ISOLATION AND CHARACTERIZATION OF ENDOFYTIC BACTERIA INDIGENUS POTENTIALLY PRODUCING IAA (Indole Acetic Acid) IN WEST SUMATERA AND THEIR EFFECT ON NURSERY PALM OIL (Elaeis guineensis Jacq)

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Abstract. The IAA potentials of endophytic bacteria indigenous isolated from roots palm oil in west Sumatra. Isolates were characterized on the base of visual observation, gram staining, hypersensitive reaction and IAA producing bacteria. Subsequently, effect on plant growth was tested by nursery palm oil used random design block. Out of 82 isolates, base on gram test 12 isolates were gram negative and 70 isolates grams positive and resulted on the reaction hypersensitive test (HR test) there were 8 isolates that positive which means the isolate cannot be applied on the soil and plant. Eighty isolates selected were able to produce IAA that was between ppm 0.30ppm - 3.65ppm. Seven isolates showed clearly the growth promoting plant under field condition. Isolates are promising plant growth promoting isolates showing multiple attributes that can significantly influence the nursery palm oil. The resulted of research, treatment SBS2.E.34 has higher plant most than other treatment i.e. 21.93 cm and number of leaves i.e. 4.33 leaves.

Keywords: endophytic; bacteria; indogenous; IAA; palm oil

1. Introduction

Palm oil plantation companies are a commodity in Indonesia who donated the country largest foreign exchange area palm oil plantations in Indonesia on the 2016 about 11.914.499 ha with production 33.229.381 tons. Palm oil plantations area in Sumatera island 7.191.738 ha. Extensive benefits of the plantation dispersed at ten provinces in the island of Sumatera one of them in the province of west Sumatera. West Sumatera having area oil palm plantations in 2015 383.385 ha with production 926.618 2.978 kg/ha. Palm oil plantation in 2016 area around 399.728 ha, with production 988.133 tons and productivity 3.089 kg/ha (Direktorat Jenderal Perkebunan, 2016).

Productivity palm oil in the province of west Sumatera are low than productivity palm oil in the of Riau province and north Sumatera greater than four tons/ha, that require input fertilizer high that more productive because palm oil plantation land were dominated by marginal soil with a kind of ultisol. Hakim (2006) said that ultisol having a low ph smaller than 5.5, toxicity aluminum, and deficiency fospor. Ultisol must given lime and given input fertilizer. Pahan (2011) stated that the problem cultivation palm oil is fertilizing, and more than 60 % maintenance costs palm oil used for fertilizing.
The use of chemical fertilizers constantly sure inefficient because it can be result in negative impact on the environment. Based on the issue needs to substitute alternative fertilizer that can enhance plant growth palm oil is it self in marginal soil. Proper Alternative is using Endophytic bacteria indigenous from the root of palm oil from west Sumatera that acts as pgpr or plant promoting rhizobacteria capable of producing plant growth hormones as IAA, and solubility phosphate.

Endophytic bacteria is saprophyte bacteria lives and associated with the plant tissue healthy (Backman & Sikora, 2008). Some Endophytic bacteria can produce plant growth hormones of plants, i.e IAA known as auxin (Spaepen et al., 2007). Endophytic bacteria in addition to promote the growth of plants, if able to mobilize phosphate and become biological control (Hallman, 2001). Bacteria producing IAA capable of producing fitohormon to speed up the growth of plants. Hormone IAA is auxsin endogenous role in enlargement cells, hinder bud growth side, stimulate absisi, role in the formation of xylem and phloem, and also had influence on the development and lengthening roots (Aryantha et al., 2004)

2. Materials and Methods

Materials and Instrument

This experiment used the soil samples and plant roots oil palm healthy in several regions optimal production center in the west Sumatera, plastic, label stationery, medium nutrients that (Na), KOH 3%, cotton, aluminum foil, plastic wrap, tissue paper, paper, label plant at four, and aquaest.

