

EFFICIENCY OF NITROGEN-FIXING BACTERIA *BRADYRHIZOBIUM* SP. AND PHOSPHATE-SOLUBILIZING BACTERIA *PSEUDOMONAS* SP., *PANTOEA* SP. ON THE GROWTH OF MAIZE [*ZEA MAYS* L.]

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ARTICLE INFO	ABSTRACT
<p>Received: 11/01/2023</p> <p>Revised: 27/4/2023</p> <p>Published: 28/4/2023</p>	<p>This study investigated the effects of nitrogen-fixing bacteria <i>Bradyrhizobium</i> sp. TTB2.1 and phosphate-solubilizing bacteria <i>Pseudomonas</i> sp. BKD2.1, <i>Pseudomonas</i> sp. TTB4.4, <i>Pantoea</i> sp. TTB4.1 on the growth and development of corn under field conditions. The results showed the yield in the treatments with phosphate-solubilizing bacteria <i>Pantoea</i> sp. TTB4.1 25.64 tons/ha (first crop) and 27.22 tons/ha (second crop), <i>Pseudomonas</i> sp. BKD2.1 25.71 tons/ha (first crop) and 26.98 tons/ha (second crop). Criteria of plant height, fruit length, leaf length, number of fruits per tree and chlorophyll content in leaves are better in the bacterial irrigation treatments than in the negative control. Criteria of nitrogen, soluble phosphorus, and microbial density in the soil changed before sowing and after harvest. Two strains of phosphate solubilizing bacteria <i>Pseudomonas</i> sp. BKD2.1 and <i>Pantoea</i> sp. TTB4.1 has the best effect on the growth and development of the corn.</p>
<p>KEYWORDS</p> <p>Corn</p> <p>Bacteria</p> <p>Dissolve phosphorus</p> <p>Microbial fertilizer</p> <p>Nitrogen fixation</p>	

HIỆU QUẢ CỦA VI KHUẨN CỐ ĐỊNH ĐẠM *Bradyrhizobium* sp. VÀ VI KHUẨN HÒA TAN LÂN *Pseudomonas* sp., *Pantoea* sp. LÊN SỰ SINH TRƯỞNG CỦA CÂY BẮP [*Zea mays* L.]

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THÔNG TIN BÀI BÁO	TÓM TẮT
<p>Ngày nhận bài: 11/01/2023</p> <p>Ngày hoàn thiện: 27/4/2023</p> <p>Ngày đăng: 28/4/2023</p>	<p>Nghiên cứu được thực hiện nhằm khảo sát tác động của các dòng vi khuẩn cố định đạm <i>Bradyrhizobium</i> sp. TTB2.1 và hòa tan lân <i>Pseudomonas</i> sp. BKĐ2.1, <i>Pseudomonas</i> sp. TTB4.4, <i>Pantoea</i> sp. TTB4.1 lên sự sinh trưởng, phát triển của cây bắp trong điều kiện ngoài đồng. Kết quả cho thấy năng suất ở các nghiệm thức tưới vi khuẩn hòa tan lân <i>Pantoea</i> sp. TTB4.1 đạt 25,64 tấn/ha (vụ 1) và 27,22 tấn/ha (vụ 2), <i>Pseudomonas</i> sp. BKĐ2.1 cho kết quả đạt 25,71 tấn/ha (vụ 1) và 26,98 tấn/ha (vụ 2). Các chỉ tiêu về chiều cao cây, chiều dài trái, chiều dài lá, số trái trên cây và hàm lượng chlorophyll trong lá ở các nghiệm thức tưới vi khuẩn cao hơn đối chứng âm. Các chỉ tiêu về hàm lượng đạm, lân hòa tan, mật số vi sinh vật trong đất có sự thay đổi giữa trước khi gieo hạt và sau thu hoạch. Hai dòng vi khuẩn hòa tan lân <i>Pseudomonas</i> sp. BKĐ2.1 và <i>Pantoea</i> sp. TTB4.1 có tác động lên sự sinh trưởng và phát triển của cây bắp tốt nhất.</p>
<p>TỪ KHÓA</p> <p>Cây bắp</p> <p>Cố định đạm</p> <p>Hòa tan lân</p> <p>Phân bón vi sinh</p> <p>Vi khuẩn</p>	

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1. Introduction

Corn (*Zea mays* L.) is the world's major cereal crop, contributing greatly to the economy of many countries, so it has been planted popular and widely [1]. In addition, corn is used as a food by the people. Today, with the development of many chemical fertilizers, it is easier to grow high-yielding corn. However, the use of many chemical fertilizers combined with pesticides greatly affect the quality of the fruit, as well as the arable land. The obvious consequence, is the residue of chemicals in the fruit, making the land infertile, costly and causing serious environmental pollution [2].

