Current Research in Agricultural Sciences
2024 Vol. 11, No. 2, pp. 56-63
ISSN(e): 2312-6418
ISSN(p): 2313-3716
DOI: 10.18488/cras.v11i2.3945

Enhancing Broccoli growth by *bacillus rhizosphere* bacteria

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Article History
Received: 15 July 2024
Revised: 26 September 2024
Accepted: 7 October 2024
Published: 21 October 2024

Keywords
Bacillus
Broccoli
Growth curves
Nutrients
Phytohormones
Plant growth
Biofertilizer.

ABSTRACT

This study examines the enhancement of broccoli growth by *Bacillus Rhizosphere. Bacteria Broccoli* is a nutrient-rich vegetable known for its high content of polyphenols, flavonoids, vitamins, minerals, fiber, and low calorie count, making it a recommended daily food. Tropical regions grow broccoli in mountainous areas. Sustainable agriculture emphasizes minimizing chemical fertilizers, and biofertilizers like Plant Growth Promoting Rhizobacteria (PGPR), such as *Bacillus*, can reduce their use. *Bacillus* improves plant growth by fixing nitrogen, solubilizing phosphate and potassium, and producing phytohormones. Understanding *Bacillus* growth dynamics is vital for optimizing its agricultural application. This study aimed to analyze growth curves of different *Bacillus* species and investigate their potential role in promoting broccoli growth. This study was carried out in January–April 2024 at the Soil Biology Laboratory, Faculty of Agriculture, Universitas Padjadjaran, and Bumi Agro Technology Company, Lembang. The research was divided into 3 stages: growth curve determination, *Bacillus* liquid inoculant preparation, and application of treatment to plants. The findings showed that isolates of *B. safensis* strain MDL5, *B. altitudinis* strain RPW2, B. subtilis strain YPS4, and *Bacillus* sp. strain SZ057 reached the highest point in their growth curves after 4 and 5 days of incubation. After that, the curves slowly went down. Inoculating *Bacillus* into broccoli plants resulted in an increase in plant height by 43.28–58.75% compared to the control.

Contribution/Originality: The study adds to what is known about using biofertilizers on broccoli in tropical mountainous areas. It shows that adding Bacillus leads to a significant increase in productivity, which in turn leads to plants that are 43.28–58.75% trailer. This underscores Bacillus' potential in reducing chemical fertilizer use and promoting sustainable agriculture.

1. INTRODUCTION

Broccoli is a nutrient-rich vegetable widely cultivated for its health benefits and economic value [1]. In the past decade, broccoli consumption has increased significantly due to awareness of its high content of polyphenols, flavonoids, and fiber, as well as its low calorie count. Broccoli contains high levels of vitamins, antioxidants, and anticarcinogenic compounds; therefore, all food authorities worldwide recommend its daily consumption [2]. Mountainous areas in tropical regions grow broccoli. Fertilization effectively achieves high broccoli production. In sustainable agriculture, it is crucial to minimize the use of chemical fertilizers. Several studies report that the use of biofertilizer can reduce the dose of chemical fertilizer [3-5]. Biofertilizer can be Plant Growth Promoting

