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Assessing plant growth and infection in *Avicennia marina* (Forssk.) Vierh following inoculation with bacterial endophytes

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ABSTRACT. Endophytic bacteria play an important role for plants in assisting the efficient absorption of nutrients from the surrounding environment. Studies on endophytic bacteria in *Avicennia marina* plants are still relatively rare**.** This research aims to determine abilities of three bacterial endophytes and a control *Bacillus subtilis* infecting the host plant *A. marina* and conducted in ekowisata mangrove Gunung Anyar, Surabaya. Each endophyte isolates at the concentration of 10^2, 10^3, and 10^4 CFU/mL were used for inoculation, whereas each concentration was applied to 3 plants (triplicate). Every ± 1 m tall *A. marina* plant was injected with 2 × 500 µL of bacterial solution. Another three plants were chosen and injected with Lactose-Broth (LB) sterile medium. The leaf length and width of 2-4 leaves per plant were measured at day 0 before inoculation and after two weeks of inoculation. Data analysis using paired t-test resulted in no change in leaf area average before and after treatments. Despite the result, we observed that some of the treated plants produced flowers, whereas no flower was observed on control plants. In addition, there were infection zones detected around the infection area. Furthermore, we have observed Indole Acetic Acid (IAA) and siderophore production in all bacterial isolates. The results of this study show that acceleration in the development of *A. marina*, evidenced by a phase shift from vegetative to generative occurring within a two-week timeframe.

Keywords: *Avicennia marina*; *Bacillus* spp.; endophytic bacteria; IAA; siderophore

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INTRODUCTION

Endophyte encompasses all microorganisms residing within plant tissues without causing harm, including bacteria, fungi, actinomycetes, and mycoplasma (Strobel, 2018; Anyasi & Atagana, 2019). Endophyte roles are diverse, especially in the agricultural world, such as regulating the plant immunity system (Dini-Andreote, 2020) by producing compounds (Gouda *et al.*, 2016) and subsequently improving the yield (Harman & Uphoff, 2019). Mangrove plants have a huge benefit to maintaining the estuary's ecosystem. The mangrove plants could help ward off the tides and mitigate the tsunami effects in severe cases. The roots of the mangroves could preserve the soil from seawater abrasion. Other than that, the mangrove plants' root systems are unique, functioning as 'a home' for various types of estuary flora and fauna. *Avicennia marina* is a mangrove plant that lives at the Kebun Raya Mangrove Surabaya, which is also found in almost every coastal area in Indonesia. Besides their protection function for the estuaries, there are several benefits of *A. marina*, e.g., food material for humans, animal food, food preservative, medicine, and firewood (Halidah, 2014). Due to its many benefits, some sources have informed that overexploitation has occurred to this plant (Huang *et al.*, 2014; Haris & Kusmana, 2019). Critically endangered plants, often harboring unique endophytic bacteria with potential biotechnological and medicinal applications, face the additional threat of losing these diverse microbial communities due to overexploitation (West *et al.*, 2019; Philippot *et al.*, 2021).

There are various advantages to investigating endophytic bacteria and their potential benefits for *Avicennia marina*, for example, increasing stress tolerance in *A. marina* and other plants such as tomatoes (Ali *et al.*, 2017). Another advantage is that it improves plant growth and survival

(Omomowo & Babalola, 2019), influences physiological and morphological aspects (Tripathi *et al.*, 2022), and increases disease resistance (Husnain *et al.*, 2023). Our previous study showed the association of endophytic bacteria in *A. marina* (Savitri *et al.*, 2023). Three samples of bacterial endophytes have been isolated from mangrove plants *A. marina*. The biochemical tests showed that all three isolates belong to the genus *Bacillus*. Furthermore, the 16S rDNA sequence analysis showed that one of the three isolates was defined as *Bacillus* sp. (98 % similarity), while others were described as *Bacillus subtilis* (98-99 % similarity) (Savitri *et al.*, 2023).

