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Rhizosphere Bacteria Increased the Height and Root Number of Chili Plants (Capsicum annuum L.)

Nova Anjani[a*], Triastuti Rahayu[b], Yasir Sidiq[c*]

[a] Department of Biology Education, Faculty of Teacher Training and Education, Universitas Muhammadiyah Surakarta, Indonesia
[b] Department of Biology Education, Faculty of Teacher Training and Education, Universitas Muhammadiyah Surakarta, Indonesia
[c] Department of Biology Education, Faculty of Teacher Training and Education, Universitas Muhammadiyah Surakarta, Indonesia

*Corresponding author: Jl. A. Yani Tromol Pos 1, Pabelan, Kartasura, Surakarta, 57162, Jawa Tengah, Indonesia. E-mail addresses: ys120@ums.ac.id

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The need for potential bacteria as plant-promoting agents is still in high demand. Previous reports suggested five potential rhizobacteria isolated from the frangipani plant (Pulmeria accuminata) and producing indole acetic acid (IAA). However, evidence of its potential as a plant growth promoter has not been obtained. Therefore, this study aims to test the effect of rhizosphere bacterial inoculation on chili plants (Capsicum annuum L.). The method was a quantitative experimental method, with 15 repetitions for each treatment. The data were analyzed using a 95% student t-test confidence test or a significant level of 5% (a = 0.05). This inoculation begins with the germination of chili seeds until the radicula is observed. The germinated chili seedlings were soaked in bacterial suspension for an hour at room temperature. The results showed that inoculation using P38 isolate incubated for 6 hours significantly increased plant height and number of roots. The P31 isolate that was incubated for 6 hours inhibited the height of plants and the number of leaves of chili plants. Thus, these results provide important information about the effect of inoculation of newly isolated rhizosphere bacteria.

1. Introduction

The chili plant is one of the crucial crops for food and economic needs so high productivity is needed. One of the problems in increasing chili productivity is the availability of nutrients in the soil. Nitrogen deficiency can cause stunted plant growth, yellowing of leaves, and decreased yields (Achmad, 2018). One way to promote plant growth and increase nitrogen availability for plants is by using free nitrogen-fixing bacteria that live around plant roots called rhizosphere bacteria.

Some rhizosphere microorganisms play a role in nutrient cycles and soil formation processes, plant growth, and as biological controllers against root pathogens (Prayudyaningsih, 2015). In addition, rhizosphere bacteria can affect plants' availability and nutrient cycling by maintaining the stability of soil structure (Susilawati et al., 2016). Rhizosphere bacteria are soil bacteria that approach seeds because they are attracted by root exudate (Santoyo et al., 2016).

Rhizosphere bacteria can influence plant growth and health through various mechanisms, such as nitrogen fixation, phosphate solubilization, hormone production, antagonism to pathogens, and induction of systemic resistance (Kholida & Zulaika, 2015). The activity of rhizosphere bacteria is influenced by the root exudate that plants produce (Chepsergon & Moleleki, 2023). Bacteria in the rhizosphere are called PGPR (Plant Growth Pomoting Rizhobacteria), which is a bacterium that can trigger plant growth by producing growth hormone, dissolving phosphate, and being a biocontrol agent against plant pathogens (Nuraini et al., 2020). PGPR is a root-colonizing bacterium beneficial for plant growth (Cahyaty et al., 2017).

In addition, some facts reported the advantages of rhizosphere bacteria in promoting plant growth. Selected rhizobacteria from a healthy chili plant significantly improve the growth of chili plant (Yanti et al., 2017). Isolated rhizobacteria from capsicum strongly increase the growth and yield of capsicum and the soil health (Gupta et al., 2015; Datta et al., 2011; Gupta et al., 2020; Gou et al., 2020). Besides, rhizobacteria from Curcuma longa benefit the growth of Capsicum annum and suppress the Fusarium wilt (Passari et al., 2018). Several rhizobacteria improved the growth of the chili plant. However, none of the rhizobacteria was isolated from the frangipani plant in the graveyard.

