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ISOLATION AND CHARACTERISTICS OF ENDOPHYTIC BACTERIA FROM PALM OIL (Elaeis guineensis L.) ROOT ON GROWTH OF SWEET CORN (Zea mays saccharata Sturt)

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ABSTRACT

Sweet corn (*Zea mays saccharata* Sturt) is one of the crops that demanded by Indonesian people. The demand for sweet corn in Indonesia has increased along with the increasing population and the need for sweet corn to be consumed directly. Production of sweet corn in Indonesia has decreased from year to year. In 2012, sweet corn production was 19.3 million tons, while in 2013 it was 18.5 million tons. To overcome this condition, there is an effort to replace the role of inorganic fertilizer as a nutrient supplier for plants. Endophytic bacteria is one of biological fertilizers, that is living microbes given to the soil as inoculants to help plants provide certain nutrients. This study use Non Factorial Completely Randomized Design with 6 treatments. The results showed the presence of endophytic bacteria in palm oil roots. Endophytic bacteria that were successfully isolated showed diversity based on morphology, namely 5 different isolates. These five endophytic bacteria showed no significant effect on the growth of corn.

Keywords: endophytic bacteria, palm oil roots (*Elaeis guineensis* L.), Sweet Corn (*Zea mays*).

INTRODUCTION

Sweet corn (Zea mays saccharata Sturt) is one of the crops that demanded by Indonesian people. This is because sweet corn has a sweeter taste compared to ordinary corn. Sugar content in sweet corn endosperm ranges from 5-6% while in ordinary corn is only around 2-3% [1]. The demand for sweet corn in Indonesia has increased along with the increasing population and the need for sweet corn to be consumed directly. Nevertheless, Production of sweet corn in Indonesia has decreased from year to year. In 2012, sweet corn production was 19.3 million tons, while in 2013 it was 18.5 million tons [1]. This amount still cannot meet the national sweet corn demand as much as 20 million tons [2].

The cause of sweet corn low productivity in Indonesia is cultivation carried out on low fertility land [3]. Farmers often applying fertilizers, both organic and inorganic, to improve soil fertility. Excessive use of inorganic fertilizers can cause several negative impacts, such as reducing soil organisms activity, changing soil physical properties [4], causing the accumulation of heavy metals in the soil and plant systems and depleting the availability of micro nutrients for plants.

Endophytic bacteria are one of biological fertilizers that can be used to reduce inorganic fertilizers. Endophytic bacteria live in plant tissues that neutral or beneficial to the host plant without damaging the host [5,6]. The role of endophytic bacteria in enhancing plant growth is thought to be supported by the ability to produce growth hormones such as indole-3- acetic acid (IAA), abscisic acid (ABA), gibberallic acid (GA) and cytokinin (CTK) [5]. In addition, endophytic bacteria can also increase plant growth by inhibiting N_2 (diazotrophic bacteria) [7,8], increasing plant productivity by producing growth hormones such as auxin, as biocontrol agents [9], mobilize phosphate, and also its ability to increase plant resistance to pests and diseases [9,10].

Kannahi & Ramya (2015) found that biofertilizers and Azospirillum application has the highest height in tomato, namely 15.9 cm, compared to controls which were only 8 cm [11]. In addition, Widawati (2015) also stated that bacterial inoculation had a positive effect on the stem diameter of turi tiller (4.3 mm) compared to control treatments (2.2 mm) [12]. Hidayati (2014) also showed that application of endophytic bacterial inoculums isolated from rubber tree is potential as growth promoting bacterium because able to increase the growth of Hevea brasiliensis rootstock [13].

Susilowati *et al*. (2003) as cited by [14] show the presence of endophytic bacteria in rice and corn that able to fix N_2 in the air and produce IAA hormone. Isolation such bacteria found 5 isolates in rice and 5 isolates in corn. Selected isolates in corn which inoculated against corn plants on at laboratory scale proved able to improve main roots and corn fibers development.

Sweet corn (Zea mays saccharata Sturt) is one of the crops that demanded by Indonesian people. However, production of sweet corn in Indonesia has decreased from year to year. In 2012, sweet corn production was 19.3 million tons, while in 2013 it was 18.5 million tons. The cause of sweet corn low productivity in Indonesia is cultivation carried out on low fertility land. To overcome this condition, there is an effort to replace the role of inorganic fertilizer as a nutrient supplier for plants.

