International Journal of Agricultural Technology 2025 Vol. 21(4):1261-1276
DOI: https://doi.org/10.63369/ijat.2025.21.4.1261-1276
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

Yield enhancement efficacy of *Bacillus velezensis* CE 100 biofertilizer on Pineapple (*Ananas comosus* L. Merr.) producion in Prachuap Khiri Khan, Thailand

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Duangkaew, P., Kim, K. Y. and Monkhung, S. (2025). Yield enhancement efficacy of *Bacillus velezensis* CE 100 biofertilizer on Pineapple (*Ananas comosus* L. Merr.) producion in Prachuap Khiri Khan, Thailand. International Journal of Agricultural Technology 21(4):1261-1276.

Abstract Biofertilizers are the best choice to promote organic and sustainable agriculture by enhancing productivity and reducing chemical fertilizer use. The results showed that T4 bacterial pure culture significantly improved plant height (96.83±0.50 cm) and D-leaf length (84.06±1.55cm) at the third month of application ($P < 0.05$). In addition, T4 significantly enhanced fruit weight (1.12±0.07 kg/fruit) and fruit yield (66.3 t/ha) compared to other treatments ($P < 0.05$). However, the fruit characteristics including fruit lengths, perimeters, citric acid contents, and total soluble solids (TSS) of pineapple fruits were not significantly differed. Therefore, these findings indicated the efficacy of *B. velezensis* as a biofertilizer is enhanced pineapple productivity.

Keywords: Plant-growth-promoting bacteria, Organic agriculture, Tropical fruits

Introduction

The pineapple (*Ananas comosus*) is an herbaceous perennial plant of the family Bromeliaceae. Pineapple is native to tropical and subtropical America (Britannica, 2024). Nowadays, pineapple is one of the economically important tropical fruits cultivated and consumed worldwide. Along with its taste, pineapple fruit has health benefits since it contains many minerals and vitamins (Ali *et al*., 2020). Therefore, it has gained more attention lately and ranked third after bananas and citrus for global in-demand fruits (Abraham *et al*., 2023). The fresh pulp, juice, and processed pineapple are used in many cuisines worldwide.

Thailand was ranked 7th for the pineapple producers with 1.7 million tonnes of pineapple produced in 2022 while Indonesia was the lead country with 3.2 million tonnes. World production of pineapples increased gradually year by year.

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Pineapple production was 29 million tonnes in 2022 compared with 15.7 million tonnes in 2002. In the past twenty years, the world area harvested pineapple has expanded from 754,472 hectares to 1,059,203 hectares (FAOSTAT, 2024).

Pineapple cultivation relies mainly on conventional production practices (Gunawardena and Lokupitiya, 2024). The high production of pineapple subsequently increased the amount of chemical fertilizers, fungicides, and pesticides spreading in the fields. Conventional cultivation with improper use of chemicals is known to cause several negative effects such as environmental pollution, microbial imbalance, resistant pathogens, soil nutrient imbalance, soil damage, toxicity to non-target organisms, and is prone to cause toxicity to humans (Castillo *et al*., 2006; Liang *et al*., 2022; Sharma and Singhvi, 2017; Smaill and Walbert, 2013). Global concerns about those adverse effects enhance the demand for sustainable and eco-friendly technologies to produce organically grown crops (Rahman *et al*., 2021).

Biofertilizers have emerged as an innovative and eco-friendly technology that uses beneficial plant microbiomes to fertilize the soil and improve crop productivity (Adesemoye *et al*., 2009; Fasusi *et al*., 2021; Kumar *et al*., 2022; Murgese *et al*., 2020). Biofertilizers have been getting more attention as a viable alternative to hazardous chemical fertilizers for organic production and promoting sustainable agriculture (Nosheen *et al*., 2021).

Beneficial bacterial communities have been highlighted in improving crop productivity for sustainable agriculture (Kumar *et al*., 2022). Microorganisms within the plant's microbiome that play a beneficial effect in promoting plant growth and productivity are known as "Plant growth-promoting bacteria (PGPB)" (Bakker *et al*., 2013; Fasusi *et al*., 2021). PGPB plays a key role in biofertilizers. PGPB promotes plant growth either by direct mechanisms such as increasing soil nutrient availability and producing plant growth-stimulating hormones (i.e., IAA, gibberellins, and cytokinins) or through indirect mechanisms such as producing biocontrol agents and mitigating abiotic stresses (Kumar *et al*., 2022). Among the PGPB, *Bacillus* spp. gained much attention and have been widely studied due to their plant growth hormone production activity, nutrient availability enhancement, diverse antagonistic activity, and ability to survive and reproduce in the soil environment (Chen *et al*., 2019; Choub *et al*., 2021a; Gomaa, 2012; Huang *et al*., 2017; Khan *et al*., 2016; Kumar *et al*., 2022; Park *et al*., 2017).

