3) endophytic bacteria *Mesorhizobium soli* NHI-8, 4) endophytic bacteria *Bacillus pacificus* EB422, 5) a combination *B. parabrevis* GF13 + *M. soli* NHI-8, and 6) a combination of *Br. parabrevis* GF13 + *B. pacificus* EB422. The highest increase in total production of fresh (32.8%) and dry (25.86%) herbs were found in treatment 4 (endophytic bacteria *Bacillus pacificus* EB422). The treatment caused the plant to have the highest nutrient uptake 0.124 N g/plant, 0.051 P₂O₅ g/plant, and 0.172 K₂O g/plant. The treatment increased soil chemical properties.

**Keywords:** Stevia, endophytic bacteria, rhizosphere bacteria, nutrient, increase, yield.

1. **Introduction**
Stevia (*Stevia rebaudiana* Bertoni) is a plant native to North and South America and belongs to the *Asteraceae* (*Compositae*) family. Its leaves contain stevioside, a glycoside compound with sweetness 200-300 times is higher than sugar cane or sucrose[1]. Thus, it could substitute sugar because it contains low calories. Therefore, stevia is good for a person with diabetes. In addition, stevia leaves contain active substances such as alkaloids, flavonoids, and tannins which possess anti-plaque and antibacterial activity [2]. The main bioactive compound of stevia is steviol and its derivatives, such as stevioside.

Currently, the number of people suffering from diabetes mellitus (DM) disease is quite high, and DM prevalence increases every year. For example, in 2010, the prevalence of DM patients at the 20-79 years old was 6.4%, and it was predicted to increase to 7.1% in 2030 [3]. The high prevalence rate in DM patients should be minimized, for instance, using natural sources for sweeteners such as stevia.

The extension of stevia cultivation might be important, considering it has many benefits for human health. However, its cultivation in the tropical regions will face some obstacles due to the characteristic environmental differences to its natural habitat. Thus, the plants will experience a stress-causing unsatisfactory environment.
Generally, introduction plants from subtropics climate regions like stevia can be grown in the tropical highlands. However, Azkiyah and Tohari (2019) revealed that when stevia was grown in the tropical highlands, it would delay harvesting time, but it had higher production than when it was cultivated in the tropical lowlands [4]. Unfortunately, stevia extension in the tropical highland will compete with the other plants which have high economic value, such as vegetable crops. Moreover, the cultivation area in the highland is limited. Thus, efforts should be made to cultivate stevia in the lowland.

Several efforts have been made to increase stevia productivity in the lowlands, including applying NPK fertilizer [5], managing harvesting time and shading [6,7], combining suitable planting media [8], and using mulch type [9]. Therefore, aside from adding inputs to support nutrient availability, these stresses should be overcome, such as using bacteria as a growth-promoting organism. Bacteria are able to protect plants from environmental stresses, both abiotic and biotic stresses [10,11]. For example, endophytic bacteria enhanced pepper plant adaptability to alleviate abiotic stress [12]. Furthermore, bacteria can increase plant growth, both from plant roots (rhizosphere) and from inside plant tissue (endophytic). Bacteria were beneficial in promoting plant growth through their role in fixing N [13], producing growth hormones such as IAA, GA3, and cytokinins [14,15,16], and solubilizing P [17]. This study aimed to determine the effect of rhizosphere and endophytic bacteria on the growth and production of stevia in the lowlands.

2. Materials and methods
The research was conducted at the Microbiology Laboratory and Greenhouse of the Indonesian Spices and Medicinal Crops Research Institute (240 m above sea level), Bogor, West Java, Indonesia, from June to December 2019.

The trial was arranged in a randomized block design, with six treatments and four replications. Treatment consisted of: 1) no bacteria addition (as control) (C), 2) rhizospheric bacteria Brevibacillus parabrevis strain GF13 (LT), 3) endophytic bacteria Mesorhizobium soli strain NHI-8 (LA), 4) endophytic bacteria Bacillus pacificus strain EB422 (LD), 5) combination of Brevibacillus parabrevis strain GF13 + Mesorhizobium soli strain NHI-8 (LTA), and 6) combination of Brevibacillus parabrevis strain GF13 + Bacillus pacificus strain EB422 (LTD).

2.1. The preparation of seeds and planting media
Stevia cuttings were planted in a 15 cm x 20 cm (high x wide) polybag filled with soil and manure (1:1). After stevia cutting had roots, then was transferred into polybags filled with 10 kg Ultisol soil and 0.5 kg manure.