Endophytic bacteria is one of biological fertilizers that is living microbes given to the soil as inoculants to help plants provide certain nutrients. This research was conducted with the following objectives:

a) To obtain endophytic bacteria from palm oil roots by isolating, characterizing, selecting and identifying the bacteria.

b) To study the role of endophytic bacteria from palm oil roots to trigger the growth of corn.

c) To study the diversity of endophytic bacteria found in palm oil roots.

EXPERIMENITAL

Location and Time of Research

This research has been carried out at the Sungai Putih Research Center in District of Galang, Regency of Deli Serdang. This research has been conducted in January 2019.

Research Method

This study used a Non Factorial Complete Randomized Design consisting of 6 treatments, and 5 replications, namely:

Control = No endophytic bacteria
B_1 = endophytic bacteria 1
B_2 = endophytic bacteria 2
B_3 = endophytic bacteria 3
B_4 = endophytic bacteria 4
B_5 = endophytic bacteria 5

Research Implementation

Sampling

Palm oil root samples was taken by digging the soil to a depth of 20 cm, after which the roots are cut and cleaned.

Making of Nutrient Agar

Agar was weighed as much as 15 grams, entered into an erlemeyer contained 1 liter of water then add with 8 grams of Sodium chloride, 2 grams of Bacto yeast, and 5 grams of Pepton, then boiled and stir for 17 minutes. After boiling, lift and pour into a glass bottle lid with sterile cotton and put it in aluminum foil then sterilized again using an autoclave at 121 °C at a pressure of 1 bar for 30 minutes.

Making of liquid Nutrient Agar media

As much as 2 grams of Sodium chloride, 2 grams of Bacto yeast, and 5 grams of Pepton put into an Erlenmeyer that contained 1 liter of water then boiled and stir for 17 minutes. After boiling, lift and pour into a glass bottle lid with sterile cotton and put it in aluminum foil then sterilized again using an autoclave at 121 ° C at a pressure of 1 bar for 30 minutes. This medium is used for bacterial propagation hat have been obtained from purification and will be applied to corn seeds by immersion.

Isolation of endophytic bacteria

Palm oil roots from 20 cm deep are cleaned with running water for 20 minutes. Root surface is sterilized by soaking the root pieces in 15% chlorok solution for 10 minutes, Benstar 0.1% (0.1 g /100 ml) solution for 5 minutes and 70% alcohol solution for 2 minutes then rinsed twice with sterile aquades for 3 minutes. One gram of sterile roots is mixed with 2 ml of distilled water and then crushed using sterile mortar, diluted from 10^{-2} to 10^{-9}. Then take 1 ml of each dilution and dropped into a sterile petridish then flattened and then pour the NA media that has been cooled to 50 °C into each of these petridish (± 20 ml). Cover the petridish tightly to prevent contamination and then move carefully so that the microbial cells spread evenly. After the media is compacting, the petridish is incubated in reverse for 1-2 days at room temperature. The growing bacterial colony is sub cultured into the same medium until pure culture is obtained.

After the purification bacteria has been obtained, then multiply the bacteria in the Liquid Nutrient Media which will later be applied to corn seeds. Morphological characterization of the colony can be directly observed and gram staining will serve to distinguish bacterial isolates from one another.

Gram Staining

Gram staining is carried out by taking bacterial isolates using ose then made a scratch on the glass object. Then added with 2-3 drops of Violet Crystal, let stand for 2 minutes then wash with distilled water and dry. After drying, drop 2-3 drops of Lugol, let stand 2 minutes then wash again with distilled water and dry. Then drop 2-3 drops of 96% alcohol and leave it for 30 seconds. Wash and dry. After that, drop Safranin 0.5% by 2-3 drops and leave it for 30 seconds. Wash again and dry then observed under a microscope.

