

Original Research Article

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## Physiological Characters *Bacillus* sp. on Rhizosphere and Roots of Banana (*Musa paradisiaca* L.)

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### ABSTRACT

Banana (*Musa paradisiaca* L.) as famous tropical fruit around the world. The one important and destructive disease of banana is *Fusarium* wilt caused *Fusarium oxysporum* f.sp. *cubense* (*Foc*). *Foc* as soil-borne pathogens and movement of pathogen through the rhizome and water, capable survive in waste crops at the field. The purpose research is to study physiological character of *Bacillus* sp. such as : ability of bacteria produced IAA (*Indole Acetic Acid*), gibberelin (GA3), ability phosphate solubilizing and produce salicylic acid as biological control against *Foc*. The research was conducted from March to November 2018 located in The Agricultural Biotechnology Laboratory, Center of Research Hasanuddin University, Makassar Indonesia. The result was showed *Bacillus* sp. at PAA 2 produced the highest concentration of IAA in the range approximately 3.26 mg l<sup>-1</sup>, followed by PAA 3 (2.67 mg l<sup>-1</sup>). However, PUA and PKT 3 isolates produced the lowest IAA with a value of 1.093 mg l<sup>-1</sup> and 1.037 mg l<sup>-1</sup>. Based on the analysis results was showed that the highest concentration of GA3 at PAA 3 (4.17 mg l<sup>-1</sup>), followed by PBT (4.16 mg l<sup>-1</sup>) and PMT 1 (4.15 mg l<sup>-1</sup>) while the lowest concentration of GA3 was produced by PUA (4.03 mg l<sup>-1</sup>). Based on the results showed that PBT isolates containing *Bacillus* sp. from the soil giving highest number in phosphate solubilizing (10.89 mg l<sup>-1</sup>), followed by PUA 2 (8.96 mg l<sup>-1</sup>) and PMT (8.47 mg l<sup>-1</sup>). The lowest result in phosphate solubilizing showed at PAA 3 (4.19 mg l<sup>-1</sup>). Based on the result, the concentration of *Bacillus* sp. the highest was found in PAA 3 (9.42 mg l<sup>-1</sup>), followed by PMT (7.79 mg l<sup>-1</sup>) and PKT1 (6.13 mg l<sup>-1</sup>). The lowest salicylic acid concentration was shown by PKA 3 (3.39 mg l<sup>-1</sup>). The presence *Bacillus* sp. as endophytic microbe living at rhizosphere and roots of banana giving important information for management *Foc*.

#### Keywords

Rhizosphere, Roots, Banana, IAA, Fusarium wilt, *Bacillus*

#### Article Info

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### Introduction

Banana (*Musa paradisiaca* L.) is famous tropical fruit in Indonesia, as important food in tropical and subtropical areas around the

world. Few years ago in the world wide, banana market opportunities are still large enough for the domestic and foreign markets. Domestic bananas consumption reaches 16 kg per capita per year, which continues

increasing by around 5.96% per year. In addition, Indonesian banana demand from abroad continues increasing especially from the middle East and East Asia (Nurchayani *et al.*, 2018; Hermanto *et al.*, 2013). As the center of banana production, South Sulawesi has opportunity improving local farmers income and community services. At the moment, production bananas in South Sulawesi decreased because presence wilt disease by *Fusarium oxysporum* f.sp. *cubense* (*Foc*) attacked banana plantation. The pathogens causing wilt as important major diseases at banana plantations around the world (Jumjunidang *et al.*, 2005; Pan *et al.*, 2008; Fourie *et al.*, 2009). *Fusarium* is a complex genus of ascomycete fungi that consists of plant pathogens of agricultural relevance. Controlling *Fusarium* infection in crops that leads to substantial yield losses is challenging. These economic losses along with environmental and human health concerns over the usage of chemicals in attaining disease control are shifting focus toward the use of biocontrol agents for effective control of phytopathogenic *Fusarium* spp. (Harper, 1950; Khan *et al.*, 2018; Deltour *et al.*, 2017; Warman and Aitken, 2018).

*Foc* as soil-borne pathogens and movement of pathogen through the rhizome and water, capable survive in waste crops at the field. Management presence of *Foc* is largely restricted to excluding the pathogen from non-infested areas and using resistant cultivars where pathogen is established. *Foc* at banana plantation cause disease in all of banana stage development. The pathogens is very difficult to control, because the complexity of the pathogen (consisting of many races and ability survive in soil). The pathogenic fungi can persist in a period of 40 years without its host plant (Akila *et al.*, 2011). The effective biological, chemical and cultural measures not yet available.