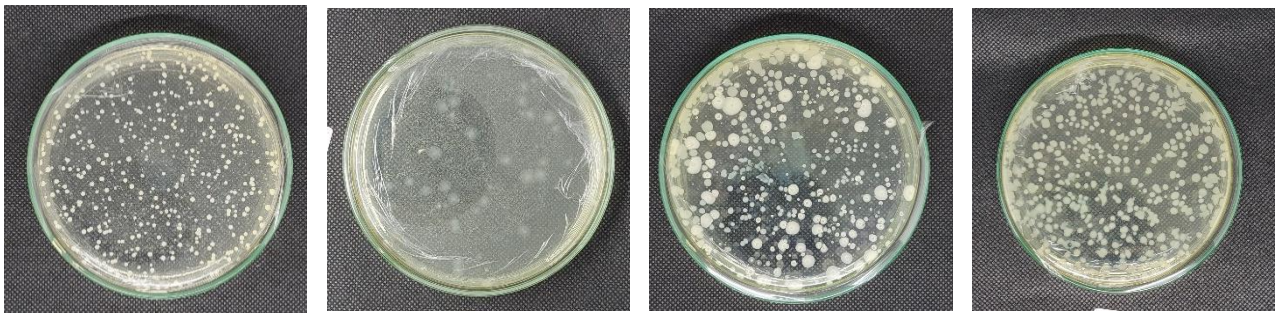
Rhizobacteria (PGPR) inoculant, which can increase plant growth. ne of the prominent PGPRs (Plant Growth Promoting Rhizobacteria) in agriculture is *Bacillus*. *Bacillus* lives in various plant rhizospheres, including food crops. The genus *Bacillus*, comprising a diverse group of Gram-positive, rod-shaped bacteria, has received significant attention in microbiological and agricultural research [6]. *Bacillus* as PGPR can boost plant growth through various mechanisms: nitrogen fixation, phosphate solubilization, potassium solubilization, phytohormone production, and as biocontrol agent [7]. *Bacillus* produces nitrogenase, which catalyzes the conversion of molecular dinitrogen (N2) to ammonia (NH3), allowing plant roots to absorb it. Additionally, *Bacillus* excretes five organic acids, namely acetic, gluconic, succinic, lactic, and propionic acids, that helps solubilize phosphate [8]. *Bacillus* species are renowned for their ability to form endospores. *Bacillus* form spores when conditions are unfavorable for growth. The spores are resistant to heat, cold, radiation, desiccation, and disinfectants [9]. A sporulating bacterium develops these spores inside its mother cell, which breaks apart and releases them into the environment. These spores are highly resistant to various environmental stressors and serve as a survival mechanism [10].

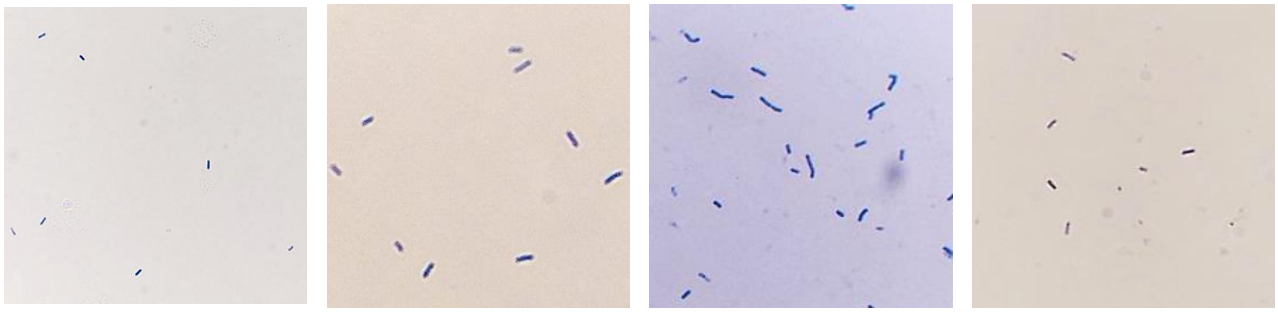
Bacillus is able to produce phytohormones such as auxin, cytokinin, gibberellin, and abscisic acid [11]. Auxin is a hormone that triggers tissue differentiation, cell elongation, and cell division in plants [12]. Gibberellin plays a role in root and shoot elongation, seed germination, flowering, and fruit pattern [13]. Cytokinins play an important role in physiological processes [14]. Abscisic acid plays a role in stomata closure, fruit delivery, and seed germination [15]. Understanding the characteristics and growth dynamics of *Bacillus* is crucial for optimizing their application in agricultural practices. Growth curve analysis helps in determining the optimal conditions for bacterial growth that are essential for maximizing their beneficial effects on plants. Biofertilizer inoculant production requires knowledge of the bacterial growth phases. Mostly bacterial inoculants are collected from the exponential phase, where the bacteria grows rapidly. In Indonesia, broccoli is high-economic-value vegetable crop that is consistently grown using chemical fertilizers. There has been limited research on the use of biofertilizers in high-economic-value vegetable crops in Indonesia. Thus, this study focuses on analyzing the growth curves of different *Bacillus* species and evaluating their essential role in promoting broccoli growth in a pot experiment.