Application of Endophytic Bacteria

Application of endophytic bacteria was carried out on corn seeds, soaked with a bacterial solution that had been diluted 10^6 for 12 hours. Corn seeds are grown for 3 weeks and placed in the greenhouse of the Sungai Putih Research Center in District of Galang, Regency of Deli Serdang. Seeds soaked with distilled water are used as controls. Each treatment is repeated 5 times. Each treatment has 2 seeds that have been soaked in a bacterial solution and then planted on soil media in a polybag. Growing sprouts were observed every 3 days for 3 weeks.

Parameters Observed

The parameters observed were:

A. Morphological characteristics of Endophytic Bacteria

- Shape;
- surface;
- Edges and Color.

B. Gram Staining and observation of Cell Forms

- Color;
- Cell Form;
- Gram Negative or Positive.

C. Growth of Corn

- Plant height;
- Length of leaves;
- Root Length;
- Wet and Dry Weight of Roots and Stems.

RESULTS AND DISCUSSIONS

Morphological Characteristics and Gram Staining and Shape of Endophytic Bacteria Cells

Observation of bacteria morphology and gram staining was carried out after isolates purification from palm oil roots isolation. Based on purification, there are 5 different bacterial isolates. According to Cappuccino and Sherman (2001), morphological characterization aims to observe both colony and bacterial cell morphology in selected bacterial isolates [15].

Observations results of morphological characteristics of endophytic bacteria can be seen in Table-1, while observation of gram staining and cell shape can be seen in Table-2.

Table-1. Results of morphological observation for endophytic bacteria.

No.	Isolate Code	Colony morphology			
		Shape	Surface	Edge	Color
1.	B_1	Irregular	Embossed flat	Jagged	Yelowish
2.	B_2	Irregular	Embossed flat	Jagged	Red
3.	B_3	Irregular	Flat into the media	Intact	Milky white
4.	B_4	Rounded	Embossed flat	Intact	White
5.	B_5	Rounded	Flat	Jagged	White

Macroscopically observation on the morphology of bacterial isolates showed that the five bacterial morphologies above have irregular shapes and are rounded in shape. Endophytic bacterial populations grow rapidly on TSA (Trypic Soy Agar) media and various types of endophytic bacteria that grow to colonies are distinctive in appearance [16]. Colony surface in isolate 1 (B_1), isolate 2 (B_2) and isolate 4 (B_4) were embossed flat while on isolate 5 (B_5) is flat. But there is a little different in isolate 3 (B_3) which has a flat surface but located in the media. Observation of the colony edge looks jagged and intact, whereas there are 2 isolates of bacterial colony that are white, 1 yellowish, 1 red and 1 milky white (Figure-1). Yulianti (2012) as cited in [13] stated that endophytic microorganisms are usually extracted from plant tissues (intercellular) and originating from the rhizosphere or filosphere.

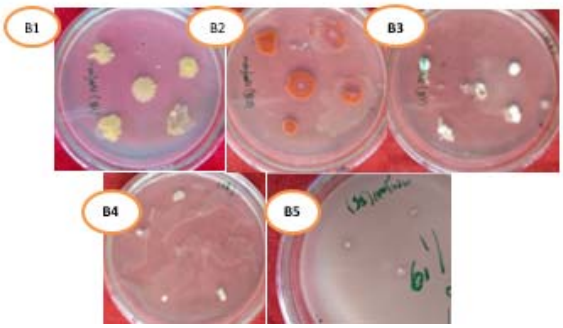

Figure-1. Observation of colony morphology at 2 days after incubation.

Bacterial staining aims to distinguish bacteria into two groups, namely gram positive and gram-negative bacteria. The difference in staining results is due to differences in the structure of two groups of bacteria so that there is a difference in the permeability. Staining is based on the thickness or thinness of peptidoglycan layer in the cell wall and the amount of fat layer contained in bacterial cell membrane. According to Sutedjo *et al*., (1991) gram positive has thicker and more peptidoglycan compounds cell walls than in gram negative [17, 18].

Table-2. Observation of gram staining and cells shape of endophytic bacteria.

