Hyposensitivity test of *Lactobacillus fermentum* InaCC B1295 probiotic bacteria on the growth of mustard greens (*Brassica juncea* L.)

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**Abstract.** The purpose of this study was to (1) determine whether the probiotic bacteria *Lactobacillus fermentum* InaCC B1295 were able to adapt (compatible) to mustard greens (*Brassica juncea* L.). (2) Knowing the effect of the probiotic bacteria *Lactobacillus fermentum* InaCC B1295 on the growth of mustard greens (3) Obtain the best concentration for the growth of mustard greens. This was an experimental study and used a completely randomized design (CRD) with 4 treatments and 4 replications to obtain 16 experimental units. The data were analysed using SPSS version 9.1.3 and ANOVA, then followed by Duncan's New Multiple Range Test (DNMRT) at 5% level. The results showed that the application of the probiotic *Lactobacillus fermentum* InaCC B1295 was significantly different from the control. So it can be concluded that the probiotic bacteria *Lactobacillus fermentum* InaCC B1295 are able to adapt their life (compatible) to mustard plants, and have a positive effect on the growth of mustard greens. The $10^5$ CFU/mL dilution is a good treatment to stimulate the growth of mustard plants.

1. Introduction

According to FAO/WHO, probiotics are adequate amounts of live microorganisms ($10^6$-$10^8$ CFU/mL) that provide health benefits to their host. Probiotics can improve health because these bacteria can suppress the growth of pathogenic bacteria in the digestive tract of humans or animals and stimulate an immune response. Just like humans and animals, plants also benefit from probiotic bacteria, including: absorbing nitrogen, absorbing soil nutrients, producing growth hormone, increasing germination, producing antibiotics and immunity against pathogenic bacteria, dissolving soil phosphate [1]; [2]; [3] as well as increasing farmers' incomes by reducing production costs, environmental protection, and food security [4].

*Lactobacillus fermentum* InaCC B1295 are probiotic bacteria isolated from curd, fermented buffalo milk which are typical of Riau and West Sumatra Province. *Lactobacillus fermentum* InaCC B1295 itself has never been studied for biological control in plants, however several researchers have found several species of Lactobacillus are able to stimulate an increase in plant height growth [5], increase in leaf number [6] and to increase the total plant wet weight [7].

*Lactobacillus fermentum* InaCC B1295 are bacteria that have great potential as probiotic for humans and animals, but are also thought to have potential as probiotic for plants. Because *Lactobacillus fermentum* InaCC B1295 are bacteria that originate from milk growing media, it is necessary to test the compatibility of *Lactobacillus fermentum* InaCC B1295 in plants and their effect on plant growth.

Mustard greens (*Brassica juncea* L.) is a vegetable crop commodity that has high economic value, although its selling value is strongly influenced by the quality of its visual appearance. The last few
years, mustard production in Pekanbaru City, Riau Province, has decreased. Mustard production in 2013 was 3,484 tons, in 2014 was 3,190 tons and in 2015 was only 1,540 tons [8].

Based on the explanation above, then the aims of this study are to (1) determine whether the probiotic bacteria Lactobacillus fermentum InaCC B1295 were able to adapt (compatible) to mustard greens (Brassica juncea L.). (2) Knowing the effect of the probiotic bacteria Lactobacillus fermentum InaCC B1295 on the growth of mustard greens (3) Obtain the best concentration for the growth of mustard greens.

2. Methods

The materials used were Lactobacillus fermentum InaCC B1295 isolate, mustard seeds, MRS-B media, soil, manure and sterile distilled water. While the tools used were test tubes, laminar air flow, test tubes and polybags.

This study consisted of several stages included (1) Bacterial propagation; (2) Dilution of $10^5$, $10^6$, $10^7$ CFU/ml. (3) Preparation of soil medium, (4) Nursery, (5) Planting, and (6) Application to mustard plants by evenly spraying the suspension as much as 10 mL/polybag on the 1st week, then 30 mL/polybag on the 2nd week and 60 mL/polybag on the 3rd week (7) Observation and data analysis.

This study used a completely randomized design (CRD), with 4 treatments and 4 replications to obtain 16 experimental units, namely water (control), dilution of $10^5$ CFU/mL, dilution of $10^6$ CFU/mL and dilution of $10^7$ CFU/mL. The parameters observed included (1) plant height, (2) number of leaves and (3) total wet weight of mustard greens. Data were analysed using SPSS version 9.1.3 and ANOVA then followed by Duncan’s New Multiple Range Test (DNMRT) at 5% level and presented in a table.

3. Result and Discussion

The data obtained in this study were (1) plant height (2) number of leaves and (3) total wet weight. Plant height was measured from the base of the stem to the end of the growth point of the sample plant. Plant height measurements were carried out once a week until the mustard greens was harvested [9]. The application of Lactobacillus fermentum InaCC B1295 affected the height of mustard greens (Table 1).