2. MATERIALS AND METHODS

The experiment was carried out in January–April 2024 at the Soil Biology Laboratory, Faculty of Agriculture, Padjadjaran University, and CV. Bumi Agro Technology, Lembang District, West Bandung, West Java. The altitude of this tropical mountainous area was 1.200 m above sea level, and the average temperature during the experiment was 17–29 °C.

The bacterial isolates used in this experiment consisted of *Bacillus* safensis strain MDL5, *Bacillus* altitudinis strain RPW2, *Bacillus* subtilis strain YPS4, and *Bacillus* sp. strain SZ057. The Soil Biology Laboratory at Padjadjaran University provided the four bacterial isloates. Figure 1 shows images of the *Bacillus* isolates used in the experiment.





Bacillus safensis strain MDL5 | *Bacillus altitudinis* strain RPW2 | *Bacillus subtilis* strain YPS4 | *Bacillus* sp. strain SZ057

Figure 1. Bacillus isolates used in the experiment.

2.1. Growth Curve Determination

This stage begins with refreshing the bacterial isolate onto Tryptic Soy Agar (TSA) slant agar media, incubating at 30 °C for 48 hours. To make a liquid culture, inoculate 0.5% starter into new Tryptic Soy Broth (TSB) media and incubate on a shaker. Bacterial liquid culture measured its population, Optical Density (OD), and pH for 7 days, then a curve was made. Bacterial population in TSB media was counted using serial dilution plate method.

2.2. Bacillus Liquid Inoculant Preparation

To make the liquid inoculant for plants, prepare 600 mL of new TSB media for each isolate. Then 0.5% liquid culture was inoculated into new TSB medium and incubated on a shaker at 180 rpm for 72 hours.

2.3. Experimental Design

The experimental design was Randomized Block Design to test four types of *Bacillus* inoculant and one control treatment, including:

A = Control (without *Bacillus* inoculation).

B = *Bacillus safensis* strain MDL5.

C = *Bacillus altitudinis* strain RPW2.

D = *Bacillus subtilis* YPS4.

E = *Bacillus* sp. strain SZ057.

F = Konsorsium *Bacillus*.

Each treatment was replicated 7 times. In control plants, a 100% dose of Nitrogen (N), Phosphate (P), dan Potassium (K) fertilizer was applied, while in plants treated with *Bacillus* incubation, 75% N, 75% P, and 100% K were applied.

2.4. Experimental Setup

The broccoli plants were grown in pots as shown in Figure 2. This experiment used planting media in the form of a mixture of soil and manure in a ratio of 2:1. Planting was carried out by transplanting 4 week-old broccoli seedlings. Bacterial inoculation treatment is given through soil application by spraying 25 mL of bacterial suspension onto the soil three days before planting and a week after planting, respectively. Broccoli was planted for up to 3 weeks. Plant height and number of leaves were measured once a week. Meanwhile, stem diameter was measured 3 weeks after planting. All plant growth data were analyzed using analysis of variance ($p<0.05$). If the treatments had a significant effect on the parameters, the Duncan Multiple Range Test was conducted. IBM SPSS Statistic software performed the analysis.

Figure 2. 3 weeks old broccoli in pot.

3. RESULTS

The four *Bacillus* isolates on TSB media were measured for growth curves, optical density at 600 nm, and media pH for 6 days. T indicates the bacterial culture's incubation time.