No.	Isolate Code	Gram	Cells Shape	Colony Color
1.	B1	Positive	Coccus (Rounded)	Yelowish
2.	B2	Positive	Bacil (Rod)	Red
3.	B3	Negative	Coccus (Rounded)	Milky white
4.	B4	Positive	Coccus (Rounded)	White
5.	B5	Positive	Bacil (Rod)	White

Observation of gram staining shows that gram positive bacteria are more dominant than gram negative which are only found in isolate 3 (B_3). The cell shape of the five bacterial isolates is rod (bacillus) and rounded (coccus). The shape of bacterial cells is very important in characterizing the morphology of a species. The difference in color in gram-positive and gram-negative bacteria indicates that there are differences in cell wall structure between two types of bacteria. Cell wall structure of gram-positive bacteria has thick peptidoglycan content (about 90% of the cell wall) and the remaining is teikhoat acid molecules. Gram-negative bacteria have a cell wall structure with high lipid content [19] and have a double membrane system in which the plasma membrane is enveloped by a permeable outer membrane. This bacteria has a thick cell wall in the form of peptidoglycan, which is located between the inner membrane and the outer membrane.

Fardiaz (1989) also explains that in gram staining, cells that cannot release color and will remain colored like violet crystals namely blue-purple are called gram positive bacteria, while cells that can release violet crystals and bind safranin and has pink color is called gram negative bacteria. Principle of gram staining is ability of the cell wall to bind the basic dyes (violet crystals) after washing with 70% alcohol (Figure-2). This condition is related to constituent compounds of cell wall [17].

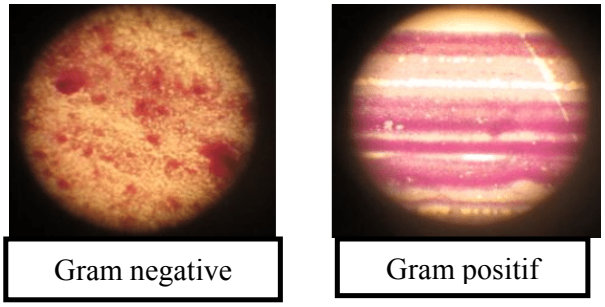

Figure-2. Observation of gram negative and positive staining.

Effect of Endophytic Bacteria on Corn Growth

Parameters observed in this study is include plant height, leaf length, root length, wet and dry weight of roots and stems. The results of statistical data analysis can be seen in Appendix 2-33. It can be seen that endophytic bacteria have no significant effect on each parameter observed. Endophytic bacteria are live in plant tissues without harming the host. Endophytic bacteria can be isolated from root, stem and plant tissue [20]. According to Aly et al [21], endophytic microorganisms, such as fungi and bacteria, form a mutualistic, commensalistic, and parasitic symbiotic relationship with endophytic hosts plants. Plants will transfer sugar types such as sucrose and glucose from their bodies and then use it as an energy source for endophytic bacteria [16].

The difference in growth rate is influenced by type and number of bacteria [22]. Beside nutrients availability, bacterial cell growth is also influenced by many factors such as microbial type, condition and number of initial cells before and after application [23]. The amount of bacteria concentration before it was applied to corn seeds can be seen in Table-3.

Table-3. The amount of bacteria concentration.

Endophytic bacteria	Concentration(cfu/ml)
Bacteria isolate 1 (B_1)	277 x 10^6
Bacteria isolate 2 (B_2)	303 x 10^6
Bacteria isolate 3 (B_3)	269 x 10^6
Bacteria isolate 4 (B_4)	256 x 10^6
Bacteria isolate 5 (B_5)	10^6

Endophytic bacteria that live in plant tissue can act as plant growth booster. Corn that were treated with endophytic bacteria had an increase after 4th observation or 12 days after planting (DAP) until 7th or 21 DAP compared to control.

This is presumably because producing IAA endophytic bacteria that was applied to corn seeds began to have an effect on the plants, although not yet significant. For more details, see Table-4.

Table-4. Average of corn height (cm) by application of edophytic bacteria.

Treatment	Observataion - ...						
	I	II	III	IV	V	VI	VII
Control	2.68	5.94	7.38	9.94	11.00	11.60	13.00
B_1	2.20	5.64	6.80	9.80	11.20	12.10	13.30
B_2	1.76	5.48	7.00	9.20	10.54	11.40	13.20
B_3	2.00	5.92	7.02	9.90	10.92	12.00	13.60
B_4	1.60	5.30	6.60	9.30	10.08	11.40	12.20
B_5	2.10	5.44	6.80	10.10	10.90	11.90	13.30

Structure of endophytic bacteria is influenced by abiotic and biotic genetic factors such as environmental factors (interactions between microbes and plants) [13].