An instrument used is a cup, Erlenmeyer (Pyrex) 250 ml, a Petri dish (pyrex), ose needles, of the object glass, measuring glass (pyrex) 250 ml, autoclave, laminar flow water cabinet, bunsen, colony counter, a shaker, vortex, pipette micro, zip lock polyethylene bag, a mortar porcelain and mortal, sentrifuge, test tube, camera, and stationer.

Methods

The soil samples taken as purposive randomized sampling method of palm oil production center in West Sumatera. The soil samples brought to laboratory for isolated and observed morphology and tested patoghenity. Subsequently, effect on plant growth was tested by nursery palm oil used random design block methode. Data of each variable from two sets of experiments were pooled together, averaged and subjected to analysis of variances. Means of treatments were compared using Least Significant Difference at P <
A two-way analysis of variance (ANOVA) was performed to determine the significance level (*\(p < .05\)). The mean values of each group were then subjected to honesty significant different Test (BNJ) using Statistic 10 at \(p < .05\).

**Sample Collection**

Samples were collected from roots around rooting plants healthy of Palm oil of West Sumatera province at Sinjunjung district. Root samples were taken and kept in a sterile zip lock polyethylene bag. Samples were taken to the laboratory maintaining the aseptic conditions.

**Isolation of Endophytic Bacteria**

Isolation endophytic bacteria indigenous using methods Yanti et al (2016), the soil samples are weighed 1 (one) gram, by using vortex (3-5 minutes) is suspended in 10 ml aquadest sterile until homogeneous. Suspension used dilution early (\(10^{-1}\)). Then from dilution early taken 1 (one) ml and admitted to test tube next containing 9 ml aquadest sterile, then vortex again until homogeneous. This suspension called dilution both (\(10^{-2}\)). Dilution is carried to \(10^{-6}\). Of the last two dilution yaitu\(10^{-5}\) and \(10^{-6}\) will be isolated in a way : take 0,1 ml (100 \(\mu\)l) dilution \(10^{-5}\) then put it in testube who already contains the liquid nutrient agar media then vortex and pour it into a petri dish glass, petri dish labeled and cooled. Turn petri dish and been incubated for 48 hours at room temperature. Similarly treated on \(10^{-6}\). The colony that grow and form clear zone is isolatebacteria. The isolate and grow again (rejuvenated) on a new media. Then in identification morphologically (color the colony, surface of the colony and the form of the outskirts of the colony).

**Characteristics Morphology of Endophytic Bacteria**

Bacterial subjected to gram staining according the methode of Schaad et al, (2001), one drop of KOH 3% on top the object glass using pippete drops and took pure culture bacteria indigenous age 2x24 hours using a needle ose and mixed with KOHsolution. If there is clumping, the bacteria is gram negative, but when not happens clumping of does is a gram positive. Then Hypersensitive reactions (HR test) aims to know of bacteria that including pathogenic or not. The probe done with plant use indicators : flowers at four (*Mirabilis jalapa*). Suspension bacteria indigenous with density of populations \(10^8\) cfu/ml infiltration in intercellular on a network the under surface leaves with a syringe until saturated and incubation for 2x24 hours. Reaction characterized when appear symptoms necrotic on the leaves infiltrate bacteria (Klement et al., 1990). If there is symptoms necrotic means the bacteria is are pathogenic and not be used for plant.
Determine of IAA Produce

Determine of IAA produced used calorimetric technique was performed with van urk salkowski reagent using the salkowski’s method. Broth cultures were sentrifuged and supernatants were mixed with Salkowski reagent in a ratio 1 : 2 (Patten and Glick, 2002). The mixture was allowed to stand for 30 minutes at room temperature in dark for color production. Isolates showing pink to red color were selected as IAA producers and were used in further experiments. The amount of IAA was measured by spectrophotometre at 535 nm. Then concentration was calculated using standard curve of IAA.