According to Saravanan *et al.*, (2004); Svabava and Lebeda (2005) reported that few species of bacteria such as *Pseudomonas fluorescens* as alternative biological agent control *Foc* by in vitro selection. Pan *et al.*, (2008) state the possibility control *Foc* used endophytic bacterium *Burkholderia cepacia*. This methods will improve plant health and safety environment.

*Bacillus* sp. is a gram-positive bacteria that lives mostly in the soil. *Bacillus* is one genus of antagonistic bacteria which reported able increasing plant resistance to disease. In general, *Bacillus* sp. has the ability forming defensive structure i.e endospores as protector from extreme environmental conditions. In other hand, *Bacillus* sp. able producing antifungal compounds causing swelling of pathogenic fungus hyphae in vitro. This is effective against *Foc* caused wilt of banana (Hadiwiyono *et al.*, 2013).

Plant root as the important plant part contain many of microbe. The high population of microbes living in the root and rhizosphere because they contain many amino acid and sugar as source of nitrogen and carbon needed for microbial development. Plant root also reported containing several types of endophytic bacteria living in plant tissue. Harni *et al.*, (2012) define endophytic bacteria as microbe living or colonize plant internal tissue but do not cause interference with the host. Plant root have the highest population density of endophytic bacteria than stem and leaves. *Bacillus* sp. is a bacteria living in the rhizosphere and plant root with ability as biological agents and control the pathogenic microbial attack on plants. The important indicator use identified potential of *Bacillus* sp. as a biological agent or antagonist is to test their physiological characters (Nel *et al.*, 2006; Kumar *et al.*, 2012; Lin *et al.*, 2014; Sukmadewi *et al.*, 2015; Ploetz, 2015; Anusha *et al.*, 2019). The purpose research is to study

physiological character of *Bacillus* sp. such as: ability of bacteria produced IAA (*Indole Acetic Acid*), gibberelin (GA3), ability phosphate solubilizing and produce salicylic acid as biological control against *Foc*. The useful of the research is providing information about the potential of *Bacillus* sp. in increasing plant resistance against *Foc*.

## **Materials and Methods**

The research was conducted from March to November 2018 located in The Agricultural Biotechnology Laboratory, Center of Research Hasanuddin University, Makassar Indonesia.

### ***Bacillus* sp. isolation from rhizosphere and roots of banana**

Samples were taken from the soil at rhizosphere of healthy banana plants from various varieties produce flowers/ fruits (reproductive phase). Samples from rhizosphere about 200 g from soil depth 20-30 cm. Each soil sample weighed about 1 g, put in test tube, added 10 ml of distilled water mixed with 0.01 M<sub>2</sub>0MgSO<sub>4</sub>.720 M. Dilution was carried out until 10<sup>-5</sup>, by an eppendorf pipette, each solution was taken 100 µl then put in a petridish containing Nutrient Glucose Agar (NGA) as medium for bacteria and flattened using a spatula. The single colony obtained was purified on the same medium.

*Bacillus* sp. also isolated from root of the same banana plant for soil rhizosphere. Bacterial isolation in banana plant roots was carried out based on the method of Handayani *et al.*, (2017) with modification. Approximately 1 g root has been washed thoroughly in running water then sterilized using 70% alcohol for 1 minute. The roots was soaked with 2% NaOCl for 2 minutes, soaked with distilled water three times for 1 minute. Banana root samples was planted on

NGA to determine the presence of microbial contaminants. If within 3 days the root sample remains healthy, the sample was crushed and added 5 ml of sterile distilled water. A total of 1 ml of the root sample solution was put into a test tube containing 9 ml of sterile distilled water was previously mixed with MgSO<sub>4</sub>.7H<sub>2</sub>O 0.01 M, diluted to 10<sup>-5</sup>. Using an eppendorf pipette 100 µl of solution was taken then put in a petridish containing NGA and flattened using a spatula. A single colony of *Bacillus* sp. obtained is transferred and purified using the same growing media.

### **Ability of *Bacillus* sp. produced Indole Acetic Acid (IAA)**

Physiologi characterization of *Bacillus* sp. includes the Indole Acetic Acid (IAA) production test. IAA production was measured using the standard method by culturing *Bacillus* sp. derived from the rhizosphere and roots of various types of bananas. All isolates were added with 0.1 g L-tryptophan then incubated for 48 hours in dark room with a room temperature around 27°C. IAA produced by *Bacillus* sp. filtered using filter paper Whatman No. 1 then centrifuged at 3000 rpm for 30 minutes, added 2 drops of orthophosphate acid and 4 ml of Salkowski reagent, stored in a dark room for 24 hours. It is expected that the color change of the solution to pink indicates the isolates tested was produced IAA. The IAA from *Bacillus* sp. was measured its absorption by using a spectrometer (Spectronic 20) with a wavelength of 530 nm (Jumjunidang *et al.*, 2011). IAA concentrations are measured and compared with IAA standard curves. Regression curves are used observe the absorption of isolates that have been tested. Auxin concentration was measured using the IAA standard curve  $Y = 0.064 x + 0.09$  where the value of R = 0.995 came from the IAA stock solution.