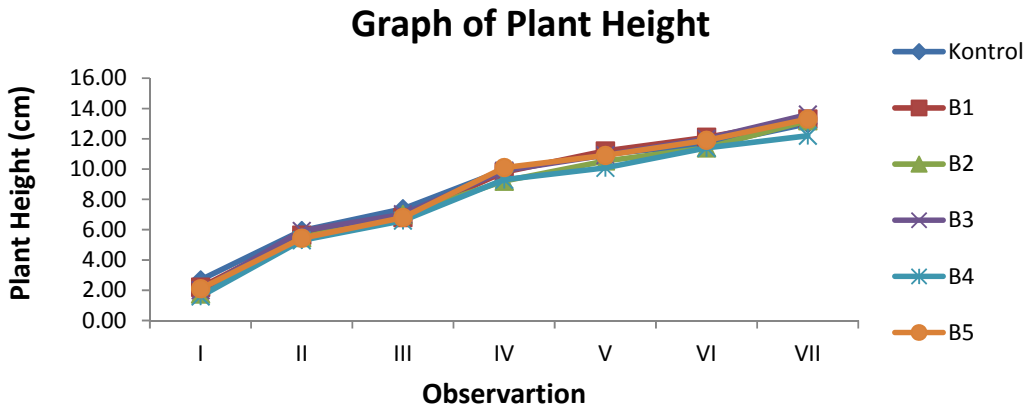

Figure-3. Graph of plant height (cm) by application of edophytic bacteria.

Table-5. Average of leaf length (cm) by application of edophytic bacteria.

Treatment	Observation- ...						
	I	II	III	IV	V	VI	VII
Control	0	7.84	17.44	26.70	30.10	31.24	32.56
B_1	0	7.14	16.96	26.70	29.40	32.90	33.60
B_2	0	7.24	16.22	26.12	28.56	30.70	31.98
B_3	0	7.10	17.38	27.74	30.22	32.30	33.80
B_4	0	6.76	16.24	26.06	27.94	30.60	31.80
B_5	0	6.72	16.28	28.70	30.90	33.90	34.60

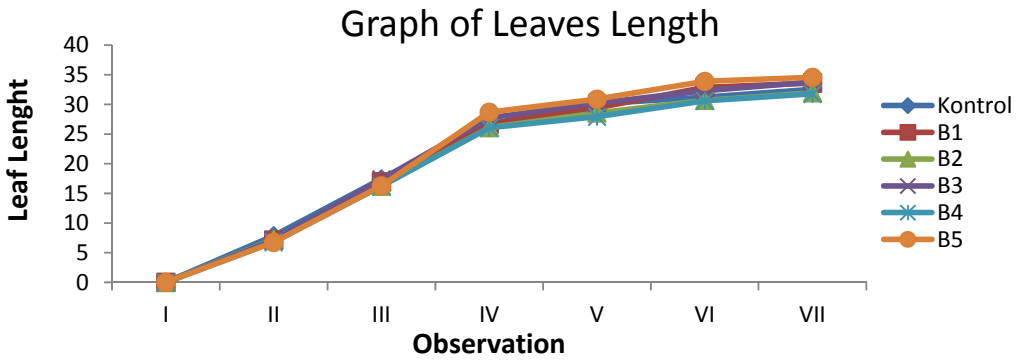

Figure-4. Graph of leaves length (cm) of corn plants on endophytic ba by application of edophytic bacteria.

Table-6. Average of root length and root and stem wet weight by application of edophytic bacteria.

