EVALUATION OF THE ABILITY OF IAA BIOSYNTHESIS AND THE EFFECT TO GERMINATION ON MAIZE KERNELS OF HALOTOLERANT PHOSPHATE-SOLUBILIZING BACTERIA *Pantoea* sp. TTB4.1

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ABSTRACT

Plant Growth-Promoting Bacteria (PGPR) are a group of bacteria beneficial to crops, among which Phosphate-Solubilizing Bacteria (PSB) are of interest due to their ability to support plant resistance to salt stress and produce plant growth-promoting substances. This study aims to investigate the synthesis capability of the plant growth-promoting substance indole acetic acid (IAA) by the salt-tolerant phosphate-solubilizing *Pantoea* sp. TTB4.1 strain and its effect on the germination ability of corn seeds immersed in bacterial suspension. The research findings indicate that over a 10-day cultivation period, the *Pantoea* sp. TTB4.1 strain was able to synthesize IAA in quantities ranging from 1.163 to 3.916 µg/mL, with the highest IAA content observed on day 4. Concurrently, this study also evaluates the impact of the salt-tolerant phosphate-solubilizing *Pantoea* sp. TTB4.1 strain on the germination ability of corn seeds through experiments involving immersion in bacterial suspension for 30, 60, 90 minutes. The results showed that root length, shoot length, number of roots, and germination rate of the corn seeds were all higher compared to the control experiment (without bacterial immersion).

ĐÁNH GIÁ KHẢ NĂNG SINH TỔNG HỢP IAA VÀ HIỆU QUẢ ĐẾN SỰ NẨY MẦM HẠT NGÔ CỦA VI KHUẨN HÒA TAN PHOSPHATE CHỊU MẶN *Pantoea* sp. TTB4.1

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TỪ KHÓA

Hạt ngô
Indole acetic acid (IAA)
Kích thích tăng trưởng
Sự nảy mầm
Vi khuẩn *Pantoea*

TÓM TẮT

Vi khuẩn thúc đẩy sinh trưởng thực vật (PGPR) là nhóm vi khuẩn có lợi cho cây trồng trong đó nhóm vi khuẩn hòa tan lân (PSB) được quan tâm bởi khả năng hỗ trợ cây trồng chống chịu với stress mặn và sản sinh chất kích thích sinh trưởng thực vật. Nghiên cứu này nhằm khảo sát khả năng tổng hợp chất kích thích sinh trưởng thực vật indole acetic acid (IAA) của chủng vi khuẩn hòa tan lân chịu mặn *Pantoea* sp. TTB4.1 và khảo sát ảnh hưởng đến khả năng nảy mầm của hạt ngô khi ngâm trong dịch huyền phù vi khuẩn. Từ kết quả nghiên cứu, trong 10 ngày nuôi cấy chủng vi khuẩn *Pantoea* sp. TTB4.1 có khả năng tổng hợp IAA với hàm lượng từ 1,163 - 3,916 µg/mL, trong đó hàm lượng IAA đạt cao nhất vào ngày 4. Đồng thời, nghiên cứu này cũng đánh giá tác động của chủng vi khuẩn hòa tan lân chịu mặn *Pantoea* sp. TTB4.1 đến khả năng nảy mầm của hạt ngô bằng các nghiệm thức ngâm trong dịch huyền phù vi khuẩn trong 30, 60, 90 phút và các kết quả chiều dài rễ, chiều dài mầm, số rễ và tỷ lệ nảy mầm của hạt ngô ghi nhận đều cao hơn khi so sánh với nghiệm thức đối chứng (không ngâm vi khuẩn).

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1. Introduction

Agriculture faces challenges from the rising global demand for food, the scarcity of arable land and resources, and various environmental pressures [1]. In recent years, the Mekong Delta has experienced increasingly severe saltwater intrusion and heightened salinity levels [2], [3]. Numerous studies have compiled and reported on plant growth promoting rhizobacteria - PGPR's high salt tolerance and its promotion of good plant growth in salt-affected soils, among them are phosphate solubilizing bacteria (PSB) [4]-[7].

