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Efficacy of Endophytic Bacteria *Pantoea dispersa* Strain DSM 30073 on The Growth of *Ipomoea reptans*

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Abstract *– Pantoea dispersa strain DSM 30073 is an endophytic bacterium isolated from the Ambon banana plant. From in vitro tests, this strain shows characteristics such as PGPB (Plant Growth Promoting Bacteria) and can support the growth of Cavendish banana plants, but its effect on Ipomoea reptans is unknown. The purpose of this study was to determine the efficacy of endophytic bacteria Pantoea dispersa strain DSM 30073 against the growth of Ipomoea reptans. The research design used was a one-factor Complete Randomized Design (CRD), namely inoculation frequency (P): control (P0), one-time inoculation (P1), and two-time inoculation (P2). The inoculum was confirmed to be pure by gram staining and cultured on NB medium with a 24-hour shake at room temperature. After 24 hours, the cells were harvested, and a suspension was made by adding Ringer's solution until it reached an OD600 nm of 0.8 – 1. Seeds were sterilized using 70 % alcohol followed by 1 % NaOCl for 1 minute each, then washed using sterile aquadest 3 times. Sterile seeds were inoculated with an inoculum suspension for 30 minutes (P1). After inoculation, seeds are planted on a sterile soil medium. Repeated inoculation is carried out on the 11th day (P2). The growth of Ipomoea reptans is observed: plant height (cm), number of leaves (strands), leaf stretch (cm), wet weight of the plant (g), root length (cm), and number of roots (total). After 30 days, data on plant height 16.1 cm (control) – 17.4 cm (inoculation), wet weight of plants 1.1 g (control) – 1.4 g (inoculation 2 times), number of leaves 7 strands (control) – 8 strands (inoculation 2 times), and leaf stretch 10.2 cm – 10.9 cm (inoculation 2 times). Inoculation of Pantoea dispersa strain DSM 30073 had no effect on plant height growth, wet weight, leaf number, and leaf stretch but was significantly different in root parameters. It can be concluded that the Pantoea dispersa was not seen to support the growth of Ipomoea reptans significantly until 30 days after inoculation.*

Keywords: *endophytic bacteria, inoculation frequency, Pantoea, PGPB*

INTRODUCTION

Pantoea dispersa strain DSM 30073, is an endophytic bacterium isolated from Ambon banana plants. This strain has been characterized as PGPB (Plant Growth Promoting Bacteria) and shows its ability to fix nitrogen by producing NH3, producing siderophores and IAA (Indole Acetic Acid) 65.45 ppm, and has antifungal activity against Fusarium. In planta strain *Pantoea dispersa* strain DSM 30073 has been tested on Cavendish banana plants and black rice plants. The results of the study showed that the strain significantly supported the growth of Cavendish banana plants, but when inoculated into black rice plants, it inhibited the growth of black rice (Rahayu et al., 2021).

Endophytic bacteria and host plants have specific interactions (Afzal et al., 2019; Iyer & Rajkumar, 2017; Mina et al., 2020). This means that a type of plant will associate with a certain type of bacteria. Some bacterial genera such as *Pseudomonas* and *Bacillus* dominate the banana plant Kluthuk cultivar compared to the Ambon cultivar (Rahayu et al., 2021). Rice plants also show specific interactions between endophytic bacteria and the roots of adam hawa (*R. discolor*) (Pradana et al., 2015). Thus, plants from different families will also interact with different types of bacteria although some types have a wide host range, such as *Bacillus polymixa* isolated from Anuma plants (Desriani et al., 2014).

Pantoea is reportedly associated with several types of plants, such as chickpeas (Panwar et al., 2016), potatoes (Jiang et al., 2019); wheat (Chen et al., 2017), and rice (Kunda et al., 2018) but no publication has been found that conveys any association

between *Pantoea* and kale plants (*Ipomoea reptans).*

Ipomoea reptans are cultivated vegetable plants that are short-lived and often consumed by the community. Kale belongs to the family Convolvulaceae, which can be classified as a vegetable plant. This plant originated in India which then spread to the territory of Australia, Africa and Asia. The nutritional content of kale is quite complete, namely protein, calcium, carotene, carbohydrates, phosphorus, fiber, and iron (Putri et al., 2017). Based on the place of life, kale consists of water kale (*Ipomoea reptans* Forsk.) and ground kale (*Ipomoea reptans* Poir.). The presence of endophytic bacteria can affect kale growth because this group is known for its ability as PGPB. *Pantoea dispersa* strain DSM 30073 will be inoculated into land kale plants and is expected to support the growth of kale plants.