### **Ability of *Bacillus* sp. produced GA3**

Gibberelin acid (GA3) as one of important hormone produced by *Bacillus* sp. based on standard methods (Hadiwiyono *et al.*, 2013). The similar source of *Bacillus* sp. in the IAA test was grown in NA media and incubated at room temperature around 27°C for 7 days. Furthermore, the media contained isolates of *Bacillus* sp. centrifuged at a speed of 8000 rpm for 10 minutes, pipetted as much as 15 ml into a test tube and added 2 ml of zinc acetate. After 2 minutes 2 ml of potassium ferrocyanide was added, then centrifuged at 8000 rpm for 10 minutes. Approximately 5 ml of the supernatant added to 5 ml of 30% HCl, incubated at room temperature 27 ° C for 75 minutes. The absorbency is measured using a UV-VIS spectrophotometer 254 nm. GA3 concentration was measured using a standard GA3 curve with regression  $Y = 0.888 X + 0.441$ ,  $R^2 = 0.921$  made from GA3 stock solution.

### **Ability of *Bacillus* sp. as phosphate solubilizing bacteria**

Ability of *Bacillus* sp. as phosphate solubilizing bacteria was observed according to the method of Kristiawati *et al.*, (2014) using Pikovskaya media. The petridish contains Pikovskaya media added with colony of *Bacillus* sp. to be tested. The media contain *Bacillus* sp. incubated for 3-7 days at room temperature. The ability to solubilize phosphate from the tested isolates was evaluated qualitatively based on the formation of clear zones around bacterial colonies.

### **Ability of *Bacillus* sp. produced salicylic acid**

Analysis of salicylic acid concentration was conducted by method of Gondor *et al.*, (2016). The absorbance was measured at wavelength 278 nm. Salicylic acid stock

solution is made with a concentration of 0.1 mg/ml and subsequently used to make 0.001% salicylic acid stock solution. Salicylic acid measurements were carried out by weighing the soil and roots of each variety of banana plant by 0.50 g, mashed then adding chloroform. After that, added distilled water until 100 ml. The absorbance measurements were carried out with wavelength 278 nm.

### **Data analysis**

Data from observations were analyzed by Combinat Analysis of Variance method in a Randomized Group Design. Data analysis techniques using combined analysis accommodate the influence variety of banana, effect of repetition, treatment and interaction between treatments. Further tests needed if the results analysis of variance indicate the interactions is real, then further tests are carried out on the interaction factors with Duncan's Multiple Range Test. If the interaction is not real then further tests are carried out on the single factor.

### **Results and Discussion**

#### **IAA Concentration from *Bacillus* sp. isolation at banana rhizosphere and roots**

In vitro antagonistic bacterial testing is a common procedure performed in selecting potential strains of bacteria controlling plant diseases, especially the activity of *Bacillus* sp. in suppressing *Foc*. Qualitative IAA results on various isolates of *Bacillus* sp. presented in Table 1:

IAA production from *Bacillus* sp. with incubation period about 24 hours produced different concentrations (Table 1). PAA 2 produced the highest IAA in the range of 3.26 mg<sup>l</sup><sup>-1</sup> and PAA 3 (2.67 mg<sup>l</sup><sup>-1</sup>). However, PUA and PKT 3 produced the lowest IAA with a value of 1.093 mg<sup>l</sup><sup>-1</sup> and 1.037 mg<sup>l</sup><sup>-1</sup>. It is

suspected that difference is influenced by the ability of *Bacillus* sp. derived from different varieties of banana isolates results in converting tryptophan content into IAA. Putra and Giyanto (2014) reported some microbes produce hormones similar to producing by plants: auxin, cytokinins and gibberelin.

Some genera of bacteria can produce auxin or IAA, such as: *Pseudomonas*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Lactobacillus*, *Paenibacillus*, *Enterobacter*, *Serratia* and *Klebsiella*. Sudirman *et al.*, (2011) and Sukmadewi *et al.*, (2015) reported that tryptophan is a precursor has functions for biosynthesis of auxins in microbes and plants. *Bacillus* sp. produce auxin and plants used improve the quality of their growth. The results of the analysis have been carried out indicate the *Bacillus* sp. known as antagonistic bacteria because inhibits the growth of pathogens as well as being endophytic.