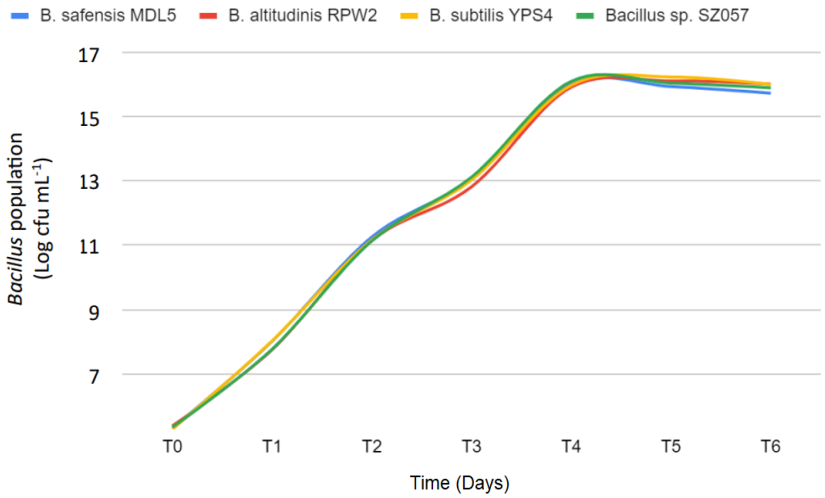


Figure 3. Bacillus growth curve at 0 – 6 days after incubation.

Figure 3 shows that the growth curves of the four *Bacillus* bacterial isolates have almost the same curve pattern. The four bacterial isolates in the lag phase had a population of 105. Isolates of B. safensis MDL5 and *Bacillus* sp. SZ057 reached peak growth at T4, while B. altitudinis RPW2 and B. subtilis YPS4 reached peak growth at T5.

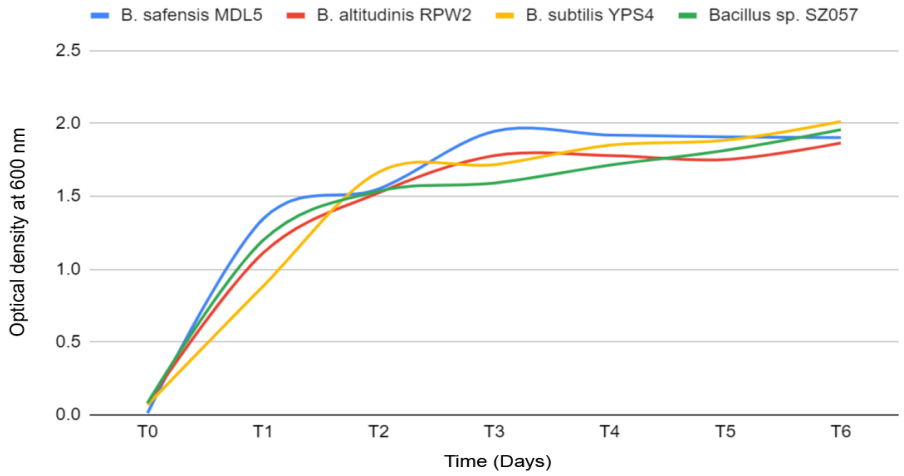


Figure 4. Optical density of bacillus growth curve at 0 – 6 days after incubation.

Figure 4 shows the optical density (OD) measurement of the bacterial growth curve. The OD value on the bacterial growth curve tends to increase over time. The curve experienced a drastic increase at T1 for the four bacterial isolates, then rose slowly thereafter.