Bacillus velezensis CE 100 has been recently investigated for biocontrol and biofertilizer activity. *B. velezensis* CE 100 was reported for their first isolation from the tomato rhizosphere by Choi *et al*. (2020). The authors revealed that *B. velezensis* CE 100 can suppress several pathogenic fungi, destroy root-rot nematode eggs, and promote tomato shoot growth (Choi *et al*., 2020). The culture

broth of *B. velezensis* CE 100 contains hydrolytic enzymes (chitinase, protease, and beta-l,3-glucanase), produces indole-3-acetic acid (up to 1.4 µg/mL), and exhibited the potential for ammonium production and phosphate solubilization. The field application of *B. velezensis* CE 100 culture broth demonstrated biocontrol against anthracnose disease and biofertilizer activity to promote walnut growth by 1.5-fold compared to conventional treatment (Choub *et al*., 2021a). *B. velezensis* CE 100 has been shown to suppress root rot *Phytophthora* spp. and increase the survival rate of Japanese Cypress (*Chamaecyparis obtuse*) seedlings significantly compared to control and fertilizer treatment (Moon *et al*., 2021). In addition, the potential to enhance strawberry growth and fruit production of *B. velezensis* CE 100 was reported under the greenhouse condition (Hong *et al*., 2022). Similarly, the plant growth promotion ability of *B. velezensis* CE 100 was noted to improve seedling growth of the Korean fir (*Abies koreana* E.H. Wilson) by increasing nutrient availability through ammonia–nitrogen production and phosphate solubilization and production of IAA (Choi *et al.*, 2024). Although the biocontrol and biofertilizer efficacy of *B. velezensis* CE 100 has been reported in many crops such as strawberry, walnut, Korean fir, and Japanese Cypress, there was no evidence of the field application on farm crops including pineapple in the tropical area.

Therefore, the objective aimed to investigate the growth-promoting biofertilizer efficacy of *B. velezensis* CE 100 on pineapple planted in Thailand. The plant growth, photosynthesis, fruit characteristics, and effect on soil pH were determined.

Materials and methods

Plant materials and study site

Pineapple (*Ananas comosus* L. cv. Patavia) was used in this study. Pineapple suckers were brought from a local commercial supplier. The field study site was located at Hin Lek Fai Sub-district, Hua Hin District, Prachuap Khiri Khan Province, Thailand (12°36'32.4" N 99°51'38.4" E) (Figure 1A). The experiment was performed during January to August 2023.

Preparation of Bacillus velezensis CE 100 Biofertilizer

The *B. velezensis* CE 100 bacteria used in this study were obtained from Purne Inc. (Jangseong, Korea). It was originally isolated from the rhizosphere soil of a tomato cultivated in Korea as previously described (Choi *et al*., 2020). The bacterial strain was inoculated and cultured in a plastic tank with aeration

pump. Briefly, dried powder of *B. velezensis* CE100 was added to 500 L of water (2 g/L) containing colloidal chitin 1%, sucrose 2 kg, NPK fertilizer (16–16–16) 3 kg, KH_2PO_4 100 g, $MgSO_4$ 100 g, $CaCO_3$ 50 g, NaCl 50 g (Choub *et al*., 2021a). The bacterial culture was grown at room temperature with an aeration pump for 7 days to be ready for spraying either directly as a culture broth or diluted with tap water. The batch was continuously cultured and was used for spraying until the end of the experiment.