Table 1. Average Height of Mustard Greens (Cm) per Week with Various Treatments Dilution of Lactobacillus Fermentum Inacc B1295

| Treatment                  | Height of Mustard Greens (cm) |
|----------------------------|--------------------------------|
|                            | Week 1 | Week 2 | Week 3 |
| Control (Water)            | 8.9    | 18.55a | 26.67a |
| Dilution of $10^5$ CFU/mL  | 8.9    | 25.17c | 34.25c |
| Dilution of $10^6$ CFU/mL  | 8.92   | 24.77c | 33.15c |
| Dilution of $10^7$ CFU/mL  | 8.95   | 21.55b | 31.25b |

Table 1 shows that the application of Lactobacillus fermentum InaCC B1295 has an effect on the height of mustard greens. Treatments using Lactobacillus fermentum InaCC B1295 bacteria were significantly different from the control. Stimulation of plant growth by microbes (bacteria) includes biological N2 fixation, production of phytohormones, such as Indole Acetic Acid (IAA), cytokines, auxins and gibberellins [10], biocontrol of phytopathogens through antifungal or antibacterial production, production of siderophores and by increasing bioavailability of minerals [11]. Lactobacillus fermentum InaCC B1295 is thought to stimulate plant height by producing IAA which is a natural auxin phytohormone and acts as a plant growth enhancer [12], dissolving phosphate to support growth increase [13]. This is in line with research [14] that lactic acid bacteria, isolate BL12 showed the highest IAA production (5.46 μg mL−1), of the eight lactic acid bacteria tested with isolate BL06 dissolved in phosphate, with high PSI (>3). Besides that, Figure 1 shows that at the $10^5$ dilution, the mustard greens are higher in height than the control and other dilutions. This is because the lower the dilution level, the more bacteria will be available, so the mustard greens will grow better.
Data of the leaf number was obtained by counting the number of leaves that grow in 1 week. The counting was performed once a week, starting from the first week until the harvest time [15]. Data of *Lactobacillus fermentum* InaCC B1295 application on the number of mustard leaves (Table 2).

**Table 2.** Average Number of Mustard Leaves (leaves) per Week with Various Treatments Dilution of *Lactobacillus Fermentum* InaCC B1295 Number of Mustard Leaves

| Treatment         | Week 1  | Week 2  | Week 3  |
|-------------------|---------|---------|---------|
| Control (Water)   | 5.0     | 10.50 b | 16.75 b |
| Dilution of $10^5$ | 5.0     | 15.75 b | 24.25 b |
| Dilution of $10^6$ | 5.0     | 14.50 b | 23.50 b |
| Dilution of $10^7$ | 5.0     | 12.00 b | 20.00 b |

Table 2 shows that the application of *Lactobacillus fermentum* InaCC B1295 has an effect on the number of mustard leaves. The treatment using *Lactobacillus fermentum* InaCC B1295 bacteria was significantly different from the control. The lower the dilution level of *Lactobacillus fermentum* InaCC B1295, the higher the number of mustard leaves produced. This is presumably due to the growth regulator (Zat Pengatur Tumbuh/ZPT) produced by *L. fermentum* InaCC B1295. The application of probiotic bacteria to plants can increase the number of leaves due to the presence of ZPT IAA and gibberellin content. Gibberellin is a growth regulator substance that plays a role in leaf growth, while the IAA hormone is a growth hormone that plays a role in controlling plant physiological processes including cell enlargement and division [16]. Figure 1 shows that the number of leaves in dilution $10^5$ was more than the number of leaves in the control and other dilutions. This is because the lower the level of dilution, the higher the bacterial density, so that the stimulus for leaf growth will increase and the number of mustard leaves will also increase.

Data of wet weight of mustard greens were obtained by weighing the plant as a whole from the tip of the leaves to the roots [17]. The application of *L. fermentum* InaCC B1295 has an effect on the wet weight of mustard (Table 3).

**Table 3.** Average Wet Weight of Mustard Greens (gram) per Week with Various Treatments Dilution of *Lactobacillus Fermentum* InaCC B1295

| Perlakuan         | Wet Weight (gram) |
|-------------------|-------------------|
|                   | Week 3            |
| Control (Water)   | 163.80 b          |
| Dilution of $10^5$| 228.78 b          |
| Dilution of $10^6$| 221.48 b          |
| Dilution of $10^7$| 215.25 b          |

Table 3 shows that the application of *Lactobacillus fermentum* InaCC B1295 has an effect on the number of leaves of the mustard greens. The treatment using *Lactobacillus fermentum* InaCC B1295 was significantly different from the control. The lower the dilution level of *Lactobacillus fermentum* InaCC B1295, the higher the wet weight will be. This is because *L. fermentum* InaCC B1295 is thought to be able to provide nutrients to mustard greens. The interaction between plants and microbes plays an important role through the synthesis of plant hormones (auxin and gibberellin), nitrogen fixation, dissolving inorganic phosphate, and mineralization of organic phosphates, so that these elements are available to the plants. In addition, probiotic bacteria are also able to suppress the growth of disease-causing pathogens [18] in mustard greens. Because the nutrients are fulfilled, the plant growth and the number of leaves will increase so that the wet weight of the plant will also increase.

In addition, the higher the plant height and the number of leaves, the more the wet weight of the plant will increase. This is in line with [19] which stated that fresh weight of a plant is influenced by
plant height and leaf area, the taller the plant and the larger the leaf area, the higher the plant’s fresh weight.

4. Conclusion
Based on the results of data analysis and discussion, the following conclusions can be drawn. The *Lactobacillus fermentum* InaCC B1295 probiotic bacteria are able to adapt their life (compatible) to mustard greens. *Lactobacillus fermentum* InaCC B1295 probiotic bacteria can have a positive effect on the growth of mustard greens. The 10^5 CFU/mL dilution is a good treatment to stimulate the growth of mustard plants.

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