Effect of IAA Producing Endophytic Bacteria Indegenous on Plant Growth

Suspension bacteria moved into erlenmeyer already contains 100 ml coconut water sterilized, and homogenous with mcfarland solution with 8 scale. If turbidity it’s same, it was assumed that the density of populations of bacteria cells. $10^8$ / ml. Then soak seed of palm oil in solution bacteria isolates ±15 minutes. After that, dryed seed before to planting in soil sterilized (Yanti., et al 2013). Then, 10 ml solution bacteria splashed in the rooting plant. After seed age 4 month, splashed 10 ml solution bacteria to rooting again. Pots were irrigated with sterile distilled water every day and kept from sunligt until 3 month. Plant was growth were measured for hight plant and number of leaves with 1 month intervals until 8 month.

3. Results and Discussion

Characteristics Morphology of Endophytic Bacteria Indegenous From West Sumatera

The results of the isolation of bacteria endophytic rooting oil palm plantations in West Sumatera obtained 82 isolates. The nature of morphology results isolation bacteria endophyticpada roots palm oil in west Sumatra province having the diameter, colony the form of, the margin, elevation and the color of the colony that varied. The observations of the nature of morphology endophytic bacteria can be seen in Table 1.

Table 1 . Characteristics morphology of colony bacteria endophytic on a plant oil palm

| Isolate  | Diameter | Shape     | margin  | elevation | color   | Isolate  | Diameter | Shape     | margin  | elevation | color   |
|----------|----------|-----------|---------|-----------|---------|----------|----------|-----------|---------|-----------|---------|
| SBS1.E.1 | 0,7 cm   | filamento | filiform| flat      | beige   | SBS2.E.5 | 0,3 cm   | circular  | entire  | Raised    | yellow  |
| SBS1.E.2 | 1,4 cm   | irregular | undulate| umbonate | white   | SBS2.E.6 | 0,3 cm   | filamentous| filiform| flat      | white   |
| SBS1.E.3 | 0,4 cm   | irregular | undulate| raised   | yellow  | SBS2.E.7 | 0,5 cm   | circular  | entire  | umbonate  | yellow  |
| SBS1.E.4 | 1 cm     | Irregular | undulate| raised   | beige   | SBS2.E.8 | 0,5 cm   | circular  | entire  | raised    | yellow  |
| SBS1.E.5 | 0,7 cm | Irregular | undulate | raised | beige | SBS2.E.9 | 0,7 cm | circular | entire | crateriform | beige |
| SBS1.E.6 | 1,1 cm | Irregular | undulate | raised | beige | SBS2.E.10 | 0,5 cm | irregular | lobate | flat | white |
| SBS1.E.7 | 0,9 cm | Irregular | undulate | raised | beige | SBS2.E.11 | 0, cm | irregular | undulate | flat | white |
| SBS1.E.8 | 0,8 cm | Irregular | undulate | umbonate | white | SBS2.E.12 | 1 cm | irregular | undulate | umbonate | white |
| SBS1.E.9 | 1,4 cm | Irregular | undulate | raised | white | SBS2.E.13 | 0,8 cm | rhizoid | filiform | flat | white |
| SBS1.E.10 | 1,1 cm | Irregular | lobate | flat | beige | SBS2.E.14 | 2,2 cm | irregular | undulate | flat | white |