Field experimental conditions

Pineapple suckers were used as propagating material (Figure 1C). Plantation was started in January 2023. The experiment was designed using a completely randomized design (CRD). The treatment was set into 4 groups:

Treatment 1 (T1) – Control without *B. velezensis* spray
Treatment 2 (T2) – 1:4 v/v diluted *B. velezensis* culture
Treatment 3 (T3) – 1:2 v/v diluted *B. velezensis* culture
Treatment 4 (T4) – *B. velezensis* culture without dilution

Treatments were arranged as shown in Figure 1B. All treatments were carried out with 3 replications. Each plot consisted of 20 suckers planted in a total area of 4 m^2 (1 m wide × 4 m long) and was separated with a 50 cm buffer zone (Figure 1D). The culture broth was sprayed onto the pineapple plants at a rate of 5 L per plot every 2 weeks from February to August 2023. For the control group, pineapple plants were sprayed with an equal volume of water to the volume used in the bacterial treatment. Plants were regularly watered twice a month. Ethephon was applied to induce pineapple flowering in March.

Plant growth determination

After *B. velezensis* spraying, some growth parameters including plant height, d-leaf width, d-leaf length, and canopy width were measured every month from February to April to determine the vegetative growth of pineapple plants. Chlorophyll fluorescence (F_v/F_m: maximum quantum yield of PSII) was analyzed using a Handy-PEA chlorophyll fluorimeter (Hansatech Instruments, United Kingdom) to estimate photosynthetic efficacy from February to June. The measurement was done during the daytime. Five plants were randomly measured for each treatment replication. All treatment was done in triplicates.

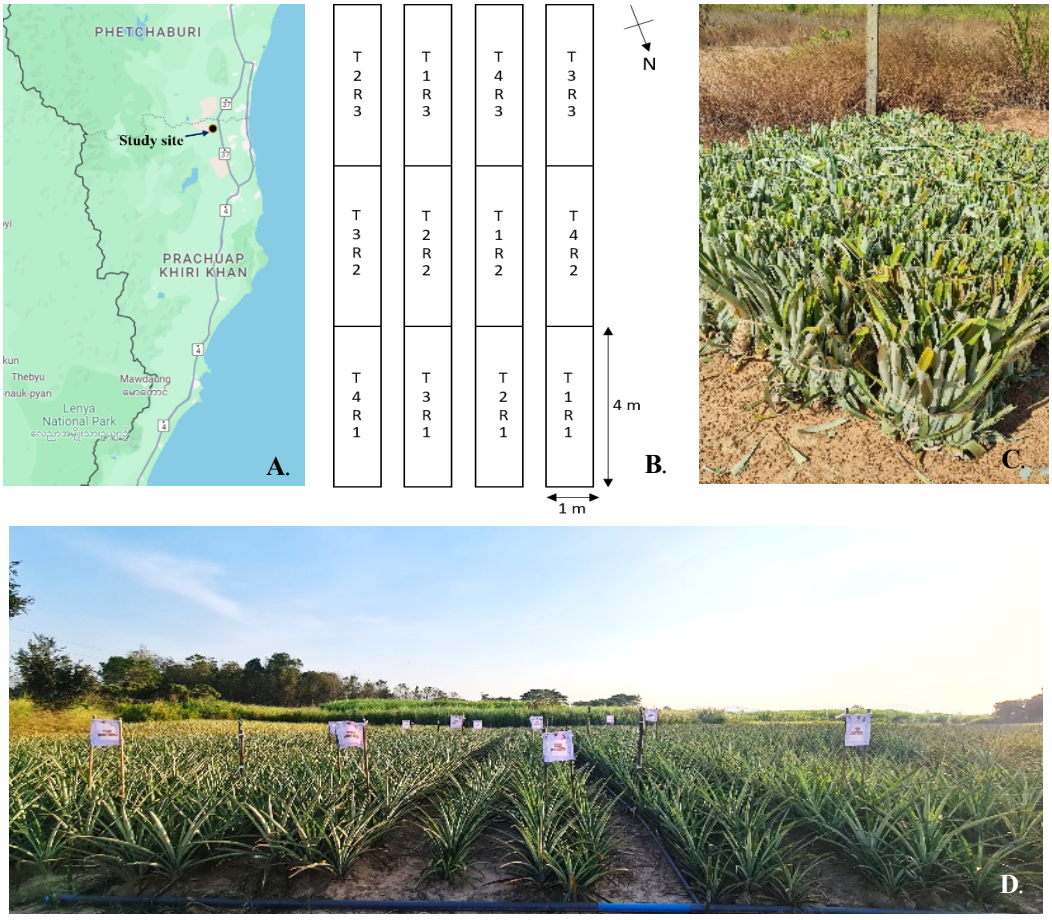

Figure 1. Study location of pineapple field experiment (A), arrangement of the treatment plot (B), pineapple suckers (C), and the growing pineapple on the field experimental site (D)

Yield measurement

Pineapple fruits were harvested at the early ripening stage (10 – 20% yellow) in mid-August 2023. The harvesting process was done in the morning. Twenty harvested pineapple fruits from each treatment replication were weighted and calculated for yield expressed in the t/ha unit.

