Endophytic Bacterial Isolates Improve the Growth of Chili Seedlings (*Capsicum annum*) as A Support towards Sustainable Agriculture

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Abstract. Several studies have shown the role of endophytic bacteria in enhancing plant growth. This is partly because these bacteria produce Indole Acetic Acid (IAA). Previous research had isolated endophytic bacteria encoded by K2 and K8, which produced IAA of 73.50 and 59.5 ppm respectively. These isolates have been inoculated into the rice and maize (monocot) plants and tomato (dicot) plants. Interestingly, K2 and K8 improve the growth of tomato plants but not rice and maize plants. Therefore, this experiment aimed to determine the potential benefits of endophytic bacteria to support the expansion of chili (Capsicum annum) seedlings which are dicot plants. This research is an experimental study following a 2-factor completely randomized design. Endophytic bacterial inoculation uses the root inoculation method. Chili seeds are soaked for 30 minutes with endophytic bacteria isolated and then sowing in sterile soil media. The measured parameters in this study were plant height, roots number, leaves count, root length and fresh weight. The data were evaluated at a five percent significance level of t-test. This study found that endophytic bacteria isolates K2 and K8 were proven to significantly improve chili seedlings' growths compared to the control. The endophytic bacteria K2 isolate significantly increased plant height, while the endophyte K8 isolate significantly increased plant height, leaves, and root growths. This research contributes to information about the potential of endophytic bacteria to improve the dicotyl plants such as chili plants.

1 Introduction

Sustainable agriculture guarantees adequate food production while minimizing adverse environmental impacts. Because chemical fertilizer is often associated with a decrease in soil health (Abebe et al., 2022), limiting the use of chemical fertilizer can help achieve sustainable agriculture. According to Tayade et al. (2024), biofertilizer can help keep the plants' production and soil healthy. A study that lasted more than forty years found that endophytic bacterial species may serve as a beneficial trait for the biofertilizer creation (Zhao et al. 2024).

Endophytic bacteria inhabit the internal tissues of plants without adversely affecting their growth or productivity.

Endophytic bacteria contribute significantly to plant growth promotion through the production of various phytohormones, one of the most crucial being Indole acetic acid (IAA), a primary auxin (Raimi & Adeleke, 2023). IAA is regarded as one of the most physiologically active growth regulators, ruling a central factor in plant growth. Its involvement in cellular processes is extensive and multifaceted. Additionally, IAA increases the permeability of the plasma membrane to water, which is vital for maintaining cell turgor pressure and overall plant hydration (Zhao, 2010). IAA also plays a critical role in maintaining cell wall integrity by reducing mechanical stress and promoting the synthesis of cell wall components, which are essential for maintaining structural rigidity during growth (Zhao, 2010). Furthermore, it regulates the production of specific proteins that contribute to the cell wall's remodeling and strengthening (Bashki et al., 2021). By mediating these processes, IAA aids in optimizing plant responses to environmental stimuli, such as drought and nutrient availability, thus contributing to improved overall plant health and productivity.

A prior investigation isolated 21 endophytic bacteria from Klutuk banana plants. Subsequently, two isolates were chosen for their elevated IAA content: K2 and K8, which exhibited IAA levels of 73.5 and 59.5 ppm, respectively (Rahayu et al., 2023). The K2 and K8 isolates were inoculated into the roots of rice plants (Oryza sativa), but no growth improvement was observed (Annisa et al., 2024). Conversely, these two isolates markedly enhanced the tomato plant's growth (Solanum lycopersicum) (Sidiq et al., 2024). Previous studies indicate that K2 and K8 isolates have the potential to enhance dicotyl plants, while their effects on monocot plants are not observed. This study sought to investigate the ability of endophytic bacteria to increase the chili plants' (Capsicum annum) growth, a species classified within dicotyledonous plants. This study demonstrated the potential of K2 and K8 isolates to enhance the growth of dicotyledonous plants, given that specific endophytic bacteria are compatible only with certain plant species.

2 Methodology

2.1 Materials

This experiment involved two strains of endophytic bacteria, which were separated from Klutuk banana roots (Rahayu et al., 2024). The bacterial strains were labeled K2 and K8. To cultivate the bacteria, Nutrient Agar (NA) and Nutrient Broth (NB) were employed. Chili plants (Capsicum annuum) were used as the experimental subjects, and the seeds were procured from a local market.