Phytohormone indole-3-acetic acid (IAA) is one of the most physiologically active auxiliary substances produced by rhizobacteria, the major contribution towards plant growth promotion. IAA contributes to root initiation, helps loosen plant cell walls to release exudates, stimulates the overproduction of root hairs, increases lateral root formation, acts as a signaling molecule for both the plant and bacteria, and aids in root elongation and developmental responses to stress, and other environmental stimuli. Therefore, it has potential applications for agriculture [8]-[10].

Simultaneously, to enhance seed quality, different seed treatment technologies are now available; in particular, pre-sowing seed treatments arouse great interest because of their efficacy and environmental benefits [11]. Pre-sowing seed treatments with beneficial microorganisms have relatively low application costs, as they require a single treatment and the active ingredients are applied at low dose rates [12]-[14].

To build upon the research results conducted by Tran et al. [5] and Nguyen et al. [15] regarding the application of salt-tolerant phosphorus-solubilizing bacteria strain *Pantoea* sp. TTB4.1 in agriculture to address climate change in the Mekong Delta, this study aims to assess the production of IAA (indole-3-acetic acid) by the bacteria and examine its impact on the germination of maize kernels.

2. Methods

2.1. Materials

The bacterial strain *Pantoea* sp. TTB4.1, which is salt-tolerant and phosphate-solubilizing, is stored at the Environmental Microbiology Laboratory, Institute of Food and Biotechnology (Can Tho University). The corn variety used in the experiment is CX247 (Southern Crop Variety Company). Chemicals: NBRIP bacterial culture medium for phosphate solubilization, standard IAA, H_2SO_4, $FeCl_3$, distilled water and alcohol.

2.2. Methods

2.2.1. Evaluation the ability to biosynthesize IAA of the halotolerant phosphate-solubilizing bacteria strain Pantoea sp. TTB4.1

The ability to synthesize IAA from the bacteria *Pantoea* sp. TTB4.1 was determined by using the Salkowski method [16]. Bacteria *Pantoea* sp. TTB4.1 was cultured in the liquid LB medium after 18 hours. The absorbance of the enrichment fluid at 600 nm was adjusted to 0.8 [17]. Then, 0.2 mL of bacterial suspension (OD_{600} = 0.8) was inoculated into 20 mL of liquid NBRIP medium, and the falcon tubes were covered with a dark color to prevent IAA produced by bacteria from being decomposed by light. The IAA concentration synthesized by bacteria was measured after 2, 4, 6, 8, and 10 days of culture. Measure as follows:

- Build a standard curve: weigh 0.0016 g of synthetic IAA and dissolve it in 10 mL of phosphate buffer to get a stock with a concentration of 160 µg/mL. Then, dilute the stock with phosphate buffer to concentrations of 0, 2, 4, 6, 8, 10 µg/mL and withdraw 1 mL into a test tube, then add 2 mL of Salkowski reagent. The solutions in the test tubes were mixed with a vortex machine and left for 15 - 30 minutes at room temperature, then spectrophotometrically with a wavelength of 530 nm.

- The bacterial solution cultured in the falcon tube is withdrawn and centrifuged at 5.500 rpm for 5 minutes. Prepare test tubes and add 1 mL of sample and 2 mL of Salkowski reagent to each tube, respectively. Mix the solution in the test tubes with a vortex machine and leave for 15 - 30 minutes at room temperature, then perform spectrophotometric measurements at 530 nm.
- Measuring IAA concentration: IAA concentration is determined by measuring color absorption (Abs) at wavelength 530 nm. Based on the standard curve equation and the OD value of the sample, calculate the IAA concentration (µg/mL) in the bacterial samples.

2.2.2. Evaluation of the effects of the halotolerant phosphate-solubilizing bacteria strain Pantoea sp. TTB4.1 affects the germination of maize kernels

To determine the effects of the bacteria *Pantoea* sp. TTB4.1 on maize kernels germination. Maize kernels were soaked for 3 – 5 hours with warm water (about 42°C) with a ratio of 2 boiling: 3 cold. *Pantoea* sp. TTB4.1 was cultured in liquid NBRIP medium [18] to a density of 10^8 CFU/mL and the experiment was arranged as follows:
- Treatment 1: control (not soaked in bacterial solution),
- Treatment 2: soaked in bacterial solution for 30 minutes,
- Treatment 3: soaked in bacterial solution for 60 minutes,
- Treatment 4: soaked in bacterial solution for 90 minutes.