Bacillus species comprises siderophore producers (Kesaulya *et al.*, 2018; Ghazy & El-Nahrawy, 2021). These substances are low molecular weight compounds that can chelate iron (Fe^{3+}) and are responsive to bacterial cell defense under an iron-limited environment (Ferreira *et al.*, 2019). There are two types of well-described siderophore, catecholate and hydroxamate (Prihatiningsih *et al.*, 2017). Siderophore-producing bacteria can transport iron-siderophore complexes with the help of specific proteins, thereby increasing the concentration of iron in the soil absorbed by plants (Maheshwari *et al.*, 2019). Iron is one of the essential elements in activating enzymes that support resistance plants against pathogens (Rizzi *et al.*, 2019). Thus, siderophore production in bacteria provides the advantage of promoting bacterial colonization and ecological competition to eliminate other microorganisms (Khan *et al.*, 2016). Furthermore, it has been known that *Bacillus* species are also good indole-3-acetic acid (IAA) producers (Bhutani *et al.*, 2018). In general, IAA production is one of the characteristics associated with endophytic bacteria (Turbat *et al.*, 2020). IAA is one of the phytohormones that plays an essential role in plant growth and development (Khan *et al.*, 2016) In addition, IAA also plays an important role as a directing molecule involved in plant and microbial interactions (Brígido *et al.*, 2019). Endophytic bacteria in host plants showed positive results in increasing plant biomass and root growth (Chen *et al.*, 2018). The concentration of IAA produced by various endophytic bacteria varies significantly, depending on their interaction with the host plant (Jasim *et al.*, 2014). Hence, a study investigating the production of siderophores, and IAA is useful to indicate the role of endophytic *Bacillus* in the plant's growth.

Recently, there have been few extensive investigations on the effects of endophytic bacteria on the development and health in many of the host plants (Choudhary *et al.*, 2023), including *Avicennia marina*. Thus, the research aimed to investigate the infection effect of endophytic bacteria on *A. marina*'s plant stem through the plant host's growth and development. This study will be helpful to describe the infection's effects on plant growth. The benefit of this research is to give information about the bacterial endophytes isolated from the Indonesian estuary that could contribute to the world's biodiversity. Other than that, this research is helpful to learn the effect of endophytic bacteria on *A. marina*'s physiological function. In the future, the result of the experiments will be beneficial as a reference to understand the impact of bacterial endophytes application in various valuable crops plants. However, growing *Avicennia marina* in a laboratory environment (greenhouse) is a particular challenge, due to the specific requirements for salinity, water content, light intensity, and environmental humidity (Sulaiman *et al.*, 2018; Budiadi *et al.*, 2022). Therefore, the endophyte inoculation treatment in this study was carried out in its natural habitat, to obtain preliminary data regarding the effect of endophytes on the growth of *A. marina*. The data obtained will be useful for further studies regarding the effects of endophytes on *A. marina* in a controlled environment.

MATERIALS AND METHODS

Study area. Mangrove plants *Avicennia marina* as host plants were located at the Kebun Raya Mangrove Surabaya (Fig. 1). The gold color indicates the location of the mangrove plants used as samples at the Kebun Raya Mangrove Surabaya. The green color indicates the area of Kebun Raya Mangrove Surabaya which is located at the southeast part of Surabaya.