| SBS1.E.11 | 0,9 cm | Irregular | undulate | flat | white | SBS2.E.15 | 1,6 cm | irregular | undulate | umbonate | white |
| SBS1.E.12 | 0,4 cm | Irregular | undulate | flat | beige | SBS2.E.16 | 0, cm | circular | entire | raised | beige |
| SBS1.E.13 | 0,7 cm | Irregular | undulate | umbonate | beige | SBS2.E.17 | 0,8 cm | irregular | undulate | umbonate | white |
| SBS1.E.14 | 0,4 cm | Irregular | entire | umbonate | beige | SBS2.E.18 | 0,9 cm | irregular | curled | crateriform | white |
| SBS1.E.15 | 0,5 cm | Irregular | entire | crateriform | white | SBS2.E.19 | 0,9 cm | irregular | undulate | umbonate | white |
| SBS1.E.16 | 0,5 cm | Irregular | entire | crateriform | beige | SBS2.E.20 | 1,2 cm | circular | entire | umbonate | beige |
| SBS1.E.17 | 0,8 cm | Irregular | lobate | umbonate | beige | SBS2.E.21 | 0,3 cm | irregular | umbonate | umbonate | red |
| SBS1.E.18 | 1,3 cm | Irregular | undulate | flat | beige | SBS2.E.22 | 0,4 cm | circular | entire | raised | beige |
| SBS1.E.19 | 0,8 cm | Irregular | lobate | flat | beige | SBS2.E.23 | 1,3 cm | irregular | undulate | crateriform | white |
| SBS1.E.20 | 1,3 cm | Irregular | undulate | raised | beige | SBS2.E.24 | 1,1 cm | irregular | undulate | crateriform | white |
| SBS1.E.21 | 2,2 cm | Irregular | lobate | crateriform | beige | SBS2.E.25 | 0,5 cm | circular | entire | umbonate | yellow |
| SBS1.E.22 | 1,4 cm | Irregular | undulate | flat | beige | SBS2.E.26 | 0,4 cm | circular | entire | raised | orange |
| SBS1.E.23 | 2,2 cm | Irregular | undulate | flat | beige | SBS2.E.27 | 0,4 cm | irregular | undulate | crateriform | white |
| SBS1.E.24 | 1,2 cm | Rhizoid | filiform | flat | beige | SBS2.E.28 | 0,2 cm | circular | entire | raised | yellow |
| SBS1.E.25 | 1,1 cm | Irregular | undulate | umbonate | beige | SBS2.E.29 | 0,7 cm | irregular | lobate | flat | white |
| SBS1.E.26 | 0,3 cm | Circular | entire | umbonate | yellow | SBS2.E.30 | 0,4 cm | circular | entire | raised | beige |
| SBS1.E.27 | 1 cm | Irregular | lobate | raised | beige | SBS2.E.31 | 0,4 cm | circular | entire | umbonate | yellow |
| SBS1.E.28 | 2 cm | Filamentous | filiform | flat | beige | SBS2.E.32 | 0,6 cm | circular | entire | raised | beige |
Variations morphological features endophytic bacteria found in rooting palm oil from West sumatera. The morphology of the bacteria colonies endophytic found varying diameter colonies around 0.2 - 7.0 cm, and variation color of colonies bacteria i.e yellowish white color, beige, orange, white and red. Before the 82 isolates done to testing the ability in producing IAA necessary hipsensitif test (HR test) to know pathogenic to plants. If isolates bacteria on the HR test showed necrosis (positive value ) will be used in this experiment. The nature of physiology isolate endophytic bacteria can be seen in table 2.