Keep the maize kernels moist in sterilized paper until they germinate (about 16 - 24 hours) and measure the length of the roots and sprouts after 2, 3, 4 days (each treatment tested on 15 maize kernels) and repeated 3 times.

Analytical criteria: Root length (cm), sprout length (cm), germination rate, number of roots.

2.2.3. Data Analysis

Data are calculated, presented as mean ± standard deviation and presented in the form of graph from Microsoft Excel 2019. Statistical analysis of ANOVA using software Minitab 16, analyze variance ANOVA One-way, comparing differences between treatments using the Tukey HSD method at reliability.

3. Results and discussion

3.1. Evaluation of the ability to biosynthesize IAA of the halotolerant phosphate-solubilizing bacteria strain Pantoea sp. TTB4.1

From the results of Table 1 on the IAA biosynthesis ability of the halotolerant phosphate-solubilizing bacteria *Pantoea* sp. TTB4.1 over 2, 4, 6, 8, 10 days shows that the bacterial strain is capable of synthesizing IAA content, the amount of IAA synthesized ranges from 1.163 µg/mL to 3.916 µg/mL, in which the highest amount of IAA synthesis was on day 4, reaching 3.916 µg/mL, gradually decreasing on the following days and significantly different from the remaining days at the 95% confidence level. From there it can be concluded that the halotolerant phosphate-solubilizing bacteria strain *Pantoea* sp. TTB4.1 has the ability to biosynthesize IAA.

Table 1. *Effect of incubation time on IAA concentration by Pantoea sp. TTB4.1 synthesized*

Incubation time (days)	IAA concentration (µg/mL)
2	2.456 ± 0.120^{c}
4	3.916 ± 0.297^{a}
6	2.991 ± 0.132^{b}
8	2.768 ± 0.171^{bc}
10	1.163 ± 0.188^{d}

The values from the table are the mean of 3 replicates ± standard deviation. Different letters in the value column represent a statistically significant difference at the 95% confidence level.

Culture time is also one of the important factors affecting the growth rate and biological activity of bacteria. In another research on two phosphate solubilizing bacterial showed that

Pantoea agglomerans exhibited the highest IAA production within the first 24h; whereas *Burkholderia anthina* exhibited the highest IAA production within the first 48h followed by reduction as incubation progressed [19]. In the study of Khuong et al. [20], it was shown that bacterial strains CL06L3 and LP05L3, isolated from rhizosphere of *Polyscias fruticosa* (L.) Harms in An Giang province, had the highest ability to synthesize IAA, with concentrations of 1.00 and 0.94 mg/L, respectively.

Some studies show that there are factors that affect the synthesis of IAA by bacterial strains such as L-tryptophan, temperature, pH, culture time... Because L-tryptophan acts as a precursor of IAA and likely directly affects IAA production. The plant growth-promoting endophytic bacterium *Pantoea agglomerans* CPHN2 isolated from *Cicer arietinum* produced 224.95 µg/mL IAA with 100 µg/mL L-tryptophan and very less amount of IAA in absence of L-tryptophan (21.671 µg/mL) [8].

3.2. Evaluation of the effects of halotolerant phosphate-solubilizing bacteria Pantoea sp. TTB4.1 affects the germination of maize kernels

The results clearly showed that soaking maize kernels with bacterial solution during 30, 60 and 90 minutes (T2, T3, T4) helps seed germination rate faster, root length, sprout length and number of roots are longer and larger, made a clear difference compared to the control sample not soaked in bacterial solution (T1). Demonstrating that the bacteria *Pantoea* sp. TTB4.1 during the soaking process, creates active ingredients that stimulate germination and promote growth in maize kernels, helping to increase and strongly develop root length, sprout length and number of roots at the same time forms root hairs that help maize kernels absorb the most nutrients.

The root lengths of T2, T3, and T4 that were soaked in bacterial solution were all longer and larger than those in T1 (not soaked in bacterial solution), with an average length ranging from 1.402 cm to 3.031 cm from days 2 to 4, of which T4 (soaked in bacterial solution for 90 minutes) had the longest root length, reaching an average of 3.031 cm on day 4 after sowing and was significantly different from the treatment control at 95% confidence level (Table 2).