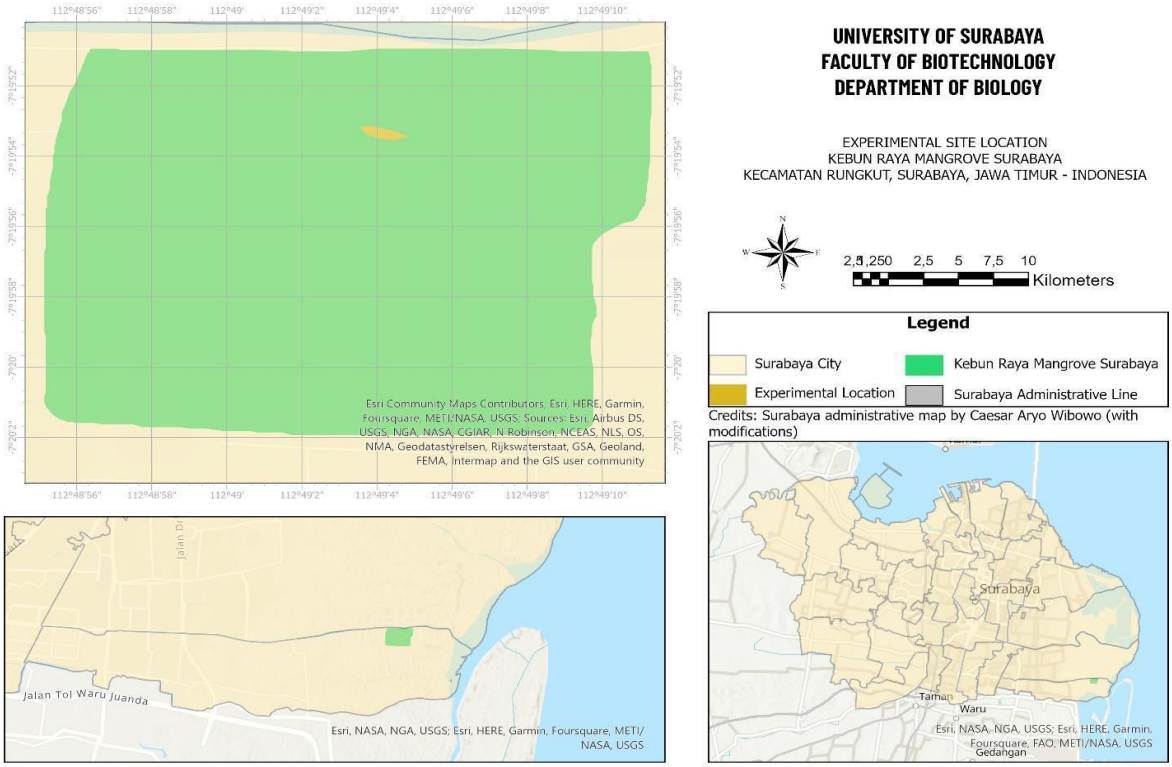

Fig. 1. The experimental site location at the Kebun Raya Mangrove Surabaya

Isolates preparation for inoculation. Our previous study (Savitri *et al.*, 2023) obtained three endophytic bacteria isolated from leaf tissue of *Avicennia marina*, originating from Kebun Raya Mangrove Surabaya, East Java, Indonesia. Based on molecular detection using 16S rDNA, the endophytic bacteria strains have the following identities: *Bacillus subtilis* YRL02 (A); *Bacillus subtilis* HYM07 (B); and *Bacillus* sp. (C). Furthermore, *Bacillus subtilis* (Laboratory of Microorganism Biotechnology isolate) was used as a control strain (K). Each culture was taken from glycerol stock, then inoculated on 100 mL Lactose Broth (LB) (Merck®/catalog no. 70142) and incubated at 37°C for 20-24 h. Subsequently, based on the number of bacteria using the total plate count (TPC) technique, the three endophytic bacterial strains and control strain to be inoculated in the infection test were 10^2, 10^3, and 10^4 CFU/mL, with a volume of 100 mL in each *Avicennia marina* test plant.

Plant materials. As many as 39 *A. marina* plants were chosen as samples. The selected plants were ± 1 meter tall from the soil surface. These samples were naturally grown at the Kebun Raya Mangrove Surabaya, East Java, Indonesia.

Inoculation. Two kinds of drill tools were prepared for the inoculation, manual drill (Hss Twist Drill®) tool (diameter of 1 and 1.2 mm) and battery-driven drill (Fisch and Fisch®) tool (diameter of 1.5 mm). Two holes were made for each plant for the inoculation. The total inoculation volume for each plant was 1 mL. The inoculations were done aseptically, using 70% ethanol solution, which was then sprayed to clean the stem area and drill bits. In addition, a gas lighter was used to provide clean air. Also, sterile 0.5 mL disposable syringes (OneMed®) were used to transfer the bacterial solution.