Physical characteristics of pineapple fruits

The head crowns of all harvested fruits were counted and recorded for each treatment. Six pineapple fruits were randomly picked from each plot (72 samples from 3 replications x 4 treatments) and directly transported to the laboratory for

physical and chemical analysis. Each fruit was measured for fruit weight, length, and perimeter. The flesh color was analyzed using MiniScan EZ 4500L Portable Spectrophotometer (HunterLab, USA) and results were expressed as lightness (L*), redness (a*), and yellowness (b*).

Total soluble solid (TSS) and titratable acid (TA) content

After physical characteristic measurement, the pineapple fruits were peeled and cut longitudinally. A flesh sample (100 g) of each pineapple fruit was chopped, homogenized, and filtrated. The filtered extract (pineapple juice) was directly used for TSS and acidity content determination.

TSS was measured using a hand-held refractometer (ATAGO, Japan). One drop of fresh juice was placed into the sample hole and the reading value was expressed in %Brix. Acidity content was determined by titration of the fresh pineapple juice (10 ml) with standardized 0.1 N NaOH to the end-point of the pale pink color of the phenolphthalein indicator. Since the predominant acid in the pineapple fruit is citric acid, the equivalent weight of citric acid was used to calculate total titratable acidity using the formula (AOAC, 1990):

$$\% \text{ Titratable acidity} = \frac{(\text{V of NaOH})(\text{ N of NaOH})(\text{mg Eq.wt of citric acid})}{\text{V of sample}} \text{ x100}$$

Where; V of NaOH = volume of NaOH (ml)
N of NaOH = normality of NaOH
V of sample = volume of sample used (ml)
mg Eq. wt of citric acid
= equivalent weight of citric acid in mg = 0.064

Soil pH measurement

Soil pH was determined according to FAO (2021) with some modifications. Soil samples were air-dried and ground. Ten grams of ground soil sample was dissolved with 100 ml of distilled water (soil-to-water ratio 1:10 w/v). The suspension was shaken thoroughly for 30 min and allowed to set for 30 min. The suspension was stirred briefly for 10 seconds before measuring the pH at room temperature using the pH meter ADWA AD12 (ADWA, Hungary). All treatments were done in triplicates.

Statistical analysis

All data were analyzed for the variance (ANOVA) using R statistical software version 4.2.1. Means were compared using Tukey's Multiple

Comparison Test of analysis of variance (ANOVA) at P<0.05. The results are reported as mean ± standard deviation.

Results

Effect of B. velezensis biofertilizer on pineapple growth

The effect of bacterial spray on plant growth and photosynthesis efficacy was determined monthly from February to June. However, the plant height, D-leaf size, and canopy size were not applicable after April since the farmer had to trim off the pineapple canopy and the plant started to form the fruit.

Biofertilizer application at 100% pure culture broth (T4) significantly promoted plant height compared to T1 control in February and April (P<0.05) as shown in Table 1. Although the plant height in March did not differ statistically, it seemed that T4 had higher plant height when compared to the T1 control. A significant promotion was also found in the D-leaf length of the T4 group in April (P<0.05) (Table 1). There were no differences among groups on the D-leaf width, canopy size, and chlorophyll fluorescence (F_v/F_m).