Rhizosphere bacteria can synthesize the growth hormone IAA (natural auxin), which regulates plant growth and development. In addition to producing IAA, rhizosphere bacteria can dissolve phosphates and act as biocontrol agents by inducing the plant's immune system (Marista et al., 2013). IAA is a major member of the auxin group that controls many important physiological processes, including cell enlargement and division, tissue differentiation, and response to light and gravity. IAA produces more lateral roots, root hairs, and root hair branches (Rini et al., 2020). However, the ability of rhizosphere bacteria can vary depending on the biotic and abiotic conditions where the bacteria originate (Lengkong et al., 2022). The presence of certain bacteria that can produce IAA causes an increase in plant growth (A. W. Putra & Advinda, 2022).

Previous research conducted by (S. S. Putra et al., 2023) showed that the isolation of IAA-producing bacteria obtained the results that 5 isolates are proven to have very high IAA content. The five isolates include P26 isolate with an IAA concentration of 9.81 ppm, P31 isolate with a concentration of 28.14 ppm, P36 isolate with a concentration of 12.21ppm, P37 isolate with a concentration of 83.09 ppm, and P38 isolate with a concentration of 65.50 ppm. However, the study's results have not been tested on plant objects. This study aims to test the effect of rhizosphere bacterial inoculation on chili plants using 5 isolates shown to have very high IAA content. This research is expected to obtain information about rhizosphere bacteria isolate that affect the growth rate of chili plants.

2. Method

The employed materials in this study were five isolates of rhizosphere bacteria isolated from frangipani roots with codes P26, P31, P36, P37, and P38. The bacterial isolates were grown on Nutrient Agar (NA) and Nutrient Broth (NB) for culture preservation and inoculation, respectively. Inoculation of plant growth is carried out using chili seeds (Capsicum annum, L.) King Seed Hanna08. Other materials used are 70% alcohol, erlenmeyer, sterile petri dishes, sterile tweezers, sterile aquades, 1% NaOCl, Laminar air flow, test tubes, and plastic polybags.

The starter was prepared with bacterial isolate from frangipani roots P26, P31, P36, P37, and P38. Then take the bacterial isolate with an ose needle from an oblique agar inoculated into a test tube containing NB media as much as 5 mL (aseptic). Do the same with other isolates, then test tubes containing NB media inoculated with isolate are inserted into plastic to make it safer when homogenized using a shaker. Incubate for 6 hours at room temperature with a shaker. Then, use a bacterial suspension of as much as 5% (0.5 mL) in a test tube containing NB 10 mL (aseptic) media. Do the same with all other bacterial isolates. Test tubes containing NB media are inserted into the plastic to make it safer. Incubate for 6 hours at room temperature with a shaker, then store it in the refrigerator.

Chili seeds are soaked with warm water for 5 minutes. The seeds chosen for sowing are drowned seeds (Mustaqimah et al., 2020). The seeds are taken to LAF, then the soaking water is removed, 70% alcohol is added in a ratio of 1: 1, and homogenized for 1 minute. After that, alcohol is removed and rinsed using sterile equates. Added 1% NaOCl solution and allowed to stand for 1 minute. Then, rinse it again with sterile, which equates to 3 times. Sterile chili seeds are stirred into sterile clock bottles, given sterile aquades at a ratio of 1: 1, and allowed to stand at room temperature for up to 24 hours.

Germinated chili seeds for approximately 3 days. This inoculation is done by soaking chili seeds in a bacterial suspension. The trick is to soak the seeds in a solution of microorganisms whose concentration is known (Lopes et al., 2021). The chili peppers that have appeared roots are then transferred to sterile petri dishes with sterile tweezers. Inoculation using chili seeds is placed on a bacterial suspension for 1 hour. The ratio of the bacterial suspension 10 mL: 10 seeds (A. W. Putra & Advinda, 2022). Chili seeds are put into polybags measuring 20x20 cm. Each polybag contains 3 seeds. Hot pepper seeds already planted in pots are watered with water daily. The treatment is stopped after the plant is 28 days old by taking into account plant height (cm), number of leaves (strands), number of roots (total), and root length (cm). Collecting plant growth research data uses quantitative experimental methods with 15 samples per treatment (n=15). Then, the data from observing plant growth parameters were analyzed using the 95% student t-test confidence test or with a significant level of 5% (a = 0.05).