Due to the influence of chemical fertilizers, agricultural land faces a severe problem of lack of beneficial natural microorganisms. Microbial products are increasingly exciting and play an important role in improving soil fertility, and enhancing crop yield and nutrient content in harvested products [3]. Bio-fertilizer products are formulations that include beneficial microorganisms that play an important role in the ecosystem. The use of beneficial bacteria groups to promote the growth and development of plants has been applied as a sustainable and long-term method [4] - [7]. When applied to seeds, soil, or seedlings, probiotics inoculants directly or indirectly provide plant nutrients and promote plant growth [8].

Nitrogen is a critical element affecting corn plants' growth, development, and yield. Full of nitrogen, corn plants will grow quickly, stems and leaves develop well, tassels are large with lots of chalk, and fruits with many seeds. If there is a lack of nitrogen, the plant's growth is slow, the fruit is small, many seeds are flat (especially at the top of the fruit), and the leaf area is reduced, making the plant less photosynthetic [9].

Most of the nutrients for plants, including phosphorus (P) are insoluble in the soil, and P deficiency occurs in 67% of the world's total arable land [10]. A large proportion of inorganic phosphorus is applied to the soil as a fertilizer that quickly resists immobilization and becomes less useful to plants [11].

2. Materials and Methods

2.1. Materials

Corn kernels:

Glutinous corn hybrid F1 CX 247 was bought from Southern Seed Corporation. Corn kernels soak for 3 hours with warm water (about 42°C), then brew in a moist towel until crack.

Bacteria culture:

Four bacterial strains are stored in the laboratory of Environmental Microbiology, Institute of Food and Biotechnology, Can Tho University: *Bradyrhizobium* sp. TTB2.1, *Pseudomonas* sp. BKD2.1, *Pseudomonas* sp. TTB4.4 và *Pantoea* sp. TTB4.1.

Nitrogen-fixing bacteria were grown proliferatively in liquid free-nitrogen Burk medium and phosphate-soluble bacteria were grown in liquid NBRIP medium on a shaker to get a density of about 10^8 CFU/mL.

2.2. Experimental design

Each treatment is sown in rows, each row is 70 cm apart, each hole is 16-20 cm apart, 2 seeds are sown in each hole until the plant has 2 cotyledons, then 1 tree is removed. The area of each treatment is about 5 m².

Bacteria were irrigated at a dose of 800 liters/ha, after 7, 21, 34, 48 days of sowing.

The experiment was conducted in a completely randomized design with 3 replications, and experimental units were set in the same positions in 2 crops.

Treatment 1: *Bradyrhizobium* sp. TTB2.1 (NT1)

Treatment 2: *Pseudomonas* sp. BKD2.1 (NT2)

Treatment 3: *Pseudomonas* sp. TTB4.4 (NT3)

- Treatment 4: *Pantoea* sp. TTB4.1 (NT4)
 Treatment 5: Combination of all 4 bacterial strains (NT5)
 Treatment 6: Negative control (no bacterial, no chemical fertilizers, only watering) (NT6)
 Treatment 7: Positive control (chemical fertilizer application rate (180 kg N – 100 kg P₂O₅ – 60 kg K₂O)/ha) [12] (NT7).

2.3. Measurement of corn growth parameters

After 65 days of sowing, the plant's parameters were taken: stem height, leaf sheath length, fruit length, fruit weight, and chlorophyll content in leaves.