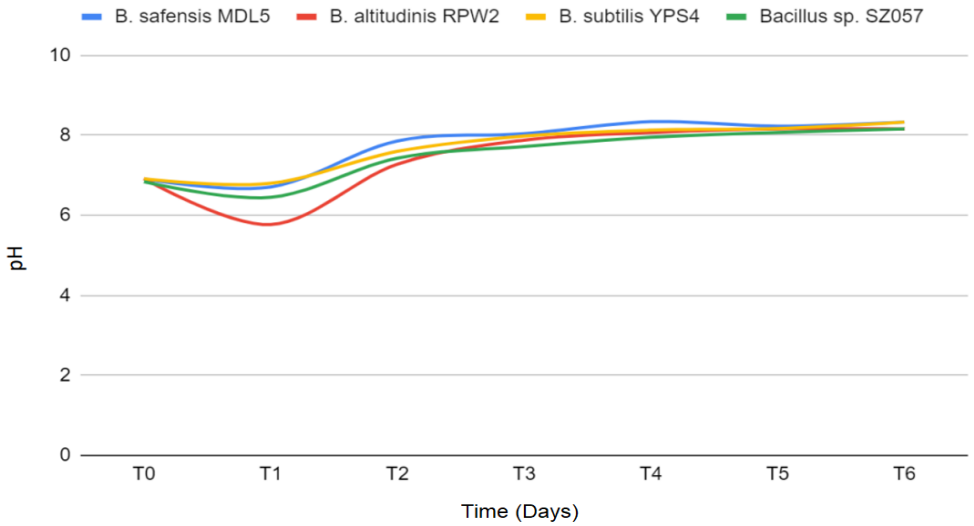


Figure 5. Bacillus pH curve at 0 – 6 days after incubation.

Figure 5 depicts bacterial growth's pH curve. At T0, the four bacterial isolates showed a neutral pH, caused by the pH of the TSB medium, which was also neutral (7.08). At T1, the pH decreased in four bacterial isolates. Then, at T2 onwards, the pH of the four bacterial isolates increased slowly.

Table 1. Height of broccoli plant grown with bacillus inoculation at 1 – 3 weeks after planting (WAP).

Treatment	Plant height (cm)		
	1 WAP	2 WAP	3 WAP
A = Control	6.33 ± 1.53	7.37 ± 1.00 a	13.84 ± 5.65
B = *Bacillus safensis* strain MDL5	7.71 ± 1.05	9.33 ± 2.08 ab	13.64 ± 3.84
C = *Bacillus altitudinis* strain RPW2	7.81 ± 0.77	11.03 ± 3.67 b	13.99 ± 5.66
D = *Bacillus subtilis* strain YPS4	7.79 ± 2.08	10.56 ± 2.75 b	15.73 ± 2.60
E = *Bacillus* sp. strain SZ057	8.57 ± 1.78	9.57 ± 1.52 ab	14.79 ± 4.01
F = *Bacillus* consortium	8.03 ± 1.50	11.70 ± 2.45 b	17.87 ± 5.30

Note: Numbers followed by the same letter are not significantly different according to Duncan's multiple range test at the 5% significance level.

The results of analysis of variance showed that *Bacillus* inoculation had a significant effect on broccoli plant height at 2 WAP (Table 1). *Bacillus* inoculation was able to increase plant height by 43.28–58.75% compared to the control, as shown in the inoculation treatment of *Bacillus* altitudinis strain RPW2, *Bacillus* subtilis strain YPS4, and *Bacillus* consortium.

Table 2. Number of leaves of broccoli plant grown with bacillus inoculation at 1 – 3 weeks after planting (WAP).

Treatment	Number of leaves		
	1 WAP	2 WAP	3 WAP
A = Control	3.29 ± 0.76	4.29 ± 0.49	5.57 ± 1.27
B = *Bacillus safensis* strain MDL5	3.29 ± 0.49	4.14 ± 0.69	5.71 ± 1.11
C = *Bacillus altitudinis* strain RPW2	3.43 ± 0.98	4.43 ± 0.53	5.86 ± 1.21
D = *Bacillus subtilis* strain YPS4	3.14 ± 0.69	4.14 ± 0.38	6.29 ± 1.70
E = *Bacillus* sp. strain SZ057	3.43 ± 0.53	4.43 ± 0.53	6.14 ± 1.46
F = *Bacillus* consortium	3.71 ± 0.95	4.71 ± 0.76	6.57 ± 0.79

Note: Numbers followed by the same letter are not significantly different according to Duncan's multiple range test at the 5% significance level.

Analysis of variance showed that *Bacillus* inoculation had no significant effect on the number of broccoli plant leaves (Table 2). At 1-3 weeks after planting, the number of leaves of plants inoculated with *Bacillus* was almost the same as control plants.