Treatment	Parameter		
	Root Length (cm)	Root Wet Weight (gr)	Stem Wet Weight (gr)
Control	36,50	0.20	2,33
B_1	44.60	0.18	2.18
B_2	45.40	0.19	1.81
B_3	45.60	0.25	2.30
B_4	42.20	0.25	1.71
B_5	48.48	0.27	2.30

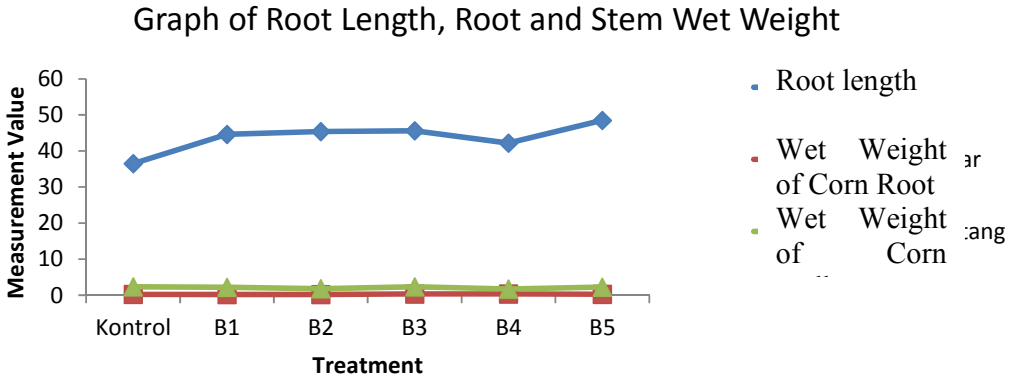

Figure-5. Measurement of root length, root and stem wet weight by application of endophytic bacteria.

Table-7. Average of roots and stems dry weight by application of endophytic bacteria.

Treatment	Parameter	
	Root Dry Weight	Stem Dry Weight
Control	0.12	0.29
B_1	0.11	0.31
B_2	0.10	0.24
B_3	0.12	0.31
B_4	0.12	0.25
B_5	0.14	0.33

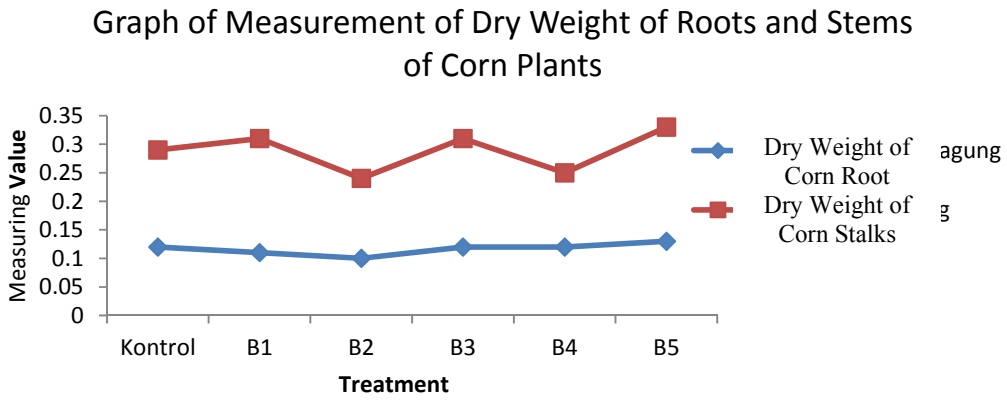

Figure-6. Graph of roots and stems dry weight by application of endophytic bacteria.

Data as shown at Table-4 to Table-7 show that application of endophytic bacteria to corn seeds has no significant effect among treatments. Observations made from 3 DAP to 21 DAP (vegetative phase) indicate that endophytic bacteria have no effect on plant height, leaf length, root length, wet and dry weight of root and stem in this phase. It is suspected that application by immersion in corn seeds is less effective. Application after planting by watering it to the roots at age between 18-35 DAP (Phase V6-V10) is needed. It is because plants begin to absorb nutrients in higher amounts, and application of endophytic bacteria at this phase is needed to meet nutrient requirements for plants[24]. Endophytic bacteria from palm oil roots have no effect or are not effective on the growth of corn. However, Sapak *et al*,[25] stated that endophytic bacteria isolated from palm oil root tissue is potential to inhibit the spread of Ganoderma boninense Pat and palm oil germination.

CONCLUSIONS

This study found five isolates of endophytic bacteria from palm oil root and showed morphology diversity. These five endophytic bacteria showed no significant effect on the growth of sweet corn. Further research is needed in searching other endophytic bacteria that affect on corn growth and the application is also added by watering to the roots after planting or in the vegetative phase.

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