Indole Acetic Acid (IAA) detection. One inoculum loop of the pure isolate was grown on Nutrient Broth (NB) (Merck®/ catalog no. 70122). Then it was incubated for 24 h in an orbital shaker incubator (New Brunswick Innova® 40, Eppendorf®) at a speed of 100 rpm and a temperature of 37 °C. Next, 1 mL of the isolate was transferred to NB + Tryptophan medium (Merck®/catalog no. T2610000) and incubated again for 24 h. After incubation, 10 mL of the microbial solution was added to a 15 mL graduated centrifuge tube (Vitlab®) and centrifuged (HERMLE Z 326 K, HERMLE Labortechnik®) at 5000 rpm for 20 min. Measurement of IAA concentration was carried out by

reacting 1 mL of the supernatant and 4 mL of Salkowski's reagent in the dark for 15 min. Then the Optical Density (OD) was observed using a Genesys 10 UV/Vis spectrophotometer (Thermo Fisher Scientific®) at 530 nm. The concentration of IAA was measured by comparing the results with a standard curve made using standard IAA solutions at different concentrations of 10, 20, 30, 40, and 50 ppm (Gordon & Weber, 1951; Fatma *et al.*, 2014).

Siderophore detection. Modified King B media (Merck®/ catalog no. 60786) was used for the siderophore production test. This test was carried out by inoculating bacterial cultures into Tryptone Soya Broth (TSB) media (Merck®/ catalog no. 22092), then incubated at 25°C for 1-2 days, continued with OD checking. Ten mL of the concentrated culture was then taken and streaked on modified King B media. Afterward, they were incubated for 2-3 days at 25°C. A positive result is indicated by the formation of an orange zone around the bacterial colony (Sukweenadhi *et al.*, 2019).

Data collection. Data was collected before and two weeks after the inoculation. Typically, endophyte colonization in host plants was studied for seven to thirty days (Posthangbam *et al.*, 2017). The data collected included the average length and width of leaves, the morphological change on treated and control plants, as well as the flowering occurrence in each sample plant. Leaves' length and width were measured by using a digital caliper (Taffware®). For leaf length, the measurement was from the leaf base to the apex. Leaf width was measured at the widest part of the leaf.

Data analysis. Paired sample t-test was used to analyze data of leaves length and width before and after treatment. The analysis was performed by using IBM SPSS ver. 26.

RESULTS AND DISCUSSION

Leaves length and width. Length and width of leaves before and after treatment were measured in treated plants and negative control plants. Each treatment consisted of 3 plants. Paired t-test analysis was conducted to treated plants (K, A, B, and C) and negative control plants (LB). Based on the four results, it can be concluded that the inoculation treatment did not give a significant change in leaf area after two weeks of observation (Table 1). The conclusion for these tests is the same for two variables that there is no significant difference between before and after treatments.

Table 1. T-paired test result on leaf length and width

Variable	Alpha	Correlations significance	Paired samples test significance	Conclusion
Leaf length	0.05	0.000	0.458	There is no significant difference between leaf length in the pretest and post test data
Leaf width	0.05	0.052	0.304	There is no significant difference between leaf width in the pretest and post test data

Plant growth could be measured in many ways (Hilty *et al.*, 2021). We chose leaves length and width measurement in this experiment because it is the easiest and quickest way to perform in the field. However, our method has at least two shortcomings, e.g., the difficulty in choosing the perfect leaves and the full leaf development; picking the leaves that are good in shape, not damaged by being eaten by insects, a difficult task in the field. Although the youngest leaves are usually the easiest to measure for growth, their shapes have often not been perfected yet. Thus, we chose the second, third, and sometimes fourth-youngest leaves. The problem with this choice is the third and fourth leaves may have already reached their maximum growth. So, no change can be observed after two weeks of treatment. Wang *et al.* (2021) apply this method to eggplant and other single leaves.

Nevertheless, our study demonstrated for the first time the ability of endophytic bacteria (*Bacillus subtilis*) to induce flowers in host plants. Some of the treated plants showed flower growth (Fig. 3).