2.2. Propagation of and application of bacteria isolates
Bacteria were propagated using TSA media and incubated for 48 hours. The bacteria density applied was 10^8 CFU/ml and was poured into the soil in the liquid form at the rate of 50 ml/polybag. The bacteria were applied every week from 2 weeks after planting (WAP) to 4 WAP.

2.3. Observation and data analysis
Plant growth was observed every week until eight weeks after planting (WAP), and plants were harvested twice. The first harvest was at 8 WAP, and the second harvest was at eight weeks after the first harvest. The growth parameters observed were plant height, leaf number, leaf area (measured using a leaf area meter) at harvesting time, and chlorophyll content (measured using a chlorophyll meter spad-05). The production parameters observed were the fresh and dry weight of biomass. The observation was also done on soil nutrients (N, P, K, pH, and C-organic), nutrient content in plant tissue (N, P, and K). N was measured following the Kjeldahl method, P content was determined using spectrophotometry, whereas K with AAS, and pH with a pH meter. In addition, N, P, and K uptake were calculated after finishing the field trial based on NPK content from plant tissue analysis results. Finally, the collected data were analyzed using ANOVA and further tested with LSD at a 5% level.
3. Result and discussion

3.1. Plant growth

The pattern of plant growth at 2-14 WAP was similar, indicating an increase in plant height and leaf number (Figure 1, 2). Moreover, at 12 WAP, the application of plant growth-promoting bacteria significantly increased the branch number, leaf area, and plant height compared to control. In contrast, leaf number and total chlorophyll did not show any significant differences among the treatments. *Bacillus pacificus* strain EB422 (LD) application produced the highest branch number, plant height, and leaf number, while the application of *Mesorhizobium soli* strain NHI-8 (LA) only gave the highest leaf area (Table 1).

![Figure 1. Plant height of stevia at several bacteria applications at 2-14 weeks after planting.](image1)

![Figure 2. Leaf numbers of stevia at several bacteria applications at 2-14 weeks after planting.](image2)

Note: C= no bacteria addition (as control), LT= rhizospheric bacteria *Brevibacillus parabrevis* strain GF13, LA=endophytic bacteria *Mesorhizobium soli* strain NHI-8, LD=endophytic bacteria *Bacillus pacificus* strain EB422, LTA=combination of *Brevibacillus parabrevis* strain GF13 + *Mesorhizobium soli* strain NHI-8, and LTD=combination of *Brevibacillus parabrevis* strain GF13 + *Bacillus pacificus* strain EB422.
Table 1. The effect of several bacteria applications on stevia growth at 12 weeks after planting.

| Treatments | Branch number | Plant height (cm) | Leaf number | Total chlorophyll | Leaf area (mm²) |
|------------|---------------|-------------------|-------------|-------------------|----------------|
| C          | 14.00 b       | 35.85 b           | 83.95 b     | 39.76 a           | 94.83 b        |
| LT         | 13.55 b       | 35.98 b           | 90.65 ab    | 40.28 a           | 104.22 ab      |
| LA         | 15.60 b       | 35.53 b           | 89.10 b     | 40.63 a           | 124.76 a       |
| LD         | 19.30 a       | 41.55 a           | 98.90 a     | 41.42 a           | 111.62 ab      |
| LTA        | 15.55 b       | 36.52 b           | 96.70 a     | 40.37 a           | 95.68 b        |
| LTD        | 17.20 ab      | 39.61 ab          | 92.75 ab    | 42.70 a           | 97.03 b        |

Note: Numbers followed by the same letter in each column are not significantly different at 5% DMRT.

The stevia cultivated in the lowlands (167 m asl) had higher plant height but lower leaf number than those in the highland (897 m asl) [4]. In this study, the plant height was in the range of 35-40 cm, and it was similar to a previous study [4]. In contrast, the leaf number was higher than in the highlands. The addition of growth-promoting bacteria generated good plant vigor. This result indicated that the plant has good adaptability in the lowland (240 m asl) with the addition of growth-promoting bacteria (Figure 3).

Figure 3. Plant performance of stevia at 240 m asl.

3.2. Plant yield

The fresh and dry weight of stevia biomass with bacteria utilization was higher than control at the second harvest. However, the endophytic bacteria application has not been yet affected the yield in the first harvest. Among all the treatments, the application of *Bacillus pacificus* strain EB422 (LD) produced the highest biomass in the second harvest and its total yield (Tables 2 and 3).