- Measurement of chlorophyll content

Leaf samples were collected in all treatments, stored in plastic bags, and stored in an ice box to avoid direct sunlight. Remove the leaf collar, midrib, and leaf border, then weigh 0.1 g of leaf blade extracted with 10 mL of the liquid mixture (acetone 80%:95% ethanol, volume ratio 2:1). Samples were measured for OD at 645 nm and 663 nm after being stored in the dark for 24 h at 37°C. Chlorophyll a (Chl a), chlorophyll b (Chl b), and total chlorophyll (Chl total), calculated by the formula:

$$\text{Chl a (mg/g)} = (12,7 \times \text{OD}_{663} - 2,69 \times \text{OD}_{645}) \times V/W/1000 \quad (1)$$

$$\text{Chl b (mg/g)} = (22,9 \times \text{OD}_{645} - 4,68 \times \text{OD}_{663}) \times V/W/1000 \quad (2)$$

$$\text{Total Chl (mg/g)} = (20,2 \times \text{OD}_{645} + 8,02 \times \text{OD}_{663}) \times V/W/1000 \quad (3)$$

In there:

V: the volume of extraction solution (mL)

W: weight of extracted sample (g) [13]

2.4. Analysis of soil parameters before and after the experiment

Soil samples were collected at 5 points of each replicate in NT 5, a combination of bacterial strains, to test the adaptation and impact of nitrogen-fixing and phosphate-solubilizing bacteria on soil improvement.

- **Determination of pH**

Weigh 5 g of soil into an Erlenmeyer flask and add 45 mL of sterilized distilled water (121°C, 1 atm). Shake on a shaker for about 45 minutes. Let it settle for 2 hours. Measure the pH with a pH meter and read the result after it has stabilized for about 30 seconds.

- **Determination of NH₄⁺ content** [14]

Using Indophenol blue method - determine NH₄⁺ content by spectrophotometer at 636 nm. This method is based on the reaction of phenol with NH₃ with the participation of hypochlorite oxidizing agent to form a blue complex under alkaline pH conditions in principle.

- **Determination of P₂O₅ content** [15]

Using the Molybdate blue method with ascorbic acid-ammonium molybdate-potassium antimony tartrate reagent, colorimetrically measured on a spectrophotometer at 880 nm.

- **Determination of the total number of microorganisms, nitrogen-fixing bacteria, phosphate solubilizing bacteria**

By spreading agar plate and counting colonies: determining the total number of microorganisms on the PCA medium, determining the density of nitrogen-fixing bacteria on the non-nitrogen Burk medium, and determining the density of phosphorus-soluble bacteria on NBRIP medium.

3. Results and discussion

3.1. Results of yield and other growth parameters of corn plants

Microbial use for corn plants had a significant effect on fruit weight, contributing to an increase in the quality of corn [16]. In the first crop, treatments NT2 and NT4 resulted in fruit

weight 14.72% and 14.39% higher than the negative control, but compared to the positive control and the rest of the treatments, the difference was not significant about statistically significant. However, after the second crop, there was a clear difference in fruit weight between treatments, NT2 and NT4 treatments gave the highest fruit weight (Table 1), more than 30.05% and 31.23% compared to the negative control, and at the same time higher than the positive control in these two treatments, 5.16% and 6.11% respectively. Particularly for the remaining bacterial treatments NT1, NT3 and NT5, the fruit weight was lower than that of the first crop but still higher than that of the negative control. This may be due to the poor adaptation of these bacterial groups to the natural soil environment.

In treatment NT5, the 4 bacterial strains have to compete for nutrients and living environment. Therefore, these bacteria could not perform at their maximum ability.



Fig 1. Comparison of corn products between treatments

Fertilization and spraying the nitrogen-fixing and phosphorus-dissolving bacteria on corn have made it produce more than 1 ear per plant. The negative control (NT6) produced 1.07 ears of corn (first crop season) and 1.11 ears (second crop season), this result is consistent with the study of Cao Ngoc Diep and Tran Minh Thien [16]. Ear length is also an inevitable part of determining the yield of corn, in addition, it is greatly influenced by the characteristics of the experimental corn variety.