In planta assay using bacteria can be done by several methods, one of which is by soaking seeds. In this study is a *Ipomoea reptans* for a certain period so that endophytic bacteria will seep into the seed, and can be stored in the plant (Yasin, 2018). Soaking the seeds with a hard type of seed skin can stimulate the entry of water on the skin called imbibition which can spur metabolic enzymes so that the seeds become soft and enzymes become active to convert fat into energy (Indah, 2019). Inoculation of endophytic bacteria can be repeated when the seeds have germinated to increase the density of bacterial cells. The density of bacterial cells as inoculum will affect its role as a plant growth-promoting bacteria (Natsagdorj et al., 2019). In this study, inoculation of the endophytic bacteria *Pantoea dispersa* strain DSM 30073 was carried out repeatedly when the seeds were still germinating.

The purpose of this study was to determine the efficacy of endophytic bacteria *Pantoea dispersa* strain DSM 30073 against the growth of land kale.

MATERIALS AND METHODS

This research was carried out at the Biology Laboratory of the Biology Education Department, Faculty of Teacher Training and Education, Universitas Muhammadiyah Surakarta. The study was conducted from March to July 2022.

1. Materials

The tools used in this study were laminar airflow (LAF), Erlenmeyer 100 mL, beaker glass 100 mL, hand sprayer, tweezers, ose, spatula, measuring cup 100 mL, autoclave, digital analytical balance (*Durascale DAB-E223*), ruler, spirit burner, 15 mL conical tube, petri dish, micropipette, spectrophotometer, blue tip centrifuge machine, binocular microscope (*Olympus CX-22*), hairdryer, object glass, cover glass, stationery and documentation tools. The materials used are isolates of endophytic bacteria *Pantoea dispersa* strain DSM 30073 (collection of Biological Laboratory FKIP UMS), soil media *(Greenmedia), Ipomoea reptans* seeds (*Bharata*), aquades, 1 % NaOCl solution, 70 % alcohol, medium nutrient agar (NA) (*Himedia*), sterile filter paper, medium nutrient broth (NB) (*Himedia*), ringer solution, Gram dye set.

2. Method and research design

The research design used was a one-factor Complete Randomized Design (CRD): the frequency of inoculation (P) of kale seeds using endophytic bacteria *Pantoea dispersa* strain DSM 30073. The research treatment consisted of three treatments, namely control (P0/no inoculation), once-inoculation (P1), and twice-inoculation (P2). Each treatment was repeated five times.

3. Procedures

The endophytic bacterium *Pantoea dispersa* strain DSM 30073 confirmed its purity by Gram-staining and then observed under a binocular microscope (*Olympus CX-*

22) with a magnification of 10x40. Sterile soil media is prepared by weighing humus soil as much as 150 g per polybag. Soil sterilization was conducted at a temperature 121^{0}C 1 atm for 20 minutes, repeatedly every 2 days. Bacterial suspension as inoculum uses NB medium incubated at room temperature, 120 rpm for 24 hours. After 24 hours, bacterial cell density was measured before cell harvesting using a spectrophotometer at a wavelength of 600 nm. Bacteria cells were harvested by centrifugation at 6000 rpm for 10 minutes. The supernatant is removed, then pellets of bacterial cells are flushed with sterile aquadest and centrifuged again. Ringer's solution was added to the pellet, and cell density was measured at a wavelength of 600 nm. Then, it was adjusted so that the cell turbidity value was between 0.8 –and cell density was measured at 600 nm. Then, the cell turbidity value was adjusted to between 0.8 and *1* (Rahayu, 2022). *Ipomoea reptans* seeds are well selected and sterilized using 70 % alcohol in a ratio of 1:1 and then shaken for 1 minute. After that, sterilization is continued by replacing 70 % alcohol with 1 % NaOCl solution for 1 minute (Nursyamsi & Toaha, 2017). To remove the disinfectant solution, the seeds are washed using sterile aquadest 3 times by shaking for 1 minute aseptically. Sterile seeds are inoculated with inoculum suspension with a ratio of bacterial suspension: seeds = 5 mL: 50 seeds (Tefera & Vidal, 2009) for 30 minutes with a shake. After inoculation, the seeds are drained and ready for planting in a sterile soil medium. Repeated inoculation is carried out on the 11th day. After 30 days of inoculation, the growth of kale plants was observed, which included: plant height (cm), number of leaves (strands), leaf stretch (cm), wet weight of the plant (g), root length (cm), and number of roots (total). Assessment of the root count parameter uses the following criteria:

+++ : very lush
++ : lush
+ : a little lush

4. Data analysis

In this study, the data analysis used was quantitative descriptive analysis to determine the efficacy of endophytic bacteria *Pantoea dispersa* strain DSM 30073 on the growth of kale plants (*Ipomoea reptans*) based on data on plant height, number of leaves, leaf stretch, wet weight of plants, root length and number of roots. Data is processed using the SPSS 20.0 Program. The data analysis technique used is the Anova Test (Analysis of Variance), using a confidence limit of 95 % or $\alpha = 0.05$. If there is a noticeable difference, it will be continued with further tests, namely by using the Smallest Real Difference Test (BNT) with a level of 5 %.

RESULT AND DISCUSSION

Endophytic bacteria *Pantoea dispersa* strain DSM 30073 morphologically confirmed colonies indicated by yellow, shiny, and round colonies (Yang et al., 2022) (Figure 1A), including the Gram-negative group with bacilli cell shape (Figure 1B).

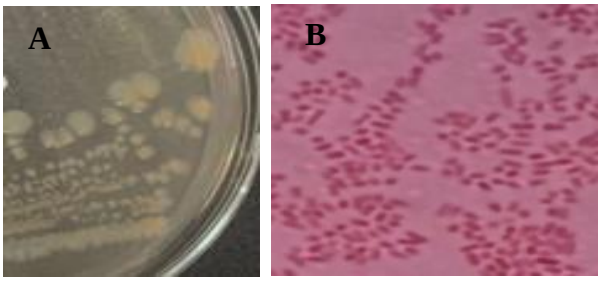

Figure 1. Bacterial colony *Pantoea dispersa* strain DSM 30073 (A) and Gram staining results (B)

Based on the results of observations of kale growth conducted for 30 days, the average results of plant height, wet weight, number of leaves, leaf stretch, and root length were obtained in the control treatment, inoculation once and inoculation 2 times the endophytic bacteria *Pantoea dispersa* strain DSM 30073.

Table 1. Growth of ground *Ipomoea reptans* with different frequencies of inoculation of *Pantoea dispersa* bacteria strain DSM 30073

Treatment	Average Growth of ***Ipomoea reptans***					
	PH (cm)	WW (g)	NL (strand)	LS (cm)	RL (cm)	NR
P0	16.1± 3.5 a	1.1± 0.3 a	7± 0.9 a	10.5± 1.1 a	11.5± 2.0 b	+
P1	17.3± 2.6 a	1.1± 0.2 a	7± 1.5 a	10.2± 1.2 a	12.8± 3.0 b	+
P2	17.4± 8.8 a	1.4± 0.9 a	8± 2.6 a	10.9± 2.6 a	17.1± 1.6 a	++

The same letter behind the mean and SD values shows no real difference between treatments in the same column

+ = small number of roots

++ = medium root count

PH : plant height; WW : wet weight; NL: number of leaves; LS : leaf stretch; RL : root length; NR : number of roots

The growth parameters of land kale plants, including plant height, wet weight, number of leaves, and leaf stretch, did not show any significant difference between treatments, but root parameters showed a significant difference. Plant height ranged from 16.1 cm (control) – 17.4 cm (inoculation), wet weight of plants ranged from 1.1 g (control) – 1.4 g (inoculation 2 times), leaf count between 7 strands (control) – 8 strands (inoculation 2 times), and leaf span between 10.2 cm – 10.9 cm (inoculation 2 times) (Table 1). did not affectThe inoculation of endophytic bacteria in the Pantoea dispersa strain DSM 30073 had no effect on plant height growth, wet weight, leaf count, or kale leaf stretch. Although there is no noticeable difference compared with controls, in plants, inoculated endophytic bacteria show higher values, especially in twice inoculation.

The data showed that the plants gave a positive growth response to inoculation of endophytic bacteria from *Pantoea dispersa strain* DSM 30073. This strain of bacteria shows several characteristics as *plant growth promoting bacteria* (PGPB), namely able to fix nitrogen, produce IAA 65.45 ppm, produce siderophore compounds, and can inhibit the growth of *Fusarium* (Rahayu, 2022). Nitrogen is a macronutrient that is a constituent of proteins that are indispensable for growth and chlorophyll. Nitrogen deficiency can result in decreased growth, chlorosis (yellowish leaf discoloration) and inhibit the emergence of lateral shoots (Leghari et al., 2016). Pantoea's ability to fix nitrogen detected from in vitro tests is thought to play a role in increasing the growth of land kale.