**Characteristics Physiology of Isolates Endophytic Bacteria**

The characteristics physiology the endophytic bacteria in isolation rooting palm oil in the west sumatera can be seen observation and characteristics physiology showed in Table 2.

| Isolat | uji Gram | Uji HR | Isolat | uji Gram | Uji HR | Isolat | uji Gram | Uji HR |
|--------|----------|-------|--------|----------|-------|--------|----------|-------|
| SBS1.E.1 | + | - | SBS1.E.29 | + | - | SBS2.E.20 | + | + |

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Based on table 2 it can be seen that the physiology 82 bacteria colonies endophytic varies from west sumatera, there are 12 bacteria gram negative and 70 bacteria gram positive. But based on the results of the bacterial isolates from HR test 82 there are 8 isolates positive to isolate the HR that could not be applied to land and crop. Isolate not used was SBS2.E.7, SBS2.E.16, SBS2.E.19, SBS2.E.20, SBS2.E.21, SBS2.E.24, SBS2.E.39 and SBS2.E.45. Comes down to about 74 isolates bacteria will be the ability produce IAA.

**Ability Endophytic Bacteria in Producing IAA**

The ability endophytic bacteria in producing IAA varied based on the type of isolate, as shown on a chart below. The distribution of the bacteria endophytic in producing IAA can be seen on a Graph 1.
Based on Graph 1 show that 74 isolate endophytic bacteria from West Sumatra capable of producing IAA 0,30ppm - 3,65ppm. The IAA is variated and will be treating to palm oil seed because every plant needs IAA according with their need.

Endophytic bacteria can be isolated from the surface plant tissue sterile or extracted from the plant tissue part in. Specifically, bacteria in to the network through a network that germinates, roots, stomata and the damaged tissue (Zinniel et al, 2002). The synthesis IAA by microbes depends on the path of tryptophan where tryptophan used as precursor and a network of plant taxonomy diverse and metabolic different. Some bacteria endophytic potentially to synthesize IAA to raise or stimulate the growth during the colonization with endophytic (Shi et al., 2009).

**Effect of IAA Producing Endophytic Bacteria Indegenous on Plant Growth**

The ability endophytic bacteria in producing IAA influence growth of palm oil. Eighty Different isolates are has been found seven different isolates are affecting the plant’s growth in the field. Base of statistical analysis and further testing Honestly Significance Difference to a level of five percent that a treatment given height and number leave of plant higher than other treatmen.
Table 3. Height and number leave of palm oil seed age 3 month

| Treatment | Height plant (cm) | Number of leave (leave) |
|-----------|------------------|-------------------------|
| SBS1.E.1  | 15.77ab          | 3.33abc                 |
| SBS1.E.2  | 14.57ab          | 3.33abc                 |
| SBS1.E.3  | 14.83ab          | 3.00bc                  |
| SBS1.E.4  | 16.13ab          | 3.00bc                  |
| SBS1.E.5  | 14.53ab          | 3.33abc                 |
| SBS1.E.6  | 12.93ab          | 3.00bc                  |
| SBS1.E.7  | 12.30ab          | 3.00bc                  |
| SBS1.E.8  | 17.40ab          | 3.00bc                  |
| SBS1.E.9  | 13.43ab          | 3.00bc                  |
| SBS1.E.10 | 11.90ab          | 3.00bc                  |
| SBS1.E.11 | 11.17ab          | 3.00bc                  |
| SBS1.E.12 | 11.77ab          | 3.00bc                  |
| SBS1.E.13 | 12.73ab          | 3.33abc                 |
| SBS1.E.14 | 13.37ab          | 3.00bc                  |
| SBS1.E.15 | 14.10ab          | 3.00bc                  |
| SBS1.E.16 | 12.63ab          | 3.33abc                 |
| SBS1.E.17 | 12.50ab          | 3.00bc                  |
| SBS1.E.18 | 12.77ab          | 3.33abc                 |
| SBS1.E.19 | 15.00ab          | 3.00bc                  |
| SBS1.E.20 | 15.67ab          | 3.00bc                  |
| SBS1.E.21 | 16.20ab          | 3.00bc                  |
| SBS1.E.22 | 16.93ab          | 3.33abc                 |
| SBS1.E.23 | 17.87ab          | 3.00bc                  |
| SBS1.E.24 | 13.00ab          | 3.00bc                  |
| SBS1.E.25 | 19.83ab          | 3.00bc                  |
| SBS1.E.26 | 9.70b            | 3.00bc                  |
| SBS1.E.27 | 11.77ab          | 3.00bc                  |
| SBS1.E.28 | 14.87ab          | 3.00bc                  |
| SBS1.E.29 | 16.97ab          | 3.00bc                  |
| SBS1.E.30 | 13.43ab          | 3.00bc                  |
| SBS1.E.31 | 13.60ab          | 3.33abc                 |
| SBS1.E.32 | 15.17ab          | 3.33abc                 |
| SBS1.E.33 | 19.83ab          | 3.33abc                 |
| SBS1.E.34 | 19.23ab          | 3.00bc                  |
| SBS1.E.35 | 12.57ab          | 3.33abc                 |
| SBS1.E.36 | 15.60ab          | 3.00bc                  |
| SBS1.E.37 | 16.00ab          | 3.33abc                 |
| SBS2.E.1  | 12.53ab          | 3.00bc                  |
| SBS2.E.2  | 16.40ab          | 3.00bc                  |
| SBS2.E.3  | 11.27ab          | 3.33abc                 |
| SBS2.E.4  | 19.33ab          | 3.33abc                 |
| SBS2.E.5  | 21.90a           | 4.00ab                  |
| SBS2.E.6  | 16.47ab          | 3.33abc                 |
| SBS2.E.8  | 18.37ab          | 3.33abc                 |
Table 3 described the isolates of endophytic bacteria that significantly improved the seed palm oil growth. This phenomenon can be attributed to ability of the isolates to produces IAA, as IAA positively influences height plant and number of leave. The best isolates is SBS2.E.34, the isolate givent heigt plant 21.93 cm and number of leave 4.33. Isolates endophytic bacteria SBS2.E.34, had the abilty to produce IAA 0, 48 ppm IAA. The IAA in plants must in accordance with their needs plant, according to Campbell et al., (2002) that highly concentration IAA caused in other plants synthesize substances growing the ethylene who work against the function IAA.