Table 1. Corn weight (g), number of corn, corn length (cm) and productivity (tons/ha) over two crops averaged over 15 different corn plants

Treatment	NT1	NT2	NT3	NT4	NT5	NT6	NT7	CV(%)
First Crop								
Corn weight	350.83 ^a	357.08 ^a	353.75 ^a	356.04 ^a	348.33 ^a	311.25 ^b	352.50 ^a	1.11
Number of corn/plant	1.51 ^a	1.73 ^a	1.52 ^a	1.89 ^a	1.53 ^a	1.07 ^b	1.69 ^a	8.69
Corn length	18.39 ^a	17.98 ^{ab}	17.37 ^{ab}	18.63 ^a	17.27 ^{ab}	16.92 ^b	17.85 ^{ab}	2.76
Productivity	25.26 ^a	25.71 ^a	25.47 ^a	25.64 ^a	25.08 ^a	22.41 ^b	25.38 ^a	1.11
Second Crop								
Corn weight	328.67 ^c	374.66 ^a	296.66 ^d	378.07 ^a	333.69 ^c	288.09 ^d	356.29 ^b	1.38
Number of corn/plant	1.87 ^a	1.58 ^{bc}	1.44 ^c	1.80 ^{ab}	1.33 ^{cd}	1.11 ^d	1.56 ^{bc}	6.46
Corn length	17.50 ^a	18.17 ^a	18.32 ^a	18.56 ^a	17.81 ^a	17.80 ^a	17.86 ^a	1.88
Productivity	23.66 ^c	26.98 ^a	21.37 ^d	27.22 ^a	24.03 ^c	20.74 ^d	25.65 ^b	1.38

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

The yield in the first season did not have a significant difference between treatments, the treatment with groups of bacteria and the positive control performed similarly at more than 25 tons/ha, and the difference was statistically significant compared to the control group (control group: 15%). In the second season, a significant difference was observed, the NT2 and NT4 treatments were 30.09% and 31.24% higher than the negative control. The NT2 and NT4 were reported to have a higher yield than the positive control of 5.19% and 6.12%, respectively. In these two treatments, the yield of the second season was higher than that of the first season, treatment NT2 increased by 4.94%, and treatment NT4 increased by 6.16%. However, treatments NT1, NT3 and NT5 had a decrease in yield. This shows that the ability to adapt to the natural conditions of the bacterial groups is different. This is why the effects of bacteria are slow and long-lasting, so the addition of these groups of bacteria to the soil greatly contributes to the improvement of deteriorated soil, reducing the amount of fertilizer for plants. Therefore, reduce production costs, improve the quality of farming products, and reduce environmental pollution.

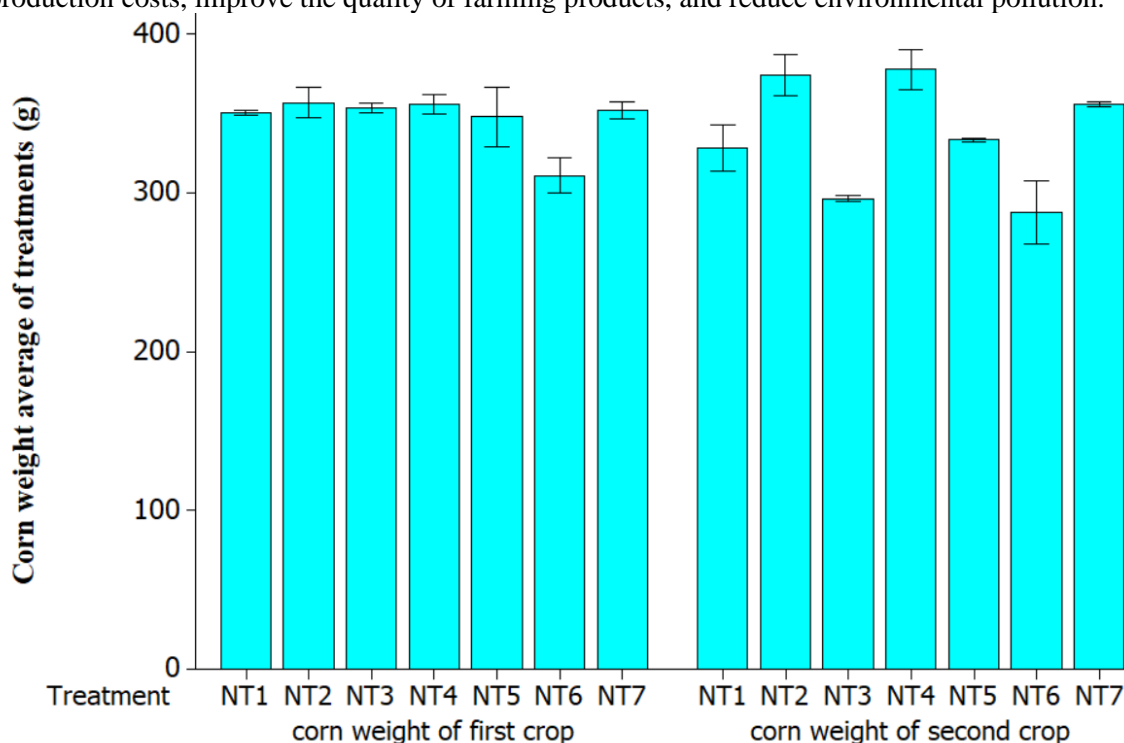


Fig 2. The chart compares the average weight of 15 fruits between the treatments after two crops