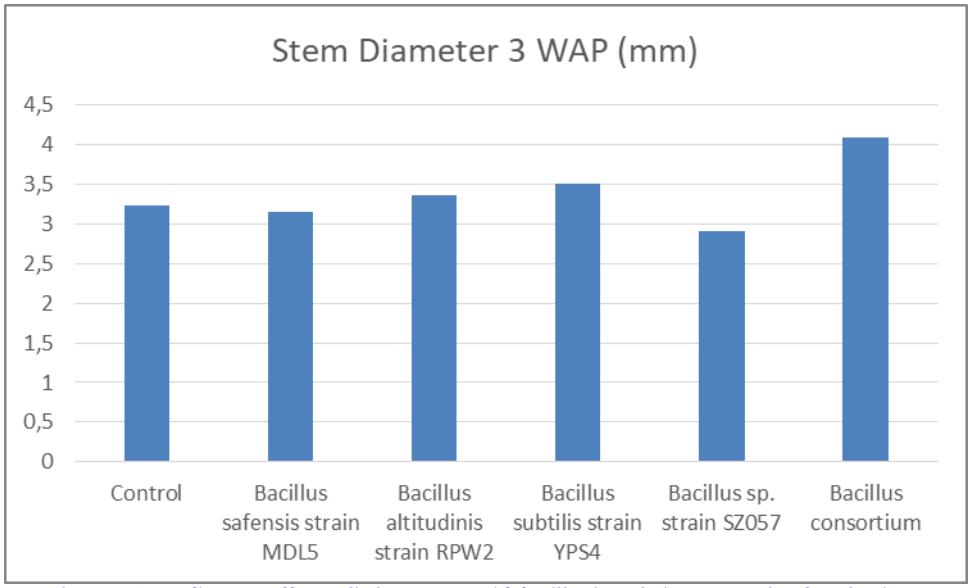


Figure 6. Stem diameter of broccoli plant grown with bacillus inoculation at 3 weeks after planting.

The results of analysis of variance showed that *Bacillus* inoculation had no significant effect on stem diameter at 3 WAP (Figure 6). There was no difference in stem diameter between the *Bacillus* inoculation treatment and the control.

4. DISCUSSION

Bacterial growth curves describe the dynamics of bacterial populations in a specific environment condition. Bacterial growth curves usually consist of several phases that reflect changes in bacterial numbers over time. The bacterial growth curve is divided into four main phases, namely the lag phase, exponential phase, stationary phase, and death phase [16]. The *Bacillus* population in this experiment is known to continue to increase until the exponential phase, then experience constant growth (stationary phase), and slowly decrease towards the death phase. The culture medium directly influences the growth rate, which is slower in nutrient-poor conditions and faster in nutrient-rich environments [17].

Bacillus is able to influence the pH of the growth medium due to several factors: the initial pH and composition of the medium, the growth phase of bacteria, and the physiology and optimum pH of the bacterium [18]. In this experiment, the pH of the medium decreased at T1. This can be caused by metabolites produced by *Bacillus*, such as indole acetic acid and amino acids. Next, the pH of the medium increases slowly. This increase is thought to be related to the carbon source in the media. Bacteria that use citrate carbon sources have the potential to cause the alkalinization of the medium [18].

Bacillus inoculation significantly increased plant height [19, 20] at 2 WAP. The ability of Bacillus to provide plants with nutrients like N and P can explain this increase. Nitrogen is a constituent component of various plant molecules such as amino acids, chlorophyll, nucleic acids, adenosine triphosphate (ATP), and phytohormones [21, 22]. The nutrient P in plants is known to play an important role in cell elongation, cell differentiation, and cell wall thickening [23]. Aside from that, *Bacillus* also produces plant growth hormones. Cytokinins take part in a variety of plant growth processes, including photosynthesis, chloroplast differentiation, cell division, regulation of leaf senescence, nutrient metabolism, and increasing shoot growth [24, 25]. Gibberellin is a hormone that is important for organ elongation and expansion through cell growth [13]. On the other hand, the number of plant leaves and

stem diameter, which do not increase, are allegedly caused by the distribution of nutrients and photosynthate, which is not dominant there.