Table 1. Effect of *B. velezensis* CE 100 biofertilizer application rates on some growth parameters of pineapple plants

Parameters	Time	Treatments [1/, 2/]			
		T1	T2	T3	T4
Plant height (cm)	February	90.41±0.44[a]	91.17±0.97[ab]	92.53±1.24[ab]	94.34±2.15[b]
	March	91.53±1.26	92.65±2.02	93.44±2.21	95.53±1.12
	April	93.17±1.80 [a]	94.11±0.59 [ab]	94.28±1.36 [ab]	96.83±0.50 [b]
D-leaf length (cm)	February	78.11±2.02	79.63±2.91	80.02±0.60	81.47±1.18
	March	79.89±1.73	80.83±1.53	81.05±1.13	82.98±0.40
	April	80.56±0.42 [a]	82.56±0.35 [ab]	83.00±1.59 [ab]	84.06±1.55 [b]
D-leaf width (cm)	February	5.09±0.15	5.13±0.18	5.16±0.21	5.26±0.11
	March	5.14±0.13	5.10±0.17	5.06±0.38	5.26±0.25
	April	5.14±0.23	5.17±0.36	5.14±0.05	5.22±0.13
Canopy (N – S) (cm)	February	122.96±11.2	124.04±3.96	121.44±3.60	120.91±7.97
	March	132.12±6.90	125.50±7.20	126.28±3.97	131.95±8.56
Canopy (E – W) (cm)	February	114.50±12.9	123.39±8.65	122.75±1.23	118.80±9.12
	March	124.99±8.22	122.52±6.42	133.17±5.48	131.76±6.75
F_v/F_m	February	0.708±0.009	0.701±0.017	0.666±0.031	0.688±0.034
	March	0.692±0.084	0.706±0.017	0.647±0.061	0.673±0.037
	April	0.625±0.087	0.628±0.027	0.607±0.060	0.611±0.057
	May	0.689±0.060	0.668±0.076	0.753±0.003	0.727±0.029
	June	0.699±0.072	0.667±0.043	0.692±0.032	0.698±0.024

[1]/Value are mean ±SD of triplicates.

[2]/Different letter indicates significant differences in mean within the same row (P<0.05).

Pineapple fruit yield

The pineapples were harvested when they reached a mature stage, with 10-20% yellow color on the fruit. The external appearance of harvested pineapples from each treatment is shown in Figure 2. After 8 months of *B. velezensis* CE 100 biofertilizer sprayed, the results showed that the T4 pure culture broth biofertilizer significantly increased the fruit yield ($P<0.05$) by approximately 13% (Table 2).

Table 2. Effect of *B. velezensis* CE 100 biofertilizer application rates on pineapple yield

Treatments	Yield (t/ha) [1/, 2/]
T1	58.53±2.61 [a]
T2	61.33±1.26 [ab]
T3	63.67±2.02 [bc]
T4	66.37±2.18 [c]

[1]/Value are mean ±SD of triplicates.
[2]/Different letter indicates significant differences in mean ($P< 0.05$).