3. Result and Discussion

Germination is the initial stage of seed plant growth, namely embryonic growth, which begins again after water absorption or imbibition. In this stage, the embryo in a dormant condition changes shape and becomes a new plant (Sondang et al., 2020). Germination includes four main processes: imbibition, enzyme system formation, initiation of organ formation, and bud formation until it appears to the soil surface (Steinbrecher & Leubner-Metzger, 2017). The parameters of this study are plant height, number of leaves, root length, and number of roots. The following picture results from the inoculation of rhizosphere bacteria in chili plants 28 days after inoculation.

Based on data from research conducted on chili plants, P31 and P38 isolates inoculated on chili plants showed a significant difference in plant height (Figure 1). P31 isolate inhibits the height growth of chili plants, while P38 increases the height growth of chili plants. Other isolates such as P26, P36, and P37 also increased the height growth of chili plants compared to the control treatment but did not have a significant difference as P38 isolates. Rhizobacteria directly or indirectly contribute to plant growth (Sembiring & Sumanto, 2021). Bacteria in the rhizosphere are called PGPR (*Plant Growth Pomoting Rizhobacteria*), which is a bacterium that can trigger plant growth by producing growth hormone, dissolving phosphate, and being a biocontrol agent against plant pathogens (Nuraini et al., 2020). The concentration of IAA in the P38 isolate (65.50) was higher than that of P31 (28.14) (S. S. Putra et al., 2023).