5. CONCLUSION

The experiments concluded that isolates of B. safensis strain MDL5, B. altitudinis strain RPW2, B. subtilis strain YPS4, and *Bacillus* sp. strain SZ057 reached the peak of the growth curve at T4 and T5, after which they slowly decreased. The pH of the medium increases slowly due to the use of carbon by the *Bacillus*. *Bacillus* inoculation into broccoli plants was able to increase plant height by 43.28–58.75% compared to the control because of its capacity to provide nutrients and produce phytohormones. *Bacillus* inoculation did not significantly increase the number of leaves or stem diameter.

Funding: This research is supported by PT Pupuk Kujang (Grant number: 5200024201).
Institutional Review Board Statement: Not applicable.
Transparency: The authors state that the manuscript is honest, truthful, and transparent, that no key aspects of the investigation have been omitted, and that any differences from the study as planned have been clarified. This study followed all writing ethics.
Competing Interests: The authors declare that they have no competing interests.
Authors' Contributions: All authors contributed equally to the conception and design of the study. All authors have read and agreed to the published version of the manuscript.

REFERENCES

[1] R. U. Syed *et al.*, "Broccoli: A multi-faceted vegetable for health: An in-depth review of its nutritional attributes, antimicrobial abilities, and anti-inflammatory properties," *Antibiotics*, vol. 12, no. 7, p. 1157, 2023. https://doi.org/10.3390/antibiotics12071157

[2] G. S. Nagraj, A. Chouksey, S. Jaiswal, and A. K. Jaiswal, *Broccoli. In Nutritional composition and antioxidant properties of fruits and vegetables*. Dublin: Academic Press, 2020.

[3] B. N. Fitriatin, N. S. Ariani, H. P. Kusumo, and M. R. Setiawati, "The effect of hydrogel-based biofertilizer and p fertilizer on the growth and yield of corn on inceptisols from Jatinangor," *Soilrens*, vol. 20, no. 1, p. 1, 2022. https://doi.org/10.24198/soilrens.v20i1.41350

[4] R. Hindersah, A. R. Syntianis, A. Setiawan, and R. Devnita, "Role of biofertilizer to increase caysim yield, n and p residues and their plant uptake," *Agricultural*, vol. 33, no. 2, pp. 93-102, 2021. https://doi.org/10.24246/agric.2021.v33.i2.p93-102

[5] B. P. Kubheka, M. D. Laing, and K. S. Yobo, "Combinations of a biofertilizer with micro-dosed chemical fertilizers increased yield of maize in a high acid saturated soil," *Rhizosphere*, vol. 13, p. 100189, 2020. https://doi.org/10.1016/j.rhisph.2020.100189

[6] S. C. Parija, *Bacillus. In Textbook of Microbiology and Immunology*. Singapore: Springer Nature Singapore. https://doi.org/10.1007/978-981-19-3315-8_29, 2023.

[7] A. R. Khan *et al.*, "Bacillus spp. as bioagents: Uses and application for sustainable agriculture," *Biology*, vol. 11, no. 12, p. 1763, 2022. https://doi.org/10.3390/biology11121763

[8] A. Saeid, E. Prochownik, and J. Dobrowolska-Iwanek, "Phosphorus solubilization by Bacillus species," *Molecules*, vol. 23, no. 11, p. 2897, 2018. https://doi.org/10.3390/molecules23112897

[9] P. C. B. Turnbull, *Bacillus. In Medical Microbiology*, 4th ed. Galveston: University of Texas Medical Branch, 1996.