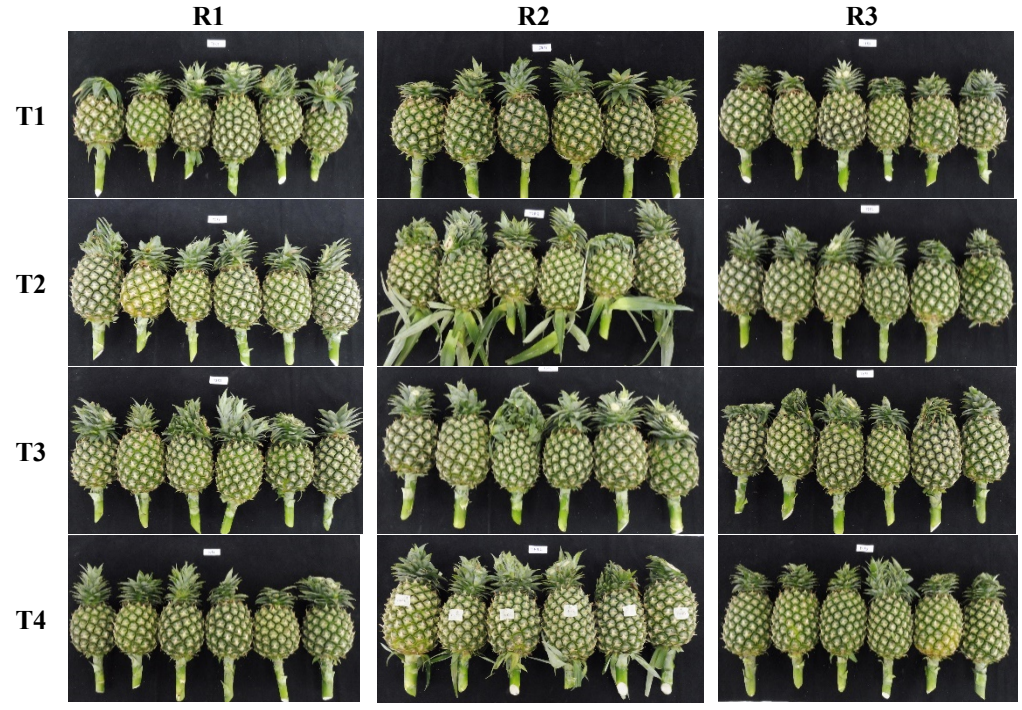

Figure 2. Harvested pineapples from each treatment

Fruit characteristics

The fruit weight of T4 was superior to other treatments (P<0.05). The fruit length and perimeter of the pineapple from T4 seemed higher than T1; however, there were no statistically significant differences. The biofertilizer treatments T3 and T4 showed a higher percentage of fruit with multiple crowns than T1 and T2 (Table 3, Figure 3). Furthermore, the application of *B. velezensis* biofertilizer did not affect internal fruit characteristics including the flesh color, TSS, and citric acid content (as calculated from %TA).

Table 3. Effect of *B. velezensis* CE 100 biofertilizer application rates on fruit characteristics

Parameters	**Treatments** [1/, 2/]			
	T1	**T2**	**T3**	**T4**
Fruit weight (kg)	0.82 ±0.12 [a]	0.90 ±0.08 [ab]	0.95 ±0.07 [ab]	1.12 ±0.07 [b]
Fruit length (cm)	11.82 ±0.58	12.20 ±0.13	11.79 ±0.46	13.51 ±1.50
Fruit perimeter (cm)	31.09 ±0.72	32.07 ±2.55	31.26 ±0.93	32.99 ±2.89
TSS (%Brix)	11.34 ±1.10	12.16 ±0.86	11.34 ±1.08	11.51 ±0.78
Acidity (mg citric acid/ml)	0.72 ±0.03	0.71 ±0.02	0.71 ±0.04	0.74 ±0.02
Fresh color				
a*	-0.25 ±0.11	-0.26 ±0.11	-0.27 ±0.01	-0.19 ±0.13
b*	16.40 ±1.72	16.75 ±0.75	16.11 ±0.30	17.00 ±1.14
L*	74.93 ±1.80	72.85 ±2.93	75.78 ±0.25	72.88 ±3.09
Multiple crowns (%)	29.86	28.16	45.43	46.84

[1]/Value are mean ±SD of triplicates.
[2]/Different letter indicates significant differences in mean within the same row (P < 0.05).

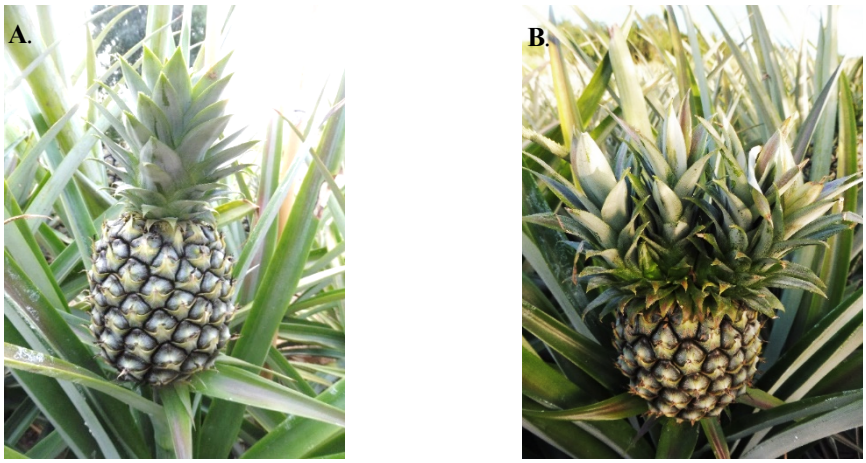

Figure 3. Fruit with a single (A) and multiple (B) crown

Soil pH after cultivation

At the end of the experiment, the soil from each plot was collected and determined for the pH value to examine the effect of biofertilizer on the soil pH. The result revealed that the pH value decreased gradually with an increased percentage of culture broth. The pH significantly dropped from 7.11 in T1 to 5.37 in T4 ($P<0.05$) (Table 4).