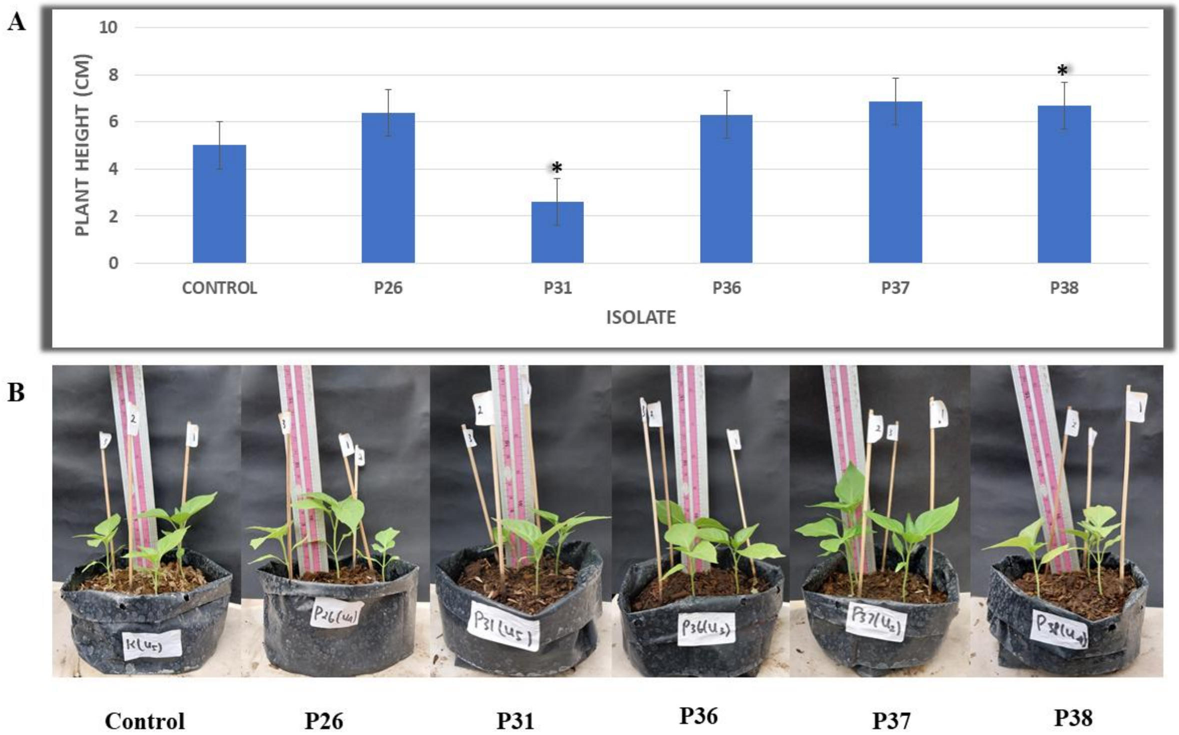

Figure 1. Day 28 chili plant height with inoculation treatment isolates rhizosphere bacteria. (A) The results of the students' t-test showed that treatment with P31 and P38 isolates showed a marked difference in plant height. The bars indicate the standard deviation. The asterisk (*) showed a significant difference compared to the control with a confidence level (α) 0.05. (B) Representative photograph of all treatments

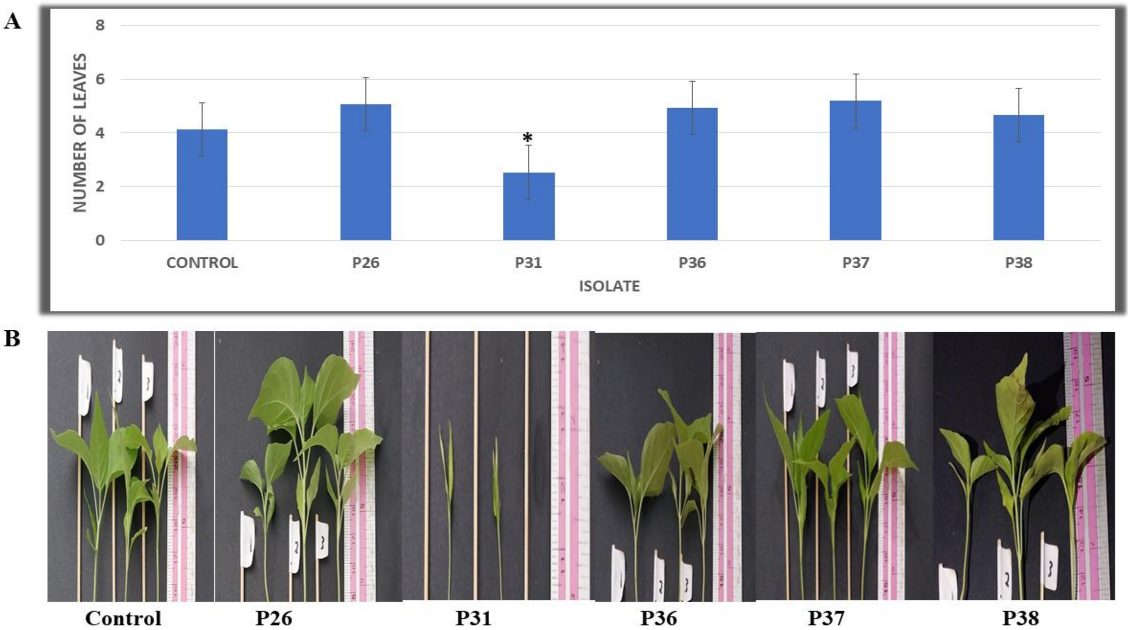

Figure 2. A number of leaves of chili plants on day 28 with inoculation treatment isolate rhizosphere bacteria. (A) The results of the students' t-test showed that treatment with P31 isolate showed a noticeable difference in the number of leaves. The bar chart shows the standard deviation. The asterisk (*) indicates a significant difference compared to the control with a confidence level (α) 0.05. (B) Representative photographs of all treatments

P31 isolate inoculated in chili plants shows a significant difference in the number of leaves (Figure 2). Isolate P31, in addition to inhibiting the growth of the number of leaves, also inhibits the growth of plant height. Lack of nutrient availability can affect the growth of the number of leaves. Sufficient nutrients allow the photosynthesis process to occur optimally, and the resulting assimilate can be used as food reserves; large food reserves allow the formation of many leaves (Darise et al., 2023). Previous research stated that the addition of husk charcoal has a real effect on plant growth and inhibits fungal growth because it functions as organic matter that can improve the physical, chemical, and biological properties of the soil (Djajakirana & Sijabat, 2022). This is in line with (Yulia et al., 2020), where IAA treatment did not affect the number of leaves in their research on Cymbidium orchid plants. An increase also influences the increase in the number of leaves in the rate of photosynthesis, where the rate of photosynthesis will be followed by the results of photosynthate. The results of this photosynthate play a role in the formation of plant structure (Suleman et al., 2019).