[10] P. Setlow, "Germination of spores of Bacillus species: What we know and do not know," *Journal of Bacteriology*, vol. 196, no. 7, pp. 1297-1305, 2014. https://doi.org/10.1128/jb.01455-13

[11] C. K. Odoh, "Plant growth promoting rhizobacteria (PGPR): A bioprotectant bioinoculant for sustainable agrobiology. A review," *International Journal of Advanced Research in Biological Sciences*, vol. 4, no. 5, pp. 123-142, 2017. https://doi.org/10.22192/ijarbs.2017.04.05.014

[12] D. Miljaković, J. Marinković, and S. Balešević-Tubić, "The significance of Bacillus spp. in disease suppression and growth promotion of field and vegetable crops," *Microorganisms*, vol. 8, no. 7, p. 1037, 2020. https://doi.org/10.3390/microorganisms8071037

[13] J. Binenbaum, R. Weinstain, and E. Shani, "Gibberellin localization and transport in plants," *Trends in Plant Science*, vol. 23, no. 5, pp. 410-421, 2018. https://doi.org/10.1016/j.tplants.2018.02.005

[14] W. G. Brenner and T. Schmülling, "Summarizing and exploring data of a decade of cytokinin-related transcriptomics," *Frontiers in Plant Science*, vol. 6, p. 29, 2015. https://doi.org/10.3389/fpls.2015.00029

[15] T. Yoshida *et al.*, "The role of abscisic acid signaling in maintaining the metabolic balance required for Arabidopsis growth under nonstress conditions," *The Plant Cell*, vol. 31, no. 1, pp. 84-105, 2019. https://doi.org/10.1105/tpc.18.00766

[16] R. M. Maier and I. L. Pepper, *Bacterial growth. In Environmental Microbiology*. Elsevier. https://doi.org/10.1016/B978-0-12-394626-3.00003-X, 2015.

[17] P. Pletnev, I. Osterman, P. Sergiev, A. Bogdanov, and O. Dontsova, "Survival guide: Escherichia coli in the stationary phase," *Acta Naturae*, vol. 7, no. 4 (27), pp. 22-33, 2015. https://doi.org/10.32607/20758251-2015-7-4-22-33

[18] R. Sánchez-Clemente, M. I. Igeño, A. G. Población, M. I. Guijo, F. Merchán, and R. Blasco, "Study of pH changes in media during bacterial growth of several environmental strains," presented at the Environment, Green Technology, and Engineering International Conference, Basel Switzerland: MDPI, Oct. 2018, 2018.

[19] B. Li *et al.*, "Bacillus subtilis promotes cucumber growth and quality under higher nutrient solution by altering the Rhizospheric microbial community," *Plants*, vol. 12, no. 2, p. 298, 2023. https://doi.org/10.3390/plants12020298

[20] M. d. C. d. Fonseca *et al.*, "Bacillus subtilis inoculation improves nutrient uptake and physiological activity in sugarcane under drought stress," *Microorganisms*, vol. 10, no. 4, p. 809, 2022. https://doi.org/10.3390/microorganisms10040809

[21] C. R. Frink, P. E. Waggoner, and J. H. Ausubel, "Nitrogen fertilizer: Retrospect and prospect," *Proceedings of the National Academy of Sciences*, vol. 96, no. 4, pp. 1175-1180, 1999. https://doi.org/10.1073/pnas.96.4.1175

[22] N. Crawford and B. Forde, "Molecular and developmental biology of inorganic nitrogen nutrition," *The Arabidopsis Book*, vol. 1, pp. e0011-e0011, 2002. https://doi.org/10.1199/tab.0011

[23] F. Khan *et al.*, "Effect of different levels of nitrogen and phosphorus on the phenology and yield of maize varieties," *American Journal of Plant Sciences*, vol. 5, pp. 2582-2590, 2014. https://doi.org/10.4236/ajps.2014.517272

[24] S.-M. Li, H.-X. Zheng, X.-S. Zhang, and N. Sui, "Cytokinins as central regulators during plant growth and stress response," *Plant Cell Reports*, vol. 40, pp. 271-282, 2021. https://doi.org/10.1007/s00299-020-02612-1

[25] T. Arkhipova, E. Prinsen, S. Veselov, E. Martinenko, A. Melentiev, and G. Kudoyarova, "Cytokinin producing bacteria enhance plant growth in drying soil," *Plant and Soil*, vol. 292, pp. 305-315, 2007. https://doi.org/10.1007/s11104-007-9233-5