Table 4. Soil pH after pineapple cultivation

Treatments	Soil pH
T1	7.11±0.18 [a]
T2	6.28±0.48 [ab]
T3	5.57±0.20 [bc]
T4	5.37±0.39 [c]

[1]/Value are mean ±SD of triplicates.
[2]/Different letter indicates significant differences in mean ($P<0.05$).

Discussion

PGPB has been demonstrated as viable biological microorganisms that can promote plant growth and, in many cases, suppress plant diseases (Adesemoye *et al*., 2009). PGPB has been applied as a biofertilizer to boost plant growth through several mechanisms such as nitrogen fixation, phosphate, and potassium solubilization or mineralization, the production of plant hormones, the production of antibiotics, and the biodegradation of organic matter in the soil (Basu *et al*., 2021; Kumar *et al*., 2022; Sharma *et al*., 2022).

In the current study, the efficacy of PGPB, *B. velezensis* CE 100, as a biofertilizer was investigated in the field production of pineapple. The results demonstrated that the bacterium pure culture spray improved the plant height, fruit weight, and fruit yield significantly compared to the control group. The growth and yield enhancement could be due to the effect of IAA, ammonium-N production, and phosphate solubilization activity of *B. velezensis* CE 100 (Choi *et al*., 2024; Choub *et al*., 2021a; Hong *et al*., 2022; Moon *et al*., 2021).

IAA belongs to the auxin group of plant hormones. IAA regulates growth and developmental processes such as cell division and elongation, tissue differentiation, apical dominance, and responses to light, gravity, and pathogens (Fu *et al*., 2015). However, there are some contradictions about the role of IAA in plant-microbe interaction. Beneficial bacteria utilize IAA to promote plant growth, mitigate abiotic stresses, and enhance nutrient use efficiency. While, the interaction between plants and phytopathogenic microbes contributes to the disturbance of the plant's IAA dynamic balance leading to the disorder of plant

development and causing tumors and gall (Etesami and Glick, 2024; Fu *et al*., 2015).

Nitrogen is a vital macronutrient of plants. Nitrogen is a crucial component of proteins, enzymes, nucleic acid, and many plant metabolites including chlorophyll molecules, an essential factor in photosynthesis for absorbing sunlight energy and promoting plant growth and grain yield (Zayed *et al*., 2023). Nitrogen in the pineapple plants' nutrition is important for the high growth rate and good fruit yields (Razaq *et al*., 2017). Nitrogen is required in low amounts during the early vegetative phase but higher at four months after planting until the flower induction stage (Choo *et al*., 2022). Nitrogen deficiency can reduce leaf number and average leaf size, causing low fruit weight (Boussadia *et al*., 2010). Thus, the *B. velezensis* CE 100 spraying until the flower induction stage provides a beneficial effect in increasing available nitrogen to the pineapple plants resulting in a high growth rate and fruit yield.

Phosphorus is essential for diverse metabolic and physiological processes such as energy metabolism, cell division, DNA synthesis, and phospholipid biosynthesis (Isidra-Arellano *et al*., 2021; Lambers, 2022). Phosphate usually stays insoluble in the soil which is unavailable for plants' absorption. Phosphate-solubilizing bacteria include *Bacillus* Spp., *Pseudomonas* Spp., and *Aspergillus* Spp. convert insoluble phosphates into soluble forms by releasing organic acids, chelation, and ion exchange reactions (Sharma *et al*., 2022). The organic acids released by the bacteria lower the soil pH to dissociate phosphate into soluble forms readily to be absorbed by plants (Itelima *et al*., 2018). This is supported by the data in Table 4 (pH of 5.37 vs. 7.11), which shows a significant decrease in soil pH in treatment T4 compared to the control group. The phosphate solubilization activity of *B. velezensis* CE 100 ensures phosphorus is available for pineapple during their growth. Therefore, apart from the IAA effect, an increase of available nitrogen and phosphorus by *B. velezensis* CE 100 should be addressed as one of the growth-promoting activities of *B. velezensis* CE 100 on pineapples.