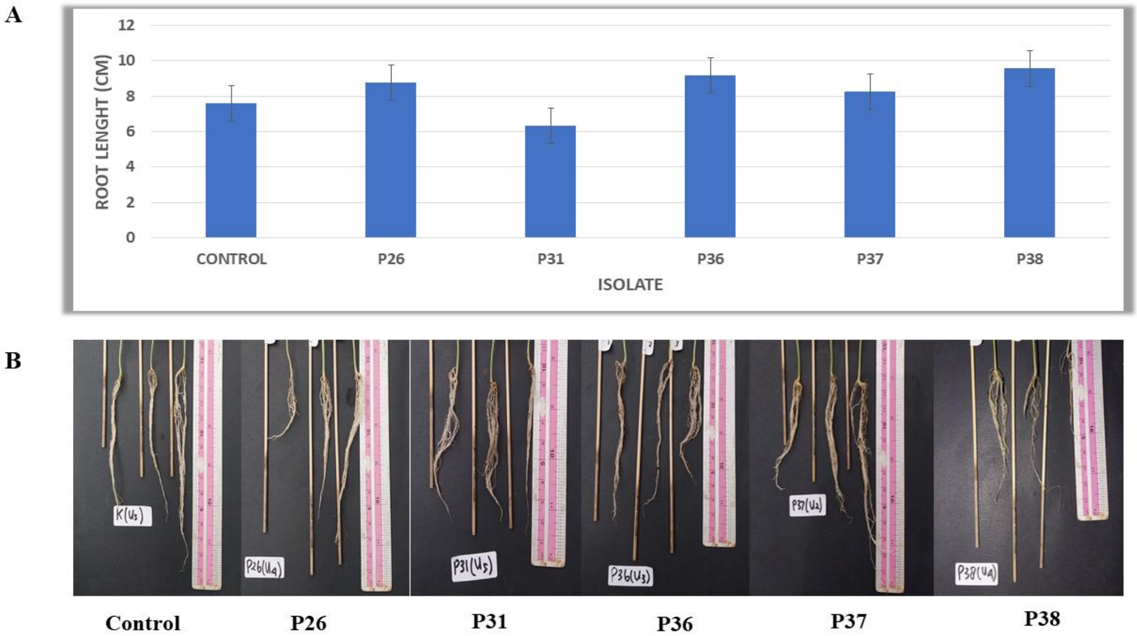

Figure 3. Root lenght of chili plants day 28 with inoculation treatment isolate rhizosphere bacteria. (A) The results of the t-test showed that treatment with P26, P31, P36, P37, and P38 isolates showed no noticeable difference in root length

Five isolates of rhizosphere bacteria inoculated in chili plants did not provide significant differences markedly (Figure 3). This is in line with (Saridewi et al., 2020), the application of bacteria that can produce IAA does not affect the length of eggplant plants, allegedly because the influence of the volume of plant media allows limited nutrients that plant roots and space for movement of plants can absorb. Low IAA concentrations cause the elongation of roots and shoots; if the concentration of IAA is higher, then the elongation of shoots and roots becomes inhibited (Herlina et al., 2016). Giving IAA-producing isolate to the length of the sprouted root has no effect; this is because other hormones, such as ethylene, in the roots produced in large quantities can inhibit root development (Suwarni & Advinda, 2021). One of the mechanisms of plant growth is the production of the hormone IAA by microorganisms (Ismawanti et al., 2022). The metabolic process in the plant body occurs due to the utilization of IAA produced by bacteria, thus helping the growth process of height, stem diameter, number of leaves, and area of plant seeds (Puspita et al., 2019).