Fig. 3. A 2-week treated *A. marina* plant developed flowers. The left arrow shows flower buds, and the right arrow shows flowers.

Moreover, to determine the influence of endophytes on the development of *A. marina*, flower occurrence was carried out which represents the change from vegetative to generative phase. Eight plants showed this response as shown in Fig. 4, namely two plants (66.7%) that received control bacteria treatment at a concentration of 10^2 CFU/mL (K10-2), all plants (100%) that received *B. subtilis* YRL02 bacterial treatment at a concentration of 10^2 CFU/mL (A10-2), one plant (33.3%) that obtained treatment of *B. subtilis* YRL02 at a concentration of 10^3 CFU/mL (A10-3), one plant (33.3%) treated with *B. subtilis* HYM07 at a concentration of 10^2 CFU/mL (B10-2), and one plant (33.3%) treated with *Bacillus subtilis* HYM07 at a concentration of 10^3 CFU /mL (B10-3). In contrast, the treatment with *Bacillus* sp. (C) did not show any flower or fruit in plants, the same as in control plants treated only with LB solution (LB).

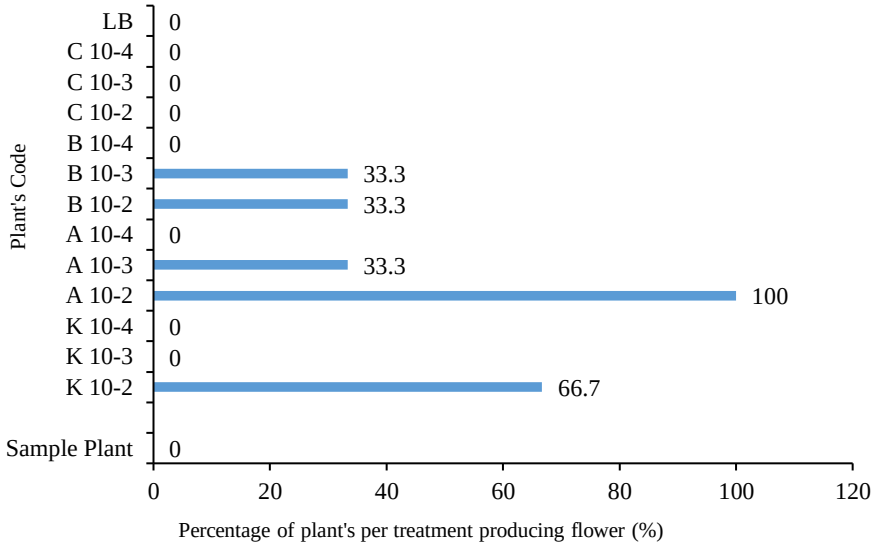

Fig. 4. Flowers occurence in treated and control plants. Note: K= plant treated with control strain of *Bacillus subtilis* (positive control); A= plant treated with *Bacillus subtilis* YRL02; B= plant treated with *Bacillus subtilis* HYM07; C= plant treated with *Bacillus* sp.; LB= plant treated with Lactose Broth (negative control)

Flower production. The production of flowers and fruits in plants signifies that the plants are going through the generative phase of reproductive development (Poethig, 2013). *Bacillus* application on plants generally has specific effects on vegetative growth. *Bacillus* can act as plant growth-promoting rhizobacteria (PGPR) that increase plant growth significantly and represent microbe-plant interaction mutually. The plant growth improved by PGPR in many ways, e.g., inducing systemic resistance against pathogens (biotic agents) and increasing the tolerance towards the harsh environment (abiotic). Other researchers (Park *et al.*, 2015) reported that *Bacillus* spp. including *Bacillus subtilis* GB03 given at a concentration of 10^8 CFU/mL to *Kalanchoe daigremontiana* increased asexual reproduction up to 2-fold after three weeks of treatment. In addition, *Bacillus subtilis* shows a biocontrol mechanism both directly and indirectly to prevent diseases caused by pathogens (Hashem *et al.*, 2019). *Bacillus* can convert the complex form of essential nutrients, such as P and N, and alter them into a more straightforward form that plant roots can absorb directly (Radhakrishnan *et al.*, 2017).