Figure 2. Average plant height, number of leaves, root length and number of roots of kale plants

Information:
A = P0 (Control)
B = P1 (One-time inoculation)
C = P2 (2 times inoculation)

IAA has many benefits for plant growth, such as affecting the process of cell elongation and division, differentiation, increasing root development, controlling vegetative growth processes, photosynthesis, and pigment formation (Glick, 2012). *Pantoea*'s ability to produce high concentrations of IAA may also play a role in the growth of kale plants. Similarly, its character in producing siderophore compounds will help plants fulfill in meeting the needs of Fe ions by plants because iron in nature is available in an undissolved state. Fe ions are micronutrients but are very important because they are directly involved in the processes of respiration and photosynthesis (Glick, 2012).

The PGPB character of *Pantoea dispersa* strain DSM 30073 showed a positive response in *planta* tests to kale plants but did not differ significantly in either the frequency of inoculation once or twice, but inoculation twice showed better data (Table 1). The allegation is related to these results because the number of inoculum Pantoea dispersa strain DSM 30073 is insufficient to affect kale plants' growth. In research by Natsagdorj et al. (2019), getting data that the density of bacterial cells as inoculum will affect its role as a plant supporter.

Growth parameters of root-related kale plants showed significantly different data from controls, specifically in twice inoculation, reaching a length of 17.1 cm compared to controls only 11.5 cm (Table 1). This data differs from the growth parameters of kale plants located in the aboveground part (plant height, number of leaves, and leaf span) which show no noticeable difference. These results show conformity with Herlina et al., 2016. This is because the root is one of the organs most sensitive to IAA fluctuations and is responsible for increasing the amount of exogenous IAA, which is useful for primary root elongation, lateral root formation and adventitious roots. Based on research Munthe (2018), IAA produced by endophytic bacteria can stimulate root hair formation, increasing nutrient absorption. *Pantoea dispersa* strain DSM 30073 which was used as an inoculum, was known to produce high IAA of 65.45 ppm (Rahayu, 2022). With these characteristics, this IAA produced by bacteria will be used in plant physiological processes, causing plants to be more sensitive in forming lateral roots and adventitious roots. The IAA produced can also be used in the process of plant metabolism, which will help increase height, wet and dry weight, and root length.

Other studies reported that endophytic bacteria use roots as the main point of plant entry (Allard-Massicotte et al., 2016; Kumar et al., 2020; Prieto et al., 2011). The root is the part of the plant that points downwards in the media. Roots as the main vegetative organ in absorbing water, minerals and materials important for plant growth and development. The role of roots in plant growth is as important as the function of leaves. Leaves are where photosynthesis takes place, where the roots provide the nutrients and water needed in this process. The ability of plants to absorb nutrients can be seen by measuring root length, number of roots, wet and dry weight of roots (Yasin, 2018). In the root length parameter, the longest root is found in the twice-inoculation treatment, while the once-inoculation treatment and the control have almost the same length.

In this case, the density of the inoculum is Pantoea dispersa strain DSM 30073, which appears to play an important role in its support for kale plant growth. This can be seen from the kale plant growth data (Table 1), although some data are not significantly different, but in general, almost all data show improvement in twice inoculation treatment. The same results were shown in the study Natsagdorj et al. (2019) but the strain used is *Variovorax* sp. To convince this argument, further research is needed to study the relationship between the density of *Pantoea dispersa* strain DSM 30073 and its character as PGPB.

Growth data and statistical calculations prove that *Pantoea dispersa* strain DSM 30073, isolated from Ambonese banana plants, can also support the growth of land kale plants. The genus *Pantoea* is reported to be able to colonize some plants such as chickpeas (Fam Fabacaea) (Panwar et al., 2016), wheat (Chen et al., 2017) (Fam Poacea), potato (Fam Solanacea) (Jiang et al., 2019), and bananas (Fam Musacea) (Rahayu, 2021, unpublished). Land kale plants belong to the family Convolvulaceae (Wikipedia, 2022). Thus, the Pantoea dispersa strain DSM 30073 has the potential to be an inoculum that supports several types of plants.

CONCLUSION

Based on the data and discussion, it can be concluded that the Pantoea dispersa bacteria strain DSM 30073 was not seen to support the growth of *Ipomoea reptans* significantly until 30 days after inoculation. Twice inoculation treatment showed a better growth response of land kale plants and a real response to root organs.

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