From Table 2, in general, the leaf length of the bacterial treatments was not statistically different from that of the positive control. Using bacterial strains contributed to increases in plant height, and leaf length, affecting plant photosynthesis and fruit pollination. Particularly, the height of the stalk also affects the resistance and fall of the tree. When the tree has an outstanding height, it is easy to fall [17]. However, in the experiment in all treatments, the tree height was still at a suitable level, so the fall of trees rarely occurred. This contributes to improving the quality of seeds, strongly affecting the fruit formation process and fruit yield later on.

Table 2. Stem height (cm) and leaf length (cm) over two crops and they were averaged over 15 different corn plants

Treatment	NT1	NT2	NT3	NT4	NT5	NT6	NT7	CV(%)
First crop								
Stem height	177.60 ^a	179.82 ^a	172.22 ^a	191.10 ^a	168.93 ^a	162.96 ^a	176.73 ^a	8.29
Leaf length	76.89 ^{ab}	78.29 ^{ab}	71.67 ^{ab}	85.13 ^a	70.02 ^b	73.29 ^{ab}	70.78 ^b	6.45

Treatment	NT1	NT2	NT3	NT4	NT5	NT6	NT7	CV(%)
Second crop								
Stem height	199.30 ^a	205.80 ^a	188.41 ^b	203.66 ^a	200.53 ^a	182.93 ^b	205.71 ^a	1.81
Leaf length	90.78 ^a	90.53 ^a	87.71 ^{ab}	90.59 ^a	89.62 ^a	83.70 ^b	89.72 ^a	2.14

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

The content of chlorophyll a is an important factor in accelerating the photosynthesis of plants, and this is also a decisive factor for photosynthesis, while chlorophyll b is an active factor to stimulate chlorophyll a activity. Light intensity also greatly affects the photosynthetic capacity of corn plants [13]. Adding bacterial strains increased the efficiency of water absorption and nutrient uptake by plants but did not affect the photosynthetic capacity of plants [18]. From the results of Table 3, in general, the chlorophyll content in the corn leaf samples changed over the two cultivation seasons and there was a difference between the treatments. Adding nitrogen-fixing bacteria *Bradyrhizobium* sp. TTB2.1 and phosphate-solubilizing bacteria *Pseudomonas* sp. BKD2.1, *Pseudomonas* sp. TTB4.4, *Pantoea* sp. TTB4.1 gave results on the content of chlorophyll a, chlorophyll b and total chlorophyll content that were higher than the negative control and the difference was not statistically significant compared with the positive control. In summary, chlorophyll content greatly impacts fruit formation, yield, and postharvest quality.

Table 3. Chlorophyll content in leaf corn (mg/g)

Treatment	NT1	NT2	NT3	NT4	NT5	NT6	NT7	CV(%)
First crop								
Chl a	3.39 ^a	3.31 ^{ab}	3.17 ^b	3.28 ^{ab}	3.32 ^{ab}	2.28 ^c	3.39 ^a	2.21
Chl b	1.93 ^a	1.85 ^{ab}	1.28 ^b	1.45 ^{ab}	1.58 ^{ab}	1.32 ^{ab}	1.87 ^{ab}	14.30
Chl total	5.33 ^a	5.15 ^a	4.45 ^b	4.73 ^{ab}	4.90 ^{ab}	3.60 ^c	5.26 ^a	5.04
Second crop								
Chl a	3.11 ^{ab}	2.95 ^b	2.99 ^{ab}	3.14 ^{ab}	3.14 ^{ab}	2.51 ^c	3.22 ^a	3.15
Chl b	1.24 ^{ab}	1.10 ^b	1.22 ^{ab}	1.35 ^{ab}	1.25 ^{ab}	0.72 ^c	1.39 ^a	8.01
Chl total	4.35 ^{abc}	4.04 ^c	4.21 ^{bc}	4.49 ^{ab}	4.38 ^{abc}	3.22 ^d	4.61 ^a	3.17