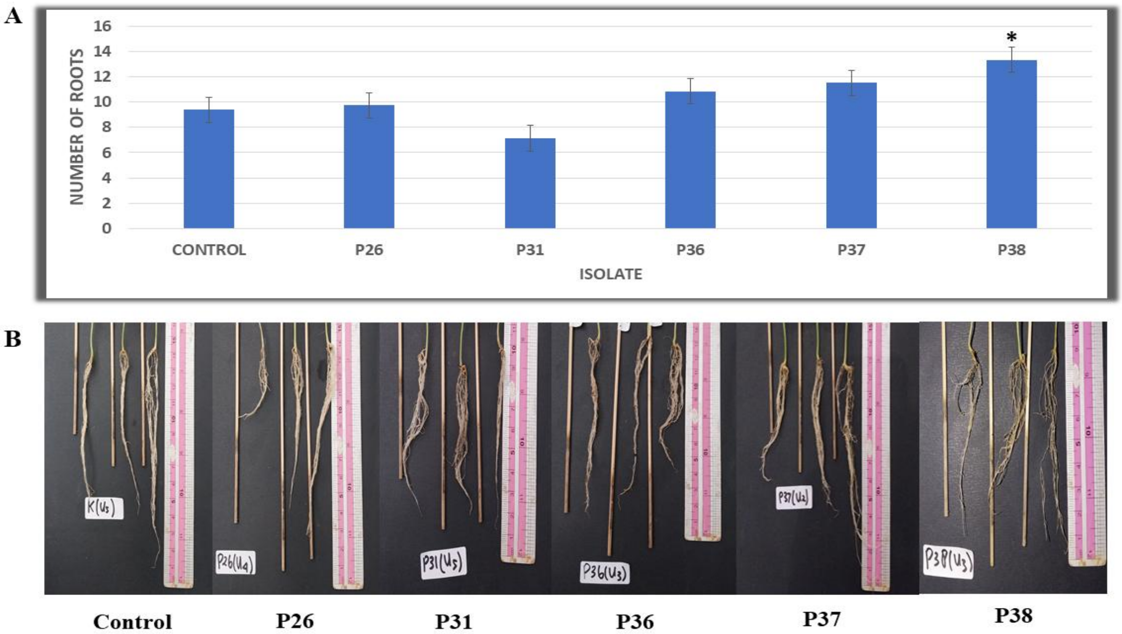

Figure 4. The number of roots of chili plants on day 28 with inoculation treatment isolates rhizosphere bacteria. (A) The results of the students' t-test showed that treatment with P38 isolate showed a noticeable difference in the number of leaves. The bar chart shows the standard deviation. The asterisk (*) indicates a significant difference compared to the control with a confidence level (α) of 0.05. (B) Representative photographs of all treatments

Chili plants inoculated with P38 isolate significantly affected root growth (Figure 4). IAA administration affects the number of lateral roots but does not affect the length of sprouts. This IAA content will affect the length of the roots, the surface area of the roots, and the number of root tips. Low IAA concentrations cause the elongation of roots and shoots; if the concentration of IAA is higher, the elongation of shoots and roots becomes inhibited (Herlina et al., 2016). Auxin hormone is more important in forming plant roots (Yulia et al., 2020)

4. Conclusion

Among five potential bacterial isolates, the P38 isolate can significantly increase the height of chili plants and the number of chili plant roots. Meanwhile, isolate P31 inhibits plant height and the number of chili plant leaves. The findings support evidence of the potential of bacteria from frangipani. Isolate P38 appears to have good potential in increasing the growth of chili plants in terms of plant height and number of roots. Therefore, isolate P38 can be used as a biological agent to increase the productivity of chili plants. In contrast, isolate P31 showed an inhibitory effect on plant height and leaf number of chili plants. Therefore, this isolate should be avoided for use on chili plants or needs to be studied further to understand its inhibitory mechanism and potential use on other plants.

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