2.2 Bacterial suspension

To prepare the bacterial starter culture, the isolates K2 and K8 from the Klutuk banana roots were transferred aseptically into tubes containing 6 mL of NB medium. For the other isolates, the NB medium was also placed in tubes and agitated. The tubes were kept warm for six hours with 100 rpm speed of agitation. A bacterial suspension was prepared by adding 5% volume of the culture into ten mL of fresh NB medium. The procedures were duplicated for the remaining isolate. After incubation on the shaker, the cultures were stored in a refrigerator. The optical density (OD) at 600 nm was measured using a spectrophotometer.

2.3 Sterilization of chili seeds's surface

Chili seeds were immersed in warm sterile-water for 6 hours. Seeds that sank during this time were selected for planting, following the method outlined by Sidiq et al. (2024). After soaking, the seeds were transferred to a laminar flow cabinet, where the soaking water was discarded. A 70% ethanol solution, prepared in a 1:1 ratio, was used to disinfect the seed surface, and the mixture was agitated for one minute. Following this, the ethanol was rinsed and the seeds were cleaned with sterilized water. A 1% NaOCl solution was applied for one minute before transferring the seeds to a fresh container. We rinsed the seeds 3x using sterilized water, one minute agitation in a sterile bench. They were then soaked in a sterile jar containing sterile aquades and kept at room temperature for 6 hours (Annisa et al., 2024). The soaked seeds then incubated on the moist paper towel for three days.

2.4 Seedling preparations and bacterial inoculation into the seedling roots

The chili seeds were first soaked in water for 6 hours, then put on a sterile dish lined with a moist paper towel for 3 days to allow germination. Once roots began to develop, the seedlings were carefully moved to new cleaned dishes using sterilized tweezers. To inoculate the seeds, they were immersed in a bacterial suspension with an OD of 0.8 for 30 minutes, with a ratio between bacterial suspension and the seeds were 10 mL per 10 seeds, respectively (Annisa et al., 2024). This ensured a consistent number of bacterial cells across all inoculations. After inoculation, the seeds were planted in sterile soil within plastic polybags, with three seedlings per pot. Each pot was watered daily with 150 mL of water. The experiment continued until the plants reached 28 days after planting (dap). Twelve replicates were included in each treatment.

2.5 Data collection

Observation of plant growth parameters began at 28 dap, focusing on five variables: plant height (cm), leaf count, root length (cm), root count, and fresh weight (gram). The seedlings height was measured from the base of the stem to tips of the newly grown leaves. The leaf count considered all leaves present on the plant at 28 dap. The total number of root branches was counted to determine the root number. Root length was measured by washing the roots thoroughly with water to avoid losing any roots. The longest root from each sample was then measured. We examined the data using t-test, with a five percent significance level.

3 Results and Discussion

This present experiment inoculated an equal quantity of two endophytic bacteria into the roots of chili seedlings. Following the inoculation, the growth indicators of chili were assessed at 28 days after plantation. This study conducted t-test of the data, revealing the influence of root inoculation on the chili seedlings. The findings of this experiment indicate that the K2 and K8 isolates have a beneficial effect on four out of five parameters related to plant growth. The chili plant seedling showed different growth indicators including the plant height, leaves number, root length and numbers. It can be seen in Figure 1 as a representative photograph. It indicated a significant difference on those mentioned growth parameters.

a b c

Figure 1. A representative photograph of inoculated chili seedlings compared to the control, uninoculated plants. (a) uninoculated plants, (b) chili seedling with K2 isolate inoculation, while (c) the seedling with K8 isolate inoculation.

Correspondingly, statistical analysis with 12 replicates of experiment samples indicated the significant improvement on the plant height, leaves number, root length, and root numbers (Figure 2A, 2B, 2D, and 2E). Even though the fresh weight was not statistically different between inoculated plant and the control, the slight improvement was observed (Figure 2 C).