| Isolate   | Height (cm) | Leaves   |
|-----------|-------------|----------|
| SBS2.E.9  | 16.70ab     | 3.00bc   |
| SBS2.E.10 | 21.10ab     | 3.67ab   |
| SBS2.E.11 | 17.80ab     | 3.33abc  |
| SBS2.E.12 | 16.50ab     | 3.00bc   |
| SBS2.E.13 | 16.90ab     | 3.00bc   |
| SBS2.E.14 | 14.57ab     | 3.00bc   |
| SBS2.E.15 | 14.50ab     | 3.00bc   |
| SBS2.E.17 | 18.73ab     | 3.33abc  |
| SBS2.E.18 | 17.97ab     | 3.00bc   |
| SBS2.E.22 | 19.73ab     | 3.00bc   |
| SBS2.E.23 | 21.57ab     | 4.00abc  |
| SBS2.E.25 | 15.27ab     | 3.00bc   |
| SBS2.E.26 | 19.90ab     | 3.00bc   |
| SBS2.E.27 | 17.57ab     | 3.33abc  |
| SBS2.E.28 | 14.83ab     | 3.00bc   |
| SBS2.E.29 | 16.40ab     | 3.00bc   |
| SBS2.E.30 | 16.33ab     | 3.33abc  |
| SBS2.E.31 | 19.27ab     | 3.00bc   |
| SBS2.E.32 | 13.43ab     | 3.33abc  |
| SBS2.E.33 | 16.07ab     | 3.00bc   |
| SBS2.E.34 | 21.93a      | 4.33a    |
| SBS2.E.35 | 17.50ab     | 3.33abc  |
| SBS2.E.36 | 13.60ab     | 3.00bc   |
| SBS2.E.37 | 15.83ab     | 3.33abc  |
| SBS2.E.38 | 19.23ab     | 3.33abc  |
| SBS2.E.40 | 17.77ab     | 3.00bc   |
| SBS2.E.41 | 17.57ab     | 3.33abc  |
| SBS2.E.42 | 15.40ab     | 3.00bc   |
| SBS2.E.43 | 19.00ab     | 3.33abc  |
| SBS2.E.44 | 13.73ab     | 3.00bc   |
| Control   | 13.50ab     | 2.33c    |

CV = 24.78%  CV = 11.19%
HSD = 11.59  HSD = 1.05

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4. Conclusion

It can be concluded from this study that at 74 isolates endophytic bacteria indigenous from root palm oil West Sumatera had ability to produce IAA 0,30 ppm - 3.65ppm. The best treatment is SBS2.E.34. The Isolates endophytic bacteria SBS2.E.34 had the ability to produce IAA 0.48 ppm IAA, given the height plant 21.93 cm and number of leaf 4,33.

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