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Growth of Endophytic Bacteria from Klutuk Banana Plant (*Musa balbisiana* Colla) with Inoculation Trial on Rice Plants (*Oryza sativa*)

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Abstract: *Four potential bacteria have been isolated from Klutuk banana plant. They exhibited IAA production. Since the bacterial inoculation to plant should be performed in exponential phase of bacterial growth, information of the growth is crucial. This study aimed to analyze the growth rate of eight isolates and examine the effect of bacterial inoculation by initial trial on rice plants. For this purpose, four endophytic bacteria from Klutuk banana were cultured and examined using 600nm spectrophotometer. The growth of the isolates was calculated every two hours for 16 hours bacterial growth in the nutrient broth medium. The inoculation trial of bacteria to rice plants was conducted with three replicates. This inoculation was begun by germinating the rice seed and the radicle was observed. Then, the germinated rice seedlings were soaked in the bacterial suspension for 5 minutes in room temperature. The results showed that the exponential phase of all isolates was observed 6 hours after shaking. Inoculation using 6-hour-incubated K7 isolate significantly improved the length of root of rice plants. Additionally, this isolate improved the number of root and shoot length without significant difference. Thus, these results provide important information of the growth phase and inoculation effect new isolated endophytic and rhizosphere bacteria.*

Keywords: *endophytic bacteria; rhizosphere bacteria; IAA; growth rate; rice plant*

1. INTRODUCTION

The growth curve is useful for knowing the growth speed of bacterial cells and the influence of the environment on the speed of growth. Because each bacterium has a different time in each phase of growth (Mahjani & Putri, 2020). There are 4 phases of bacterial growth, namely: lag phase, log / exponential phase, stationary phase, and death phase (Bertrand, 2019). The lag phase is referred to as microbial adaptation to a new environment (Setyati & Martani, 2015). Influenced by the composition of the media, the number of cells in the initial inoculum, temperature, pH conditions and physiological properties of microbes in the previous medium. The lag phase lasts a few minutes to several hours (Risna et al., 2022). The exponential phase occurs the growth of bacteria takes place very quickly (Mardalena, 2016). The mass of the cell doubles as the cells divide at a constant rate (Sutrisna et al., 2013). After accretion cells tend to stagnate, called the stationary phase (Kusumaningati et al., 2013). The stationary phase does not have a significant decrease or increase in the number of cells, so the growth rate of the culture is zero or equal. However, the process of biosynthesis and energy metabolism of cells continues (Wahyuningsih & Zulaika, 2019). In this phase, the available nutrients will decrease (Irdawati et al., 2018). If there are fewer growth support

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factors, then the bacterial cells enter the death phase. Characterized by the death of cells in large numbers and no cell division (Prayitno, 2016).

Previous research has carried out isolation of bacteria whose ability to produce IAA can increase plant growth, for that it is necessary to know the period of bacterial growth to optimize the method of inoculation into plants. The isolates derived from kluthuk banana roots in regosol soil obtained the results of 21 isolates, selected 4 isolates of K2, K7, K8, and K117 as endophytic bacteria containing IAA of 73.50; 16.20; 59.5; and 42.60 (Rahayu, 2022). Endophytic bacteria live and associate with healthy plant tissues without causing symptoms of disease (Ramadhan & Hastuti, 2017).

The eight isolates were selected based on moderate to very high IAA results. Indole Acetic Acid (IAA) is one of the phytohormones that play a role in the development of roots, flowers and the growth of plant seeds (El-Mergawi & Abd El-Wahed, 2020). So that the presence of certain bacteria producing IAA can cause increased plant growth (Putra & Advinda, 2022). This study aimed to analyze the growth rate of eight isolates and examine the effect of bacterial inoculation by initial trial on rice plants.