Interestingly, some reports describe a positive effect of auxin to induce flower and fruit production in plants. Although they are not directly related to *A. marina*, the application of auxin has succeeded in reproductive induction in some plants, including *Capsicum annuum* L. (Tiwari *et al.*, 2012) and tomato (Liu *et al.*, 2018). These reports may explain why the reproductive organs are produced in our treated plants because the endophytes produced IAA. Then, the IAA triggered the alteration of treated plants from vegetative to generative phase. In addition, there is a report explaining that auxin-mediated histone acetylation and deacetylation regulated flowering initiation (Wakeel *et al.*, 2018).

Infection zones. Some changes of color in the area around the inoculation site could be observed. The plant's stem diameter average was also so small, between 4-6 mm. Thus, taking a representative picture of this infection zone was challenging to achieve. However, we tried to take a good picture (Fig. 5) of how the area was formed in the stem. In addition, no infection zone was observed in control plants. The formation of an infection zone is a sign of bacterial metabolism, reproduction, and invasion inside the plant's cell and tissue. In the future, more research into endophyte colonization on stem tissues is required to ensure that endophyte metabolism occurs within the host plant.

Fig. 5. Infection zones formation on the infected stem. The left arrow shows the infection zone, while the right arrow shows the infection spot.

IAA and siderophore detection. All bacterial endophytes, including the control bacteria, produced IAA and siderophore (Table 2). The concentration of IAA between the four bacteria varied, with the highest achieved by *Bacillus subtilis* YRL02 with a concentration of 4.9 ppm. The next lower concentration of IAA production was produced by *Bacillus subtilis* HYM07 (3.7 ppm) and *Bacillus* sp. (2.2 ppm) subsequently. Moreover, IAA at the concentration of 5.8 ppm was also observed as a control. All the isolates are observed to produce siderophores with a different growth pattern for the siderophores production, as illustrated in Fig. 6.

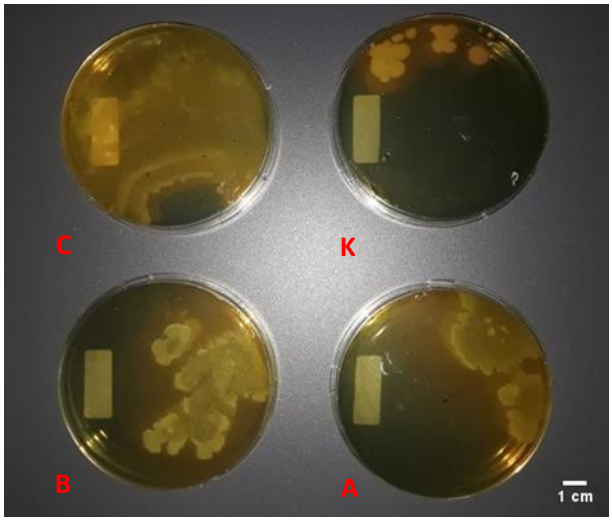

Fig. 6. Sample bacteria showing siderophore production: A. *Bacillus subtilis* YRL02 (A) ; B, *Bacillus subtilis* HYM07 (B); *Bacillus* sp. (C); and *Bacillus subtilis* (control) (K)

IAA producing *Bacillus* has been demonstrated in many publications. For instance, *Bacillus subtilis* (Mt3b) made 322.6 µg/mL IAA after 24h of incubation (Wagi & Ahmed, 2019). Moreover (Yanti *et al.*, 2017) explained that *Bacillus subtilis* BSn5 and *Bacillus subtilis* CIFT-MFB-4158A produced 29.25 ppm and 8.30 ppm IAA, respectively. However, the IAA production is relatively low if compared with that research. We observed the IAA production between 2.2-4.9 ppm from the lowest to the highest concentration. The precursor and growth medium determine the production of IAA by *Bacillus*. Tryptophan is a good precursor for *B. subtilis* (Mt3b) and *B. cereus* (So3II) because its availability can improve IAA production by 39% and 205%, respectively. Although IAA can still be produced without tryptophan, this amino acid is considered suitable (Mohite, 2013) and primary precursor (Shao *et al.*, 2015) for IAA production in *Bacillus*. In addition, yeast extract mannitol broth (YEMB) is a satisfactory medium for both bacteria, because the growth of the bacteria on the medium can improve IAA production (Wagi & Ahmed, 2019).