Table 2. *Effect of seed-soaking time in bacteria solution on root length*

Treatment	Root length (cm)		
	Day 2	Day 3	Day 4
T1	1.300 ± 0.029^{b}	1.428 ± 0.021^{c}	1.777 ± 0.040^{c}
T2	1.402 ± 0.013^{b}	1.595 ± 0.013^{bc}	1.957 ± 0.060^{c}
T3	1.628 ± 0.110^{a}	1.824 ± 0.109^{ab}	2.355 ± 0.126^{b}
T4	$1.786^{a} \pm 0.102^{a}$	1.946 ± 0.141^{a}	3.031 ± 0.127^{a}

The values from the table are the mean of 3 replicates± standard deviation. Different letters in the value column represent a statistically significant difference at the 95% confidence level.

Similar to root length, the sprout length of maize kernels in T2, T3, and T4 soaked with bacterial solution all sprouted longer and earlier than the control T1, ranging on average from 0.612 cm to 3.546 cm after sowing from day 2 to day 4, in which T4 was recorded to have the longest sprout length reaching an average of 3.546 cm on day 4 and was significantly different from the control treatment in terms of 95% confidence (Table 3).

Table 3. *Effect of seed-soaking time in bacteria solution on sprout length*

Treatment	Sprout length (cm)		
	Day 2	Day 3	Day 4
T1	0.534 ± 0.058^{b}	0.760 ± 0.040^{b}	1.148 ± 0.038^{c}
T2	0.612 ± 0.082^{b}	0.804 ± 0.076^{b}	1.320 ± 0.050^{c}
T3	0.804 ± 0.233^{ab}	1.093 ± 0.258^{ab}	1.655 ± 0.193^{b}
T4	1.008 ± 0.071^{a}	1.284 ± 0.153^{a}	3.546 ± 0.094^{a}

The values from the table are the mean of 3 replicates± standard deviation. Different letters in the value column represent a statistically significant difference at the 95% confidence level.

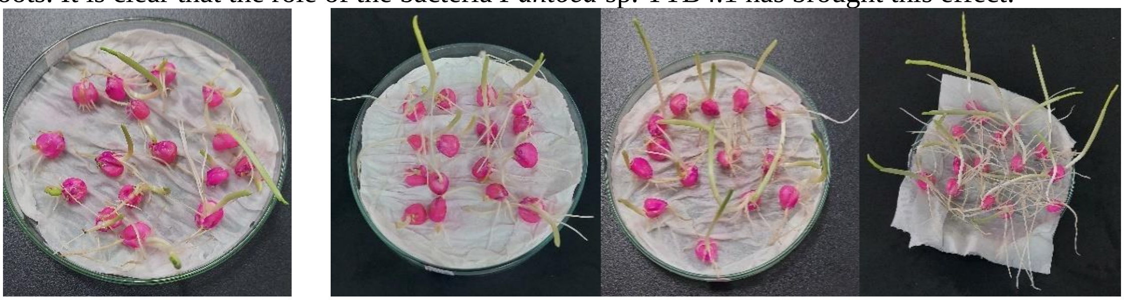
Regarding the root number indicator, it can be clearly seen that for treatments soaked with bacterial solution, the number of roots is greater and root hairs form earlier than in treatments without soaking in bacterial solution. The average number of roots of treatments soaked in bacterial solution from 30 minutes to 90 minutes (T2, T3, T4) ranged from 1.244 to 5.333 from day 2 to day 4 after sowing, and root formation was highest on day 4 with an average value of 5.333 and was significantly different at the 95% confidence level for the treatment without soaking in bacterial solution (Table 4).

Table 4. *Effect of seed-soaking time in bacteria solution on roots number*

Treatment	Roots number		
	Day 2	**Day 3**	**Day 4**
T1	1.222 ± 0.192^{b}	2.111 ± 0.138^{b}	3.600 ± 0.115^{c}
T2	1.244 ± 0.203^{b}	2.644 ± 0.582^{b}	4.000 ± 0.305^{bc}
T3	1.511 ± 0.154^{b}	2.911 ± 0.300^{b}	4.155 ± 0.101^{b}
T4	3.177 ± 0.315^{a}	4.111 ± 0.167^{a}	5.333 ± 0.133^{a}

The values from the table are the mean of 3 replicates± standard deviation. Different letters in the value column represent a statistically significant difference at the 95% confidence level.