The new finding of this study was the growth rate of potential endophytic bacteria which the IAA production was done in the previous study. In addition, this study observed the effect of endophytic bacterial inoculation in rice plants. Overall results contributed novel information of the potencies of endophytic bacteria toward the rice plant growth and the optimum growth phase of endophytic bacteria for inoculation.

2. MATERIAL AND METHOD

Materials and tools

The study used four endophytic bacteria isolated from klutuk banana roots. The four endophytic bacteria were coded K2, K7, K8, and K117. The growth of these bacteria in Nutrient Agar (NA) and Nutrient Broth (NB) for culture preservation and inoculation respectively. Measuring bacterial concentration using Shimadzu 1280 UV-Vis spectrophotometer.

Initial inoculation of plant growth was carried out on rice plants (*Oryza sativa*, L.) cultivar Inpari-32. Seeds are obtained from the general market.

Experimental design and procedures

This study was an experimental deasign with two replicates (n=2) for bacterial growth analysis and three replicates (n=3) for bacterial inoculation in rice plants.

Analysis of bacterial growth

Analysis of bacterial growth was initiated by making a starter with bacterial isolates from banana roots kluthuk following Wahyuning and Zulaika (Wahyuningsih & Zulaika, 2019). Then the resulting bacterial isolate was inoculated in the NB media with a volume of 6 mL and was incubated for 8 hours at room temperature at a speed of 100 rpm. The next step was pouring 1.5 mL starter into 50 mL NB then incubating with agitation. To gain the data of the bacterial growth, the Optical Density (OD) of each isolate was measured with λ 600 nm spectrophotometer (Respati et al., 2017). The OD measurement was done for 16 hours at interval of every 2 hours. NB media only without inoculation of isolate was used for blanks. Finally, the OD values of the absorption result were analyzed.

Preparation of bacterial suspension

A starter was manufactured with bacterial isolates, K2, K7, K8, and K117, from banana root Kluthuk. One end of ose of the bacterial isolates from oblique agar was inoculated into 6 mL of NB media in test tubes (aseptically). Similarly, NB media was inserted into test tubes placed in plastic for safety during shaking for the other bacterial isolates. The shaker was utilized to incubate the mixture for 6 hours at room temperature, maintaining a speed of 100 rpm. Following this, a bacterial suspension was created from the starter, with 5% (0.5 mL) of the suspension transferred aseptically into 10 mL of NB media. This process was repeated for all other bacterial isolates, with NB media in test tubes placed in plastic for safety during shaking. The mixtures were incubated on a shaker for 6 hours at room temperature, running at a speed of 100 rpm, and subsequently stored in the refrigerator.

Rice seed sterilization

The rice seeds were soaked in warm water for 5 minutes. Sinking seeds were selected for seeding, as per Mustaqimah & Nurhatika, (2019). The soaked rice seeds were taken to the LAF,

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where the soaking water was removed, and a 1:1 ratio of 70% alcohol was added to sterilize the surface of the seed. They were then shaken for 1 minute and the alcohol was subsequently removed, followed by rinsing using sterile aquades. A 1% NaOCl solution was added, and the seeds were allowed to stand for 1 minute before being transferred into the reservoir. Subsequently, the seeds were rinsed three times in sterile water channels by shaking for 1 minute in an aseptic environment. They were placed in a sterile jar bottle, which contained a 1:1 ratio of sterile aquades, and left at room temperature for up to 24 hours.

Bacterial inoculation into rice plants

The rice seeds were germinated by soaking in water for 5-6 hours, and subsequently placed on a petri dish lined with moistened paper for 48 hours until the seeds sprouted and developed roots. Inoculation was conducted by immersing rice seeds in a bacterial suspension with a known concentration (Lopes et al., 2021). The rice seedlings that displayed roots were transferred to sterile petri dishes using sterile tweezers. Inoculation was performed by placing the rice seeds in a bacterial suspension for 5 minutes, with a ratio of 10 mL of bacterial suspension to 10 seeds (Putra & Advinda, 2022). Rice seeds were placed in 8 cm plastic pots, with each pot containing 3 seeds. The planted rice seeds were watered with 50 mL of water per pot daily. The treatment was ceased once the plant reached 14 days after seedling emergence (HST), paying attention to parameters such as plant height (cm), number of leaves, total number of roots, and root length (cm).