In soybean, Jiang *et al.* (2020) have reported that IAA can affect the development of stems. The concentration of IAA in soybean was varied in the mature zone, elongation zone, and apical meristem. The highest concentration was found in the mature zone, where the cells had fully developed. In contrast, apical meristem (with some leaf primordia) had the lowest concentration of IAA, analyzed by high-performance liquid chromatography (HPLC). As in our result, IAA might influence stem growth and development because of the endophytes inoculation toward the stems. IAA produced by the *Bacillus* is transported through the phloem in the stem (Wulf *et al.*, 2019). The two IAA transporters (uptake and efflux) (Damodaran & Strader, 2019) then brought the IAA to the basal of the stem to produce more roots (Zhao, 2018). The increase of roots production, in turn, will escalate nutrition absorption from the soil (Wang *et al.*, 2016). Subsequently, the growth of the plants will be improved (Baiyin *et al.*, 2021).

Healing wounds were observed on infection zones two weeks after the inoculation. It seems that IAA also plays a part in the wound healing mechanism. Hoermayer *et al.* (2020) reported that IAA concentration is increased in cells next to the wounds. The concentrated IAA will trigger excess

production of new cells to replace the damaged cells. IAA is not the only factor included in the mechanism. The change in turgor pressure is the other component that drives the wound healing system. In addition, a positive response toward IAA (availability, synthesis, signaling, and transport) would reduce the regulation of related nitrilase genes, which plays a role in IAA synthesis (Sun *et al.*, 2018; Wang *et al.*, 2022).

Table 2. IAA and siderophore production

Sample Code	Average of OD	IAA concentration (ppm)	Siderophore production
K	0.07	5.8	√
A	0.061	4.9	√
B	0.048	3.7	√
C	0.032	2.2	√

Note: K= plant treated with control strain of *Bacillus subtilis* (positive control); A= plant treated with *Bacillus subtilis* YRL02; B= plant treated with *Bacillus subtilis* HYM07; C= plant treated with *Bacillus* sp.

Siderophores are a secondary metabolite functioning in scavenging iron from the outside environment to make it available inside the plant's body (Albelda-Berenguer *et al.*, 2019). There are three types of siderophores, i.e., hydroxamates, catecholates, and carboxylates. *B. subtilis* produces a catecholate type called Bacillibactin (Patra *et al.*, 2018; Nithyapriya *et al.*, 2021). Also, it has been reported by Ferreira *et al.* (2019) that *B. subtilis* possesses several lipoprotein receptors embedded in the cell membrane to bind with the exogenous and endogenous Bacillibactin. Iron is a source of micronutrients for plants, meaning that only a tiny amount of it is needed for plant growth. However, iron insolubility in the environment leads to iron deficiency for some plants. The plants need a particular type of iron, which is Fe^{2+}. The Fe^{2+} absorbed by plants is essential for chlorophyll development and some enzymes involved in plant's respiration (Patra *et al.*, 2018). Our study results revealed that all the bacterial endophytes produced siderophores, although there is still a lack of information about their type and name. Furthermore, it has been explained that siderophore-producing bacteria could trigger bacterial colonization (Islam *et al.*, 2017). This is another proof that in our experiment, the bacterial colonization has occurred, because of the siderophore content produced by *B. subtilis* and *Bacillus* sp.