Table 2. The effect of bacteria application on the fresh weight of stevia biomass at the first and the second harvest.

| Treatments | The first harvest (g/plant) | The second harvest (g/plant) | Total yield (g/plant) |
|------------|-----------------------------|-----------------------------|-----------------------|
| C          | 9.27 a                      | 10.19 b                     | 19.46 b               |
| LT         | 8.78 a                      | 13.40 b                     | 22.18 ab              |
| LA         | 8.11 a                      | 11.90 b                     | 20.00 b               |
| LD         | 9.39 a                      | 16.45 a                     | 25.85 a               |
| LTA        | 8.92 a                      | 12.25 ab                    | 21.18 b               |
| LTD        | 10.05 a                     | 12.04 ab                    | 22.09 ab              |

Note: Numbers followed by the same letter in each column are not significantly different at 5% DMRT.
Table 3. The effect of bacteria application on the dry weight of stevia biomass at the first and the second harvest.

| Treatments | The first harvest | The second harvest | Total yield |
|------------|------------------|--------------------|-------------|
|            | g/plant          |                    |             |
| C          | 1.79 a           | 2.81 b             | 4.60 b      |
| LT         | 1.47 a           | 3.03 b             | 4.50 b      |
| LA         | 1.47 a           | 3.52 b             | 5.00 ab     |
| LD         | 1.74 a           | 4.05 a             | 5.79 a      |
| LTA        | 1.63 a           | 2.94 b             | 4.57 b      |
| LTD        | 1.96 a           | 3.18 b             | 5.15 ab     |

Note: Numbers followed by the same letter in each column are not significantly different at 5% DMRT.

Figure 4. The effect of bacteria application on soil chemical characteristics.

*Bacillus pacificus* strain EB422 (LD) application generated the highest increase in fresh and dry biomass weight, 32.83% and 25.86%, respectively. The highest yield was due to the beneficial characteristics of the bacteria as N-fixing, P-solvent, and phytohormones producer such as IAA and GA3 [13][14]. Furthermore, the bacteria application also improved the soil characteristic such as pH, N, P, and K and organic C content, which were higher than the control (Figure 4). Thus, the plant will more easily absorb the nutrients for its growth and development.

3.3. *N, P, and K Nutrients Uptake*

The uptake of NPK nutrients was enhanced in the first, second, and total harvests following the bacteria application (Figure 4). At the end of the experiment, the plants were treated with the endophytic bacteria *Bacillus pacificus* strain EB422 (LD) and the combination of rhizospheric bacteria *Brevibacillus parabrevis* strain GF13 and endophytic bacteria *Bacillus pacificus* strain EB422 (LTD) absorbed nutrients other treatments. However, the plants with single endophytic bacteria *Bacillus pacificus* strain EB422 (LD) absorbed much higher K nutrients than other treatments. Potassium has an essential role in protecting plants from environmental stresses. In addition, potassium is a cofactor and activates more than 40 enzymes involved in respiration and photosynthesis. It is the main cation in cell turgor formation and maintains cell electroneutrality, and engages in potential osmotic regulation in plants [18]. The utilization of endophytic bacteria on pepper seedlings in nurseries was also reported can increase the uptake of N, P, and K nutrients [14].
Figure 5. Effect of plant growth-promoting bacteria on N, P, and K Nutrient Uptake.

*Bacillus pacificus* strain EB422 is an endophytic bacterium, and *Brevibacillus parabrevis* strain GF13 is a rhizosphere bacterium. Both bacteria produce IAA, solubilized P, and fixed N [19]. However, the combination had an insignificant effect on the growth and yield of stevia. On the other hand single application of endophytic bacterium *Bacillus pacificus* strain EB422 generated the best growth and the highest yield.

4. Conclusion
The utilization of plant growth-promoting bacteria enhanced the growth and yield of stevia. Endophytic bacteria *Bacillus pacificus* strain EB422 gave the best results by increasing fresh and dry herb yield, 32.8% and 25.86%, respectively, and by enhancing nutrient uptake of N, P, and K. In addition, the application of *Bacillus pacificus* strain EB422 also improved soil chemical properties, such as pH, P, K, and C-organic after the experiment.

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Contributorship
Gusmaini and M Syakir were the main contributors, whereas A Kartikawati, H Nurhayati and R A Permadi were co-contributors.

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