Data analysis

The amount of bacterial growth was analyzed qualitatively based on the absorbance results of the sample from the spectrophotometer. While the observation data of plant growth parameters were analyzed with confidence, the student's t-test with a significant level of 5% ($\alpha = 0.05$).

3. RESULTS AND DISCUSSION

Results

Bacterial population growth is studied by observing growth curves in bacterial cultures. Bacterial growth from isolates of origin of banana root klutuk can be obtained by calculating the resulting absorbance value. The results of bacterial growth curve research can be seen in Figure 1 as follows :

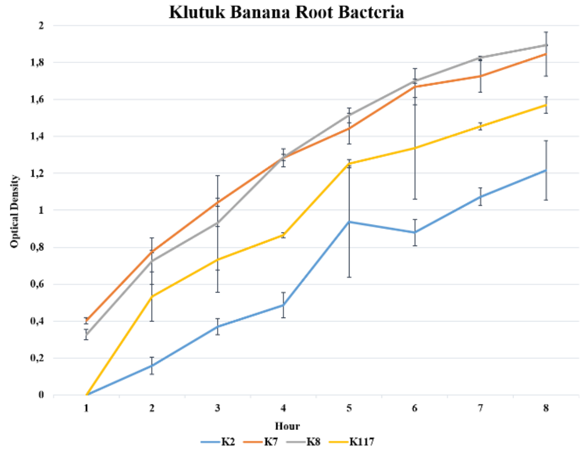

Figure 1. Potential bacterial growth curve of banana klutuk root isolate. Bars indicate the standard deviations.

The examination of endophytic bacteria whether can have an influence on the growth rate of plants was conducted. The results of research on the growth of rice plants inoculated with endophytic bacteria from banana klutuk roots can be seen in figure 2, Figure 3, Figure 4 and Figure 5 as follows:

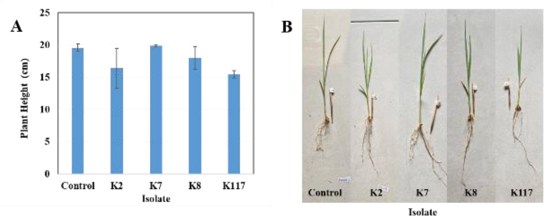

Figure 2. Day 14 rice plant height with inoculation treatment of endophytic bacterial isolates. (A) The results of the students' t-test showed that treatment with K2, K7, K8 and K117 isolates did not show a significant difference in plant height. The bar symbol indicates standard division. (B) Representative photograph of all treatments.

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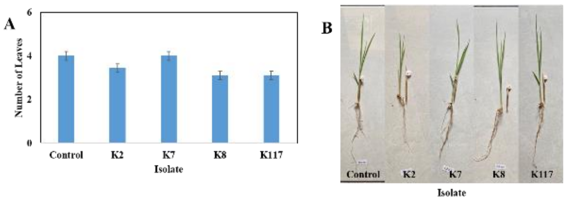

Figure 3. The number of leaves of rice plants day 14 with inoculation treatment of endophytic bacterial isolates. (A) The results of the student's t-test showed that treatment with K2, K7, K8 and K117 isolates did not show a significant difference in leaf count. The bar symbol indicates standard division. (B) Representative photograph of all treatments.