*Bacillus* sp. derived from the roots and stems of banana plants, can produce IAA hormones was characterized by the formation of pink and transparent pink. The combination of Fe and sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) as a single reagent can increase sensitivity in determining IAA formation. According to Nurcahyani *et al.*, (2018) and Faridah *et al.*, (2018) reported that bacteria able to produce IAA will turn red when Salkowski reagents are dropped due to interactions between IAA and Fe to form complex compounds [Fe<sub>2</sub>(OH)<sub>2</sub>(IA)<sub>4</sub>].

The color produced by bacterial antagonist isolate *Bacillus* sp. after addition Salkowski reagents are unstable because the color will quickly form and fade. Findings of Faridah *et al.*, (2018) and Pegg *et al.*, (2019) reported that *Bacillus* sp. produced IAA and showed a deep red color, while the pink color is an indication of the high concentration of IAA

produced by them. IAA is an important part of the auxin hormone and very vital part in supporting the process of plant growth. The IAA hormone plays a very important role in plant growth because it plays an important role in the process of cell extension, apical dominance, bud growth, leaf growth/fall and flower formation.

### **GA3 Concentration from *Bacillus* sp.**

The study of plant growth and development is very important because plants are strongly influenced by the availability of phytohormones such as: auxin, gibberelin and cytokinins. Gibberelin produced by plants stimulate growth and development of roots, flowers and fruit. Beside plant, phytohormones also produced from microbes, for example bacteria (Jumjunidang *et al.*, 2011; Sukmadjaja *et al.*, 2013). The results of the GA3 hormone test on *Bacillus* sp. presented in Table 2 below:

Based on the analysis results was showed that the highest concentration of GA3 at PAA 3 (4.17 mg l<sup>-1</sup>), PBT (4.16 mg l<sup>-1</sup>) and PMT 1 (4.15 mg l<sup>-1</sup>), respectively while the lowest concentration of GA3 was produced by PUA (4.03 mg l<sup>-1</sup>). Farida *et al.*, (2018) reported the concentration of GA3 hormone produced by bacteria is still very limited related ability *Bacillus* sp. produced GA3.

In general, gibberelin produced by plants stimulating growth and development of roots, flowers and fruit. However, phytohormones also produced from *Bacillus* sp. (Jumjunidang *et al.*, 2011; Sukmadjaja *et al.*, 2013). PAA 3 isolates showed the highest concentration of GA3 produced by *Bacillus* sp. This isolate recommended as an important source of phytohormone against *Foc* caused wilt disease in banana.

**Table.1** The Indole Acetic Acid (IAA) Concentration from *Bacillus sp.*

Isolate	IAA Concentration (mg <sup>l</sup> <sup>-1</sup> )
PUA 2	21.09
PKT 3	1.04
PBT	2
PKT 1	1.76
PMT	1.8
PMT 1	1.61
PAA 2	3.26
PAA 3	2.67
PKA 3	2.06

PUA = Purple banana (root), PKT = Purple banana (rhizosfer), PBT = Barangan banana (rhizosfer), PMT = Sweet banana (rhizosfer), PAA: Ambon banana (root), PKA: Kepok banana (root)

**Table.2** The GA3 Concentration from *Bacillus sp.*

Isolate	GA3 Concentration (mg <sup>l</sup> <sup>-1</sup> )
PUA 2	4.03
PKT 3	4.1
PBT	4.16
PKT 1	4.09
PMT	4.14
PMT 1	4.15
PAA 2	4.12
PAA 3	4.17
PKA 3	4.08

PUA = Purple banana (root), PKT = Purple banana (rhizosfer), PBT = Barangan banana (rhizosfer), PMT = Sweet banana (rhizosfer), PAA: Ambon banana (root), PKA: Kepok banana (root)

**Table.3** Ability Phosphate Solubilizing from *Bacillus sp.*

Isolate	Ability Phosphate Solubilizing (mg <sup>l</sup> <sup>-1</sup> )
PUA 2	8.96
PKT 3	7.35
PBT	10.89
PKT 1	7.79
PMT	8.47
PMT 1	7.86
PAA 2	7.42
PAA 3	4.19
PKA 3	6.98

PUA = Purple banana (root), PKT = Purple banana (rhizosfer), PBT = Barangan banana (rhizosfer), PMT = Sweet banana (rhizosfer), PAA: Ambon banana (root), PKA: Kepok banana (root)