Successful infection methods. While Bacillus spp. are typically categorized as rhizobacteria, known to inhabit the soil and root zone of plants, the isolate employed in our previous study (Savitri *et al.*, 2023) was obtained from the internal tissues of surface-sterilized leaf cuttings of *A. marina*. Consequently, the initial application of the bacteria targeted the leaves. Unfortunately, they were far from a success, because the leaves developed holes in the inoculation area and subsequently withered. Another attempt was to spray the inoculum solution on the sterile *A. marina*'s seeds. However, the seeds underwent browning and then died. The propagation of *A. marina* itself is quite difficult inside the sterile condition. We have tried to bring some *A. marina* plants to the greenhouse, but their condition would deteriorate in 3-7 days. That was why those plants could not be used as the sample for experimental treatment. Our sample plants could only grow well in their natural habitat. We could not perform the infection procedure to the root system because of those difficulties in providing good sterile seedlings and young plants at the greenhouse (under controlled conditions).

Eventually, we used the stem for endophytes inoculation. Some small drills were applied to make holes inside the tiny stems. The 1 mL bacterial solution successfully went through inside the stems. There were no detrimental effects after 2 weeks of treatment observed in all plants that grow naturally in the mangrove forest. Although it is not popular, *Bacillus* inoculation through the stem has been done by some researchers. *B. amyloliquefaciens* (GB03) inoculated to the stem of cassava plants to combat *Fusarium* root rot (Freitas *et al.*, 2019). The study found that after inoculation, cassava shoots and roots grew by more than 100% when compared to untreated plants. In addition, rooted stem cuttings of *Euphorbia pulcherrima* Willd.ex Klotzsch were reported as sample propagules, dipped in some bacterial mixtures including *Bacillus megaterium* TV-91C and *Bacillus subtilis* TV-17C to observe the root formation (Karagöz, 2023). The research has shown that PGPR applying on

inoculated plants resulted in a higher shoot rate as well as higher N, P, Ca, Fe, and K contents than control plants. Some endophytic investigations also found that early flowering may occur following endophytic inoculation. For example, in chili plants (Habazar *et al.*, 2021) and lilies (Reut *et al.*, 2024). The mechanisms of floral early development may be related to the release of plant growth regulators by endophytes, which alters the balance of hormone concentrations within host plants, resulting in flowering.

The current study found that applying endophytic bacteria *Bacillus* spp. to the stems of host plants *A. marina* accelerated the development of *A. marina*, with the phase shift from vegetative to generative occurring within 2 weeks. Another significance of the experiment for future studies is the employment of *Bacillus* spp. as biocontrol agents due to their diverse chemical capabilities, as well as to preserve coastal areas from deterioration by improving ecosystem services (Dechaves *et al.*, 2022). However, our studies ascribed numerous uncontrolled variables in the natural environment, thereby affecting the significant results. As an outlook, subsequent studies should consider initiating experiments in a controlled environment (greenhouse) to minimize uncontrolled variables. Thus, the treatment effects will be better measured.

CONCLUSION

Although there was no significant change in leaves length and width before and after treatment, we observed alteration in the plant phase from vegetative to reproductive in just two weeks. IAA produced by *Bacillus* spp. probably is the reason for the alteration. The IAA and siderophores production may also trigger wound healing and enhance plant growth and development. We suggest further research of infection zone analysis to make sure that the infection zone consists of the injected bacterial endophytes.

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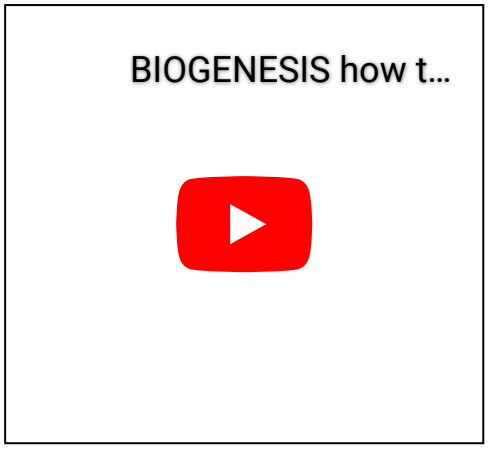

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