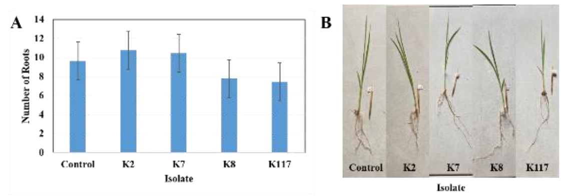

Figure 4. The number of roots of rice plants day 14 with inoculation treatment of endophytic bacterial isolates. (A) The results of the students' t-test showed that treatment with K2, K7, K8 and K117 isolates did not show significant differences in the number of roots. The bar symbol indicates standard division. (B) Representative photograph of all treatments.

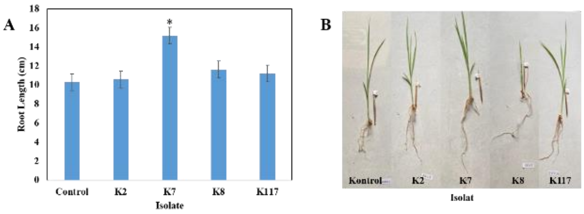

Figure 5. Root length of rice plants day 14 with inoculation treatment of endophytic bacterial isolates. (A) The results of the students' t-test showed that treatment with K7 isolates showed significant differences in root length. The bars indicate the standard deviation. The asterisk (*) showed a significant difference compared to the control with a confidence level (α) of 0.05. (B) Representative photograph of all treatments.

Discussion

This kluthuk cultivar banana was chosen because of its resistance to biotic and abiotic stress associated with its endophytic microorganisms. The Kluthuk cultivar has a BB genome that is reported to be resistant to biotic stresses such as Xanthomonas and FoC TR-4 (tropical race-4) as well as abiotic stresses such as drought (Rocha, et al., 2021).

The growth curve can be interpreted as the growth pattern of bacterial cells. Bacterial growth can be divided into 4 phases namely adaptation phase, log phase, stationary phase and death phase (Mahjani & Putri, 2020). The results of the study obtained a bacterial growth curve (Figure 1) showed that 4 selected isolates had a close lag phase at the incubation time of the first 2 hours, but in K2 isolates experienced a lag phase at 4 hours of incubation. Because K2 isolates are experiencing slow cell growth due to isolates adapting to the environmental conditions of the growing media. In the adaptive phase, slow growth occurs influenced by bacterial activity in making the process of adjusting to the conditions of the surrounding environment such as pH, temperature and nutrient conditions (Risna et al., 2022). The length or shortness of the adaptation phase is largely determined by the number of inoculated cells, the appropriate physiological and morphological conditions and the required cultivation medium (Yuliana, 2008).

Then, there is a log phase where bacterial growth takes place very quickly (Efendi et al., 2017). Bacterial isolates have a noticeable log phase characterized by an increase in the growth curve (Respati et al., 2017). Cell mass and volume increase because the nutrients in the media are sufficient so that they can grow optimally (Sudin et al., 2020). To meet the needs in the growth phase of the log cell produces many metabolites (Irdawati et al., 2018). The results of each isolate underwent a log phase at 3-14 hours of incubation. There is a log phase difference in K2 isolates, namely at 6-14 hours incubation. Furthermore, K2 isolates decreased at 12-hour incubation time with a log cell of 0.880. After that it increased again at an incubation time of 14 hours with a cell log of 1.073. The log phase undergoes very rapid synthesis of cell material with a constant amount, so in this phase bacteria are very good if used as inoculum (Sudin et al., 2020).

At an incubation time of 15-16 hours the bacterial isolate underwent a stationary phase, although there was a decrease in P31 isolate with an incubation time of 16 hours. The stationary

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phase occurs if the number of bacterial cells stops increasing. Even if there is no growth, cells can still grow and divide (Risna et al., 2022). In this phase the number of bacteria that grow with the dead is balanced. Although there is no net growth in the stationary phase, the cells still grow and divide (Bertrand, 2019). Although there was a decrease in incubation time of 14 hours in P31 isolates with a cell log of 1.6025. The trigger for this decrease is due to the presence of secondary metabolite compounds resulting from metabolic activity. Secondary metabolites are compounds resulting from stationary growth phases. These compounds are antibiotics, toxins, vitamins, pigments and alkaloids. The stationary phase shows a buildup of metabolites resulting from cell metabolic activity and nutrient content begins to run out. So there is a competition for nutrients, where some cells die and others will continue to grow (Sudin et al., 2020).