The observation that K2 and K8 isolates enhance plant growth appears to be species-specific, as evidenced by contrasting results from different studies. Root inoculation with these isolates did not lead to improved growth parameters in rice (Oryza sativa), as reported by Annisa et al. (2024), suggesting that the beneficial effects of K2 and K8 may not extend to monocot species such as rice. On the other hand, a study by Sidiq et al. (2024) demonstrated that these same isolates significantly promoted the growth of tomato plants (Solanum lycopersicum), a dicotyledonous species. This discrepancy between monocot and dicot species highlights the potential for differential responses to endophytic bacteria based on plant physiology, where certain plant taxa may harbor a more favorable environment for symbiotic relationships with specific bacterial strains. Supporting the aforementioned studies, the current work provides further evidence that K2 and K8 isolates might exhibit a marked preference for enhancing the growth of dicotyledonous plants. This aligns with previous research indicating that endophytic bacteria often display host-specific interactions, which are influenced by factors such as plant tissue compatibility, root exudates, and the presence of specific plant growth regulators (Alori et al., 2017; Schenke et al., 2019).

Both K2 and K8 isolates have been shown to produce high levels IAA, a well-known plant hormone that regulates cell growth and elongation (Spaepen et al., 2014). While IAA production is a common trait among plant-growth-promoting bacteria, observed beneficial effects of these isolates on plant growth seem to be contingent on the plant's inherent response to IAA and the compatibility of the bacterial strains with the host plant's cellular mechanisms. This specificity could suggest that while the isolates possess the capacity to produce growth-promoting substances, their influence on plant's growth is environment-dependent, varying with plant species and potentially other ecological features (Van Der Heijden et al., 2015).

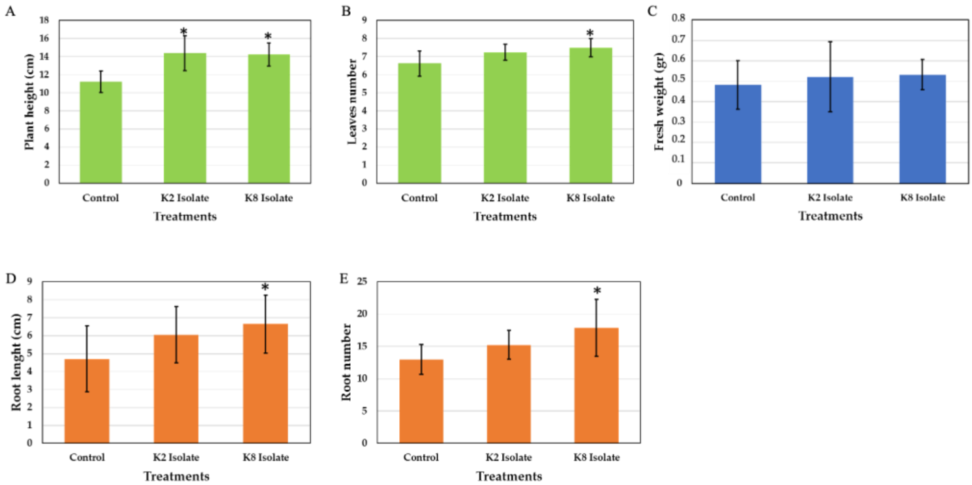

Figure 2. The result of endophytic bacterial inoculation on plant height, (B) leaf number (C) fresh weight, (D) root length, and (E) root number at 28 dap. The asterisks (*) indicate a significant difference as tested by t-test. The bars illustrate variability recorded across 12 replications.

In summary, although both K2 and K8 isolates exhibit high IAA production (Rahayu et al., 2024), their beneficial effects on plant growth appear to be tailored to specific plant species, likely favoring dicotyledons over monocotyledons. This specificity underscores the need for further research to understand the molecular and ecological mechanisms driving the plant-bacteria interactions to optimize the utilization of these isolates in farming applications.

4 Conclusions

The endophytic bacteria isolates K2 and K8 were shown to significantly promote the growth of chili seedlings compared to control treatments. Specifically, the K2 isolate had a notable effect on enhancing plant height, while the K8 isolate demonstrated broader growth-promoting capabilities, significantly improving plant height, leaves number, root length and number. These findings support the hypothesis that K2 and K8 isolates specifically enhance the growth of dicotyledonous plants, such as chili, suggesting that the beneficial effects of these isolates may be linked to their compatibility with dicot species. These findings contribute valuable insights into the benefits of endophytic bacteria on plant growth promotion and also highlight their potential applications in sustainable agricultural practices such as biofertilizer development, particularly for dicot horticulture.

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