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

3.2. Results on the content of nitrogen, soluble phosphorus, and microbial density in the soil

The fertility, ventilation and porosity of the soil greatly depend on the pH, when the pH is in the ideal range of 6.5 to 7.5, the soil is more porous. The nutrient content needed for plants is created in the most optimal way, creating conditions for the development of roots and increasing the plant's ability to absorb nutrients. In addition, if the soil pH value is higher or lower than the response threshold, the plant will become stagnant, and underdeveloped, significantly affecting yield and fruit quality [19].

Table 4. The content of nitrogen (mg/kg), soluble phosphorus (mg/kg) and microbial density in the soil log(CFU/g) before sowing and after 2 plantings

Targets in soil	Before sowing	After second crop	CV%
Total of microbial density	4.04 ^b	5.81 ^a	10.68
Nitrogen-fixing bacteria	4.24 ^b	4.76 ^a	12.91
Phosphate-soluble bacteria	3.97 ^b	4.60 ^a	13.62
pH	6.72 ^a	6.86 ^a	8.39
Nitrogen content	403.59 ^a	423.89 ^a	12.41
Phosphorus content	367.78 ^b	567.26 ^a	8.21

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

According to Cao Ngoc Diep and Tran Minh Thien [16], nitrogen is a very important factor affecting the growth, development process, the yield of corn, especially during the active growth phase of the plant. Nitrogen plays an important role in plant photosynthesis, ensuring the maintenance of chlorophyll content in leaves, and keeping the green color of leaves throughout the period from cotyledon to harvest. Therefore, a lack of nitrogen will show signs of yellowing of leaves, interrupted growth, and stunted plants, leading to a decrease in yield and even the death of plants. The nitrogen content in the soil after the second crop was higher than before sowing. The amount of nitrogen that microorganisms can fix is just enough for plants to absorb for growth and development, the excess amount remaining in the soil is not much, not enough to improve soil fertility. However, the number of nitrogen-fixing bacteria after second crop was higher than the initial one. This may be due to the process of nitrogen produced by bacteria partly being evaporated into the air, partly washed away by watering the plants as well as affected by months of heavy rain. Phosphorus is the main factor determining the flowering, fruiting, ripening of fruits and seeds, helping flowers, large fruits, and firm seeds; limiting the harmful effects of excessive nitrogen fertilization; phosphorus has a buffering effect, making plants tolerant of acidity, alkalinity, etc. Therefore, the supply of phosphorus-soluble microorganisms in the soil plays an extremely important role in the absorption of phosphorus by plants, groups of soil microorganisms help in the release of phosphorus from insoluble complexes into readily absorbable soluble forms [20]. The results of Table 4 shows that the amount of phosphorus remaining in the soil after two cultivation crops is very different from before sowing, the phosphorus content increases to 54.24% compared to the original, this is just the remaining after the plant is absorbed for growth, development and a loss due to the influence of natural factors. Here, it can be seen that the strong effect of phosphate-solubilizing bacteria strains is irrigated into the soil. These bacterial groups have adapted to the cultivation soil, the most obvious manifestation is the significantly increased number of phosphorus-soluble bacteria after 2 cultivation seasons.

4. Conclusion

The nitrogen-fixing bacteria *Bradyrhizobium* sp. TTB2.1 and phosphate-soluble bacteria *Pseudomonas* sp. BKD2.1, *Pseudomonas* sp. TTB4.4 and *Pantoea* sp. TTB4.1 have the ability to fix nitrogen and dissolve phosphorus in the arable soil environment. Of these four strains, *Pseudomonas* sp. BKD2.1 and *Pantoea* sp. TTB4.1 affect the growth, development, and yield of corn more strongly than other strains. Strains of *Bradyrhizobium* sp. TTB2.1 and *Pseudomonas* sp. TTB4.4 have an impact on the growth, development, and yield of corn but not at a high level. Therefore, two strains of *Pseudomonas* sp. BKD2.1, *Pantoea* sp. TTB4.1 have the potential for the production of microbial fertilizers.

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