Based on the results of research that has been conducted (Figures 3, 4, and 5) from four isolates inoculated into rice plants did not have a real effect with control plants on plant height growth, number of leaves and number of roots. However, some bacterial isolates increased plant height in K7 isolates (Figure 3) and root counts in K2 and K7 isolates (Figure 5) compared to control treatments, although the difference was not significant. This is different from the results of the treatment of endophytic bacterial filtrate isolate has a real effect on the height growth of potato plants (Utami et al., 2012). However, there was one isolate that showed a significantly different treatment of rice plant growth support in root length parameters, namely in K7 isolate (Figure 6). A total of 16 consortiums of endophytic bacteria can spur the growth of tomato plants that produce markedly different root lengths of control plants (Pradana et al., 2020). Four isolates of endophytic bacteria from ferns can improve rice seed germination, increase in root length, shoot length, wet weight and dry weight better than controls. Increased growth of rice plants by treatment due to endophytic bacteria can increase nitrogen fixation, photosynthetic activity, and IAA production (Asmoro & Munif, 2019). Endophytic bacterial isolates from the roots of mahogany, trambesi, agarwood and meranti plant seedlings increased the growth of tomato plants by 60% by seed soaking method (Munif et al., 2015).

K7 isolates were detected to produce IAA of 16.20 or moderate concentrations compared to K2, K8 and K117 isolates that had high IAA concentrations (Rahayu, 2022). This IAA content will affect the length of the roots, the surface area of the roots and the number of root tips (Murthi et al., 2015). The concentration of IAA produced by endophytic bacteria as an inoculum is not bending straight with the growth of rice plants. The data shows that the ability to produce IAA is not the only factor that causes rice plant growth to increase (Rahayu, 2022). At low concentrations IAA causes elongation of roots and shoots, if the concentration of IAA is higher the elongation of shoots and roots becomes inhibited (Herlina et al., 2016). It can be seen in (Figure 6) that the root length in K2, K8 and K117 isolates has a shorter root length than K7 isolates.

There were several isolates of endophytic bacteria from sago that had poor rice plant height growth compared to controls, but root length was better than controls. There are also isolates of endophytic bacteria that can have a good influence on plant growth through plant height and root length. The variation of endophytic isolates in spurring the growth of rice plants shows that each endophytic isolate has a different ability to increase plant growth through phytohormone compounds produced to spur plant growth (Yatni et al., 2018). IAA hormone belongs to the class of auxin hormones found in plants play an important role in plant growth and reproduction (Taghavi et al., 2009). Auxins play an important role in stem elongation, apical dominance, lateral root initiation, cell elongation, cell division, cell differentiation, and others. In monocotyledonous plants, auxins play a role in the formation of adventitious roots while in dicotyledonous plants play a role in the formation of lateral roots (Aji & Lestari, 2020). In addition to the IAA content of endophytic bacterial isolates, there are several factors that affect the growth of this rice plant. One of them is by setting a wider planting distance, because competition in obtaining nutrients, water, and sunlight between plants becomes lower (Amiroh et al., 2019). Abiotic factors such as soil (nutrients, heavy metals, pH, and salinity), water availability, light intensity, and temperature can affect plant-microbial interactions, as they can alter plant metabolism (Lopes et al., 2021).

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4. CONCLUSION

All the 3 isolates had an exponential phase in the first 2 hours of incubation, for K2 isolate undergoing a log phase after 4 hours of incubation. In addition, K7 isolate significantly improved the root length of rice plants. These findings supported the proof of potential bacteria from kluthuk banana.

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