The production yield of pineapple observed in this study was approximately 13% higher than the conventional control group. This biofertilizer efficacy corresponded with previous studies but had lower efficacy. *B. velezensis* CE100 has been shown to increase the total biomass of walnut trees in the field experiment by 1.5-fold and 2.0-fold compared to the conventional and control groups (Choub *et al*., 2021a). Moreover, *B. velezensis CE 100* was reported to increase collar diameter, shoot length, and root length of *Chamaecyparis obtuse* seedlings by 1.3, 1.4, and 1.5-folds under the experimental greenhouse, compared to the control group (Moon *et al*., 2021). Co-inoculation with *B. velezensis* CE 100 in plants infected with *Macrophomina phaseolina* or

Fusarium oxysporum improved strawberry yield compared to only fungal inoculation by 10.6-fold or 4.9-fold, respectively, in the greenhouse environment (Hong *et al.,* 2022). Choi *et al*. (2024) revealed an increase in shoots and roots of seedlings inoculated with *F. oxysporum* treated with bacterial culture 3.3-fold and 4.0-fold than the control (Choi *et al*., 2024). The lesser efficacy of *B. velezensis* CE 100 on pineapple may be due to the differences in the environmental fields versus control greenhouse, the climatic region of the study site, and the type of cultivation plants.

Many globally available PGPR-based biofertilizer products have been registered and applied to several crops (Basu *et al*., 2021). Most products are nitrogen-fixers and phosphate solubilizers, some are biocontrols or mixed (Basu *et al*., 2021). However, they are rarely tested on pineapple. Krishan *et al*. (2017) reported the application of bio-fertilizers on the growth and yield of pineapple in India. Two types of biofertilizers with mixed PGPBs were used including Biomix-1 (*Azotobacter, Azospirillium, Rhizobium, Bacillus, Pseudomonas,* and *Trichoderma*) applied in the soil at the time of planting, and Biomix-5 (*Azotobacter, Trichoderma,* and *Pseudomonas*) applied at the root zone of the plant. They found that the highest fruit yield was recorded with the application of Biomix-1 + Biomix-5 in the Kew variety at 31.02 t/ha and the lowest was the application of Biomix-5 in the Mauritius variety at 14.44 t/ha. In this study, the highest pineapple yield was 66.37 t/ha from the 100% biofertilizer, and the lowest was 58.53 t/ha in the control group (no biofertilizer). The productivity was higher than in the previous study by Krishan *et al* (2017). However, the fold increase was lower (1.14-fold compared to 2.14-fold). This may be due to the number and type of PGPBs used in biofertilizers, the differences in cultivars and climates, the density of planting, and other cultivation practice methods. In Thailand, Khamtib (2023) identified the phosphate and potassium-solubilizing bacteria, *Burkholderia ferrariae* PaS2(1). The test on *B. ferrariae* PaS2(1) biofertilizer activity in the presence of different amounts of chemical fertilizer on pineapple production showed that it can increase fruit weight compared to non-biofertilizer treatment. However, the limited amount of potassium and phosphorus in chemical fertilizers reduced the weight and sweetness of the fruit.

In the present study, biofertilizer treatment did not alter the internal characteristics, including TSS, citric acid content, and flesh color. This may be due to the plants having sufficient nutrients during their growth to their metabolites, as controlled by the variety's internal genetics. However, the result revealed that biofertilizer treatment at a 1:2 ratio (T3) and non-dilute culture (T4) greatly induce the production of multiple crowns on the fruit. The crown is the main source of endogenous abscisic acid (ABA) which controls internal browning (IB) in pineapple during postharvest. Detach the crown deteriorated

quality of the flesh and shortened shelf-life (Liu *et al*., 2017). Pineapples with multiple crowns are supposed to be a heritable character, found mostly in the Cayenne group (Kumar *et al.,* 2023). However, environmental factors such as imbalanced nutrients, intense sunlight, or high temperature may partly contribute to the induction. Multiple crowning made the flat and broad top of the fruit, thus unfit for canning (Kumar *et al.,* 2023).

In summary, this study revealed the efficacy of *B. velezensis* CE 100 in enhancing pineapple yield in the farmers' field. The results strengthen the use of biofertilizers to promote organic and sustainable pineapple production in Thailand. Further study should be done to optimize the application procedure and maximize the efficacy of biofertilizers.

Acknowledgments

The author would like to acknowledge Purne Inc. (Jangseong, Korea) for providing *B. velezensis* bacteria and thanks to the faculty of Animal Science and Agricultural Technology, Silpakorn University, Thailand for the laboratory facility support.

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(Received: 25 September 2024, Revised: 27 June 2025, Accepted: 3 July 2025)