**Table.4** The Concentration Salicylic Acid from *Bacillus* sp.

Isolate	Concentration of Salicylic Acid (mg <sup>l</sup> <sup>-1</sup> )
PUA 2	4.55
PKT 3	4.06
PBT	5.64
PKT 1	6.13
PMT	7.79
PMT 1	4.26
PAA 2	4.23
PAA 3	9.42
PKA 3	3.39

PUA = Purple banana (root), PKT = Purple banana (rhizosfer), PBT = Barangan banana (rhizosfer), PMT = Sweet banana (rhizosfer), PAA: Ambon banana (root), PKA: Kepok banana (root)

#### Ability *Bacillus* sp. as phosphate solubilizing bacteria

The Table 3 was showed result of *Bacillus* sp. as phosphate solubilizing bacteria at in vitro test:

Based on the results showed that PBT isolates containing *Bacillus* sp. from the soil giving highest number in phosphate solubilizing (10.89 mg<sup>l</sup><sup>-1</sup>), followed by PUA 2 (8.96 mg<sup>l</sup><sup>-1</sup>) and PMT (8.47 mg<sup>l</sup><sup>-1</sup>). The lowest result in phosphate solubilizing showed at PAA 3 (4.19 mg<sup>l</sup><sup>-1</sup>). Kristiawati *et al.*, (2014) reported that bacteria from plant roots or rhizobacteria playing role as phosphate solubilizing bacteria.

They are very important in providing plant nutrition. *Bacillus* sp. secrete various types of organic acids (such as carboxylic acid) and reduce pH especially releasing phosphate bonds such as Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub> in soil. The use of *Bacillus* sp. as an environmentally friendly biofertilizer help reduce applied of expensive phosphate fertilizers. Phosphate solubilizing bacteria such as *Bacillus* sp. improving the efficiency of nitrogen fixation, the availability of Fe, Zn through the production of plant growth promotion hormones.

#### Ability *Bacillus* sp. produced salicylic acid

The result was showed at Table 4 was showed ability of *Bacillus* sp. produced salicylic acid at in vitro test:

Based on the result, the concentration of *Bacillus* sp. the highest was found in PAA 3 (9.42 mg<sup>l</sup><sup>-1</sup>), PMT (7.79 mg<sup>l</sup><sup>-1</sup>) and PKT1 (6.13 mg<sup>l</sup><sup>-1</sup>), respectively. The lowest salicylic acid concentration was shown by PKA 3 (3.39 mg<sup>l</sup><sup>-1</sup>). Harni *et al.*, (2012) and Muslimah *et al.*, (2017) reported that salicylic acid is a phenolic compound playing role in stimulating plant growth especially some physiological activities such as: photosynthesis, nitrate metabolism, ethylene production, flowering, protecting plants from stress biotic and abiotic. Phenolic compounds are aromatic ring compounds contain hydroxyl groups or functional derivatives. Phenolic compounds including secondary metabolites has important role in the regulation of plant growth, phytoalexin as a defense against pathogens and rapidly transplanted from the location or to tissues through phloem. Rhizosphere bacteria are also reported to have the ability to produce salicylic acid is an indicator that induction of the main systemic resistance induced in the root part of the life of *Bacillus* sp. In addition

to increasing salicylic acid levels, several bacterial species can increase plant growth (plant growth promoting rhizobacteria (PGPR) including *Bacillus pumilus*, *B. subtilis*, *B. amyloliquefaciens*, *B. circulans*, *B. megaterium*, *Pseudomonas aeruginosa*, and *P. fluorescens* (*B. fluorescens*).

In conclusion based the findings of research, the conclusion is:

1. *Bacillus* sp. was produced the highest concentration IAA from isolate PAA 2 (3.26 mg<sup>l</sup><sup>-1</sup>) and the lowest PK3 (1.037 mg<sup>l</sup><sup>-1</sup>).
2. *Bacillus* sp. was produced the highest concentration GA3 from isolate PAA 3 (4.17 mg<sup>l</sup><sup>-1</sup>) and the lowest PUA (4.03 mg<sup>l</sup><sup>-1</sup>).
3. *Bacillus* sp. has highest potential in solubilizing phosphate as isolate PBT (10.89 mg<sup>l</sup><sup>-1</sup>) and the lowest from isolate PAA 3 (4.19 mg<sup>l</sup><sup>-1</sup>).
4. *Bacillus* sp. was produced the highest salicylic acid from isolate PAA 3 (9.42 mg<sup>l</sup><sup>-1</sup>) and the lowest from PKA 3 (3.39 mg<sup>l</sup><sup>-1</sup>).

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