Besides, soaking maize kernels with bacterial solution for certain periods of time also affects the kernels' ability to germinate. Specifically, after 24 hours, maize kernels soaked in bacterial solution germinated faster than maize kernels not soaked in bacterial solution. And most clearly, the maize kernels in T4 that were soaked with bacterial solution for 90 minutes also had a difference when the root length and sprout length were longer, the germination rate after 24 hours was faster and more numerous, and the quantity was longer roots, root hairs were formed and developed more strongly than in T2 (soaked with bacterial solution for 30 minutes) and T3 (soaked with bacterial solution for 60 minutes). Research results show that soaking maize kernels with bacterial solution helps increase germination rate, root length, sprout length and number of roots. It is clear that the role of the bacteria *Pantoea* sp. TTB4.1 has brought this effect.



Control sample (T1) Sample soaked in bacterial solution (T2, T3, T4)

Fig 1. *Differences in root length, shoot length and number of roots between treatments have soaked bacteria (T2, T3, T4) and without soaked bacteria after 4 days*

Bakonyi et al. [21] soaked seeds with microbial preparations, helping the seeds increase their germination rate by 20%, and the dry biomass of radicle roots and shoots increased by 7% compared to the control treatment without treatment with preparations. The study of Nguyen et al. showed that after a 24-hour incubation period, the effectiveness of two native PSBs, *Burkholderia vietnamiensis* L1.1 and *Staphylococcus hominis* T7.3, on the germination of rice seeds. Remarkably, the treatment involving *Burkholderia vietnamiensis* L1.1 yielded the highest percentage of germination, at 95.3%. Two PSB strains were tested for their effects on the vigor of rice seedlings under laboratory conditions after 20 days. The results showed that the inoculated treatments significantly outperformed the control treatment in terms of plant height, root length, number of roots, root weight, and stem weight. Plant heights, root lengths, number of roots, root biomass, and dry rice seedling biomass were all significantly higher in the co-inoculant treatment. This suggests that co-inoculation of rice seedlings with both bacterial strains greatly increases their biomass and rate of growth [4].

Similarly, various isolates from *Bacillus* species were evaluated for their ability to produce indole-3-acetic acid (IAA) and then applied to maize seeds. The germination rate of the seeds was not affected compared to the control group, but there was a significant increase in above-ground growth (fresh and dry biomass and shoot length) and root development (fresh and dry biomass and number of adventitious roots) as a result of the treatment. The specific effects varied depending on the strain of bacteria used [11]. And the research results of Adoko et al. [22] on bacterial strains of *Pseudomonas* sp. or *Bacillus thuringiensis* helps stimulate growth of corn plants and increases the biomass of roots, stems, and leaves of maize, which is the basis to prove that plants absorb adequate nutrients to ensure better productivity than treatments without bacteria in net house conditions.

From the research results of the experiment on the ability to synthesize IAA of the bacteria *Pantoea* sp. TTB4.1 can serve as a basis to prove the experiment below that the bacteria *Pantoea* sp. TTB4.1 could synthesize plant growth stimulants IAA, and IAA has been shown to help stimulate cell growth, and support cell growth and elongation, so in experiment 2 when soaking maize kernels with sp. TTB4.1 over certain periods produced useful active ingredients that help quickly accelerate the germination process, forming roots and sprouts of maize kernels as soon as possible compared to maize kernels in the untreated treatment soaked in bacterial solution. Therefore, more research on methods and applications of bacterial strains capable of synthesizing IAA should be done to treat seeds before planting to help increase productivity and ensure optimal crop quality.

4. Conclusion

The study investigated the ability to synthesize IAA of the halotolerant phosphate-solubilizing bacteria strain *Pantoea* sp. TTB4.1 was cultured in liquid NBRIP medium and color absorbance was measured after 2, 4, 6, 8, and 10 days of culture. The results showed that the bacterial strain synthesized IAA on most days, in which the IAA concentration reached the highest value of 3.916 µg/mL on day 4. At the same time, the study also evaluated the impact of the halotolerant phosphate-solubilizing bacteria strain *Pantoea* sp. TTB4.1 helped stimulate root length, sprout length, number of roots, and increased germination rate, which was clearly shown from day 2 to day 4 for treatments soaked in a bacterial solution with different time points and different periods, in which T4 (soaked in bacterial solution for 90 minutes) reached the highest average value on day 4.

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